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FUELS FROM MICROALGAE

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PREFACE

Many species of aquatic plants can provide a source of renewable energy. Some species of microalgae, in particular, produce lipids--oils that can be extracted and converted to a diesel fuel substitute or to gasoline. Since 1979 the Aquatic Species Program element of the Biofuels Program, sponsored by the U.S. Department of Energy's (DOE) Biofuels and Municipal Waste Technology Division and managed by the Solar Energy Research Institute (SERI), has supported fundamental and applied research to develop the technology for using this renewable energy resource.

This document, produced by the Solar Technical Information Program, provides an overview of the DOE/SERI Aquatic Species Program element. Chapter 1 is an introduction to the program and to microalgae. Chapter 2 is an overview of the general principles involved in making fuels from microalgae. It also outlines the technical challenges to producing economic, high-energy transportation fuels. Chapter 3 provides an overview of the Algal Production and Economic Model (APEM). This model was developed by researchers within the program to identify aspects of the process critical to performance with the greatest potential to reduce costs. The analysis using this model has helped direct research sponsored by the program. Finally, Chapter 4 provides an overview of the Aquatic Species Program and describes current research.

SUMMARY

Liquid fuels play a vital part in the economy of the United States--primarily for transportation. In fact, liquid fuels, such as gasoline and diesel fuel, made up 42% of the energy consumed in 1988. About a third of these fuels is derived from imported petroleum, contributing more than $40 billion to the trade deficit. This portion is expected to increase as domestic petroleum production continues to decline. It is clearly in our best interest to develop alternative domestic energy resources to reduce our dependence on foreign oil.

Biomass--plants such as grasses, trees, and aquatic algae--is such an alternative. Aquatic algae, in particular, show promise and form the basis of research supported by the U.S. Department of Energy (DOE). The Aquatic Species Program element, managed by the Solar Energy Research Institute (SERI), was created in 1979 to evaluate the potential of aquatic plants as a renewable energy source. Since 1982, research has investigated microalgae that produce lipids, oils that can be converted to a diesel fuel substitute or to gasoline. Research sponsored by the program has produced exciting results. With continued research and development, this technology could make a significant contribution to our fuel requirements in the next century.

Microalgae, which have been called the most productive biochemical factories in the world, grow very fast, doubling their weight three to five times a day. Species have been found that grow rapidly, produce large quantities of oils, tolerate wide variations in temperature, and can live in saline water. Because of climate and cost considerations, these characteristics make microalgae well-suited for initial deployment in the southwestern United States. The algae would be grown in saline water in large outdoor ponds, making use of the area's high solar radiation and open flat land with few competing uses (i.e., low land costs). Large resources of underground saline water are also available in the region at low cost.

Beside water and sunlight, microalgae need large additions of carbon dioxide to grow and produce lipids in the quantities required for economical liquid fuel production. This requirement provides another advantage to the technology--reducing the atmospheric gases that contribute to global warming. A microalgal facility could be sited next to a power plant or industry that burns fossil fuels and could recycle part of the carbon dioxide from flue gases into liquid fuels. This would decrease the amount of carbon dioxide released into the atmosphere.

The program element supports research at SERI, at universities, at government laboratories, and in industry. Current research is concentrated in six areas:

- Genetic engineering of microalgae for lipid production at high growth rates
- Identification of "trigger points" in biochemical pathways to turn lipid production on and off
- Development of inexpensive, large-scale, outdoor culture technologies to grow microalgae
- Evaluation of resource requirements and environmental impacts of microalgae facilities in the desert Southwest
- Development of technologies for converting microalgal lipids into high-value liquid transportation fuels, particularly diesel fuel
- Transfer of the technology to the private sector for continued development by involving industry at the earliest possible time.

Results of these activities are expected to lead to a technically and economically feasible process in the early part of the next century. Research has already reduced the projected price of diesel derived from microalgae from $18 per gallon in 1983 to less than $7 per gallon today. This is an impressive start. By 2010 we should have the technology that will allow us to produce competitively priced fuel from microalgae.

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CHAPTER 1
INTRODUCTION TO THE AQUATIC SPECIES PROGRAM

The Arab oil embargo of the early 1970s encouraged many nations to look for new sources of energy for heating, electricity, and transportation. One potential source, biomass,* is an attractive option because it is renewable and can provide all the forms of energy we need. Unlike finite deposits of coal, oil, and natural gas, biomass can be produced almost anywhere and under a wide range of environmental conditions. The first biomass sources considered for energy production were wood and agricultural products like corn. But other sources of biomass also have potential for energy production, and research has been initiated to develop these alternatives.

Considered among the most promising of the biomass alternatives are a number of aquatic species. In 1979, the U.S. Department of Energy (DOE) and the Solar Energy Research Institute (SERI) initiated research to investigate the energy potential of aquatic plants. Since 1982, the DOE/SERI Aquatic Species Program element has concentrated on evaluating the energy potential of just one group of aquatic plants, the microalgae.

INTRODUCTION TO MICROALGAE

Microalgae are the most primitive and simply organized members of the plant kingdom. Most algal species exist as single cells in aqueous habitats, but some are organized in simple, usually filamentous, colonies. A few species, the seaweeds, have developed further and have organized their cells in more complex structures resembling the leaves, stems, and roots of higher plants.

Almost 20,000 species of algae have been identified and grouped into divisions. Three of these divisions contain algae that are frequently grown in large-scale cultivation for commercial purposes and are therefore important to the program. Members of one group, the blue-green algae, have a cellular organization similar to primitive organisms like bacteria, whose cells lack a nucleus. Some blue-green algae use atmospheric nitrogen to produce inorganic compounds useful to plants and thus serve an important ecological role.

The remainder of the algae are true plants and have both nuclei and chloroplasts, the subcellular organelles that characterize plants and in which photosynthesis takes place. The green algae (*Chlorophyta*) are among the most common, especially in fresh water, and can occur in single-celled or colonial forms. The golden algae (*Chrysophyta*) have developed more complex pigment systems and appear yellow, brown, or orange. Diatoms are a particularly important group of golden algae that are distinguished by the silica shell that surrounds their cell mass. Unlike the majority of the green algae, which produce carbohydrates in the form of starches as their major storage product, golden algae form lipids. Lipids are a heterogeneous group of organic compounds that includes fatty acids and other vegetable oils. Several species of microalgae are shown in Figure 1-1.

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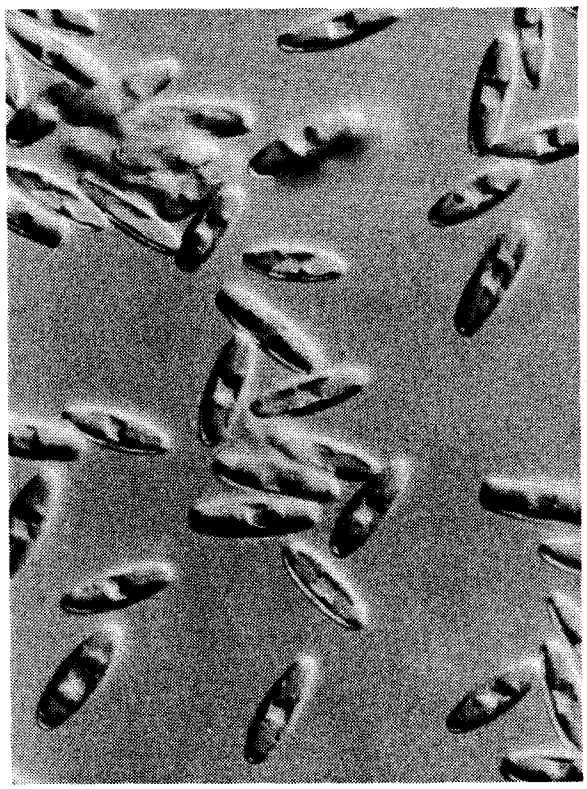
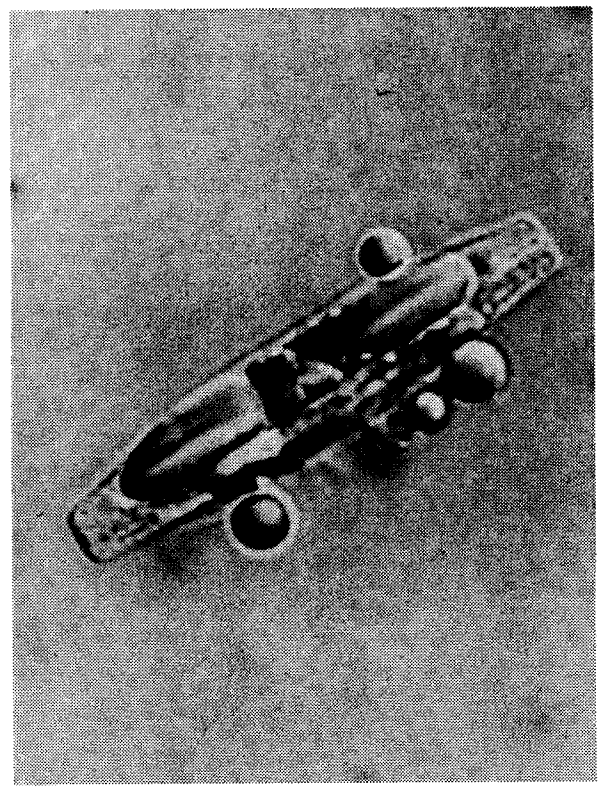
*Biomass is defined as "any organic matter which is available on a renewable basis, including agricultural crops and agricultural wastes and residues, wood and wood wastes and residues, animal wastes, municipal wastes, and aquatic plants."



Navicula (diatom)



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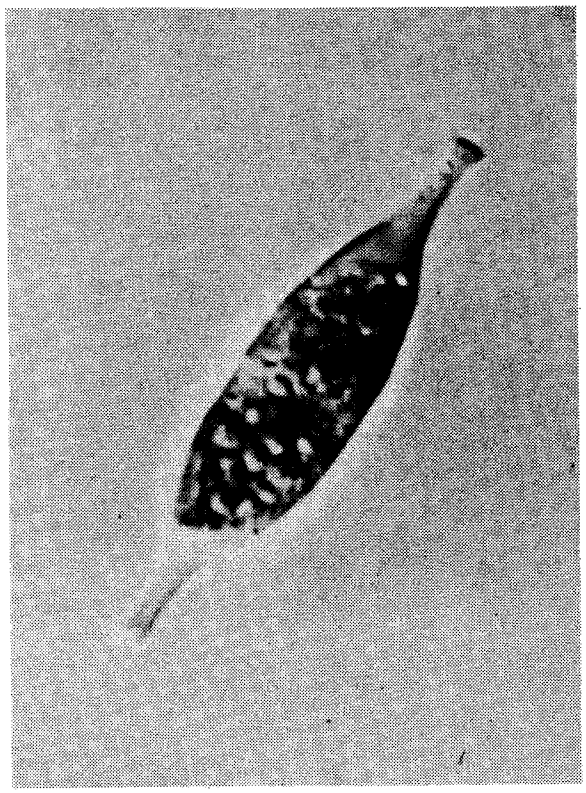

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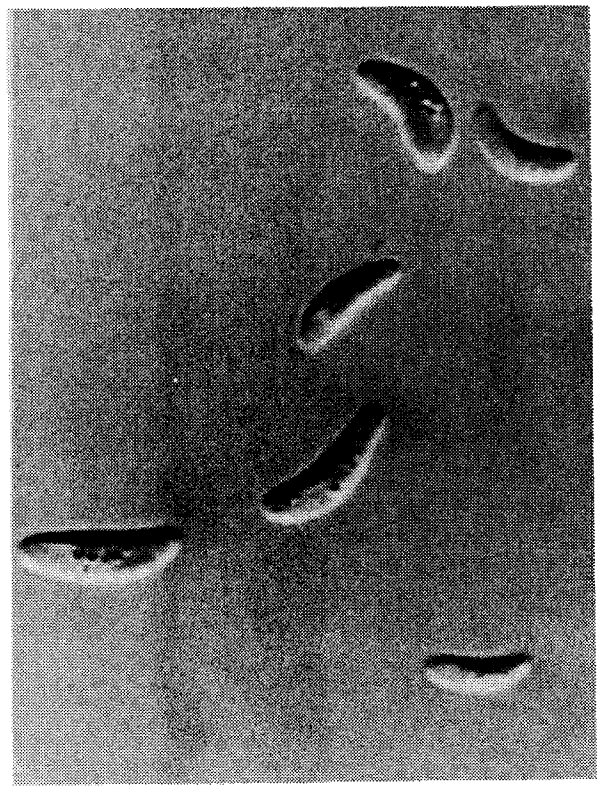

Monoraphidium

Figure 1-1. Oil-producing microalgae

As their name implies, microalgae are usually extremely small, although their size can vary widely. A few single-celled species are more than a quarter of an inch long; other species can be as small as bacteria--less than one one-thousandth of an inch. Most species are typically several thousandths of an inch in size.

The photosynthesis in algae is similar to that found in all plants, but algae are especially effective in converting carbon dioxide (CO_2) and other nutrients into organic compounds because they do not need elaborate support and reproductive structures. Under ideal conditions, microalgae can double their weight three to five times a day.

Historically, microalgae, especially in mass culture, have been seen as a promising source of low-cost protein (Benemann, Tillett, and Weissman, 1987). Research initiated by the Carnegie Institute in the early 1950s was the first to concentrate on growing microalgae in outdoor mass culture for food (Burlew, 1953). These studies provided some of the most comprehensive early reports on algal growth, physiology, and biochemistry, and led to expanded efforts by German and Israeli researchers to produce animal feed protein (Shelef and Soeder, 1980).

Microalgae are being grown in Israel, Australia, Mexico, Taiwan, and the United States for high-value health food and other products. Among these products are the filamentous blue-green alga ***Spirulina*** and the vitamin beta-carotene, obtained from ***Dunaliella salina.*** Several companies are using microalgae as a source of omega-3 fatty acids, a dietary supplement used for preventing heart disease. Blue-green algae are providing pigments for high-value fluorescent dyes. Cultivating microalgae as a soil conditioner and as a food source for shellfish is increasing in importance (Enright et al., 1986; Pipe and Shubert, 1984).

Sanitary engineers have devised the most common commercial use of microalgae as a component of wastewater treatment. Many municipal facilities in the United States use microalgae to break down unwanted organic products from sewage in oxidation ponds. This technique has also been applied to irrigation water that may contain excessive amounts of nutrients and other organic contaminants.

MICROALGAE AS A SOURCE OF ENERGY

Under normal growth conditions microalgae produce carbohydrates, proteins, and lipids. Lipids are similar in structure to many liquid fuels, although they contain more oxygen and are more viscous than crude petroleum. The lipid content of the cell normally ranges from 5%-20% of the total dry weight. This percentage can be greatly increased in certain circumstances. For example, if the cells of some species are starved for certain nutrients they will stop growing and dividing and will transfer most of their energy into producing lipids as storage products for survival. Under these conditions, some strains can accumulate more than 60% of their weight as lipids.

The idea of using microalgae for energy production is not new. Producing methane gas from the carbohydrate portion of the cell was first proposed more than 30 years ago (Meier, 1955). However, the idea of using the lipid fraction of the cell to produce liquid fuels developed more recently. The process follows four basic steps, shown in Figure 1-2. First, large quantities of microalgae are grown in ponds. The microalgae are then transferred to lipid induction ponds where cultural conditions, such as nutrient starvation, encourage lipid production. Next, the cells are harvested and the lipids extracted. Finally, the lipids are converted into high-energy fuels. The two most promising options for lipid conversion are transesterification and catalytic conversion.

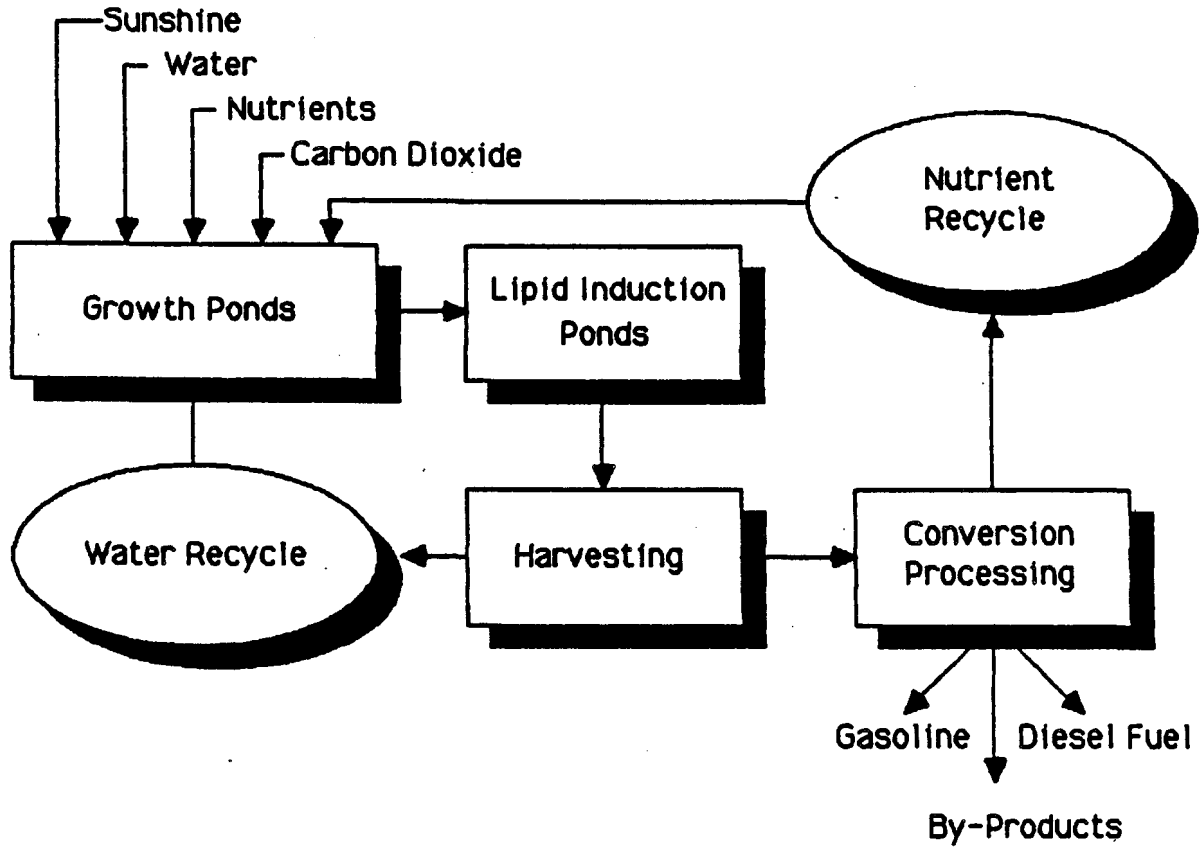

Figure 1-2. Microalgal growth and fuel conversion process

Although the lipids represent the premium energy product, the energy trapped in the remainder of the cell can be used as well. For example, the cell residue can be digested anaerobically to produce methane and CO_2, which can supply part of the energy used by the production facility.

Microalgae offer an advantage as an energy source because they are relatively easy to manage. They grow virtually anywhere and require only light, CO_2, and small amounts of mostly inorganic nutrients. Many algal species grow under harsh environmental conditions. For example, species have been found that can grow in saline water, including saturated brines (Bonin, Maestrini, and Leftly, 1981). In addition, a number of species have adapted to wide temperature ranges. Some thermophilic species can grow in temperatures above 160°F, yet can survive freezing.

The requirement for CO_2 provides another advantage to fuel from microalgae--a way to combat global warming. Scientists are concerned that the buildup of CO_2 in the atmosphere from burning fossil fuels is contributing to a global warming trend known as the greenhouse effect. Mass cultures of microalgae will require large amounts of CO_2. Studies have shown that the CO_2 produced by coal combustion will provide a plentiful resource. A microalgal facility coupled to a fossil fuel plant could provide a renewable liquid fuel and at the same time reduce the amount of CO_2 released into the atmosphere.

THE AQUATIC SPECIES PROGRAM

The goal of the DOE/SERI Aquatic Species Program is to develop the technology to produce liquid fuels from microalgae at prices competitive with conventional fuels. In pursuit of this goal, the program is focused on outdoor cultures of salt-tolerant microalgae that produce large quantities of oil. This focus offers several advantages. First, outdoor cultures reduce the costs of buildings and artificial lighting. Second, salt-tolerant microalgae can use saline water, which has few competing uses. And third, the technology can be located in the desert Southwest of the United States, which has large reservoirs of saline water; large areas of flat, inexpensive land with high solar radiation and suitable year-round temperatures; and significant resources of CO_2, necessary for cell growth.

In order to develop the technology, biological and engineering research is required for each step in the process: growth, lipid production, harvesting and extraction, and conversion (Figure 1-2). However, each step is interrelated and cannot be pursued in isolation. For example, the size and characteristics of the organisms selected for use in the system will necessarily affect the method of harvesting, and a particular extraction technique may affect the success of a subsequent conversion process.

To help integrate these elements and allocate program resources in the most effective manner, the Aquatic Species Program developed the Algal Production and Economic Model (APEM) (Hill, 1984). This computer model represents a series of physical and economic relationships important in cultivating microalgae and assessing the costs of the resulting fuel. It is designed to provide sufficient detail in critical areas of the process to suggest which elements are most important in reducing overall costs. The purpose of running the model is to identify those specific areas of research with the greatest potential for driving the program toward its overall goal. The result of this effort is that time and money are allocated to those technical issues critical for reducing the cost of producing microalgal fuels.

As a result of analysis using the APEM and conclusions of subsequent research, the program now has four primary objectives:

- Improve the yield and efficiency of microalgal-derived liquid fuel production
- Integrate biological concepts with engineering principles to develop a cost-effective microalgal culture technology
- Support technology development through assessments of costs and resources
- Transfer newly developed technology to industry.

In support of these objectives, research has addressed three general areas: optimizing the organism, optimizing the fuel conversion process, and optimizing the system design.

Optimizing the Organism

Before 1982, little research had been conducted on lipid-producing microalgae. Before improving or optimizing an organism for fuel production, it was first necessary to identify organisms from natural habitats whose performance is closest to that desired. In other words, success is more likely if we use the best nature has to offer. Therefore, beginning in 1983, microalgae strains were collected from diverse geographical locations, primarily in the Southwest.

Because the microalgae will be grown in outdoor culture where there can be wide fluctuations in temperature and salinity, the microalgae were screened to determine which grew best under these conditions. Screening and characterizing these strains for their tolerance of high light intensity, their requirements for dissolved oxygen, and their CO_2 utilization efficiency have been an important research priority. These studies have identified several species that can tolerate severe fluctuations in temperature or salinity, reproduce rapidly, or produce lipids in quantity. No single strain with all the desired characteristics has been found.

A computer model has been developed to predict the effects of environmental parameters such as salinity, pH, temperature, and nutrient concentration on lipid production. The model is being used to identify the most important variables. This information will help guide research.

One performance goal of the program is to be able to produce 60% of the total dry weight of algae grown in continuous outdoor culture as lipids. Using selected strains and manipulating culture conditions, lipid content has been increased from 20% in 1982 to 66% in the laboratory and 40% outdoors. To obtain the required performance in outdoor culture, more needs to be known about the lipid biochemistry of the organism. Specifically, the mechanism by which photosynthetically fixed carbon is partitioned into lipids must be understood. Current research is focused on determining the lipid biosynthetic pathways. Once these pathways are understood, scientists can use biochemical and genetic engineering techniques to increase the lipid yields in promising strains.

Because no single strain has been found with all of the desired characteristics, the economic feasibility of using microalgae for fuel production will likely depend on the development of strains that have been genetically altered. The collection of strains provides a gene pool for this work, but the application of classical and molecular genetics to microalgae is a relatively new field. The program is therefore laying the groundwork through research on intraspecific genetic variability and on vector and protoplast fusion methodology. The work on genetic diversity has shown wide genetic variability within a number of microalgal species selected from the screening protocol. This is encouraging because it suggests that future genetic engineering efforts have a good chance of success.

Optimizing the Fuel Conversion Process

Analysis of several fuel processing options showed that two processes were most promising for producing liquid fuels. One involves transesterification of algal lipids to produce diesel fuels; the other involves catalytic conversion to produce gasoline. The first step in either process is the extraction of the oils from the algae.

Research on optimizing the fuel conversion processes began only recently but has produced promising results. Technical feasibility of both processes has been demonstrated. Studies have shown that oils can be extracted from the algae using solvents and then converted very efficiently to a diesel fuel. Similarly, solvent-extracted oils have been converted to gasoline using a zeolite catalyst. This research will continue to address issues of improved performance and scale-up to larger sized systems.

Optimizing the System Design

Biological concepts are integrated with engineering principles by testing laboratory-selected strains outdoors in several different reactor designs and with varied culture techniques. An outdoor test facility was built in 1987 in Roswell, N. Mex.

(Weissman, Tillett, and Goebel, 1987). Two ponds, each 0.25 acre in size, are used to examine the issues associated with operating a microalgal production system at a larger scale. One of the ponds is earth-lined; the other is plastic-lined. These ponds are shown in Figure 1-3.

Experiments at the outdoor test facility are testing several issues related to outdoor culture. Parameters including temperature, depth, salinity, and mixing are examined to determine their effect on productivity and lipid content. Other experiments test harvesting techniques, such as settling or flotation, centrifugation, and filtration. The cost and performance of pond liners are also being studied.

The 0.25-acre ponds are supplemented by a smaller system of ponds, shown in Figure 1-4, used for intermediate tests between the laboratory and the larger ponds. Future plans include the fabrication and operation of a 1-acre pond at Roswell. Additional pond experiments are conducted at the greenhouse at SERI, shown in Figure 1-5.

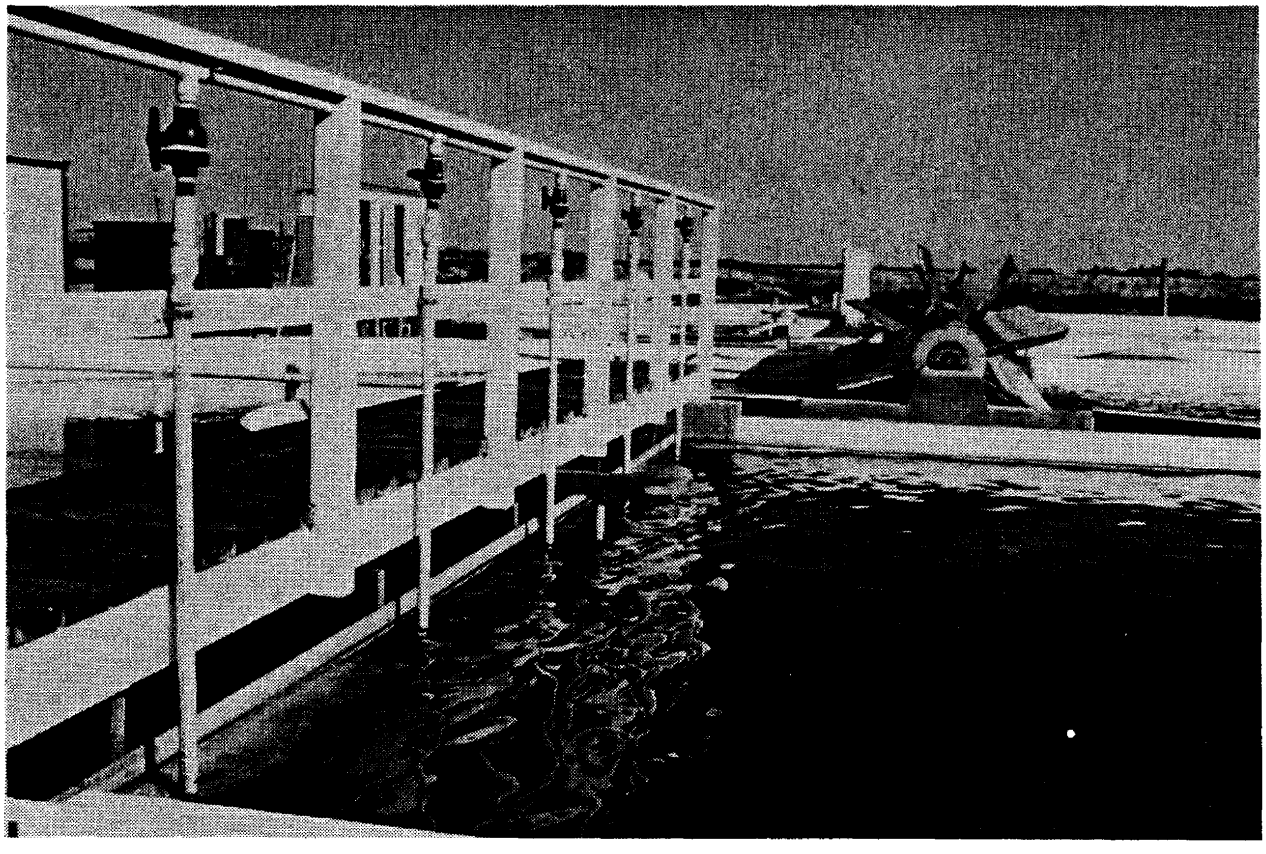

Figure 1-3. 0.25-acre ponds at the outdoor test facility in Roswell, N. Mex.

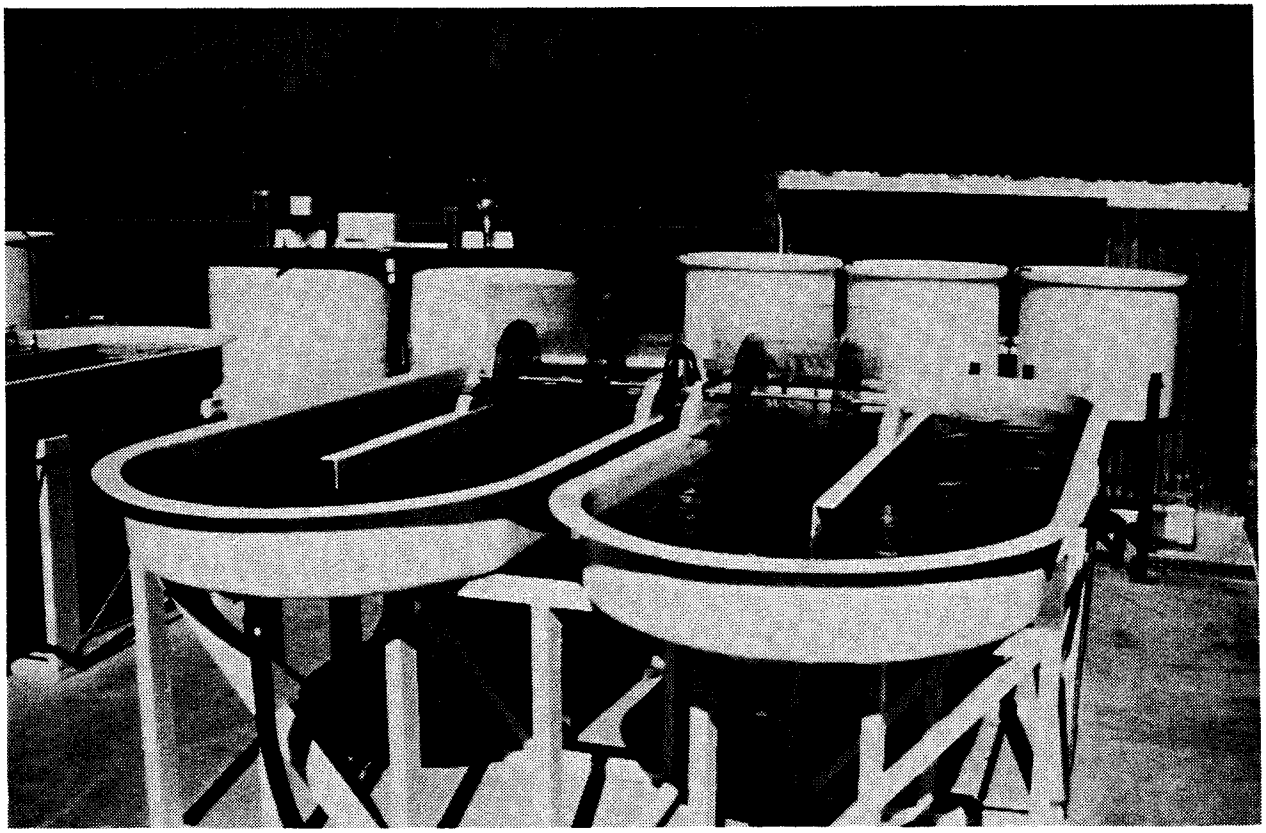

Figure 1-4. Roswell test ponds

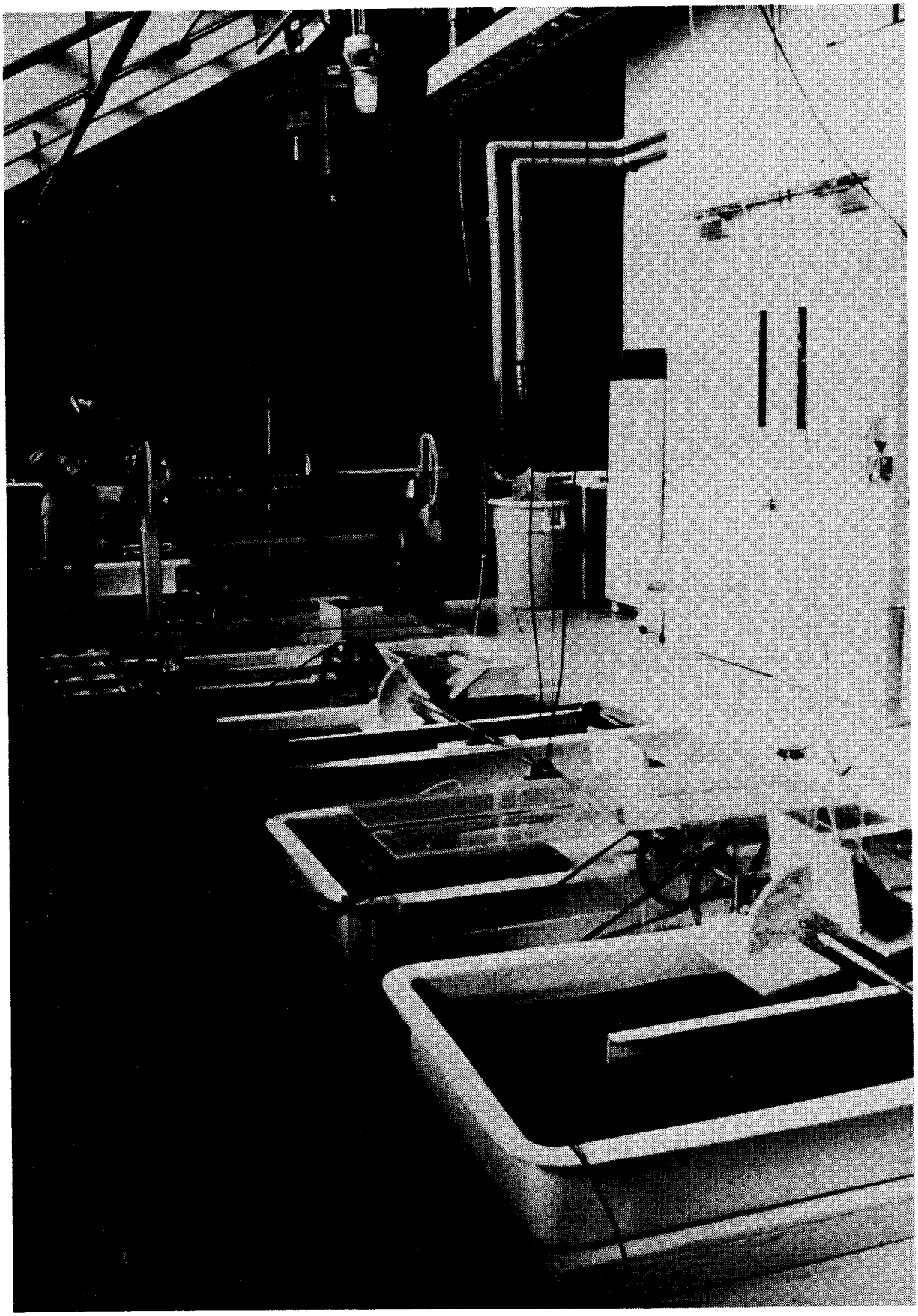

Figure 1-5. SERI greenhouse ponds

CHAPTER 2
PRINCIPLES OF MICROALGAL FUELS TECHNOLOGY

This chapter provides an overview of the basic technical and scientific issues important in growing mass cultures of microalgae and producing fuels. It is, in effect, a primer on the biological, resource, and engineering parameters that affect economic fuel production from microalgae. Additional information on these topics can be found in other SERI publications (Neenan et al., 1986).

MASS CULTURING OF MICROALGAE

Microalgae have four basic requirements for growth: light, water, carbon dioxide, and nutrients. Growing microalgae in mass culture is simply the science of supplying these four requirements in the proper proportion at the proper time.

Light

Microalgae are photosynthetic organisms, meaning they use solar energy to manufacture the cell components they need to live and grow. The basic process of photosynthesis is simple to put into words: carbon dioxide and water are transformed by energy from the sun into organic compounds and oxygen. And yet the process is profound: more than 90% of the total energy used in the world by humans for heat, light, and power is released from materials made by photosynthetic organisms.

The amount of light microalgae actually use is determined by two factors: the amount of light that reaches the cell and the efficiency with which that light is used. Incident light can be lost in many ways. For example, cover materials, reflection from the culture surface, particles suspended in the culture medium, and the organisms themselves all can interfere with light transmission. Careful design, however, can reduce or eliminate most of these factors--except for reflection, which inevitably eliminates about 10% of the available light.

The second determining factor is the photosynthetic efficiency of the organism itself. Photosynthetic efficiency is a measure of how much of the sun's energy available to the organism is actually captured in organic products. The theoretical maximum efficiency is about 23%. The practical limits are usually well below this value, especially for high-intensity light, which most plants use less efficiently than they do low-intensity light. Usually, increasing light intensity beyond about 20% of full sunlight does not lead to a proportional increase in the growth rate of cells. Algal cells *can* use high-intensity light efficiently when exposed for short periods of time. Thus, if cells in the culture are moved from the surface to the darker areas at depth, they can sustain a higher efficiency and, hence, a higher productivity. This movement can be accomplished by mixing.

Water

Two issues are important with respect to water: where does the water come from, and what is its quality? Locating culture production facilities in the desert Southwest allows use of saline water, which is available cheaply and in large quantities because it is unsuitable for agriculture or domestic use. Saline water is defined as having greater than 4000 ppm total dissolved solids (TDS), about the most that livestock can tolerate and considerably higher than most plants can tolerate. This water could come primarily from saline ground water, although some may come from irrigation returns, which often have high salt and nutrient concentrations. Preliminary studies of the quantity of saline ground water identified 230,000 acre-ft in Arizona and 1.5 billion acre-ft in New Mexico.

Eight areas in Arizona and six areas in New Mexico have been identified as suitable sites for a microalgal production facility (SERI, 1987).

The question of water quality centers around the concentration of salts. Water demand and salt balance in mass cultures are governed by the evaporation rate and the salinity of the source water. The evaporation rate depends on the climate and the design of the facility. Uncovered ponds lose water to evaporation and thus become solar concentrators of dissolved salts. Even though many algal species can tolerate salinity well in excess of that of saline ground water (4000-25,000 ppm), and some can tolerate salinity in excess of seawater (35,000 ppm), this concentration process will eventually limit the organism's ability to survive. To maintain the salt equilibrium within tolerable limits, a portion of the culture water will need to be removed and replaced by source water with a lower salt concentration. This process is called blowdown. Disposal of blowdown water is an environmental issue that must be addressed by the facility's design.

Two aspects of salt concentration influence algal growth: the total salt concentration and the concentrations of individual ions in the solution. In uncovered cultures in the desert Southwest, high average operating salinities can be expected because of high evaporation rates. However, periodic rainstorms will lower the salinity temporarily. Thus, the most desirable species will be those that tolerate large fluctuations in salinity as well as high average salinity.

The concentrations of particular ions in solution can significantly affect algal growth. The major ions in saline water (sodium, potassium, calcium, magnesium, chloride, and sulfate) are involved in algae metabolism although little is known about how their concentration affects growth. Other ions (including nitrate, nitrite, ammonium, phosphate, carbonate, bicarbonate, and iron) are present in lower concentrations and have been identified as limiting growth in natural waters. Algal requirements for these low concentration ions are well known.

Source water will probably also contain a number of trace elements vital to the survival and growth of microalgae but which can become toxic at higher concentrations. These include copper, cobalt, zinc, manganese, molybdenum, selenium, and vanadium. Virtually nothing is known about the quantitative requirements of microalgae for these trace elements. Cadmium and mercury can also be toxic. All these elements can be concentrated in the culture medium along with salts. The concentration levels at which they become toxic are not well known.

Carbon Dioxide

Carbon dioxide is the major source of carbon for the synthesis of organic compounds like carbohydrates and lipids in the cell. Because carbon demand increases in proportion to productivity, large-scale mass culturing of microalgae requires a large, reliable source of CO_2.

Atmospheric concentrations of CO_2 are extremely low, making it impossible to passively acquire the amount of CO_2 needed to sustain rapid growth. Thus, CO_2 must be added to the system. It can be added by bubbling air or concentrated CO_2 through the culture. If too much is added, the CO_2 will be lost to the atmosphere, a process called outgassing. This is inefficient and expensive. On the other hand, if too little CO_2 is added, productivity will be diminished. Figure 2-1 shows the effect of CO_2 on productivity.

The efficiency with which CO_2 is dissolved in the culture medium is also important. If CO_2 is added as bubbles, large quantities can be lost if the bubbles reach the surface and burst. CO_2 can be added instead as small bubbles in counterflow columns, where the downward movement of water offsets the upward movement of bubbles until the CO_2 dissolves.

Perhaps the most attractive and least expensive alternative for supplying CO_2 is to use dissolved forms of CO_2 (primarily bicarbonate) that can be present in the source water. Depending on the evaporation rate and the bicarbonate content, source water can supply from 2% to 20% of the CO_2 requirement.

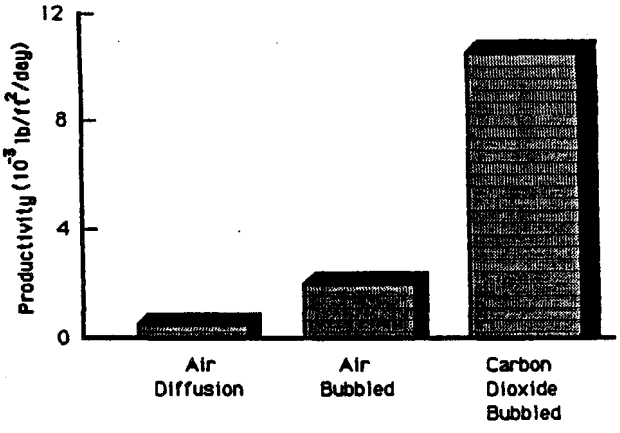

Figure 2-1. Productivity enhancement with carbon dioxide

A recent study has shown that large quantities of CO_2 should be available in the southwestern states from coal combustion in chemical process, power, and synthetic fuel plants and from depleted oil fields and natural gas wells (Feinberg and Karpuk, 1988). CO_2 might also be available from landfills and sewage treatment plants using anaerobic digestion. Predictions are that CO_2 supplies could support a very large microalgal fuels industry. Using CO_2 from these sources could have a positive effect on the environment by reducing the amount of the gas released into the atmosphere.

Nutrients

In the context of the Aquatic Species Program element, nutrients are defined as the inorganic or organic compounds--other than carbon dioxide and water--that are used by microalgae for growth and other cellular functions. The algae being studied within the program require only inorganic nutrients. Nutrients that normally limit growth in outdoor mass cultures are nitrogen, phosphorus, silicon (for diatoms), and iron. Algae can store nutrients in substantial excess of their requirements as a reserve. For example, algal cells may contain two to four times more nitrogen and ten times more phosphorus than they need.

The regulation of nutrient consumption is a major concern in algal mass culture. The consumption of nutrients can be reduced if cellular processes can be directed away from normal cellular products and toward energy-rich storage products, which typically contain smaller amounts of inorganic nutrients.

The nutrients required by algal cultures can be supplied in many forms. Some exist in solution as ions or are bound in organic compounds. Ionic forms can be supplied to the culture as either inorganic or organic salts. Nitrogen, for example, can be supplied as nitrate, nitrite, ammonium gas or liquid, or as a wide variety of organic nitrogen compounds. The selection of a nitrogen source may be significant because nitrogen is needed in large quantities and because there are so many possible sources.

Of the easily available nitrogen sources, ammonia and urea are the least expensive. Handling costs are generally low for nutrients like ammonia that can be pumped easily.

Although the cost of urea per nitrogen atom is similar to that of ammonia, urea is supplied as a crystal and requires additional handling that can add to this cost. In addition, only 47% of the weight of urea is nitrogen compared to 83% of ammonia, which means that more material by weight must be handled with urea than with ammonia. One advantage to using urea, however, is that the molecule has a carbon atom that is released as CO_2, so urea can simultaneously supply part of the carbon requirement as well.

Another potential source of nutrients is municipal wastewater, which contains nutrients in forms that are readily available to algae or become available after bacterial decomposition. These bacteria-algae interactions are well known and form the basis of existing sewage treatment methods. One problem with using wastewater, however, is that the nutrients are not concentrated enough to support intensive cultures of microalgae. Another obstacle is the absence of sizable cities to supply wastewater in the locations proposed for microalgae facilities.

Growth Conditions

Growth conditions in microalgal mass cultures can be divided into two categories: those dictated by the location of the culture facility and those based on culture management strategies. Variables related to location include the amount of sunlight available, evaporation, rainfall, temperature, and wind conditions. Variables of culture management include salinity, nutrient concentration, CO_2 concentration, culture mixing, aeration, and residence time of the culture population.

Algal growth rates are directly proportional to temperature up to a threshold level. Algae in outdoor mass cultures do not normally grow near the maximum rate dictated by the temperature but are limited by the availability of light or other factors. Properly selected species may continue growing at low temperatures and may even survive freezing. This has been demonstrated at the outdoor test facility where one green algal species survived the winter, then continued growing the following spring.

Culture aeration is another important aspect of maintaining proper growth conditions for mass culture. Although its major function is to provide CO_2 through gas transfer, other functions may also be important. For example, if oxygen, an end product of photosynthesis, builds to too high a level, it inhibits further photosynthesis. Culture aeration helps to reduce oxygen concentrations, keeping productivity high.

The operation of outdoor mass culture systems with harvesting and processing facilities can be simplified where a single algal species is maintained year-round. It is also possible, however, that a mixed culture will be used, in which different species are dominant at different times. The harvesting and processing characteristics of these different species must be similar so that equipment can be used throughout the year without modification. Ideally, seasonal crops would remain at low levels in the culture at all times and reappear as conditions became suitable. Careful culture management would avoid drops in system productivity during transitions between species or strains.

Manipulating the microalgal environment can increase lipid production. This production increase can be induced by nitrogen deprivation in most algae and by silicon deprivation in diatoms. Lipids in growing cells are mostly membrane-bound glycolipids and phospholipids. Under environmental stress, production switches from these membrane lipids to storage lipids. (See Figure 2-2.) Storage lipids are organized in the cell in the form of vesicles or globules that are much easier to extract from the cell than membrane lipids are. Furthermore, they consist primarily of triacylglycerols and other hydrocarbons, the most desirable compounds for making high-energy liquid fuels.

Algal Species Selection

The design of microalgal mass culture systems is a synergistic process in which the engineering design and selection of the species interact. Both aspects must be considered to obtain economic construction and operation of the facility. To achieve the best overall design, the organism will need to be "engineered" in some way. Through breeding and molecular genetics (including genetic engineering), selected species can be altered to enhance the desirable traits and suppress the less desirable.

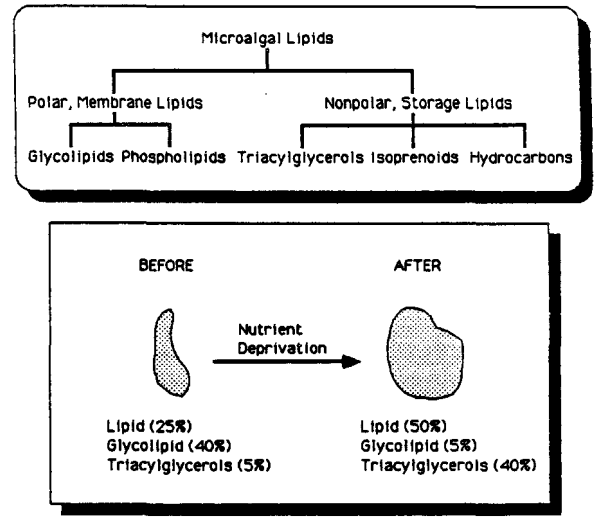

Figure 2-2. Lipid composition of a typical microalgal feedstock

The first priority in selecting species is to identify the most desirable characteristics. Among these characteristics are

- Tolerance to a wide range of temperatures (especially high temperatures)
- Tolerance to a wide range of salt concentrations (especially high concentrations)
- High productivity at high incident light intensity
- Size, buoyancy, or behavioral characteristics that make the organisms easier to harvest
- Resistance to predators, disease, and contaminants
- Absence of autotoxicity
- Suspendability (absence of sticking or settling)
- Tolerance to high nutrient concentration
- A life cycle that permits continuous culturing
- A readily inducible sexual reproductive phase to permit genetic modification through classical breeding and selection.

Species that fulfill most of the above criteria have been selected from the natural environment. However, the environment in a mass culture system differs significantly from most natural environments, and performance criteria in mass culture systems (fuel production) are the same as performance criteria in nature (species survival and reproduction). Species with the best possible combination of the desired characteristics are now being improved through classical and molecular genetics.

CULTURE PRODUCTION SYSTEMS

The first section of this chapter focused on the biological principles important in producing fuels from microalgae; this section and the next concentrate on engineering and design principles.

Engineering and Construction

Although many pond designs have been used throughout the 40-year history of microalgal mass culturing, one design is now used almost exclusively. This design uses a shallow open pond, 6-20 in. deep, laid out in either an oval or meandering shape. Water is continuously circulated through the pond to maintain culture suspension. (See Figure 2-3.)

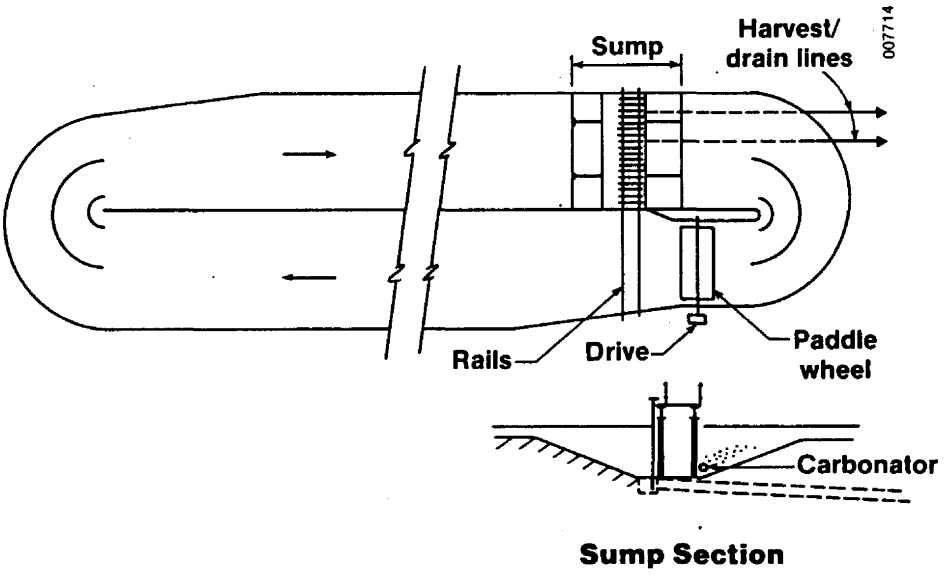

Figure 2-3. Open pond design

A motorized paddlewheel is commonly used to circulate the water because it moves water efficiently and avoids mechanical damage to the cells. Air-driven circulation systems such as airlift pumps or bubbling systems have been studied, but they operate at lower energy efficiencies than the paddlewheel.

The open pond design has been widely accepted for several reasons. The most important reasons are that the design is inexpensive to construct and circulation is efficient. Table 2-1 lists estimated construction costs for several designs. Although this open pond design is much cheaper to build than the raceway or enclosed tube designs, it is not the most productive (Terry and Raymond, 1985). Its selection is based on trade-offs between productivity and costs. It is conceivable, therefore, that research could result in a better, innovative design.

Ponds have been built with a wide variety of bottom materials, including compacted soils, crushed rock, soil cement, concrete, and plastics. These materials vary in roughness, stability, permeability, and durability. Surface roughness determines the resistance to water movement and the potential for cell aggregation. Unstable bottom materials are subject to shifting and settling that can destroy the hydraulic design of the pond. Permeability or leakage, particularly in plastics, can lead to the loss of both water and algae. Failures, including cracking of the bottom and breakdown of the sides, lead to high maintenance costs and lost production. If large culture facilities are to produce fuels economically, then developing specialized techniques for lining ponds might be justified.

Harvesting

There are a number of techniques for harvesting microalgae. If cells can be made to either float to the top or settle to the bottom, they can be skimmed off and harvested. Cells can also be centrifuged, a process similar to the spin cycle in a washing machine, although this equires substantial energy input. Another technique is to harvest cells by

Table 2-1. Estimated construction and operation costs for three engineering designs

Engineering Design	Cost ($/acre)
Open Ponds	30,000
Raceways	64,000
Enclosed Tubes	139,000

filtration. All these processes are aided by cell flocculation, in which cells are encouraged by natural or artificial means to gather in clumps. Cell clumps are more easily filtered and settle more rapidly, either by gravity or centrifugation. Cells can be made to rise to the surface of the culture by introducing small bubbles into the bottom of the culture, which float the algae to the surface.

Most harvesting techniques have been developed for freshwater or wastewater algal systems. They are based on interactions between the cells and various charged particles used as flocculants. Since the effectiveness of these flocculants is reduced in water with a high salt content, the techniques must be adapted for use in saline water. As a general rule, more flocculants must be added in saline water to produce the same effect, raising production costs. An alternative solution is to encourage autoflocculation, which is often stimulated by pH increases accompanying algal growth. But autoflocculation processes are also less effective in saline water.

There are biological aspects to harvesting technology. Harvesting microalgae depends on the organism's size. The smaller an organism, the more difficult it is to settle or filter. Cell motility (spontaneous, unpredictable movement) can also affect harvesting. Other important properties include the cell surface charge--which affects the cell's ability to aggregate--and a variety of behavioral characteristics. The most rapidly growing species are often small, motile unicells that are the most difficult to harvest. Thus, harvesting ease cannot be an absolute requirement. Trade-offs must be made between harvesting and other desirable characteristics.

FUELS FROM MICROALGAE

The primary purpose of research into fuel conversion processes is to determine the best ways to exploit the chemical composition of microalgae to produce fuel products (Feinberg, 1984). Before we look at the types of fuels that can be produced from microalgae, it is important to examine the properties of conventional fuels. This provides a basis for comparing microalgal fuels with those in the marketplace.

Characteristics of Conventional Fuels

Conventional fuels have physical and chemical properties that make them attractive to producers and consumers. Physical properties include the state of the fuel (whether it is a solid, liquid, or gas), the density or specific gravity, and the viscosity. Other important properties include boiling and freezing points, cloud point, pour point, and flash point.

The state of a fuel must be suited to its application. For example, liquid fuels make the best transportation fuels because they flow well, are stored easily, and provide a large amount of energy per unit volume. Solid fuels work best for stationary applications like power plants. Fuel density and viscosity are important when designing pumping and storage systems. Other properties must also be matched with the conditions under which the fuel will be used. For example, the pour point of diesel fuel is the point below which the fuel will not flow and burn efficiently. If the outside temperature is below this point, diesel engines will not run properly.

In terms of chemical properties, the most important distinction between fossil fuels and biomass fuels, or biofuels, is that petroleum, natural gas, and coal are all hydrocarbons--compounds composed entirely of carbon and hydrogen. In contrast, most biofuels contain oxygen as well, which changes many of the properties of the fuel. The most important change is the decreased energy available. Figure 2-4 compares the amount of energy available from gasoline with several potential biofuels. In every case, biofuels provide less energy per unit amount.

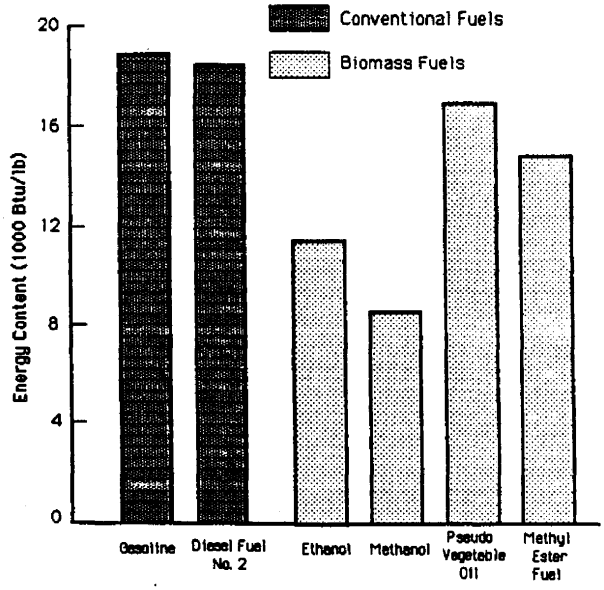

Figure 2-4. Energy content of conventional fuels compared to biomass fuels

Characteristics of Microalgal Biomass

Crude algal biomass is a thick slurry containing 10% solids by weight. It is composed of lipids, carbohydrates, protein, intermediates, and ash. Intermediates are low-molecular-weight substances that do not belong to the first three categories. Ash is the inorganic fraction, which does not contribute to the fuel value of the feedstock.

Because of their oxygen content, crude algal feedstocks cannot be used directly in conventional oil refineries, either alone or blended with crude petroleum. The carbohydrate fraction of algae contains about 50% oxygen and the lipid fraction contains about 10%. Together, a typical feedstock contains about 13% oxygen, whereas crude petroleum contains none. At the high temperatures used to distill crude petroleum, algal feedstocks would react with the unsaturated hydrocarbons to form undesirable side products. Thus, research has focused on developing processes to convert algal feedstocks to more conventional liquid fuels.

Fuel Conversion Processes

A significant amount of research outside the program has been conducted on using oilseed crops such as soybean, peanuts, and rapeseed as diesel fuels. These vegetable oils consist mostly of triacylglycerols (also called triglycerides), the storage form of fatty acids. (See Figure 2-5.) The amount of energy that can be released from burning these oils is generally about 5% less than diesel fuel. The most significant difference, however, is in viscosity at low temperatures. Fuel system modifications, such as a fuel preheater, are required to get reliable performance. These research results give some indication of the issues and challenges of using microalgal oils as a liquid fuel.

The first step in the conversion of algal oils to fuels is to extract the oil from the cell. Lipid extraction techniques have focused on the use of solvents. Recent research has tested several solvents to determine whether high yields can be achieved (Nagle and Lemke, 1988). Several solvents show promise and research is continuing.

One option for using oils from microalgae is to produce an ester fuel, which has characteristics similar to those of diesel. In this process methanol or ethanol is substituted for the glycerol component of the triacylglycerol, forming methyl or ethyl esters. This process is called transesterification. Although ester fuel has about 10% less energy than diesel, its viscosity and boiling point are closer to those of diesel than to those of refined vegetable oil. One disadvantage of ester fuel is that it begins to turn cloudy and form waxes at temperatures slightly above freezing, so it will require some fuel or fuel line preheating. Using it as a diesel-ester blend reduces this requirement.

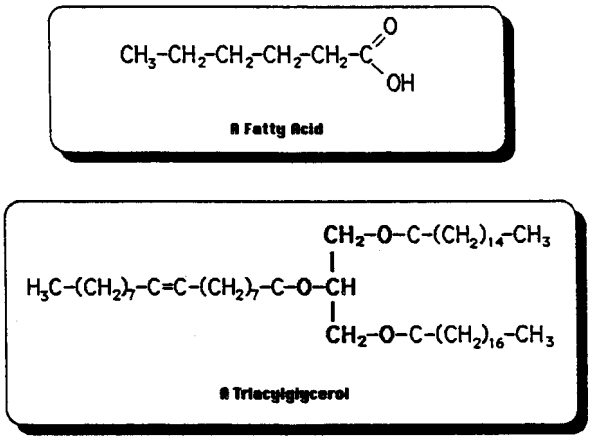

Figure 2-5. Structure of a typical fatty acid and triacylglycerol

Recent studies on transesterification of algal oils has yielded promising results (Nagle and Lemke, 1988). Two techniques, alkaline hydrolysis and acid hydrolysis, were compared. Alkaline hydrolysis has been used to produce fuels from oilseed crops. With algal oils, however, acid hydrolysis in methanol produced the best results in small-scale laboratory experiments.

Another promising process is to convert the lipids to a mixture of hydrocarbons essentially identical to those found in gasoline. The Mobil Oil Company has experimented with such a process for a number of feedstocks such as corn oil, castor oil, jojoba oil, and latex (Weisz, Haag, and Rodewald, 1979). The process was recently tested with algal oils obtained through solvent extraction (Milne and Evans, 1988). Results of this work have shown that algal oils can be converted to gasoline with high yields.

Several other fuel conversion processes are potentially available that use algal products other than lipids. For example, methane, the primary constituent of natural gas, can be produced by the anaerobic digestion of the entire algal cell mass. And ethanol can be produced from the carbohydrate fraction. Neither of these processes is likely to be the primary fuel conversion process for producing microalgal fuel, however, because of economics. Both may have potential as secondary processes, however, used with the cell residue after lipid extraction. In this case, methane or ethanol would be a by-product.

CHAPTER 3
ECONOMICS OF MICROALGAL FUELS

There is no doubt that high-energy fuels can be produced from microalgae; the challenge is to produce them at a cost low enough to compete with conventional fuels. This challenge forms the foundation of the DOE/SERI Aquatic Species Program element. To help guide research and ensure success, scientists developed the Algal Production and Economic Model (APEM) (Hill, 1984). The purpose of the model is to estimate the costs associated with the process and to identify the components that affect overall production cost the most. The results can be used to identify critical research needs and establish priorities, ultimately ensuring that research funds are allocated on those areas expected to reduce overall costs (Neenan et al., 1986).

ALGAL PRODUCTION AND ECONOMIC MODEL

The APEM is a computer model that consists of a series of linked physical and economic relationships representing the major aspects of microalgae cultivation, production costs, and economics. No attempt was made to model each aspect of the process comprehensively. Instead, the model was designed to provide sufficient detail in critical areas of the process to identify those parameters with the greatest potential for affecting the cost of production.

A schematic diagram of the model is shown in Figure 3-1. The heart of the model is the algal production system. Notice that, with the exception of nutrient and media recycling, all arrows lead away from this system to the other, peripheral subsystems modeled by the computer. This is because the operation and production levels within this system establish the input requirements and, ultimately, the production output of the facility. For example, requirements for carbon, nitrogen, potassium, and phosphorus are based on what is needed to maintain growth in the culture system. Culture growth or density, in turn, sets the requirements for the harvester subsystem, and so forth. The production model reflects steady, continuous production determined from data from field experiments. Based on the assumed chemical composition of the microalgae and the size of the facility, data on flow rates, nutrient requirements, and product yields are then calculated.

To explore the critical issues in producing microalgal fuels, the model establishes a reference or base case that corresponds to a hypothetical mass culture production facility located in the desert Southwest. The facility is assumed to be 2500 acres in size, 86% of which is dedicated to mass culture ponds. There are 43 ponds, each 50 acres in size. Ten percent of the ponds are assumed to be out of production at any time for routine maintenance or repairs. The open ponds are 6 in. deep, and water is circulated by paddlewheels. The harvesting system is two-stage. The first stage is a microstrainer capable of handling large influent flow rates and concentrating the algae as much as tenfold. The second stage is a centrifuge system designed to bring the product stream up to 10% solids.

The reference case is defined in the model as a specific set of input parameters chosen to reflect the requirements of this hypothetical facility. Each parameter is assigned a reference value within a range based on relevant experiments, engineering studies, and the judgment of scientists. Thus, the reference case is neither the best nor the worst, but rather a set of median values from the range of possible results. The parameters are broken down into four categories, representing resource, biology, facility design, and financial information. The parameters used in the APEM, along with their ranges, are included in the appendix.

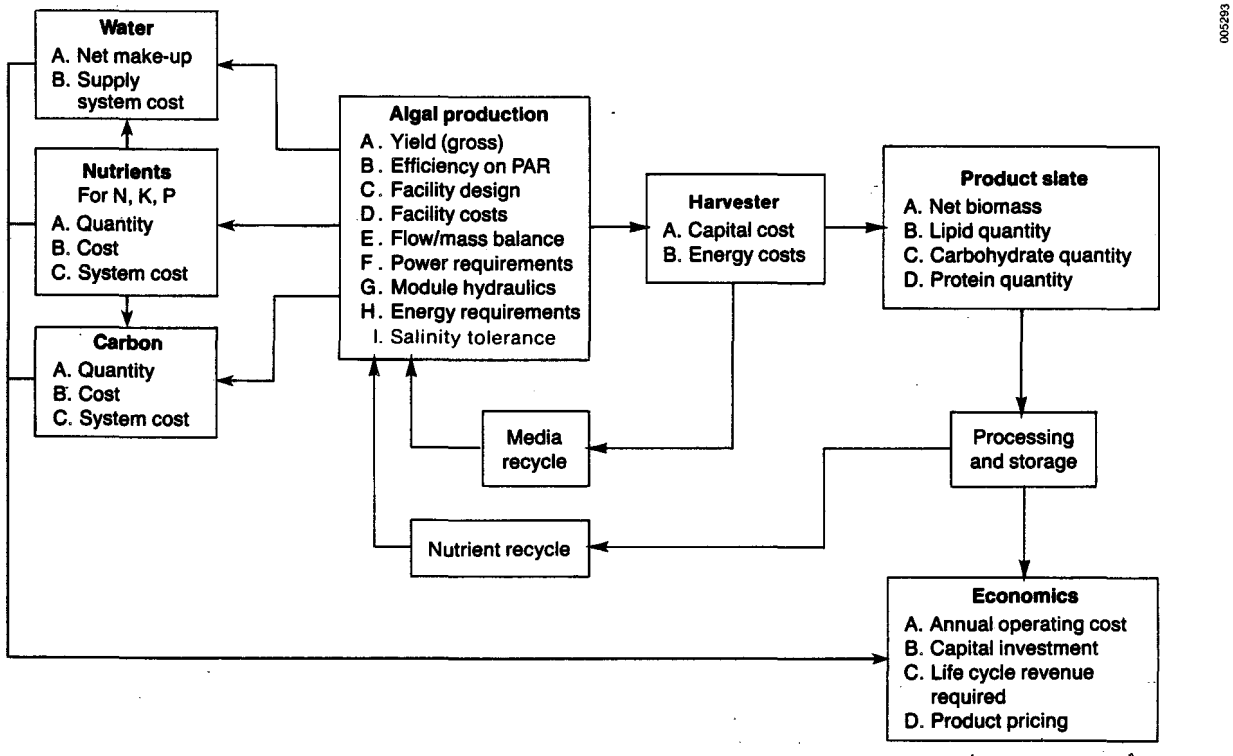

Figure 3-1. **Overview of the Algal Production and Economic Model (APEM)** (Source: Hill 1984)

The economics of the reference system are developed by two models: the micro-algal production model and a revenue requirement economic model. The production model develops first-year cost estimates for the facility and determines production levels based on defined inputs and requirements. These first-year costs and production levels are then transferred to the revenue requirement model, which determines the required selling price of the algal products to achieve a specified minimum return on equity investment. These prices are determined by annualizing the present value of all facility costs and dividing these annual costs by the production volume. All costs are reported in constant 1984 dollars.

RUNNING THE ALGAL PRODUCTION AND ECONOMIC MODEL

After running the model, the total cost for constructing and operating the micro-algal production facility can be estimated. A percentage distribution of these costs is presented in Figure 3-2.

The model goes on to evaluate what the maximum feedstock costs should be if microalgal fuels are going to be economically competitive with conventional fuels at selling prices estimated for 2010. The results of this analysis are presented in Figure 3-3 for the production of five potential fuel products from microalgae. Notice that current production costs are higher than allowable costs. Thus, the next step in the modeling process is to determine which parameters most affect the overall production

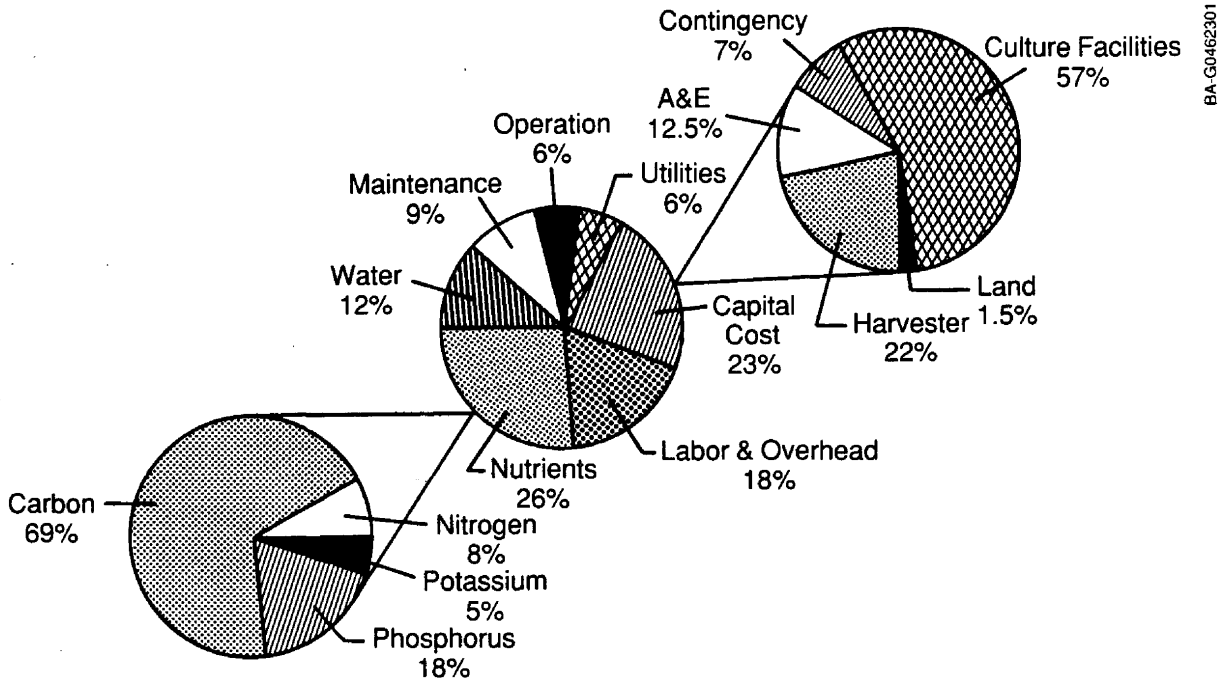

Figure 3-2. Distribution of costs for a microalgal production facility

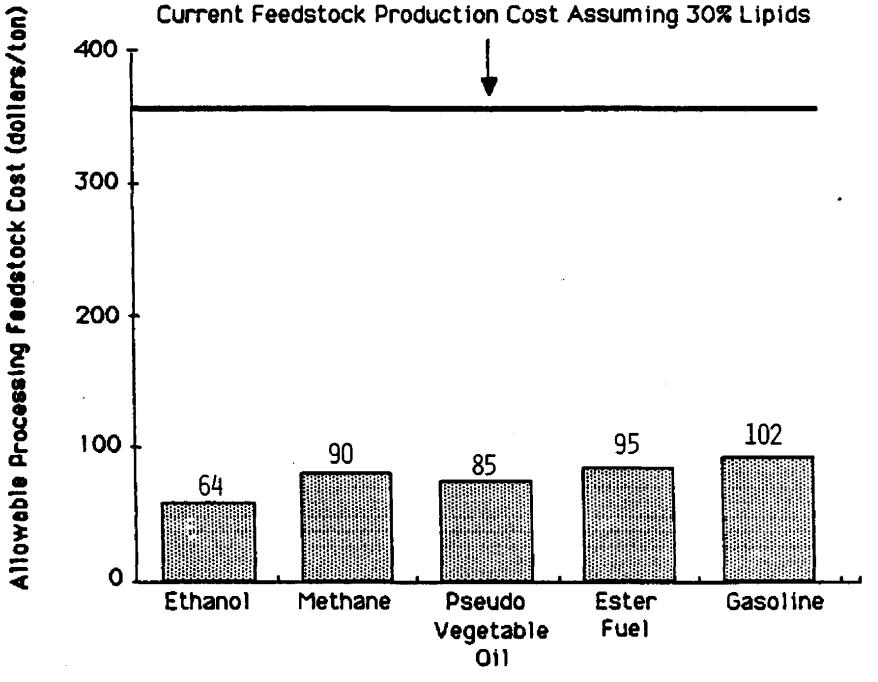

Figure 3-3. Comparison of allowable fuel processing costs for five biofuel options. All costs are calculated for the reference case and reported in 1984 dollars.

cost and to consider how changes in those parameters will narrow the gap between current and allowable costs.

To see how changes in input parameters affect the overall cost of production, each parameter listed in the appendix is varied independently in the APEM. Those that resulted in large variations in production costs were examined more closely in a second evaluation. Sensitivities were evaluated by varying the value of the parameter over its assumed range, while holding all other variables at their reference values. Interpreting the results of this analysis requires recognition of the constraints of the methodology, however, because some parameters are technically interrelated. For example, CO_2 requirements and culture productivity are linked, and any parameter changes that influence productivity will affect CO_2 requirements and costs. These adjustments were not made in the sensitivity analysis.

ANALYSIS RESULTS

The analysis using the APEM identified five issues particularly critical to cost-effective fuel production from microalgae. These are water demand, carbon dioxide demand, algal productivity and products, algal harvesting, and fuel conversion. The first four are related to algal production; the fifth is concerned with fuel processing options.

Water Demand

Water demand is a function of the evaporation rate, the salinity of the source water, and the operating salinity of the system (which is determined by the salinity tolerance of the microalgae). These parameters are linked. For example, for a given evaporation rate, either higher source water salinities or lower species salinity tolerance means that the system must undergo more frequent blowdown to maintain productivity.

The effect of evaporation rate and algal salinity tolerance on production cost is illustrated in Figure 3-4. The curves map salinity tolerance and algal production costs for three evaporation rates. The graph shows that algal salinity tolerances above 35,000 ppm TDS reduce production costs, and tolerance below 35,000 ppm TDS increase those costs. This demonstrates the importance of choosing an algal species with a high salinity tolerance.

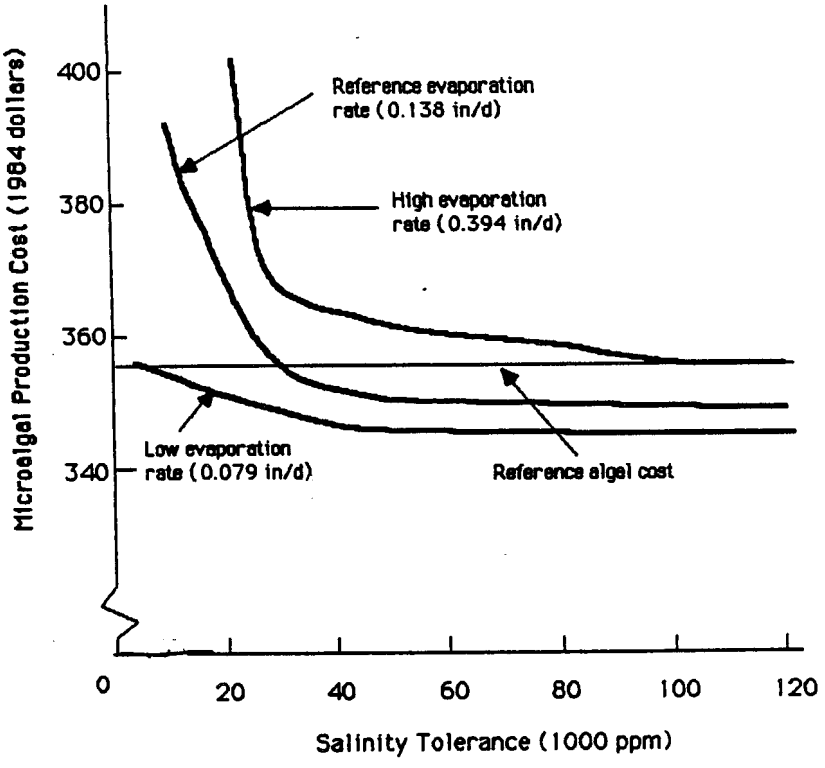

Figure 3-4. Comparing microalgal production costs with variations in evaporation rate and salinity tolerance

Reducing evaporation can have a major effect on production cost. Pond covers can help; they also protect against dust, predators, and wind. As with many other factors, however, the benefits must be balanced against the extra cost. It might be cost-effective to cover only the sections in which CO_2 is added, to conserve that valuable resource.

Another important trade-off is between the organism's ability to tolerate salinity and the cost of saline water. In general, the lower the salinity, the higher the water's cost. Therefore, a higher salinity tolerance in the organism will lower operating costs. Modeling suggests that the organism must tolerate a concentration of at least 50,000 ppm TDS to provide protection against the most extreme environmental conditions.

Carbon Dioxide Demand

Microalgae require large amounts of CO_2 because it provides the carbon for all the metabolic products of growth. Forty to fifty percent of the dry organic weight of microalgae is carbon. The algae take CO_2 into their cells directly from the culture medium. Analysis using the APEM showed that CO_2 represents a large fraction of product cost (30% in the reference case). It is important, therefore, to supply the gas efficiently to the culture with a minimum of losses. With proper control, these losses can probably be eliminated completely, although there is a trade-off between the cost of controls and the savings realized by using CO_2 more efficiently.

Other important issues are the source and cost of the CO_2. A recent study sponsored by the program has shown that the major source for CO_2 is likely to be coal combustion in chemical process, power, and synthetic fuel plants (Feinberg and Karpuk, 1988). Flue gas from coal-fired power plants contains about 15% CO_2. Recovering and purifying this gas would add to the cost of the CO_2. However, technologies under development for clean coal-burning could produce large quantities of concentrated CO_2 at low cost. In addition, concerns about the environmental effects of CO_2 in the atmosphere--the greenhouse effect--might result in the availability of CO_2 for a negative cost (i.e., a revenue instead of a cost).

Transportation of the gas also affects its cost. Supercritical pipelines are the most economical method for transporting CO_2 over the range of flow rates required for these plants.

Algal Productivity and Products

The sensitivity analysis performed with the APEM showed that lipid costs are highly sensitive to biomass productivity and lipid content. As these factors go up, the fixed costs of the plant are spread over a larger amount of product, reducing its required selling price.

Productivity is a measure of the amount of biomass produced per unit area per unit time. The value used in the reference case was 17 g/m^2-d. This rate results in a microalgal production cost of $393/ton of biomass. Varying productivity from the reference case to a value of 46 g/m^2-d resulted in a reduction in the production cost to $180/ton.

Lipid content also affects economics. The reference case assumed a lipid content of 30% by weight. This reference case results in a lipid cost of $103/barrel. Varying this parameter from 30% to 50% in the model, combined with the improvement in productivity from 17 to 35 g/m^2-d, cut the lipid cost in half, to $50/barrel.

Research results have shown that improvements over the reference case are possible. Productivity rates of 50 g/m^2-d have been achieved for short periods in controlled outdoor experiments. And laboratory experiments have achieved lipid contents exceeding 60%. These achievements have not been made using the same organism, however, so current research in classical and molecular genetics is addressing this challenge.

Algal Harvesting

The reference harvesting system consists of first-stage microstrainers, which concentrate the product stream by a factor of 10, followed by second-stage centrifugation to bring the product up to 10% solids content. This system contributes nearly 25% of the total capital investment of the mass culture facility. Increasing solids removal efficiency would decrease operating costs by allowing the use of smaller equipment and, thus, less electricity to harvest the same amount of microalgae. Another type of first-stage harvester, the belt-filter, was also considered but was found to increase product cost despite its ability to concentrate the product stream by a factor of 70.

Increasing culture density will decrease the overall concentration factor required by the harvesting system. The more expensive stage (usually the second stage) could then be scaled back, resulting in capital and operating cost savings. Operating at higher densities allows shallower pond depths, which decreases head losses and reduces power requirements for pumping. These savings must be weighed against the decreased temperature stability of shallow ponds. More operating data will help evaluate harvesting strategies to determine the best technique.

Alternative Fuel Products

In addition to evaluating microalgal production, the APEM was used to evaluate fuel processing technology. Five major fuel products were considered:

- Ethanol
- Pseudo vegetable oil (PVO)
- Ester fuel
- Methane
- Gasoline.

Some of the properties of these fuels were discussed in Chapter 2.

The analysis first determined the total revenue available for each fuel for the base case. The total revenue represents the estimated selling price of the fuel in 2010 and the value of any by-products. Next, the fuel processing costs were determined. These costs are sensitive to the total amount of algal biomass processed and to the lipid and carbohydrate content of the feedstock. The difference between total revenue and the processing cost is the allowable feedstock processing cost--the amount the facility operator can afford to pay for the feedstock and still make a profit. These allowable feedstock costs are compared to the estimated algal production costs, assuming different levels of algal productivity and lipid content.

The analysis showed that gasoline and ester fuel are the most promising fuel options. Assuming improvements in productivity and lipid content, both of these options result in fuel costs within the cost goals for 2010, when the technologies are expected to be deployed. As shown in Figure 3-5, both fuels could be made for $1.55-$1.60/gal, within the cost goal of $1.75.

The processes based on methane and ethanol resulted in much higher fuel costs. These are both based on production of a large fraction of carbohydrates in the cell instead of lipids. They result in fuel costs roughly twice the cost goals for 2010.

The prospects for pseudo-vegetable oil are more promising than those for ethanol and methane, but this option does not appear to be feasible in the near future. Even with scale economies and process improvements, the production cost exceeds the processing value by 14%.

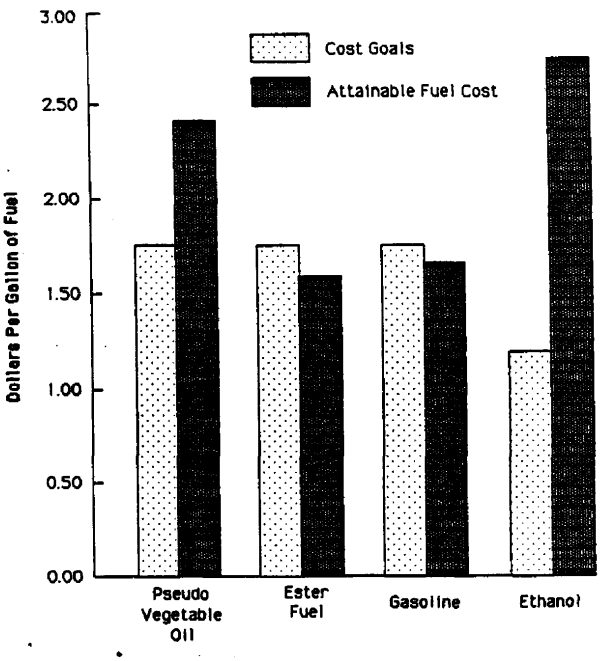

Figure 3-5. Comparison of 2010 cost goals with fuel cost projections given anticipated production and processing improvements

Thus, the most promising fuel options are gasoline and ester fuel. To achieve cost-competitiveness, however, a full set of technological improvements must be implemented. Specifically, algal production must achieve a level of 46 g/m^2-d with a lipid content of at least 50%. Moreover, the fuels must be produced with carbon dioxide and water supply costs at or below $2.83/MSCF and $3.68/MSCF, respectively. On the processing side, progress must be made in fuel conversion efficiency and in achieving scale economies.

Current research is making substantial progress toward these goals. For example, peak productivity has reached 50 g/m^2-d outdoors, and lipid content has reached more than 60% in the laboratory. Research on lipid biochemistry, genetic manipulation, and characterization--along with outdoor systems development--suggests that continued improvements are likely and that the reliable sustained productivity and lipid yields required for viable liquid fuel production from microalgae are possible.

CHAPTER 4
AQUATIC SPECIES PROGRAM RESEARCH

The primary research objective of the Aquatic Species Program is to gain the technical knowledge and capability to produce large quantities of competitively priced gasoline and diesel fuels from microalgal lipids. In pursuit of this objective, program activities are focused on five primary areas:

- Physiological research to increase lipid production in microalgae with high growth rates
- Elucidation of the biochemical mechanisms responsible for microalgal lipid accumulation
- Molecular biology research for the application of genetic engineering of microalgal species to improve desirable characteristics
- Engineering research that will provide design and operational data for large-scale outdoor production facilities
- Development of technologies for the efficient conversion of algal oil into liquid fuel products.

About half the research sponsored by the program is conducted at SERI. The other half is subcontracted to universities and small businesses. Figure 4-1 shows the funding breakdown by research areas for fiscal year 1989. Of a total budget of $600,000, 74% supported research in microalgal growth and production, 8.5% went to engineering design, and 17.5% went to analysis and management. Detailed information on Aquatic Species Program research can be found in other SERI publications (Johnson, 1987; Johnson and Sprague, 1987).

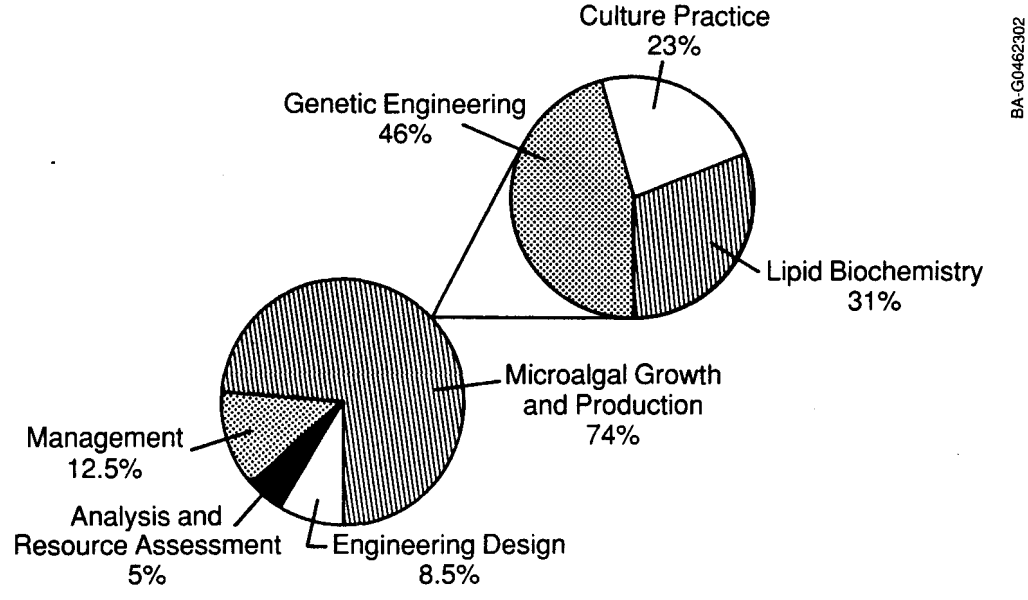

Figure 4-1. Funding breakdown for fiscal year 1989

MICROALGAL GROWTH AND LIPID PRODUCTION

Most of the microalgae research sponsored by the program since 1982 has been devoted to improving microalgal growth and lipid production. This direction was supported by analysis with the APEM, which showed that these factors can have the biggest impact on fuel cost. Thus, the focus during the first several years was on collecting and screening microalgae for desired characteristics. Attention has now turned to more basic research on the biochemistry and genetic basis for microalgal growth and lipid production. Research is also addressing lipid extraction and conversion techniques.

Species Characterization

By 1986, more than 3000 microalgal strains had been collected (Figure 4-2). Screening and characterization of these strains produced a list of the best strains. As scientists learn more from culture efforts and develop new strains through genetic engineering, the list will be modified. Work to optimize growth and lipid production is ongoing. Research on biochemistry and genetic engineering will result in a collection of organisms well-suited to production of lipid-based fuels.

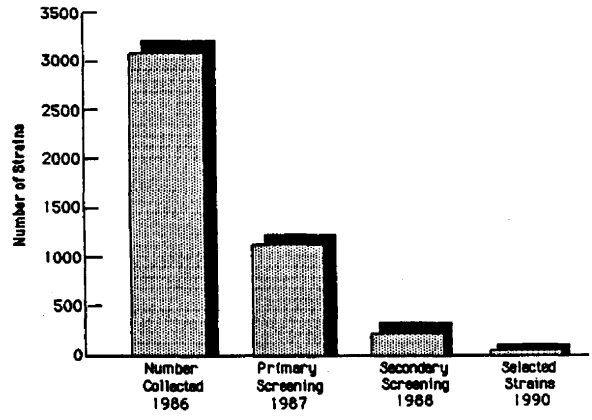

Figure 4-2. Summary of the strains of microalgae collected by the Aquatic Species Program and future screening goals

The most promising strains on the list include several that can tolerate severe fluctuations in temperature and salinity. In 1982, the best strains showed temperature tolerances of 59°-77°F and salinity tolerances of 20,000-40,000 ppm TDS. Newly identified strains have extended these ranges to 50°-95°F and 10,000-85,000 ppm TDS. One strain can even withstand freezing. Figure 4-3 shows the improvements in temperature and salinity tolerance made since 1982.

A major accomplishment was developing a quick, inexpensive method for screening those microalgae that produce large quantities of lipids. Researchers from the University of Montana, Oak Ridge National Laboratory, and SERI developed a Nile red staining technique in 1985 that stains algae but does not kill them. The amount of fluorescence detected from the stained cells is directly related to the amount of lipid.

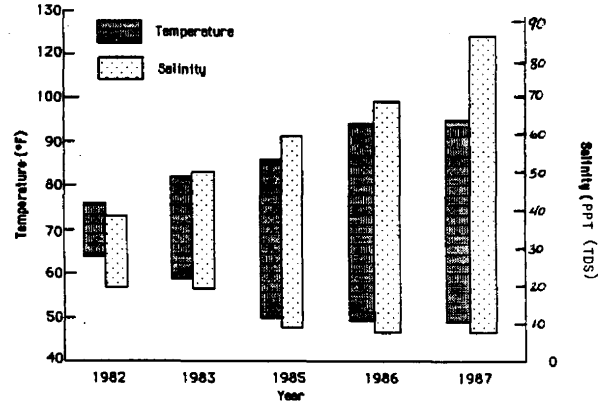

Figure 4-3. Improvements in temperature and salinity tolerances

In 1987, 131 warm-water strains from the desert Southwest--primarily

diatoms and green algae--were characterized for growth rate and lipid content (Sommerfeld, Ellington, and Tyler, 1987). Forty-nine of these strains had growth rates exceeding one doubling per day, and five exceeded two doublings per day. Experiments designed to optimize growth with 15 of the best strains indicated that the most rapid growth occurs when isolates are cultured at 77°F with urea as the nitrogen source. Seven strains had greatly improved lipid content, in some cases exceeding 400 ppm lipids. Another 55 strains were also isolated from Alabama and Mississippi, and five were characterized for growth rate and lipid content.

Several cold-water strains were characterized as well. Six strains exhibited growth rates exceeding one doubling per day at 59°F or cooler. Such strains will allow continuous operation of an outdoor facility during the cooler months of the year, which improves the economics of fuel production.

Biochemistry of Lipid Accumulation

The goal of lipid biochemistry research is to understand the mechanism by which cells accumulate lipids. This knowledge might then be applied to increase the amount of lipids in the cell. The target is 60% of the total cell biomass in outdoor culture systems. Research has increased lipid content from 20% in 1982 to more than 60% in the laboratory and 40% outdoors in 1987. This has been accomplished by selecting the appropriate organism and by learning more about environmental conditions that can induce the cell to accumulate lipids. For example, experiments have indicated that by allowing nitrogen or silicon to become deficient in the culture medium, lipid accumulation can be induced. This apparently stresses the cells, causing them to make storage lipids as insurance for survival. A computer model based on experimental data has been developed to predict these effects (Chelf, Barclay, and Lemke, 1987).

Changes in temperature, pH, inorganic carbon, and light intensity can also have an effect on lipid content. Laboratory experiments suggest that there may be universal lipid "triggers" that can be exploited to increase lipid accumulation (Guckert, Cooksey, and Jackson, 1987). They also suggest that depriving the cell of nutrients may be effective because it disrupts the cell cycle, thus alerting the cell to a potential danger and stimulating the production of survival products. If this is true, other methods of disrupting the cell cycle may also be effective lipid triggers.

Researchers are also trying to determine the lipid synthesis pathways, for diatoms in particular. Researchers have isolated the key enzyme in this synthesis pathway that performs a key role in regulating lipid production (Roessler, 1988). This enzyme is being characterized in terms of structure and specificity and may be the target of future genetic engineering studies.

Genetic Engineering

Currently, there is no single microalgal strain that has all the desired properties of wide environmental tolerances, high productivity, and high lipid yield. Yet all three properties are required if the program is to meet its goal of producing competitively priced fuels. Ideally, these characteristics should be combined in a single strain. For this reason, research has been initiated to develop techniques to genetically modify these organisms. These studies are the major focus of biological research with particular emphasis on triggering lipid accumulation.

A prerequisite to genetic engineering is the demonstration of genetic variability within groups of organisms. That is, the greater the variability of the genes in the organism's gene pool, the greater the possibility of constructing an organism with the desired characteristics. Several studies have looked at diversity within single microalgal species.

One such experiment investigated three strains of microalgae with potential for lipid production and showed that genetic diversity in microalgae is extremely high compared to other plants (Gallagher, 1987). This experiment compared physiological traits of the organism with DNA banding patterns from gel electrophoresis. (This technique exposes gels with DNA to an electric current. The DNA migrates on the gel, forming distinctive patterns of bands. These patterns can then be used to identify relationships between DNA and certain characteristics and can also be used as genetic markers in later manipulations.) A similar experiment also concluded that genetic diversity is high and that growth rate and lipid content can vary significantly between clones (Johansen et al., 1987).

Experiments performed on the diatom ***Chaetoceros muelleri*** also showed extensive diversity (Cohen, 1987). Several clones of this strain have high growth rates, broad salinity and temperature tolerance, and high lipid content. More than 200 clones were isolated and studied. Differences in DNA banding patterns were evident even in clones isolated from a single collection, which is a strong indication of genetic diversity. Correlations between morphological traits and physiological characteristics have also been noted.

The main principle of genetic engineering research is transformation--the technique of inserting desired genes into host organisms. Two variations are promising. The first uses viruses, which are commonly used to transfer genetic material. In this method, a virus attacks the alga cell and injects its DNA into the cell. The viral DNA is then replicated when the alga reproduces. Studies have shown that ***Chlorella*** is one of the few microalgae that is attacked by a specific virus.

The second technique makes use of naturally occurring or artificially constructed plasmids, which are circular segments of DNA not normally part of the cell's chromosomes. The desired gene is inserted into the plasmid, which is then introduced into the cell. If the plasmid is stable, the new genetic material is replicated when the cell reproduces and is expressed as the desired characteristic. Researchers have not yet been able to identify a plasmid in the microalgae in the program, but results for related organisms have shown that plasmids are probably widely distributed in microalgae. Research in this area is continuing along with work on constructing artificial plasmids.

Two genetic manipulation techniques support the genetic engineering research. The first involves characterization of the genetic material--or genome--of the cell nucleus and chloroplast, the organelle where photosynthesis takes place. Correlations can be made between the DNA and desirable traits in the organism, leading to selection of specific genes for insertion into recombinant organisms. Scientists have developed complete gene libraries for both nuclear and chloroplast DNA for the green alga ***Chlorella*** (Meints, 1987). This work will provide sections of DNA that might prove useful in constructing artificial plasmids. The added DNA could enhance the stability of the plasmid or the expression of the desired gene.

Selectable genetic markers form the basis of the second technique for genetic manipulation. These markers permit identification of the recombinant organisms. Genetic markers usually involve an observable change in a characteristic of the organism caused by genetic mutation. For example, a technique often used in genetic engineering

involves resistance to antibiotics. When altering the organism's DNA, the researcher endows the cell with this resistance. Consequently, any cell able to grow in media containing the antibiotic is certain to have the desired genetic alteration. This technique has been applied successfully to diatoms, providing an essential tool for genetic engineering experiments.

Engineering Design

An outdoor test facility has been built in Roswell, N. Mex., to examine the mass culture of microalgae in large outdoor ponds (Johnson, Weissman, and Goebel, 1988). The test facility will help determine what problems are likely to occur in operating and scaling up large production facilities. The site is located near a source of saline groundwater so this resource will also be evaluated.

The first stage of the outdoor test facility, shown in Figure 1-4, was completed in 1987. It consists of six open fiberglass ponds, each with an area of about 9 ft^2. These ponds are used to evaluate the growth and lipid production of various algal species. These small cultivation units are inexpensive to build and easy to operate, making them ideal for controlled, replicated experiments.

The second stage of the facility, completed in 1988, consists of two larger ponds, each covering about 0.25 acre. These ponds, shown in Figure 1-3, are being used to compare the performance of low-cost earthen liners with more expensive plastic ones. Operating these ponds should point out problems inherent in scaling up production and will provide the first estimates of average production in the desert Southwest from large, outdoor cultures. Future plans include the construction and operation of a 1-acre pond to address the problems associated with operating a full-scale facility.

In addition to the Roswell test facility, four small ponds were installed in the SERI greenhouse in 1987. As shown in Figure 1-5, the design of the SERI ponds is similar to the small Roswell ponds. They are monitored for temperature, light intensity, and pH. These ponds are used to supplement research at the Roswell site, because environmental conditions can be controlled better indoors.

Harvesting and Conversion Technology

After the microalgal cells are grown, they must be separated, or harvested, from the culture medium efficiently and economically. As discussed previously, the ease of harvesting depends primarily on the organism's size. As a general rule, the smaller the organism, the harder it is to settle or filter, and the harder it is to harvest. Common harvesting techniques include settling or flotation, centrifugation, and filtration. All these techniques are aided by cell flocculation. Flocculation can occur naturally or it can be induced by physical or chemical techniques.

Research has concentrated on finding good chemical flocculants. This is particularly important for small unicellular species that do not tend to autoflocculate. Experiments have shown that the dose of the flocculant can be reduced if the precipitant is recycled after flocculation (Johnson and Sprague, 1987). If three flocculation cycles are used, the amount of chemical required can be reduced by 75% with 90% of the microalgae recovered. Reducing the chemical dose reduces the feedstock cost.

Research on extraction and conversion of lipids to fuels has begun only recently. Experiments have examined solvent extraction and conversion to both gasoline and ester fuel. Extraction experiments have shown that n-butanol is one possible solvent with high

extraction efficiency and rapid phase separation (Nagle and Lemke, 1988). This leads to excellent recovery of both lipid and solvent.

Oils extracted with n-butanol were subjected to two techniques for producing fuels. First, researchers used alkaline and acid hydrolysis to convert the oils to methyl esters (Nagle and Lemke, 1988). Acid hydrolysis produced the best results. The second technique converted the oil to gasoline using a zeolite catalyst (Milne and Evans, 1988). In this experiment, the oils were vaporized by fast pyrolysis, a high-temperature thermal process. The vapors then passed over a shape-selective zeolite catalyst, resulting in a high yield of gasoline. This experiment was successful as well, converting the oil to gasoline with high yields. It has been suggested that the technique might work with whole algae, bypassing the extraction step entirely. Both experiments have provided preliminary indications that algal oils can be converted to high-value fuels in simple economic systems.

FUTURE RESEARCH

The focus of today's Aquatic Species Program on biotechnology will continue in the next few years. Recent progress in the characterization of algal strains, understanding of biochemical mechanisms, and development of genetic engineering techniques has demonstrated the great potential for improving algal growth and lipid production. Future research will continue to develop the understanding of the process and apply this understanding to the development of several ideal algal strains.

Research will also continue at the outdoor test facility. The outdoor ponds will continue to be used to examine operating and production issues involved in scaleup.

Research conducted to date in the Aquatic Species Program has already achieved significant results. In fact, the estimated cost of diesel fuel from microalgae is less than half what it was in 1983--reduced from $18/gal to less than $7/gal. Recent progress has shown there to be great potential for further improvement. It is expected that research will provide the foundation for a commercially ready technology to produce fuel at competitive prices by 2010.

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APPENDIX

APEM Parameter Values

Resource Parameters	Low	Reference	High
Evaporation (in/d)	0.079	0.138	0.394
Salinity of Source Water (ppt)	2.5	25.	35.
Nitrogen in Source Water (ppm)	0.	13.	96.
Phosphorus in Source Water (ppm)	0.	0.5	1.0
Potassium in Source Water (ppm)	8.	46.	400.
Carbon in Source Water (ppm)	10.	200.	420.
Land Cost ($/acre)	400.	500.	600.
Energy Cost ($/kWh)	0.040	0.065	0.100
Water Cost ($/acre-ft)	62.	83.	247.
Ammonia Cost ($/ton)	150.	168.	186.
Superphosphate Cost ($/ton)	204.	230.	254.
Potassium Cost ($/ton)	72.50	83.50	91.
Distance to CO_2 Source (mi)	0.	50.	62.
CO_2 Cost ($/MSCF)	3.68	3.68	3.68
Biology Parameters	**Low**	**Reference**	**High**
Ash Content (% dry weight)	8.	8.	8.
Lipid Content (% dry weight)	20.	30.	60.
Carbohydrate Content (% dry weight)	49.	20.	9.
Protein Content (% dry weight)	13.	32.	13.
Intermediate Content (% dry weight)	10.	10.	10.
Salinity Tolerance (ppt)	10.	35.	120.
Phosphorous Cell Content (% dry weight)	0.12	0.70	2.30
Growing Season (d)	200.	250.	365.
Photosynthetic Efficiency (%)	4.	5.	16.
Depth (ft)	0.33	0.50	1.00
Detention Time (d)	7.	7.	3.

Facility Design Parameters	Low	Reference	High
Effective Culture Area (% of total)	70.	86.	90.
Culture Downtime (% of total surface)	30.	10.	5.
Module Size (acre)	4.	8.	16.
Channel Width (ft)	20.	100.	200.
Channel Roughness (Manning's "n")	0.023	0.025	0.030
Depth of Culture (ft)	0.33	0.50	0.66
Carbon in Medium (ppm)	7.5	12.0	25.0
Nitrogen in Medium (ppm)	0.14	0.28	14.0
Phosphorus in Medium (ppm)	0.31	0.62	31.00
Potassium in Medium (ppm)	5.	25.	400.
Carbon Losses (ppm/d)	0.0	0.1	3.0
Nitrogen Losses (ppm/d)	0.0	0.1	3.0
Mixing Velocity (ft/s)	0.16	0.66	0.98
Mixing System Efficiency (%)	30.	65.	75.
Harvester Solids Removal (%)	50.	90.	98.
Financial Parameters	**Low**	**Reference**	**High**
Return on Debt (%)	3.7	3.7	5.6
Return on Common Stock (%)	6.5	6.5	9.75
Return on Preferred Stock (%)	4.5	4.5	6.75
Capital Cost Escalation (%/yr)	0.0	0.0	2.0
Operating Cost Escalation (%/yr)	0.0	0.0	2.0
Maintenance Cost Escalation (%/yr)	0.0	0.0	2.0
Cover Cost ($/ft^2)	0.00	0.00	0.16
Liner Cost ($/ft^2)	1600.	2000.	2400.

NOTE ON AVAILABILITY OF THE ALGAL PRODUCTION AND ECONOMIC MODEL (APEM)

The Algal Production and Economic Model is available to the public. Copies of the program, which is written in Basic, are available on diskette by writing:

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