# DEVELOPMENT OF MICROALGAL SYSTEMS FOR THE PRODUCTION OF LIQUID FUELS

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## ABSTRACT

The Solar Energy Research Institute, funded by the U.S. Department of Energy, is conducting research into the production of fuels from algal biomass. The current focus of the program is on the production of lipid-based oils from lipogenic microalgae. Areas of research emphasis in the program are : species collection and isolation, algal physiology and lipid biochemistry, strain improvement and genetic engineering, and outdoor production and engineering. Since the program was established in 1979, significant improvements have been made in the productivity of microalgal systems, with average productivities now reaching 35-40 g $m^{-2}$ $day^{-1}$. Research into strain improvement and genetic engineering is now commencing to develop strains where high productivities and high lipid yields can be achieved simultaneously.

## INTRODUCTION

Fuels, especially combustible volatile oils (CVO), are derived almost exclusively from fossil deposits. World resources of these oils are being rapidly depleted. Since the world-wide energy shortage of the 1970's, many countries are now investigating alternative approaches to the production of fuels and chemicals from petroleum, with a special interest in renewable resources created by solar energy. Government laboratories in the U.S.A. have concentrated on several promising possibilities for alternative fuels including : fermentation of wood to

alcohol, methane production from anaerobic digestion of organic wastes and photosynthetic production of oil by plants. Oil production by plants is attractive because it can lead to the production of CVO that can be used in conventional engines. In higher plants, oil production is generally confined to the seeds. Production of algae is preferable to that of higher plants because most algal cells are totipotent and their growth can be exponential, with doubling times in the range of 4-48 hr.

In 1979, the U.S. Department of Energy established an Aquatic Species Program under the direction of the Solar Energy Research Institute (SERI) to investigate the potential of producing liquid fuels from algal biomass (1). Since 1982, the primary focus of this program has been on the production of lipid oils from microalgae. Microalgae were selected because potentially they can exhibit very high productivities, from 30-300 metric tons $ha^{-1}$ $yr^{-1}$. They can be grown in desert regions, where solar insolation is abundant and land is generally inexpensive, and can be produced utilizing the high-salinity groundwater generally found in these arid regions. Most importantly, microalgae can accumulate up to 60 % of their ash-free dry weight as lipid oils. These oils can be readily transformed into diesel fuel through transesterification processes, or into gasoline through catalytic procedures (2).

Research in the Aquatic Species Program has been conducted in several areas including : collection and isolation of algal strains ; algal physiology and biochemistry ; genetic engineering and strain improvement ; and engineering and outdoor production. Progress that has been made in each of these areas is described briefly below, along with plans for the future research necessary to bring this technology into commercial reality.

### Species collection and screening

In the early 1980's an intensive, nationwide, collection and screening effort was initiated to isolate and identify naturally occurring microalgal strains suitable for outdoor biomass fuel production. SERI scientists and subcontract researchers collected strains of many species from a variety of geographic locations and ecological niches. New methods were developed for isolating strains with wide ranges of environmental tolerances and high lipid-production potential. As outlined in Table 1, three general types of collecting strategies were employed : (1) regional (e.g. collection of strains from deserts of the southwestern United States) ; (2) taxonomic (e.g. collection of strains of

**Nannochloropsis** spp.) ; and (3) ecological (e.g. collection of strains from very shallow inland saline habitats).

TABLE 1
Summary of the collection strategies employed by SERI scientists and subcontract researchers to isolate lipogenic strains of microalgae for biomass fuel production applications

| Collection strategy | Focus | Reference |
|---|---|---|
| Regional | California & Nevada | (3) |
| | Southwestern Deserts | (4) |
| | Gulf of Mexico | (5) |
| | Hawaiian Islands | (6) |
| Algal group | **Nannochloropsis** ssp. | (7) |
| | Chromophyta | (8) |
| Habitat | Thermal Springs | (9) |
| | Very Shallow, Inland Saline Habitats | (10,11) |

In general, all of the strategies were successful in isolating lipogenic microalgae. A total of over 2000 strains were identified, many of which, under nutrient stress, exhibited lipid contents of over 50 % of their ash-free dry weight [e.g. see (12)]. Additionally, significant progress was made in isolating strains with wide tolerance of temperature and salinity. The tolerances of two strains of **Chaetoceros** are illustrated in Figure 1. In both of these strains growth was independent of salinity at temperatures below 30°C. Excellent growth was exhibited over a range of salinities from one-third to twice that of seawater. Overall progress in the collection and screening research is illustrated in Figure 2. In 1982, when the collection program started, strains were identified which grew well only over a temperature range of 15-25°C and at a salinity near that of seawater. By 1986, strains had been isolated which exhibited excellent growth over a temperature range of 15-35°C, and in salinities from one-third to twice that of seawater. SERI is now developing screening techniques to identify the algae in this subset that produce abundant lipid under conditions of both high temperature (30°C) and high salinity (>55 mmho $cm^{-1}$). These strains will provide the gene pool for future genetic enhancement research.

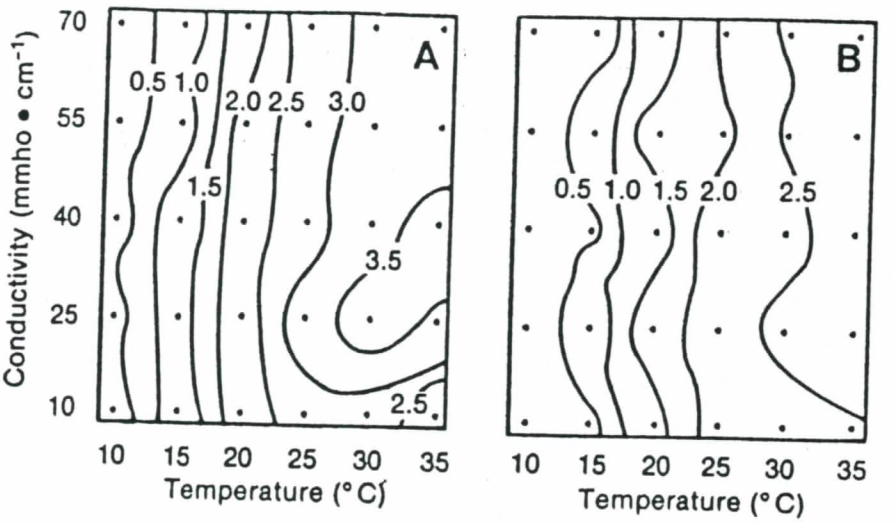


FIGURE 1
Contours of exponential growth rate (doublings $d^{-1}$) of two **Chaetoceros muelleri** strains in semicontinuous culture in Type II inland saline water
A = CHAET9, B = CHAET10, both isolated from saline waters in Utah. Contours are plotted along points representing the means of at least five separate daily growth rate determinations. [For a description of the inland saline water types see (11)].

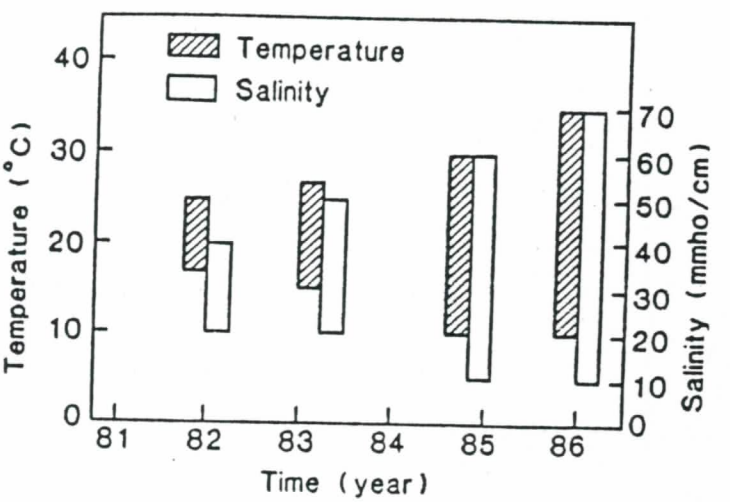


FIGURE 2
Improvements in temperature and salinity tolerances of strains of microalgae collected by the Aquatic Species Program. Each bar represents the maximum tolerance of strains in the SERI microalgal culture collection.

## Algal physiology and lipid biochemistry

Some of the initial physiological research in the Program focused on evaluating the enhancement of photosynthesis in dense cultures through fluctuating light environments. The results indicated that significant enhancements to photosynthesis could be achieved with total cycle periods of one second or more (13). Further research is necessary to facilitate the incorporation of this enhancement into outdoor culture systems.

Current physiological research is centered on providing optimum environmental conditions for the induction of neutral lipid production (14). The environmental variables under investigation include : temperature, conductivity, alkalinity, pH, light intensity, and nitrogen and silica concentrations.

Investigators in the SERI program are conducting research into the biochemistry of lipid synthesis. Research is directed toward identifying enzymes involved in lipid biosynthesis in various microalgal species, and determining the important control points in lipid biosynthesis pathways (Fig. 3) (15-17). Experiments conducted with silicon-deficient cells of **Cyclotella cryptica** (a diatom species that accumulates lipids under non-growing conditions) have indicated that lipid accumulation is due to both increased partitioning of newly photoassimilated carbon into lipids and a slow conversion of non-lipid compounds into lipids (18). There is a concomitant increase in the specific activity of acetyl-CoA carboxylase, which is usually the rate-limiting enzyme of fatty acid biosynthesis . The increase in activity can be blocked by the addition of a protein-synthesis inhibitor (e.g. cycloheximide or actinomycin D), suggesting that silicon deficiency may lead to an increase in the net rate of acetyl-CoA carboxylase synthesis, with possible control at the level of gene transcription. This information, along with results from other biochemical investigations being carried out by SERI researchers and subcontractors, will be used in future attempts to genetically modify microalgae in order to produce strains with enhanced lipid production capabilities.

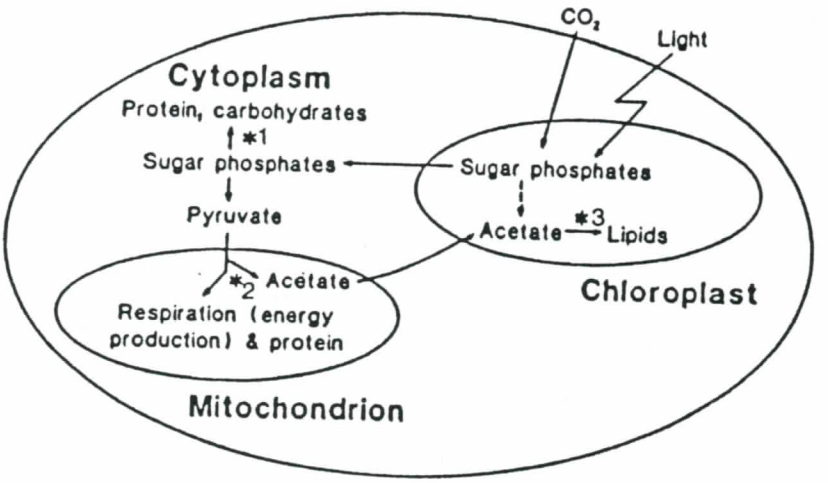


FIGURE 3
Pathways of lipid biosynthesis in microalgae. Asterisks mark portions of the pathways under investigation by SERI researchers and subcontractors.
These include : 1) carbon flow to storage polysaccharides ;
2) loss of fixed carbon to respiration ; and
3) carbon flow to neutral lipids.

### Genetic Engineering and strain improvement

Initial research in this area focused on documenting the degree of strain variability within species of lipogenic microalgae. The results suggested that there is a wide range of genotype of **Amphora** and **Nannochloropsis** (19), and these data laid the foundation for implementation of research into the genetic improvement of lipogenic microalgae. This work, currently initiated in the Program, is focused on three main areas : development of protoplasts and vectors ; strain selection through the use of flow cytometry ; and mutagenesis. In the area of protoplast and vector development, research is under way on the symbiotic **Chlorella** of green hydra. These algae, which are normally infected by a virus, can now be cultured in the laboratory. The viral genome is being characterized with the aim of using the virus as a vector for the transfer of genetically engineered DNA into algae (20). Additionally, attempts are being made to characterize the enzymes the virus employs to penetrate algal cell walls, with the hope of eventually using it to develop protoplasts of algae.

A technique has been developed for the selection of high-lipid cells by using flow cytometry and the fluorescent dye Nile Red (21). Preliminary results

indicate that high-lipid daughter cell populations, sorted by lipid content from parent cultures, are able to exhibit enhanced lipid levels for many cell generations.

### Engineering and outdoor production

Research in outdoor production has been conducted to test the productivities of new strains as they are isolated and developed by the program, and to evaluate new production systems. This research has been conducted in a shallow outdoor raceway system in Hawaii (22) and in pond systems in California and Israel (23, 24). Results from the outdoor testing of new strains indicate that overall productivities have increased from 15-20 g $m^{-2}$ $day^{-1}$ in 1982 to 30-35 g $m^{-2}$ $day^{-1}$ in 1986. This increase in productivity is in part the result of employing new strains with wider salinity and temperature tolerances, capable of sustained growth rates in fluctuating environmental conditions (25).

Research directed towrd evaluating new concepts in outdoor production systems has focused on raceways utilizing a foil array (26) to enhance vertical mixing. However, although average daily production rates of over 40 g ash-free dry weight $m^{-2}$ were achieved in the raceway over periods as long as one month (27), a preliminary economic study has suggested that the cost of the foils may exceed the economic return from increased productivity. In light of these data, outdoor production research is now centered on the design and operation of pond systems. An outdoor test facility is currently under construction in Roswell, New Mexico which will eventually enable the evaluation of algal production under actual southwestern U.S. conditions.

Additional engineering research has focused on mechanisms for harvesting microalgae. Research by Microbial Products, Inc. of Fairfield, California, has indicated that highly charged, synthetic, polycationic polymers can be utilized to agglutinate algal cells and thereby provide a cost-effective process for harvesting algae. Preliminary data suggest that at polymer costs of 0.5-5.0 $kg^{-1}$ dry mass one may achieve removal efficiencies of 85 % - 95 %.

## CONCLUSIONS

The SERI/DOE Aquatic Species Program has been able to make rapid and significant improvements in algal productivity through the application of relatively simple techniques of strain collection and selection. The program is now moving into the next research phase of strain improvement and genetic

engineering. The incremental enhancements in overall lipid productivity may advance more slowly now, since this may require the development of special genetic enhancement techniques. The wide gene pool of strains so far acquired, and the continual feedback developed between laboratory and outdoor production research, provide us with a strong foundation for future progress. We are optimistic that an economic technology can be developed for the production of liquid fuels from lipogenic microalgae.

## ACKNOWLEDGEMENTS

This work was supported by the Biofuels and Waste Technology Division of the U.S. Department of Energy under FTP 513.

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