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**Overview of the  
DOE/SERI Aquatic  
Species Program  
FY 1986**

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## PREFACE

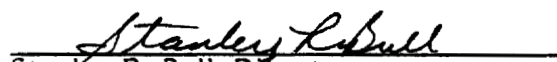
This document contains an overview of the DOE/SERI Aquatic Species Program in FY 1986. This report presents and discusses significant research advances achieved by program participants during the preceding year. The SERI Biofuels Program receives its funding through the Biofuels and Municipal Waste Technology Division of the Department of Energy.



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## SUMMARY

The goal of the Aquatic Species Program is to develop the technology to produce gasoline and diesel fuels from microalgae grown in saline waters of the desert Southwest. Microalgae are known to accumulate lipids in large quantities and can thrive in high salinity water, which currently has no other significant use. Three major task areas are important to the economical development of this technology: biology, engineering, and analysis.

Biological activities include screening, characterizing, and improving microalgae species. In 1982 we began extensive efforts to collect and screen microalgae strains that are salinity and temperature tolerant, highly productive, and that produce large amounts of lipids. More than 3000 microalgae strains have been collected to date. Species used by the program in 1982 had temperature tolerances of 15°-20°C and salinity tolerances of 20-40 mmho cm<sup>-1</sup>. With the intensive collection efforts, the program now has strains that can tolerate wide environmental fluctuations, from 10° to 35°C, and 10 to 70 mmho cm<sup>-1</sup>. Rates of productivity increased from 10-20 g dry wt m<sup>-2</sup> d<sup>-1</sup> in 1982 to greater than 50 g dry wt m<sup>-2</sup> d<sup>-1</sup> under laboratory conditions and more than 35 g dry wt m<sup>-2</sup> d<sup>-1</sup> in outdoor systems in 1986. Lipid content of the algal cells also increased significantly, from 20% in 1982 to 66% indoors and 40% outdoors by 1986. A current problem is that salinity- and temperature-tolerant species do not always have high productivity and produce large amounts of lipid. Therefore, basic research is under way in genetic engineering to put all three characteristics into one or two strains.

Engineering research focused on polymer harvesting of microalgae. All algae were harvestable but required different polymers. Harvesting was accomplished for 0.5¢-1.5¢ kg<sup>-1</sup> dry mass, with removal efficiencies greater than 85%-95%. Cross-flow microfiltration was tested and determined to be too costly. Another method of harvesting examined was flocculation. Recycling flocculants reduced costs by 100%-200%.

We performed a technical and economic analysis of a microalgae fuel production system and published it in the report entitled *Fuels from Microalgae*. The study defines performance requirements to produce gasoline and diesel fuels at prices that will be competitive with conventional fuels. Aggressive research is needed, but the improvements defined are within the bounds of attainability. A major concern has been the availability of saline water resources in the desert Southwest. It has been recently demonstrated, however, that there is sufficient saline water in Arizona and New Mexico to produce at least one quad of energy from microalgae.

Future activities of the Aquatics Species Program include: completing collection activities and focusing on characterizing species collected, consolidating all outdoor test facilities into one large (0.5-1.5 ha) system in a desert region, conducting research on harvesting and carbon dioxide supply, and converting microalgae lipids into liquid fuels. Research will continue on algal physiology, biochemistry, and genetic engineering.

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### 1.0 INTRODUCTION

In 1979, the Department of Energy (DOE) and the Solar Energy Research Institute (SERI) initiated a research program to pursue opportunities for producing liquid fuels from microalgae. Microalgae are unique photosynthetic organisms in that they are known to accumulate storage lipids in great quantities and will thrive in highly saline water. The program is focused on the production of lipids from microalgae because plant storage lipids are an attractive biomass feedstock for producing renewable, high-energy liquid fuels such as gasoline and diesel fuel. The overall process for growing microalgae and converting the lipids they produce into gasoline and diesel fuel is shown in Figure 1-1. The DOE/SERI program emphasizes the development of microalgae systems in the desert Southwest because this area offers flat land, high incident solar radiation, few competing land uses, and large reservoirs of saline water. Locating a mass culture facility in this region minimizes land costs, and using saline water, which is not suitable for agricultural, domestic, or industrial purposes, minimizes competition for the limited supplies of fresh water in the Southwest.

Microalgae can be grown in large outdoor ponds in a desert region, using the resources of sunlight, saline water, nitrogen, phosphorus, and carbon dioxide. Algae can convert these raw materials into proteins, carbohydrates, and lipids and, in the process, double their biomass three to five times a day. After a rapid growth phase, the algae are transferred to induction ponds where nutrient limitation is allowed to occur. Under these conditions, many algae stop growth and division and use all their energy to make lipids as storage products to survive. Once the cells have accumulated lipids, they are harvested and the water is recycled back into the growth ponds. The harvested cells then are subjected to an extraction process to remove the lipids. Algal lipids are primarily triglycerides with fractions of isoprenoids, phospholipids, glycolipids, and hydrocarbons. They 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.

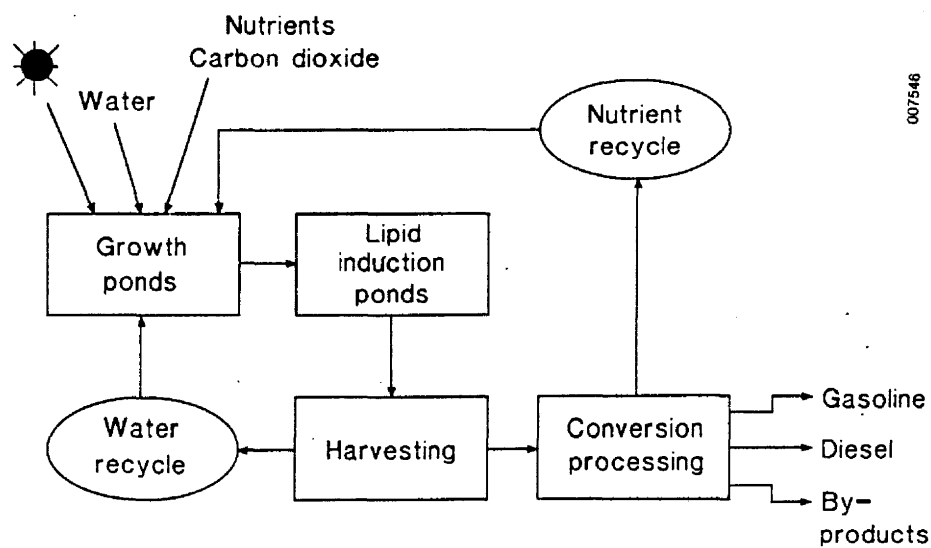


Figure 1-1. Microalgae Growth and Conversion Process

## 2.0 GOAL AND OBJECTIVES

The goal of the program is to provide the technology base for large-scale production of oil-rich microalgae and the conversion methods to convert the microalgae lipids into gasoline and diesel fuels needed for industry and transportation. It is important for the United States to develop alternative renewable oil sources since currently 41% of the energy market in the United States is for liquid fuels, and one-half of these fuels are imported.

To achieve this goal, the objectives of the program are to:

- Collect microalgae strains from many areas within the United States to provide a large number of different organisms for screening, characterization, and improvement;
- Screen microalgae to select for those species that are temperature and salinity tolerant, have high productivities, and are good lipid producers;
- 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 the saline water resources available for raising microalgae in the desert Southwest of the United States;
- Develop technologies for converting microalgae lipids into high-value liquid transportation fuels; and
- Transfer the technology to the private sector for rapid commercialization by involving industry in the research process at the earliest possible time.



### 3.0 RESEARCH AND TECHNOLOGY DEVELOPMENT

To develop the technology base to obtain liquid fuels from microalgae, three research and technology development areas have been identified. These areas and the main research activities in each area are as follows:

- Biology
  - Species Collection and Screening
  - Productivity Improvement
  - Lipid Production, Physiology, and Biochemistry
  - Genetic Engineering
- Engineering
  - Cultivation System Design
  - Harvesting Improvement
- Analysis
  - Technical and Economic Analysis
  - Saline Water Assessments

Approximately half of the research is conducted in-house by SERI, and the other half is subcontracted to universities and small businesses. Table 3-1 lists the FY 1986 budget of \$1.68 million. Biology received approximately 79% of the total budget, engineering was 0% (some engineering research was done in the outdoor culture subcontract), and analysis was 6%. Management received the remaining 15%. In addition, there is an Historically Black College and University Program (HBCU), and these subcontracts were all in the area of biology. A summary of the FY 1986 Aquatic Species Program active subcontracts is given in Table 3-2. This includes projects funded in FY 1985 and FY 1986.

The following sections describe each of these three main research areas and the major accomplishments of FY 1986.

#### 3.1 Biology

The primary focus of the research to date has been in the area of biology. A decision was made that unless it was feasible to obtain organisms that were environmentally tolerant, had high productivity, and produced large amounts of lipids, there was no reason to do large-scale engineering research. Until recently, our major focus has been on collection and screening, and collecting will be completed in FY 1987. Major advances have been made in productivity, and the program is ready to scale up the size of the test production facilities. Most of the major advances in the area of biology were made in lipid biochemistry and physiology, and work in genetic engineering is just beginning.

##### 3.1.1. Species Collection and Screening

The overall objective of collecting and screening is to identify and obtain naturally occurring microalgal strains most suitable for outdoor biomass fuel production. Specific objectives include collecting strains from diverse geographical locales and ecological niches, selecting strains with wide environmental tolerances and high production rates,

Table 3-1. FY 1986 Procurement Plan  
Summary for Aquatic Species  
Program

Task/Projects	Funding (1000\$)
Biological	
Species Selection	629
Improvement	375
Outdoor Mass Culture	205
Engineering	0
Analysis	101
HBCU	125
Management	245
Total	1680

developing rapid techniques for identifying high-lipid-producing strains, and characterizing natural phenotypic variability within microalgae species.

More than 3,000 strains have been collected by the program to date (Figure 3-1). Most of the promising strains collected are Bacillariophyceae (diatoms), Chlorophyceae (green algae), and Eustigmatophyceae (Eustigmatophytes). Collection efforts are concluding this year, and we will thoroughly screen and characterize the collected strains to obtain 10-20 strains by 1990 for intensive species improvement research.

Using innovative collection and screening processes, the program identified several strains that are very tolerant to severe environmental fluctuations in temperature and salinity (Figure 3-2). Strains of microalgae used by the program in 1982 exhibited temperature tolerances of 15°-25°C and salinity tolerances of 20-40 mmho cm<sup>-1</sup>. With the intensive collection efforts nationwide, the program now has strains that can tolerate 10°-35°C and 10-70 mmho cm<sup>-1</sup>.

For each strain that exhibits high growth and lipid production, a temperature/salinity profile is done in three different types of saline water: Type I (high in divalent ions), Type II (high in monovalent ions), and artificial seawater. Each strain may have different growth rates in the different media, and in addition, there may be wide variability between different strains of the same species. Figure 3-3 outlines the response of three strains of *Chaetoceros muelleri* to changes in temperature and salinity. The Utah and

Table 3-2. Aquatic Species FY 1986 Active Subcontracts  
(FY 1985 and FY 1986 Funding)

Title	Contractor	Date of Performance
1. Optimization of outdoor culture	University of Hawaii	5/86 - 2/87
2. Production of liquid fuels and chemicals by microalgae	Microbial Products	3/86 - 2/87
3. Adaptation of microalgal production technology	Ben Gurion University (cost shared 50:50)	6/85 - 5/86
4. Screening and characterizing oleagenous microalgae species from the Southeastern U.S.	Alabama A&M	2/86 - 1/87
5. Characterization of hydrocarbon producing strains of microalgae	Scripps Institution	11/85 - 11/86
6. Ultrastructure evaluation of lipid producing microalgae	Oak Ridge	10/84 - 11/86
7. Collection of high energy yielding strains of saline microalgae from the Hawaiian Islands	University of Hawaii	3/86 - 10/87
8. Genetic variation in high energy yielding microalgae	City College of New York (cost shared 70:30)	3/86 - 5/87
9. Collection of high energy yielding strains of saline microalgae from southwestern states	Arizona State University (cost shared 75:25)	3/86 - 3/87
10. The effects of fluctuating environments on the selection of high yielding microalgae	Georgia Tech (cost-shared 20:80)	3/85 - 2/87
11. Collection and selection of high energy thermophilic strains of microalgae	Montana State University	3/86 - 4/87

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

	Title	Contractor	Date of Performance
12.	Collection of high energy yielding strains of saline microalgae from South Florida	Harbor Branch	5/85 - 6/86
13.	Characterization of photosynthetic efficiency and growth of selected microalgae in dense culture	Martek	9/85 - 2/87
14.	Nutritional requirements for maximal growth of oil-producing microalgae	Jackson State University	9/86 - 9/87
15.	Evaluation of available saline resources in New Mexico for the production of microalgae	New Mexico State University (cost shared 50:50)	8/85 - 9/86
16.	Inventory of sources of available saline waters for microalgae mass culture within Arizona	University of Arizona	7/85 - 9/86
17.	Chrysophrysean lipids: Effects of induction strategy in the quantity and types of lipids	Selma University	9/85 - 2/87
18.	Biochemical elucidation of neutral lipid synthesis in microalgae	Montana State University	1/86 - 10/87
19.	Transformation and somatic cell genetics for the improvement of energy production in microalgae	University of Nebraska	1/86 - 2/87
20.	Biochemical elucidation of neutral lipid synthesis in microalgae	University of Nebraska	1/86 - 2/87
21.	Macroalgal genetics	Neushul Mariculture, Inc. (cost shared - 20:80)	2/86 - 1/87
22.	Cultivation and chemical analysis of microalgal species	Georgia Tech	11/84 - 2/86

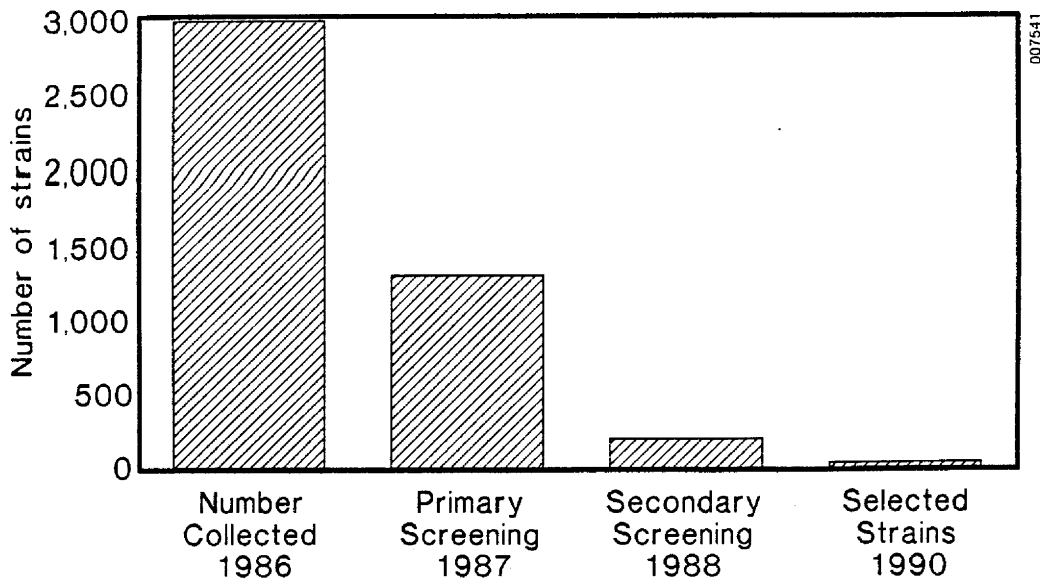


Figure 3-1. Summary of the Strains of Microalgae Collected by the Aquatic Species Program and Future Screening Goals

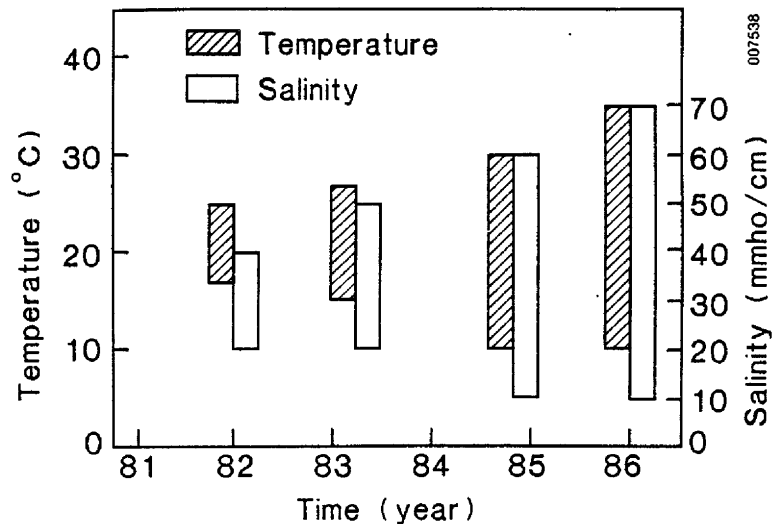
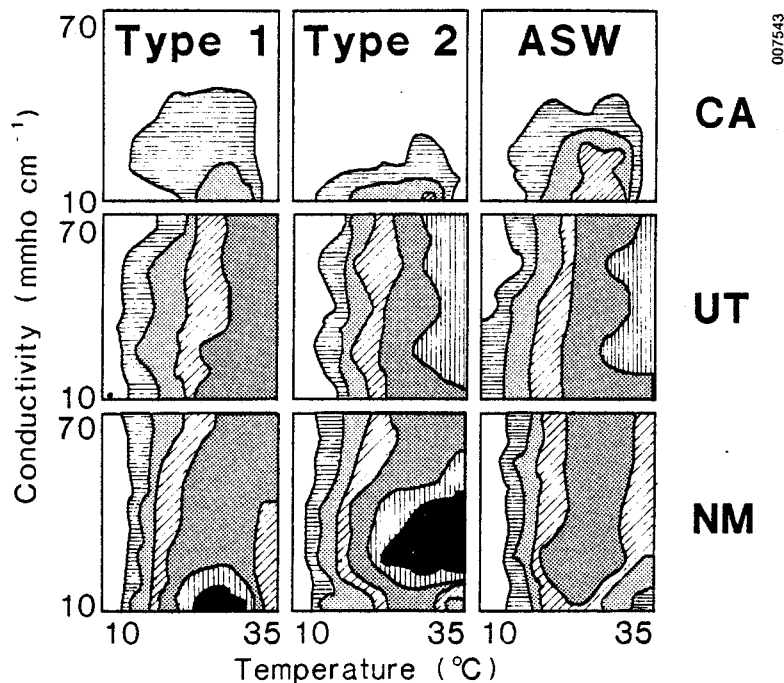


Figure 3-2. Improvements in Temperature and Salinity Tolerances of Strains of Microalgae Collected by the Aquatic Species Program. Each bar represents the maximum tolerance of a strain in the SERI culture collection.



**Figure 3-3. Temperature Salinity Media Effects on Growth of Three Strains of *Chaetoceros muelleri*.** The outer, horizontal, boundary is where growth is greater than 1.0 doublings day<sup>-1</sup>. The highest growth rate, solid area, is 3.5 doublings day<sup>-1</sup>. Increments are in 0.5 doublings day<sup>-1</sup>. Type I water is high in divalent ions, and Type II is high in monovalent ions. ASW = artificial seawater; CA = California strain, UT = Utah strain, and NM = New Mexico strain.

New Mexico strains are very euryhaline and exhibit only small variations in growth response to different media. In contrast, the California strain exhibits a strong stenohaline response and grows poorly.

Developing a quick, inexpensive method to screen for high-lipid-producing microalgae has been a major advance. A Nile red staining technique was developed by researchers at the University of Montana, Oak Ridge National Laboratory, and SERI. Nile red stains the algae but does not kill them. The amount of fluorescence corresponds to the amount of lipid in the cells. Before this technique, a long, expensive (in labor) method of chemical determination was the only way to determine lipid quantity in microalgae. This technique will facilitate a more rapid screening process for microalgal strains.

The SERI Microalgae Culture Collection, established in support of the U.S. Department of Energy's Biofuels and Municipal Waste Technology Division, provides a repository for strains identified or developed for biomass production and makes these strains readily available to the research community. The strains in the collection were selected for their potential in biomass fuel applications, and many produce significant quantities of cellular storage lipids. The 1986-1987 Culture Collection Catalog lists 39 of the program's best strains of microalgae and includes the productivity and physiological data for each strain.

### 3.1.2 Productivity Improvement

The objective of this effort is to increase the rates of productivity of microalgae to enhance yields of energy products. We are conducting research in both laboratory and outdoor cultures to identify species and develop culture management strategies that improve productivity rates.

As a result of this project area, rates of productivity increased from 10-20 g dry wt m<sup>-2</sup> d<sup>-1</sup> in 1982 to greater than 50 g dry wt m<sup>-2</sup> d<sup>-1</sup> under laboratory conditions (Figure 3-4). Sustainable rates of 35 g dry wt m<sup>-2</sup> d<sup>-1</sup> in outdoor systems were achieved in 1986, with short-term optimum reaching 50 g dry wt m<sup>-2</sup> d<sup>-1</sup>. In general, research has suggested diatoms are more stable and have substantially higher productivity in outdoor culture than green algae.

Diatoms and green algae also differ in their ability to grow well at low CO<sub>2</sub> levels. The diatoms required concentrations of almost 100 μM CO<sub>2</sub> and pH below 8.5 to attain maximal, light-limited productivity. The green algae needed only 5 μM CO<sub>2</sub> and pH of 9. However, the maximum productivity of the green algae was only 60% that of the diatoms.

One-, two-, and three-day dilution intervals were evaluated in outdoor culture systems to optimize productivity. A two-day interval resulted in higher productivities than the one-

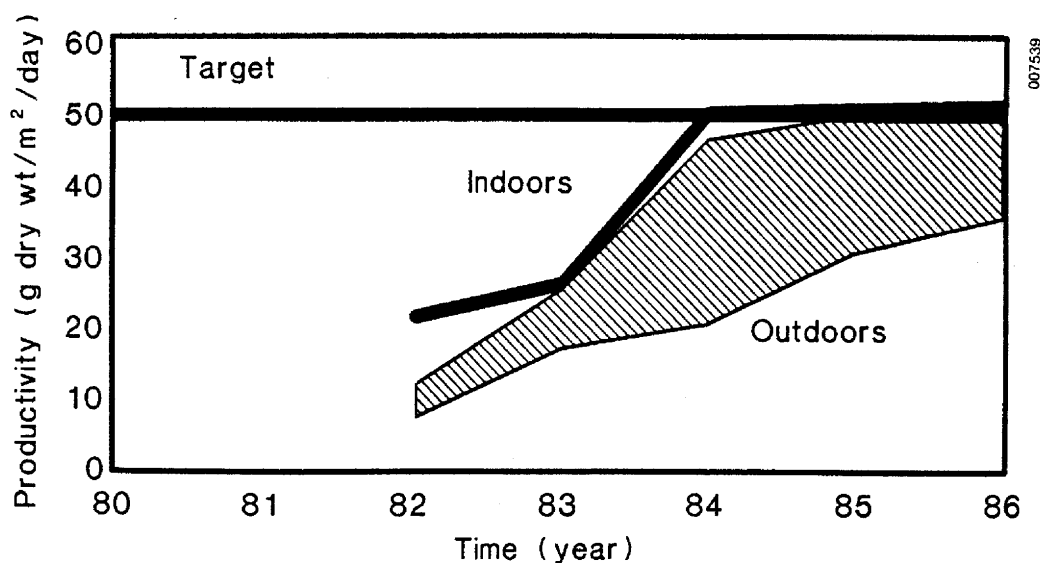


Figure 3-4. Improvements in Productivity of Microalgae Cultures under Indoor and Outdoor Culture Conditions from 1982 to 1986. For outdoor culture productivity the top of the range is the highest productivities obtained by SERI subcontractors while the bottom of the range is the productivities we can consistently get and sustain for long periods of time. The target of 50 g dry wt m<sup>-2</sup> d<sup>-1</sup> was defined by the microalgae technical and economic analysis.

and three-day intervals. Growth rates were also significantly increased when initial culture density was 5 to 400 g AFDW m<sup>-3</sup>, and the growth rates decreased rapidly at higher initial culture densities.

When mixing foils were tested in shallow raceway systems as a mechanism to increase productivity, a significant enhancement was observed, from 20 to 28 g m<sup>-2</sup> d<sup>-1</sup>. However, an independent economic analysis found that the energy cost associated with the foils and the capital cost of the foils exceeded the value of the increased production by a factor of 7.4 with the present system design. Thus, the cost effectiveness of using foils to increase productivity for microalgae does not look promising at present.

### 3.1.3 Lipid Production, Physiology, and Biochemistry

It is in the areas of lipid production, physiology, and biochemistry that the greatest advances were made in 1986. Figure 3-5 shows the significant increases in lipid content of algal cells from 20% in 1982 to 66% indoors and 40% outdoors by 1986.

Researchers examined the effects of environmental and nutrient stress on lipid induction in microalgae. Nitrogen, silica, temperature, pH, inorganic carbon (CO<sub>2</sub> and bicarbonate), light intensity, and salinity were all evaluated for their effects on lipid induction. Under silica limitation, diatoms exhibited increasing lipid yield (mg/L) with increasing bicarbonate concentration, and lipid production increased with increasing light intensity up to 1000 μE m<sup>-2</sup> s<sup>-1</sup>.

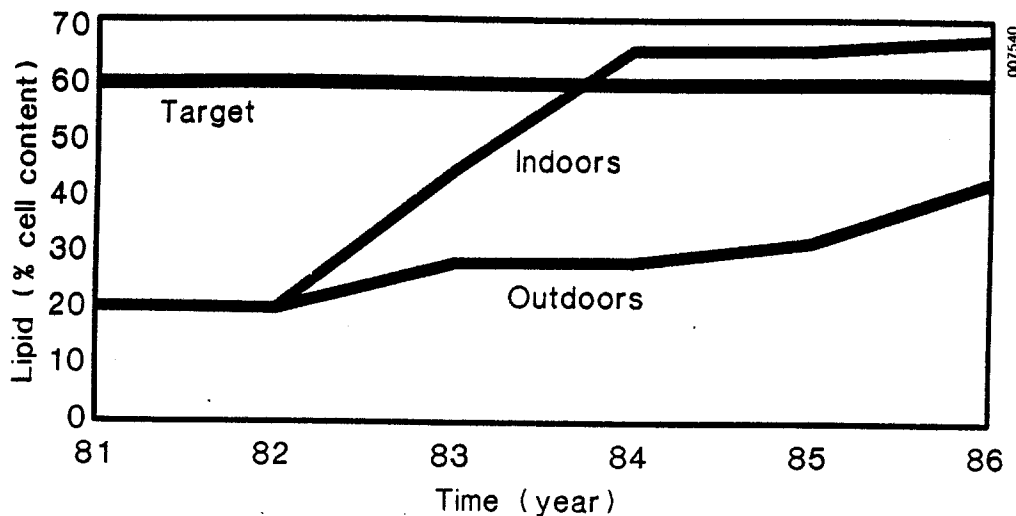


Figure 3-5. Improvements in Lipid Content of Microalgae Cells Grown under Indoor and Outdoor Conditions from 1981 to 1986. The target of 50% of cell content was defined by the microalgae technical and economic analysis.



Nile red, a fluorescent dye, was adapted with success to semiquantitatively measure the neutral lipid content in microalgae. The procedure was developed on a Turner 111 fluorometer and made available to all researchers. The method involves adding Nile red to algal cell suspensions and measuring the resulting fluorescence. This method has a great advantage over the traditional Bligh-Dyer method in that it requires only a small sample size and can be done in less than 5 min. Correlation between gravimetric lipid measurements and Nile red fluorescence was high.

Lipid accumulation in microalgae was investigated with flow cytometry (FCM) and transmission electron microscopy (TEM). Previous studies using batch cultures of algae led to the assumption that lipid accumulation in microalgae is a gradual process requiring at least several days for completion. However, FCM revealed, through changes in the chlorophyll:lipid ratio, that the time span required for individual cells to change metabolic state is short. Simultaneous FCM measurements of chlorophyll and Nile red fluorescence in individual cells of nitrogen-deficient populations revealed a bimodal population distribution as one stage in the lipid accumulation process. The fact that two discrete populations exist, with few cells in an intermediate stage, suggests rapid response to a lipid trigger. Interpretations of light and electron microscopic observations are consistent with this hypothesis. The time required for an entire population to achieve maximum lipid content is considerably longer than that required for a single cell, due to the variation in response time among cells. Cultures exhibiting enhanced lipid content could be obtained by using FCM to separate high lipid cells from the remainder of the population.

Improving lipid yields in microalgae requires an understanding of the physiological and biochemical basis for partitioning photosynthetically fixed  $\text{CO}_2$  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  $\text{CO}_2$  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, biochemical and genetic engineering techniques can be used to increase the lipid yield of promising algal strains.

#### **3.1.4 Genetic Engineering**

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 began on developing genetic engineering methods so that by the time the program reduces its strains to the best 10 to 20, by 1990, the methods to genetically modify these organisms will be available.

We are working in three areas of genetic engineering research: 1) classical genetic manipulation methods, 2) intraspecific genetic variability, and 3) vector and protoplast methodology. Each research area provides different parts of the total knowledge that we will need to genetically engineer a better organism.

The classical genetic manipulation methods include studying the heritability of morphological, physiological, and biochemical traits and using higher plant mutagens to reproduce a superior organism. Some of the work to date has focused on developing these methods for algae using gametes of the macroalgae, *Macrocystis*. These techniques are now being extrapolated for use with microalgae.

Genetic banding patterns have been identified for two species of green algae. These bands can be used as genetic markers. This preliminary genetic work, examining intra-specific variations within the species, is important for future genetic manipulation. Using these results, we can perform selective breeding experiments to improve productivity and lipid yield. These results are also the preliminary data needed to find genetic markers, which can be used in genetic engineering for strain improvements.

More than 50 algal viruses have been isolated as potential vectors for algal recombinant DNA work as a mechanism to introduce DNA from one species into another (Figure 3-6) (27,28). To date, none of the viruses isolated will replicate within algae used by the Aquatic Species Program. Protoplasts have been formed in one species of green algae, and further studies are under way to characterize the enzyme preparations that degrade the cell wall. Fusion studies of the protoplasts will be conducted in 1987. In addition, a cloning project has been initiated that will, when it is finished, result in a restriction map of the chloroplast genome. This map is a major step to be completed before we can begin to genetically engineer the recombinant DNA material.

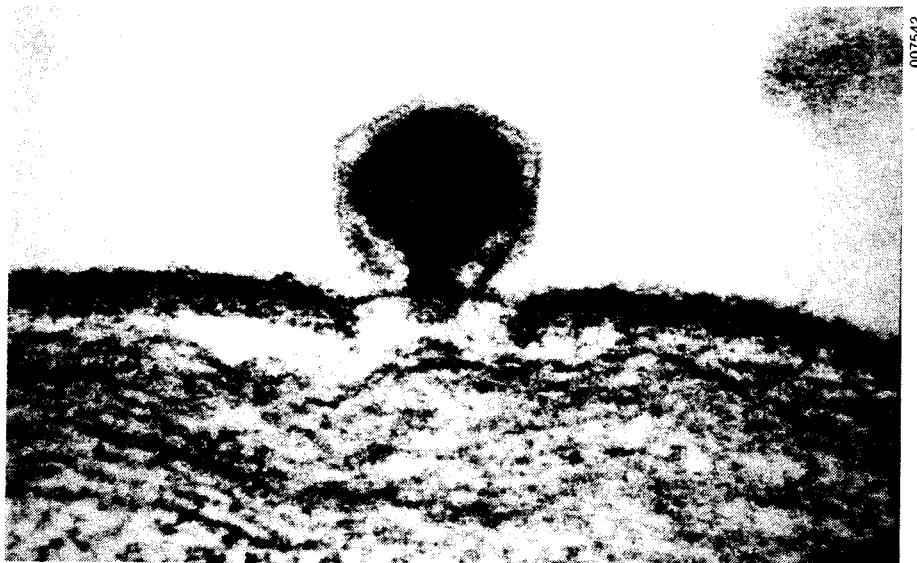


Figure 3-6. Viral Vector Releasing its DNA into an Algal Cell

### 3.2 Engineering

Engineering research has focused on harvesting microalgae. Technical feasibility and cost analyses were completed for polymer harvesting of saline microalgae. All microalgae tested were harvestable, but different algae required different polymers. The amount of polymer increases as the requirement for clarification is made 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<sup>-1</sup> dry mass, with removal efficiencies of 85%-95%. Polymers with higher rigid backbones are less affected by the salt concentration and are thus recommended as flocculants of microalgae in saline water. A cross-flow microfiltration process was tested as a harvesting method and determined to be too costly for use when considering energy production from microalgae.

Another method of harvesting examined was chemical flocculation. Flocculant dose was reduced 75% by recycling the precipitant following flocculation back into the mixing-flocculation chamber. Three flocculation cycles not only reduced flocculant dosage but also were required for a 90% removal of the microalgae from saline water.

### 3.3 Analysis

The two major subtasks in the analysis section are technical and economic analysis and saline water resource assessments. We have made significant progress in both areas.

#### 3.3.1 Technical and Economic Analysis

A technology assessment was performed that demonstrates that gasoline and diesel fuels could be produced from mass-cultured microalgae at prices that will be competitive with conventional fuels. Aggressive research is needed to fulfill the performance requirements defined by the analysis, but the required improvements are within the bounds of attainability and have been closely approached under controlled conditions. Improvements needed are the enhancement of productivity, lipid yield, and salinity and temperature tolerance of microalgae species. Engineering improvements are also needed in the cultivation system design and in harvesting. Two critical resource requirements are the abundance and availability of saline waters in the Southwest deserts and the low cost of carbon dioxide. Based on the achievement of these research goals, demonstrated performance, and availability of economic supplies of critical resources, liquid fuels that are potential direct substitutes for conventional hydrocarbon fuels could be produced from microalgae for \$1.60-\$2.00/gal by 2010.

Analysis of a number of fuel conversion options for microalgal biomass has demonstrated that the promise of microalgae for fuel production is best realized through conversion processes based on cellular lipids, an energy-rich hydrocarbon. The ability to produce lipids that can constitute 60% or more of the total biomass is a distinguishing characteristic of microalgae and makes them uniquely attractive candidates for conversion to liquid fuels. 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 could also be used; e.g., the cell residue after lipid extraction could be anaerobically digested to produce methane and carbon dioxide.

The availability of saline water will be an important factor in determining the ultimate scale of fuel production technology based on this resource. Because of high evaporation rates, water demands for uncovered cultures in this region will be extremely high. Saline

aquifers are found throughout the Southwest, but 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.

Carbon dioxide supply is the largest single contributor to the cost of liquid fuels derived from microalgae. However, the acquisition of sufficient quantities of carbon dioxide should not impose constraints on the ultimate scale of the microalgal fuels technology. Existing and proposed coal-fired power plants will produce carbon dioxide in excess of the quantity required for microalgal production. Competing demands from enhanced oil recovery are not anticipated, since most oil fields in the area are expected to be depleted by the year 2010, the expected date of emergence of an extensive microalgal mass culture technology for liquid fuel production. If methods were developed for the recovery of carbon dioxide previously injected into oil wells, these large reservoirs could supply additional abundant quantities of low-cost carbon dioxide.

The major issues to be resolved in mass culture technology are biological. For this technology to become cost competitive, the biological productivity of these systems must be improved. The production analysis indicates that photosynthetic efficiencies of 12%-16% must be attained, and that 50%-60% by weight of the biomass produced must be in the form of lipids. If higher lipid contents were achieved, lower photosynthetic efficiency would be acceptable, and vice versa.

In addition to displaying improved productivity, species must demonstrate environmental tolerance characteristics that make them suitable for outdoor culture in arid regions. The productivity analysis has identified species salinity tolerance as a particularly significant aspect of environmental tolerance since operation of cultures at high salinities is necessary to control water requirements through minimization of water consumption for blowdown.

The basic engineering needs for microalgal culture have been identified and form the basis of a significant portion of the production analysis. Considerable additional engineering research and development will be required. Important engineering issues are efficient and inexpensive harvesting methods, mechanisms to reduce evaporation, production system design, brine disposal, and carbon dioxide input systems.

The technology improvements mentioned above will reduce the feedstock cost from \$393/ton to \$192/ton (Figure 3-7). Major technology improvements can be made to reduce the costs associated with capital investments, water, labor, and operation. The major cost factor for the final feedstock will be carbon dioxide and nutrients.

### 3.3.2 Saline Water Resource Assessment

Saline water resource assessments were completed for Arizona and New Mexico. Arizona has  $2.8 \times 10^8 \text{ m}^3$  ( $2.3 \times 10^5$  acre-ft) of saline water and New Mexico has  $1.8 \times 10^{13} \text{ m}^3$  ( $15 \times 10^9$  acre-ft) of saline water. The water necessary to produce one quad of energy from microalgal lipids is  $4.7 \times 10^9 \text{ m}^3$  ( $3.8 \times 10^6$  acre-ft). Arizona identified eight areas within the state as suitable for a microalgal production system, and New Mexico identified six areas as potential sites.

#### 3.3.2.1 Arizona

- Saline Surface Water - Of the 34 sources of saline surface water identified, only 8 were judged to have a sufficient volume of water for a project. The total amount of

saline surface water from these eight sources is estimated to be  $4000 \text{ L d}^{-1}$  ( $1.1 \times 10^3 \text{ gal d}^{-1}$ ).

- Saline Ground Water - Nineteen saline ground-water plumes were identified in the six focal areas as being capable of providing greater than  $1.5 \times 10^7 \text{ L d}^{-1}$  ( $4.0 \times 10^6 \text{ gal d}^{-1}$ ). The total water storage is  $7.1 \times 10^{10} \text{ m}^3$  ( $5.8 \times 10^7 \text{ acre-ft}$ ). No information exists to date on the rate of renewal.
- Institutional Considerations - A microalgae production facility constructed in Arizona will require a permit from the Arizona Department of Environmental Quality (after 7/1/87). Prior to the issuance of a permit, it must be demonstrated that operation of a project will not adversely affect the quality of the underlying ground water. If the project is on federal land or federal funds are used, an environmental impact statement will be required.

### 3.3.2.2 New Mexico

- There is  $1.8 \times 10^{13} \text{ m}^3$  ( $15 \times 10^9 \text{ acre-ft}$ ) of unutilized saline water in New Mexico.
- Six basins were identified as having the potential for siting a microalgae production facility: Tularosa, Estancia, Crow Flats, Roswell East, San Juan, and Tucumcari. Estancia, Tularosa, and Crow Flats basins were chosen as the best three sites.
  - Estancia Basin - An area of  $274 \text{ km}^2$  ( $170 \text{ mi}^2$ ) is well suited for a microalgae production facility. There is  $3.9 \times 10^8 \text{ m}^3$  ( $3.2 \times 10^5 \text{ acre-ft}$ ) of saline water available at this site.
  - Tularosa Basin - An area of  $185 \text{ km}^2$  ( $115 \text{ mi}^2$ ) is the second-best site for the location of a microalgae facility. There is  $7.4 \times 10^9 \text{ m}^3$  ( $6.0 \times 10^6 \text{ acre-ft}$ ) of saline water available at the site.
  - Crow Flats Basin - An area of  $58 \text{ km}^2$  ( $36 \text{ mi}^2$ ) is the third best site in New Mexico for the location of a microalgae test facility. Well yields in the area are greater than  $3785 \text{ L min}^{-1}$  ( $1000 \text{ gal min}^{-1}$ ).

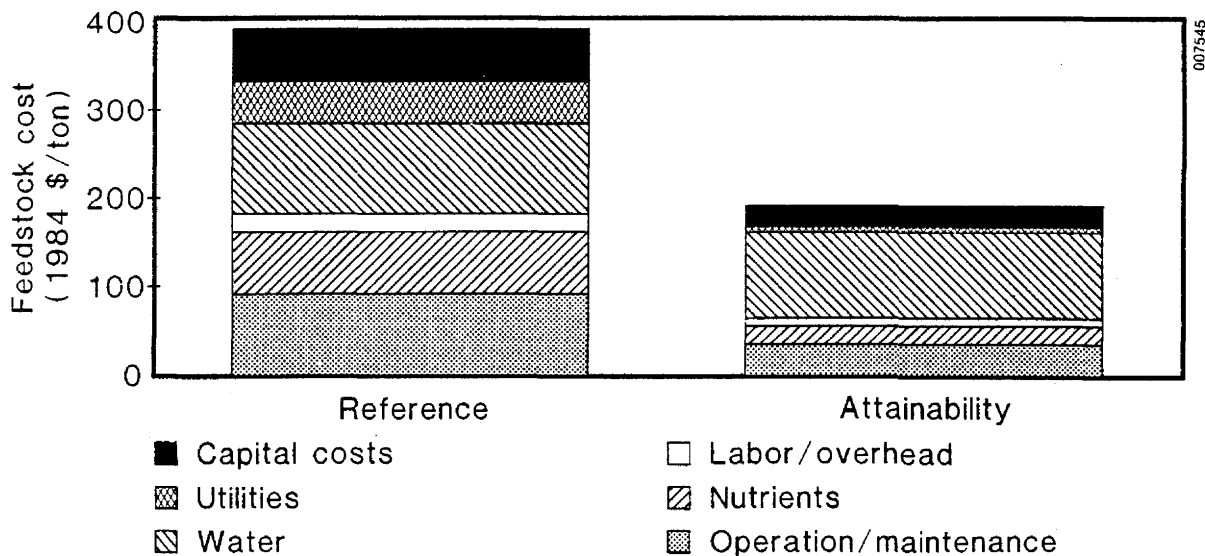
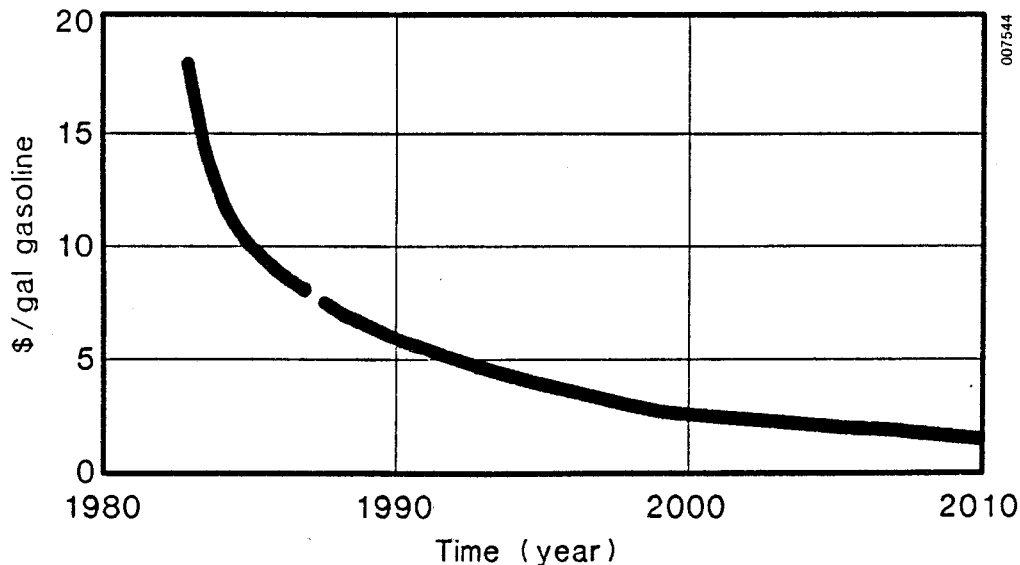


Figure 3-7. Major Cost Drivers to Produce Microalgae as a Feedstock

### 4.0 FUTURE ACTIVITIES

Rapid improvements have been made since the inception of the program in finding suitable species to produce fuels from microalgae and in improving production technologies. The net result of this research to date has been to drastically reduce the price of gasoline derived from microalgae (Figure 4-1). However, many more developments are needed in the technology in the upcoming years to reduce the price of gasoline from microalgae so it will be competitive with fossil fuels.

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 lipid 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 outdoor test facility. Samples from a number of promising species should be included, since there are strong indications that the characteristics of lipids vary widely between taxa.



**Figure 4-1.** Current and Projected Costs to Produce Gasoline from Microalgae. The first part of the curve (1982-1986) is the cost decrease obtained from improvements to date. The second part of the curve (1986-2010) shows the projected cost decreases as the technology improves.

In addition to these new activities, technology development will continue in the areas of species screening and characterizing lipid production, physiology, and biochemistry, productivity, and genetic engineering. The identification or development of microalgal strains that will meet the performance criteria of high productivity, high lipid content, and wide ranges of environmental tolerance is the single most critical research requirement for the economic viability of a microalgal fuels technology. The ability to meet these requirements must first be established in closely controlled experimental cultures, then confirmed under conditions that more closely approximate outdoor mass culture conditions. The ultimate success of the fuels-from-microalgae concept is critically dependent on the rate and degree of species improvement.

## 5.0 FY 1986 PUBLICATIONS AND PRESENTATIONS

### 5.1 Journal Articles and Abstracts

Barclay, W. R., N. Nagle, K. Terry, J. Weissman, and G. Goebel, "The Potential of New Strains of Marine and Inland Saline-Adapted Microalgae for Aquaculture Implications," *Journal of the World Aquaculture Society*, in review.

Barclay, W. R., and R. P. McIntosh, Eds., *Algal Biomass Technologies: An Interdisciplinary Perspective*, Nova Hedwigia, J. Cramer, Berlin 273 pp., 1986.

Barclay, W. R., J. Kennish, V. Goodrich, and R. Fall, "High Levels of Phenolic Compound in *Prochloron* sp.," *Phytochemistry*, in press.

Cooksey, K. E., S. A. Williams, and P. R. Callis, "Nile Red, a Fluorophor Useful in Assessing the Relative Lipid Content of Single Cells," *Biochemistry, Structure and Function of Plant Lipids*, Plenum Press, N.Y., in press.

Laws, E. A., S. Taguchi, J. Hirata, and L. Pang, "High Algal Production Rates Achieved in a Shallow Outdoor Flume," *Biotechnology & Bioengineering* 28:191-197.

Laws, E.A., S. Taguchi, J. Hirata, and L. Pang, "Continued Studies of High Algal Productivities in a Shallow Flume," *Biomass*, in press.

Neushul, M., "Macrocystis Genetics," *Aquaculture*, in press.

Neushul, M., and B. M. W. Harger, "Nearshore Kelp Cultivation, Yield and Genetics," Chapter 4 of *Biomass Production*, Elsevier Publishing Company, in press.

Owens, T. G., J. Gallagher, and R. S. Alberte, "Photosynthetic Light-Harvesting Function of Violaxanthin in *Nannochloropsis* spp. (Eustigmatophyceae)," *Journal of Phycology*, in press.

Roessler, P. G., "UDP-Glucose Pyrophosphorylase Activity in the Diatom *Cyclotella Cryptica*: Pathway of Chrysolaminarin Biosynthesis," *Plant Physiology*, in review.

Roessler, P. G., "Lipid Accumulation in Silicon-Deficient Diatoms," in *Plant Lipids: Biochemistry, Structure and Functions*, P. F. Stumpf, J. B. Mudd, and W. D. Nes, Eds., Plenum Press, N.Y., in press.

Roessler, P. G., "Characteristics of Abrupt Size Reduction in *Symedra ulna* (Bacillariophycene)," *Journal of Phycology*, in review.

Terry, K., and L. Raymond, "System Design for the Autotrophic Production of Microalgae," *Enzyme and Microbial Technology*, 7:474-487.

Van Etten, J. L., A. M. Schuster, and R. H. Meints, "Viruses of Eukaryotic *Chlorella*-Like Algae," in *Viruses of Fungi and Simple Eukaryotes*, Y. Koltin and M. J. Leibowitz, Eds., Marcel Dekker, Inc., New York, in press.

Van Etten, J. L., Y. Xia, K.E. Narva, and R. H. Meints, "*Chlorella* Algal Viruses," in *Extrachromosomal Elements in Lower Eukaryotes*, Plenum Press, N.Y., in press.



## 5.2 Technical Reports

Arad, S., (Ed.), "Development of Outdoor Raceway Culture Technologies for Oil-Rich Halotolerant Microalgae," Final Report submitted to SERI by Ben Gurion University, Israel.

Carlson, R. D., J. H. Ryther, P. D. Pendoley, P. R. Jensen, and M. Blakeslee, "Collection and Characterization of Saline Microalgae from South Florida," Final Report submitted to SERI by Harbor Branch Foundation, FL.

Lansford, R. R., J. W. Hernandez, and P. Enis, "Evaluation of Available Saline Water Resources in New Mexico for the Production of Microalgae," Final Report submitted to SERI by New Mexico State University.

Laws, E., "Productivity Optimization of Saline Microalgae Grown in Outdoor Mass Culture Systems," Final Report submitted to SERI by University of Hawaii.

Neenan, B., D. Feinberg, A. Hill, R. McIntosh, and K. Terry, *Fuels from Microalgae: Technology Status, Potential, and Research Requirements*, Solar Energy Research Institute, Golden, CO, SERI/SP-231-2550.

Olson, K. L., L. A. Wilson, M. Wallace, and M. D. Osborn, "Utilization of Saline Water Sources in Arizona for Microalgae Production," Final Report submitted to SERI by University of Arizona.

Rhyne, C., "Nutritional Requirements for Maximal Growth of Oil-Producing Microalgae," Final Report submitted to SERI by Jackson State University.

Solomon, J., and R. E. Hand, Jr., "Ultrastructural and Flow Cytometric Analysis of Lipid Accumulation in Microalgae," Final Report submitted to SERI by Oak Ridge National Laboratory.

## 5.3 Presentations

Barclay, W., N. Nagle, and K. Terry, "Environmental Control of Lipid Production in Diatom Cultures," presented at the Phycological Society of America/American Society of Limnology and Oceanography, Kingston, RI, June 22-26, 1986.

Barclay, W., K. Terry, N. Nagle, J. Weissman, and R. Goebel, "New Species of Marine and Inland Saline-Adapted Microalgae," presented at the Annual Meeting of the World Aquaculture Society, Reno, NV, January 1986.

Barclay, W., "Research on Microalgal Cultivation for Biomass Fuel Applications," presented at the University of Nebraska, Lincoln, NE, November 1985.

Benemann, D, D. Tillett, and J. Weissman, "Microalgae as a Source of Liquid Fuels-Economic Analysis and Experimental Status," presented at the Sixth Annual Solar, Biomass and Wind Energy Workshop, Atlanta, GA.

Cooksey, K. E., D. Berglund, B. Cooksey, and L. R. Priscu, "Lipid Synthesis and Straining by Nile Red in an Oleaginous Marine Alga," presented at the Phycological Society of America/American Society of Limnology and Oceanography, Kingston, RI, June 22-26, 1986.

Ellingson, S. and M. Sommerfeld, "Search for Lipid-Yielding Microalgae in Saline Waters of the Southwest," presented at the Arizona-Nevada Academy of Sciences meeting, Glendale, AZ, April 19, 1986.

Gallagher, J. C., and J. Stabile, "Variation Among Strains of *Nannochloropsis* spp.," presented at the Phycological Society of America/American Society of Limnology and Oceanography, Kingston, RI, June 22-26, 1986.

Gallagher, J., J. Lee, J. Stabile, and B. Nathanson, "Inter- and Intraspecific Variation in the diatom species *Amphora*," presented at the American Society for Limnology and Oceanography, New Orleans, LA.

Johansen, J., S. R. Rushforth, and W. R. Barclay, "*Chaetoceros muelleri*: Taxonomy and Physiology of Populations from Inland Brackish Waters of the Western United States," presented at the Phycological Society of America/American Society of Limnology and Oceanography, Kingston, RI, June 22-26, 1986.

Lewin, R.A., and L. Cheng, "Oil Production by Picoplankton," presented at the Phycological Society of America/American Society of Limnology and Oceanography, Kingston, RI, June 22-26, 1986.

Roessler, P.G., "UDP-Glucose Pyrophosphorylase Activity in the Diatom *Cyclotella cryptica*," presented at the American Society of Plant Physiologists, Baton Rouge, LA, June 1986.

Roessler, P. G., "Lipid Accumulation in Silicon-Deficient Diatoms," presented at the Seventh International Symposium on the Metabolism, Function and Structure of Plant Lipids, Davis, CA, July 1986.

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