

**SERI/TR-231-1918**  
**UC Categories: 61a, b**

# **SERI Biomass Program Annual Technical Report: 1982**

**Paul W. Bergeron**  
**Robert E. Corder**  
**Andrew M. Hill**  
**Hilde Lindsey**  
**Michael Z. Lowenstein**

**February 1983**

**Prepared Under Task No. 1356.10**  
**WPA No. 434-82**

## **Solar Energy Research Institute**

A Division of Midwest Research Institute

1617 Cole Boulevard  
Golden, Colorado 80401

Prepared for the  
**U.S. Department of Energy**  
Contract No. EG-77-C-01-4042

Printed in the United States of America  
Available from:  
National Technical Information Service  
U.S. Department of Commerce  
5285 Port Royal Road  
Springfield, VA 22161  
Price:  
Microfiche \$3.00  
Printed Copy \$11.50

#### **NOTICE**

This report was prepared as an account of work sponsored by the United States Government. Neither the United States nor the United States Department of Energy, nor any of their employees, nor any of their contractors, subcontractors, or their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness or usefulness of any information, apparatus, product or process disclosed, or represents that its use would not infringe privately owned rights.

## PREFACE

This report summarizes the progress and research accomplishments of the SERI Biomass Program during FY 1982. The SERI Biomass Program consists of three elements: Aquatic Species, Anaerobic Digestion, and Photo/Biological Hydrogen. The program is funded through the Biomass Energy Technology Division of the Department of Energy (Beverly J. Berger, Director).

SERI has adopted several strategies to ensure that the data developed by the Biomass Program is transferred to other researchers and to the private sector. Involvement of industry in the early stages of research and development will help direct the program toward the development of operational systems as well as promote the transfer of the technical data base to private industry. Publication of data in peer-reviewed journals, in SERI publications, and proceedings of regular contractor review meetings will make SERI-sponsored research available to the scientific community in the public and private sectors.



---

Michael Z. Lowenstein  
Program Coordinator

Approved for

SOLAR ENERGY RESEARCH INSTITUTE



---

Clayton S. Smith, Manager  
Solar Fuels and Chemicals Research Division

## SUMMARY

### Objective:

To summarize the progress and research accomplishments of the SERI Biomass Program during FY 1982.

### Discussion:

Most products derived from petroleum and natural gas can be produced directly from biomass. The "biomass" with which this report is concerned includes aquatic plants, which can be converted into liquid fuels and chemicals; organic wastes (crop residues as well as animal and municipal wastes), from which biogas can be produced via anaerobic digestion; and organic or inorganic waste streams, from which hydrogen can be produced by photobiological processes. The challenge is to develop technology that will be competitive with existing processes using nonrenewable resources. The Biomass Program Office supports research in three areas which, although distinct, all use living organisms to create the desired products. The Aquatic Species Program (ASP) supports research on organisms that are themselves processed into the final products, while the Anaerobic Digestion (ADP) and Photo/Biological Hydrogen Program (P/BHP) deals with organisms that transform waste streams into energy products. The P/BHP is also investigating systems using water as a feedstock and cell-free systems which do not utilize living organisms.

**TABLE OF CONTENTS**

	<u>Page</u>
1.0 Introduction .....	1
2.0 Program Element: Aquatic Species .....	3
2.1 Background.....	3
2.2 Objectives .....	8
2.3 Status .....	9
2.3.1 Biological Research .....	9
2.3.2 Engineering Research and Development .....	17
2.3.3 Technology Analysis .....	21
2.4 Summary.....	25
3.0 Program Element: Anaerobic Digestion .....	27
3.1 Background.....	27
3.2 Objectives .....	31
3.3 Status .....	32
3.3.1 Pretreatment .....	32
3.3.2 Biological Processes .....	36
3.3.3 Engineering Parameters .....	42
3.4 Summary.....	45
3.5 References .....	46
4.0 Program Element: Photo/Biological Hydrogen .....	47
4.1 Background.....	47
4.1.1 Whole-Cell Hydrogen Technologies .....	47
4.1.2 Cell-Free Hydrogen Technologies.....	49
4.1.3 Engineering Research .....	50
4.2 Objectives .....	50
4.3 Status .....	52
4.3.1 Whole-Cell Research .....	52
4.3.2 Cell-Free Research.....	61
4.3.3 Engineering Research .....	65
4.4 Summary and Forecast .....	71
5.0 Publications .....	73
5.1 Aquatic Publications.....	73
5.2 Anaerobic Digestion Publications .....	74
5.3 Photo/Biological Hydrogen Program .....	76
6.0 Meetings and Presentations .....	79
6.1 Aquatic Species Program .....	79
6.2 Anaerobic Digestion Program .....	79
6.3 Photo/Biological Hydrogen Program .....	81

**LIST OF TABLES**

	<u>Page</u>
2-1 Composition of Microalgae, Macroalgae, and Emergent Plants as Found in Natural Stands and, in the Case of Microalgae, after Laboratory Manipulation .....	4
2-2 Aquatic Species Program Tasks, Subtasks, and Projects .....	8
2-3 Yields and Photosynthetic Efficiencies of Several Microalgae at Moderate Light Intensities and Nutrient Sufficiency .....	10
2-4 Effects of Nitrogen Deficiency on Yields and Photosynthetic Efficiencies of Several Microalgae at Moderate Light Intensity .....	10
2-5 Wetland Species Comparison: Demonstration Plots .....	15
2-6 <u>Typha</u> Planting Stock Comparison .....	16
2-7 Summary of Water Hyacinth Harvest Data by Channel.....	16
2-8 Production of <u>Phaeodactylum tricorutum</u> in 50-m <sup>2</sup> Flume .....	18
2-9 Results of One-Week Productivity Studies with and without Foil Arrays.....	19
2-10 Resource and Environmental Parameters Affecting Microalgal Production Systems .....	22
2-11 Parameters Used in Stratifying the Southwest into Zones of Suitability for Microalgal Systems .....	23
2-12 Land Use/Cover Categories Used for Stratifying the Southwest .....	23
3-1 Anaerobic Digestion Program Tasks and Projects .....	31
3-2 Alkaline Pretreatments of Straw .....	34
4-1 Photo/Biological Hydrogen Program Tasks, Subtasks, and Projects .....	51
4-2 Growth of New <u>Rhodopseudomonas</u> Species on Various Substrates .....	53
4-3 Growth and H <sub>2</sub> Photoproduction by <u>Rhodopseudomonas sphaeroides</u> SCJ on Defined Substrates .....	57
4-4 Purification of Hydrogenase from <u>Chlamydomonas reinhardtii</u> .....	62
4-5 Product Yields in the Photo-Kolbe Reaction .....	63
4-6 Relative Rates of Gas Production with Photocatalytic Semiconductor Powders .....	63

**LIST OF TABLES (Concluded)**

	<u>Page</u>
4-7 (a) Conceptual Near-Term Plant Capital and Operating Costs	
(b) Research Goals for Plant Capital and Operating Costs.....	67
4-8 Thin Polymer Sheeting for Photosynthetic Bacterial Hydrogen Reactors.....	69

LIST OF FIGURES

	<u>Page</u>
2-1 Location of Saline Groundwater Reservoirs in the American Southwest.....	7
2-2 Energy Yields, Expressed as Methane, Produced from Cultures Grown in the Recycled Digester Residues .....	14
2-3 The Two Troughs of One of the Five 4.8-m <sup>2</sup> Shallow Raceways Operating at the University of Hawaii .....	20
2-4 One of the Deep Raceways Operating at the Enbio Facility .....	20
2-5 Land Use/Cover Map of Utah .....	24
3-1 Cross Section of a Reaction Vessel for Determining Autohydrolysis Decomposition Kinetics of Biomass Products.....	32
3-2 Effect of Time on Glucose Decomposition and Formation of Decomposition Products .....	33
3-3 Temperature Profile for Growth of <u>Thermobacteroides saccharolyticum</u> sp. nov .....	35
3-4 pH Profile for Growth of <u>Thermobacteroides saccharolyticum</u> sp. nov .....	35
3-5 Researchers Operating a 400-L Fermenter for the Cultivation of Large Quantities of Methanogenic Bacteria .....	36
3-6 Growth of <u>M. barkeri</u> on H <sub>2</sub> /CO <sub>2</sub> in the Presence of Pyrophosphate .....	37
3-7 Growth of <u>M. barkeri</u> on Methanol in the Presence of Pyrophosphate .....	38
3-8 An Anaerobic Chamber for the Transfer and Manipulation of Methanogens under an Oxygen-Free Atmosphere .....	40
3-9 Survival of <u>Methanococcus voltae</u> after Ultraviolet Irradiation .....	41
3-10 Survival of <u>Methanobacterium thermoautotrophicum</u> after Ultraviolet Irradiation .....	41
3-11 Diagram of a 6.3-L Anaerobic Baffled Reactor .....	42
3-12 Photograph of 3-L Continuously Stirred Anaerobic Reactors for the Study of High Solids Loading of Corn Stover .....	43

LIST OF FIGURES (Concluded)

	<u>Page</u>
3-13 Photograph of a 110-m <sup>3</sup> Dry Fermentation Reactor Showing the Distribution Grid through Which Leachate Was Recycled for Moisture Temperature Control .....	45
4-1 Z-Scheme of Photosynthesis Showing the Flow of Electrons from Water to Hydrogen Evolution or Carbon Fixation under the Influence of Light .....	48
4-2 Paul Weaver of SERI Following Hydrogen Evolution Rates from Cultures of Photosynthetic Bacteria .....	52
4-3 Lawn of Mutants on Screening Plate .....	55
4-4 Genetic Map Locations of the Genes Involved in Hydrogen Metabolism in <u>Escherichia coli</u> .....	56
4-5 (a) Effect of Oxygen Partial Pressure on Hydrogen Production in Cell Suspension (●) and Immobilized Cells (O) (b) Storage Stability of Immobilized Cells in Seawater.....	58
4-6 The Cumulative Yields of the Simultaneous Photoproduction of Hydrogen and Oxygen in Anaerobically Adapted <u>Chlamydomonas reinhardtii</u> .....	59
4-7 Photoproduction of H <sub>2</sub> by <u>R. capsulata/Cellulomonas</u> sp. Cocultures at Initial NH <sub>4</sub> <sup>+</sup> Concentrations from 0.5 to 15 mM, Incubated in Darkness.....	60
4-8 Steve Lien of SERI Assaying Hydrogenase Activity.....	61
4-9 Surface Pressure/Area Curve for Isolated Bacterial Reaction-Center Complexes Obtained on the Air/Water Interface of Langmuir Trough .....	64
4-10 (a) Uninsulated Channel Reactor (b) Deep Pond Reactor .....	66
4-11 (a) Comparison of SOLBUG Results with Test Site Data: Nighttime Temperature Profile (b) Cell Concentration as a Function of Depth at Various Light Intensities .....	68
4-12 Exposure of Various Polymer Films in a Photosynthetic Bacterial Hydrogen Reactor.....	70

## SECTION 1.0

### INTRODUCTION

Most products derived from petroleum and natural gas can be produced directly from biomass. The "biomass" with which this report is concerned includes aquatic plants, which can be converted into liquid fuels and chemicals; organic wastes (crop residues as well as animal and municipal wastes), from which biogas can be produced via anaerobic digestion; and organic waste streams or water, from which hydrogen can be produced by photobiological processes. The challenge is to develop technology that will be competitive with existing processes using nonrenewable resources. This challenge provides several important opportunities for high risk, high payoff research in biomass production and conversion technologies.

The Biomass Program Office supports research in three areas which, although distinct, all use living organisms to create the desired products. The Aquatic Species Program (ASP) supports research on organisms that are themselves processed into the final products, while the Anaerobic Digestion (ADP) and Photo/Biological Hydrogen Programs (P/BHP) deal with organisms that transform waste streams into energy products. The P/BHP is also investigating systems using water as a feedstock and cell-free systems which do not utilize living organisms.

As previously stated, virtually every organic compound utilized by industry today can be obtained from plant material. Ways that may prove practical have been identified for rapidly producing aquatic plants containing high percentages of the compounds most sought after for fuel and chemical replacements. Furthermore, a number of aquatic plants can be produced under conditions that are not suitable for conventional crops (e.g., in saline water or on low-quality arid land).

The Aquatic Species Program addresses the development of processes that utilize aquatic plants in the renewable production of fuels and chemicals. Emphasis is placed on salt-tolerant species for cultivation on poorly utilized, low-value lands, where conventional agriculture is not economical. Candidate species include microalgae—unicellular plants capable of converting sunlight into high quality oils; macroalgae—large, chemically unique plants that can be easily fermented to methane or alcohols; and emergents—plants that grow rooted in waterways and bogs, but are partially exposed above water, which can also be converted to methane or alcohols.

The anaerobic digestion process has been used for years to obtain a useful energy product (methane gas) from very wet or otherwise low-quality biomass resources. Relatively unsophisticated, low-cost processes are possible and anaerobic fermenters based on manure or other sewage feedstocks are well addressed outside the DOE program. Less well developed are fermenters utilizing crop residues and other cellulosic materials as feedstock, and these are, therefore, the main thrusts of the ADP. The bacteriological processes functioning within these systems are multistep and complex and are only now being delineated and understood. Since the development of low-cost manure fermenters is relatively far along in the commercialization stage, the primary research opportunity lies in the investigation, understanding, and biological control of the anaerobic digestion process with a view toward greater digester efficiency, better crop residue utilization, and possible energy product selectivity.

The basis for research in the P/BHP is the prediction of a significant future hydrogen demand. In fact, hydrogen use in the United States is expected to grow considerably over the next two decades. Current U. S. hydrogen use is approximately  $10^{18}$  J/yr (1 quadrillion Btu/yr). This represents the consumption of an equivalent of half a million barrels of oil per day for the production of the hydrogen which is chiefly used in petroleum refining and ammonia and methanol manufacture. The movement of the petroleum industry toward the processing of heavier sour crudes requires greater usage of hydrogen in the necessary hydrotreating and desulfurization of these heavy crudes. In addition, hydrogen will be required for the production of synfuels from tar sands, oil shale, coal, and biomass. All of these resources are deficient in hydrogen and require enormous amounts of this element for conversion into useful liquid fuels. Use of renewable hydrogen could double the yield of synthetic fuels from coal. The electric utilities have expressed an interest in moving to the use of fuel cells as load-leveling devices. Hydrogen is an ideal fuel for fuel cells. Some projections predict as much as an additional  $10^{18}$  J (1 quad) of hydrogen will be needed for electricity production using fuel cells by 1990. By 2000, the total use of hydrogen could be  $5-20 \times 10^{18}$  J (5-20 quad), much of which would be obtained from petroleum if present technology were used. If an aggressive research program were established, much of this hydrogen demand could be met through renewable resources. At least a portion of it could be produced using biological hydrogen technology. A significant added benefit is the efficient treatment of biological wastes.

The remainder of this report is organized into five sections: Section 2.0 discusses the Aquatic Species Program; Section 3.0 concerns the Anaerobic Digestion Program; Section 4.0 outlines the Photo/Biological Hydrogen Program; and Sections 5.0 and 6.0 list the year's publications and meetings, respectively.

## SECTION 2.0

### PROGRAM ELEMENT: AQUATIC SPECIES

#### 2.1 BACKGROUND

The overall objective of the Aquatic Species Program (ASP) is to support research on the utilization of plant biomass, a renewable resource, for the production of fuels and other high energy products. In particular, the plants under investigation are microalgae, macroalgae, and emergents. Microalgae are unicellular organisms about 1 to 10 micrometers in diameter that live totally submerged in water, either fresh or saline. They are capable of rapid growth, providing biomass yields that significantly surpass those attained by terrestrial plants. Macroalgae range in size from a microscopic collection of cells to giant seaweeds more than 60 m long. In fact, the giant seaweed Macrocystis is the longest known plant in the world. Macroalgae exist rooted to the ocean floor and free-floating on or suspended in marine and fresh water. Emergent plants are commonly found in bogs of fresh or marine water, rooted to the submerged ground or mud and growing erect above the water level. Familiar genera with potential for energy production include Typha (cattail), Scirpus (rush), and Phragmites (reed).

All of these plants use the sun's energy to convert water, a carbon source such as carbon dioxide, and various mineral nutrients into energy for internal consumption and living matter necessary to grow, maintain health, and reproduce. This solar utilization process is known as photosynthesis, and the myriad internal physical and chemical processes constitute the organism's metabolism. The products of cellular metabolism are proteins, carbohydrates, and lipids.

Proteins are either enzymes (catalysts involved in the cell's complex chemical reactions) or compounds of the cell's physical structure. Carbohydrates are stored for short-term energy needs (as sugars and starches) and are also integrated into the cell's structural components, its physical support and boundary system (as cellulose and hemicellulose). Lipids are a form of long-term energy storage and are found within the cell membrane (the lining just inside the cell wall) and within the cell body.

The use of proteins is similar in microalgae, macroalgae, and emergent plants. However, thus far only microalgae have been found to use lipids and carbohydrates as their energy supply. A group of microalgae called diatoms are especially prolific at synthesizing lipids. Macroalgae and emergent plants are now thought to use carbohydrates exclusively for storing energy, the more complex starch molecules being used for long-term storage and the simple sugars for short-term use. What little lipid is found in them is a cell membrane constituent. SERI is currently sponsoring research on macroalgae to determine definitively the extent of their ability to synthesize lipids. Cellulosic carbohydrate provides support for an organism's structure. There is, therefore, very little found in microalgae and macroalgae, but it comprises a significant percentage of the total weight of emergent plants, which must maintain an erect posture.

The use to which each category of plant is put depends on the natural plant composition (relative amounts of protein, carbohydrate, lipids, ash, moisture) and on how effectively the plants can be manipulated to enhance production of a specific, desirable component. Table 2-1 presents data on plant composition as found in nature and as a result of manipulation in the laboratory.

**Table 2-1. Composition of Microalgae, Macroalgae, and Emergent Plants as Found in Natural Stands and, in the Case of Microalgae, after Laboratory Manipulation**

	Microalgae		Macroalgae		Emergents
	Natural	Manipulated	Natural	Manipulated <sup>b</sup>	Natural
Carbohydrate (%) <sup>a</sup>	20-30	5-90	73-85	73-85	70-85
(Cellulose)	(6-9)	(1-5)	(3-8)	ND	(20-25)
(Starch + C <sub>6</sub> Sugars)	(12-17)	(4-73)	(67-80)	ND	(35-40)
(C <sub>5</sub> Sugars + Lignin)	(2-4)	(1-12)	ND	ND	(15-20)
	no lignin	no lignin	no lignin	no lignin	
Protein (%) <sup>a</sup>	50-60	10-60	12-20	ND	4-10
Lipid (%) <sup>a</sup>	7-25	7-95	3-7	ND	2-7
Ash (%) <sup>c</sup>	8-36	8-36	25-49	25-49	3-8
Moisture (%) <sup>c</sup>	88-92	88-92	88-92	88-92	60-70

Note: "C<sub>6</sub> sugars" refer to six-carbon sugar molecules (e.g., glucose, fructose, and sucrose) and "C<sub>5</sub> sugars" to five-carbon sugars (pentoses). ND means no data.

<sup>a</sup>Percentage of dry, ash-free weight.

<sup>b</sup>Laboratory data for macroalgae are currently being generated.

<sup>c</sup>Percentage of total weight.

The research supported by the Aquatic Species Program is organized into three elements: biological research, engineering research, and technology analysis. The first of these—biological research—involves identification, culture, evaluation, and manipulation of these three types of aquatic species.

The thrust of microalgae research has been toward developing a technology that will maximize the production of lipids. Lipids are a useful class of compounds because they have a high energy content and can be used as or converted to hydrocarbon fuels and chemicals, thus enabling direct replacement of petroleum. Table 2-1 exhibits both the naturally high lipid content of microalgae and the tremendous potential for increased lipid production by laboratory manipulation. Microalgae are also capable of producing large quantities of protein feeds, a characteristic already being exploited by a number of commercial facilities. The microalgae production facility of the future, like the petroleum refining of today, will probably deliver a variety of products including fuels, specialty chemicals, and protein feeds.

Historically, the objective of macroalgae research has been the production of biogas by anaerobic digestion. This process converts all of the plant except the ash and moisture into a mixture of methane and carbon dioxide. Work sponsored by SERI at the Harbor Branch Foundation has shown the sludge remaining after completion of the digestion process to be an effective enrichment medium for stimulating macroalgae growth. This eliminates the need for importing nutrients to the macroalgae facility. Preliminary work at the Harbor Branch Foundation is underway to investigate the potential of macroalgae for petroleum replacement products similar to those of microalgae.

Table 2-1 shows the extraordinarily high carbohydrate production potential of all three plant types. Alcohol production by microalgae is not being considered by the ASP primarily because alcohol has a lower energy content than do lipids and its utility as a petroleum replacement is more limited. Alcohol production is an obvious application for both macroalgae and emergent plant research. At present C<sub>6</sub> sugars and starches (with mild pretreatment) can be directly fermented to ethanol, and C<sub>5</sub> sugar fermentation yeasts are being developed in the lab. Cellulose is actually a polymer consisting of many C<sub>6</sub> sugar units, and it can be broken down into these fermentable C<sub>6</sub> sugar units by hydrolysis. Lignin, present as a kind of cement for the cellulose fibers where structural rigidity is necessary, cannot be fermented and, in fact, makes hydrolysis of cellulose much more difficult. Since the hydrolysis step is expensive, the ideal biomass feedstock for alcohol production contains almost all starches and sugars with very little cellulose and no lignin; in other words, macroalgae.

Research supported by the ASP is broken down into biological research, engineering research, and technology assessment. Biological research includes what has already been referred to as the "manipulation" of microalgae in the laboratory. This manipulation can take many forms, all of which are attempts to force the microalgae to produce the desired product—lipids in this case—as a larger-than-natural fraction of their cell weight. One method involves alteration of the microalgae's aquatic environment such that they naturally respond to these stimuli by producing more lipids. Subjecting certain microalgae to a deficiency in the nutrient nitrogen stimulates lipid production at the expense of proteins (which require nitrogen for their manufacture).

Alternatively, scientists can attempt to change the way the microalgae naturally respond to their environment. This can be done by tampering with the cell's genetic machinery, which is responsible for reproduction and metabolic regulation. This can be done by mutagenesis or genetic engineering. In mutagenesis, chemicals introduced into a batch of microalgae enter the cells and modify the DNA—the blueprint for all the proteins used by the cell. Since the proteins are catalysts for the chemical activities involved in cell metabolism, the hope is that the altered metabolic processes in some of the cells will subsequently increase lipid production. After mutagenesis, the batch of microalgae contains cells that are unaffected and mutant cells whose biochemistry has been changed in a number of different ways. Because of this lack of selectivity the batch must be screened to discover which, if any, of the mutants have acquired the hoped-for traits. In most instances the mutations will not last and the cell reverts back to its original state.

Mutagenesis can be the first step in the second genetic manipulation technique—genetic engineering. Using genetic engineering, scientists transfer a specific gene or a group of genes from one cell to another. Since each gene is the blueprint for one protein, genetic engineering techniques can be very selective. They are, however, very complicated and have not yet been developed for microalgae.

All of the above-mentioned techniques for microalgae manipulation can be performed in the laboratory as part of the ASP biological research. One activity assumed to be done prior to and along with the manipulatory research is species collection and screening. Through this process, scientists attempt to find species of microalgae that naturally produce significant quantities of the desired product, are amenable to manipulation for increasing the quantities of this product, and can thrive in the environment foreseen for eventual mass production. It is also necessary to define each species' nutritional needs and tolerances to variations in such environmental parameters as temperature, pH, and salinity. These tolerance levels are important because it would be too expensive or even impossible to maintain rigid control over these parameters in the field.

Engineering research is concerned with the productivity of the algae under the actual field conditions to be encountered in the mass production system. The engineer is concerned with not only the algal growth unit, but also the harvesting technique and the processing technology necessary to turn out the final product or products. The bulk of the engineering research to date has dealt with the algae growth unit, which is referred to as the "mass outdoor culture."

This research progresses from analysis of theoretical and laboratory data to conceptualization of system designs, followed by construction and operation of the various designs and, finally, performance and cost comparisons of each concept to decide which are worthy of scale-up for final debugging. The initial theoretical analysis step provides a standard against which prototype performance can be measured. Using only theoretical considerations allows definition of the maximum possible performance or yield in such terms as metric tons of lipid product per hectare per year. No limitations connected to the operation of a real system are included. Instead, only limitations imposed by nature are considered—mainly, availability of sunlight and the efficiency of its use by the organism. The introduction of laboratory data into the analysis defines a more realistic performance goal by defining the limits of manipulation and control of the algae and their environment. In the laboratory the scientist can use very sophisticated equipment on a very small scale to exert the maximum possible control over the microalgal environment. This degree of control is very expensive. When dealing with economic realities, the engineer is faced with trade-offs between cost and productivity.

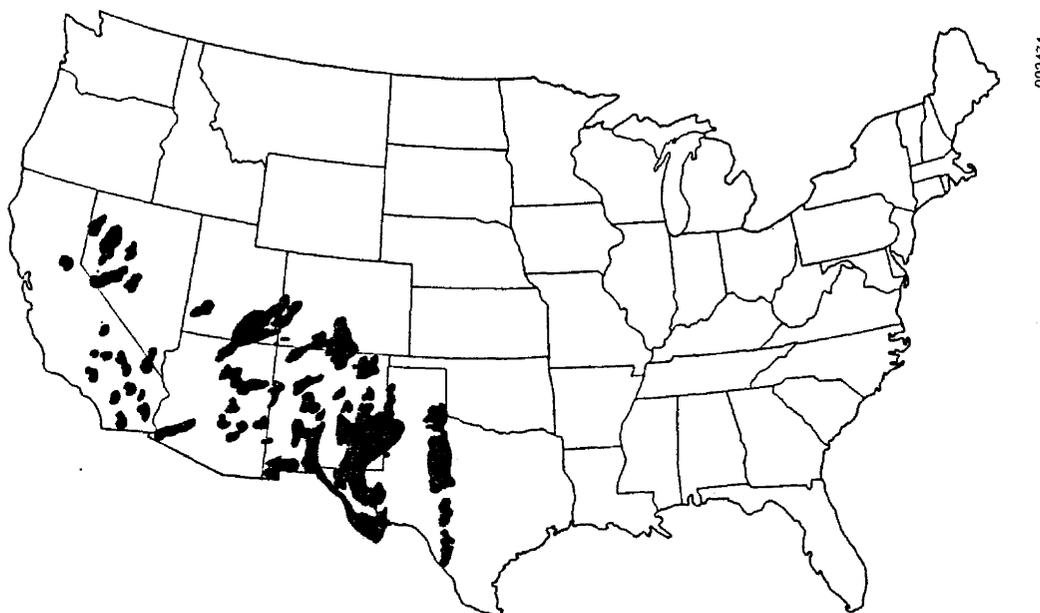
In response to this dilemma, the ASP is sponsoring engineering research using a high-cost, high-productivity prototype system and a lower-cost, lower-productivity prototype system. The first is operating at the University of Hawaii and is termed a "shallow covered raceway" (SCR) system. The second is run by EnBio, Inc., of California and is called a "deep open raceway" (DOR) system. The SCR system has a plastic cover to minimize escape of CO<sub>2</sub> and evaporative loss of water, to lessen the danger of predation (attack by predator organisms can wipe out an entire microalgae population overnight), and, depending on the cover design, to reduce or eliminate the infrared portion of the solar spectrum, which cannot be used by the algae. The SCR design is shallow to accommodate high-density cultures (millions of cells per milliliter) and better control of such operational parameters as flow, pH, and salinity. The DOR concept, on the other hand, is much less expensive and exhibits better temperature control because of the greater heat capacity of the larger volume of water and evaporative cooling.

To determine which type of design—the high-cost, high-yield or low-cost, low-yield—is more cost-effective, it is necessary to know or to be able to project the costs of building and operating each system at the time the technology is scheduled for commercialization (10 to 15 years hence for microalgae). To do this, the supply and demand of the required inputs (water, carbon, and the sundry nutrients) must be calculated, as must the market potential for the intended products. This task is the objective of the third area of research in the ASP—technology analysis. The resource assessment subtask is concerned with the availability of the required resources, and the economic evaluation subtask with cost/benefit and ultimate marketability analyses.

Sunlight, land, and water are the most basic requirements. Careful study has shown the American Southwest to be best suited to fulfill these requirements. It is an area with high insolation, a large amount of land with no competitive uses, and a vast supply of saline water available in underground aquifers (see Figure 2-1).

Carbon and nitrogen are the resources needed in the next greatest quantities. A number of potential  $\text{CO}_2$  carbon sources have been identified, including the following: commercial  $\text{CO}_2$ ;  $\text{CO}_2$  trapped in geologic formations in the Southwest; and  $\text{CO}_2$  from mining and processing of calcium carbonate in the Southwest, from power plant stack gases, from synthetic fuel plant waste streams, and from biogas resulting from anaerobic digestion of organic by-products or waste streams from algal facilities. An alternative to  $\text{CO}_2$  may be bicarbonate, depending on the ability of the microalgae species to utilize it. A thorough cost/benefit analysis of the alternative supplies of carbon and the rest of the nutritional needs of the microalgae is yet to be done.

In summary, the Aquatic Species Program supports research on microalgae, macroalgae, and emergent plants. This research is organized into three elements: biological research, engineering research, and technology analysis. It is the objective of biological research to identify, culture, evaluate, and manipulate aquatic species to enable sustained yields of high-value energy products. The engineering research is concerned with design, evaluation, construction, and testing of the components and processes that will be required to produce these yields. The technology analysis element deals with identification of the resources required and available, and evaluation of the economics of the eventual mass-production system. An additional task is technology transfer, which develops lines of communication with the private sector to aid in program element direction and planning and to facilitate the commercialization of each technology as it is developed.



**Figure 2-1. Location of Saline Groundwater Reservoirs in the American Southwest.** 86.4 million acres of land overlays these subterranean deposits and potentially could be used for production of halophylic microalgae.

## 2.2 OBJECTIVES

The overall objective of the ASP is to develop technologies for the production of petroleum replacement products from aquatic plants. Table 2-2 describes how the research is broken down into tasks and subtasks and projects. Descriptions of work in each area follow in Section 2.3.

**Table 2-2. Aquatic Species Program Tasks, Subtasks, and Projects**

---

### Task I: Biological Research

#### Species Collection and Culture

Scripps Institution of Oceanography - Collection and Selection of Oil-Producing Desert Microalgae; also, Yields and Photosynthetic Efficiency of Marine Microalgae.

#### Species Screening

##### Microalgae

Solar Energy Research Institute - Algal Oils R&D

##### Macroalgae

Harbor Branch Foundation - Cultivation and Conversion of Marine Macroalgae

##### Emergent Plants

University of Minnesota - Wetland Biomass Production

#### Species Development

Georgia Institute of Technology - Chemical Profiles of Microalgae

### Task II: Engineering Research

#### Design Coordination

Solar Energy Research Institute - Microalgal Systems Simulation

Jaycor - Biological and Engineering Parameters of Algal Mass Culture

#### Component Development

Subcontracts not yet issued

#### Experimental Facilities

University of Hawaii - Sustaining Yield Performance of Shallow Algal Culture

EnBio - Production of Liquid Fuels and Chemicals by Microalgae

Reedy Creek Improvement District - Water Hyacinth Wastewater Treatment System

### Task III. Technology Analysis

#### Resource Assessment

Solar Energy Research Institute - Resource Assessment for Aquatic Biomass Production Systems

Batelle Columbus - Identification of Resources for Aquatic Biomass Production in the Southwest

#### Economic Evaluation

Solar Energy Research Institute - Cost Budgeting of Microalgal Facilities

---

## 2.3 STATUS

### 2.3.1 Biological Research

Aquatic microorganisms such as algae predominate in the biosphere and represent a major source of photosynthetically produced biomass. Their inherent rapid growth characteristics, combined with diverse chemical compositions, present a significant resource base for the production of fuels and other chemicals.

Because of their high yields and efficiencies of light utilization compared to higher plants, microalgae are attractive potential sources of biomass for energy and protein production. Emphasis is placed on the production of lipids for conversion to hydrocarbons for fuel or petrochemical replacement. The use of freshwater algae would require massive amounts of freshwater, a potentially scarce resource with competing demands for agricultural or domestic use. Marine microalgae, on the other hand, can live in seawater and saline or brackish water, which has been found in large underground aquifers in the desert of the Southwest, where sunlight is also abundant.

#### 2.3.1.1 Species Collection and Culture

The objective of this subtask is to discover, collect, and culture in the laboratory microalgae species that are native to the geographical areas in which large-scale microalgal production is feasible. Data describing the physical and chemical environment of species collection sites will be obtained and the taxonomy and morphology of each collected species will be related to these data.

The initial research in species collection and cultivation was conducted by the Scripps Institution of Oceanography during 1980 through 1982. This two-year study investigated the yields, photosynthetic efficiencies, and proximate chemical composition of dense cultures of marine microalgae. The experimental plan, carried out with 12 marine microalgae involved growing the algae in continuous, flat-sided culture systems, where they were subjected to conditions of nitrogen deficiency to induce increased cell lipid content.

The algae studied by Scripps include Phaeodactylum tricornutum, Dunaliella primolecta, Monallanthus salina, Isochrysis, Tetraselmis suecica, Porphyridium cruentum, Rhodospseudomonas sp., Botryococcus braunii, Playtmonas sp., Carteria pallida, Chaetoceros gracilla, and Chaetoceros affinis. The selection of these algae and diatoms was based on prior research results obtained by Scripps and other research laboratories which indicated high specific growth characteristics and high lipid accumulation in the cells when grown under certain environmental conditions.

Table 2-3 indicates that the highest yield (21.5 g dry weight/m<sup>2</sup> day) and efficiency (12.2%) were obtained with Phaeodactylum at a light intensity equaling 29% of the maximum sunlight at La Jolla, Calif., and nitrogen sufficiency. Lipid and protein yields were 5.62 and 13.0 g dry weight/m<sup>2</sup> day, respectively. Comparative higher plant yields average 7.3 g dry weight/m<sup>2</sup> day. Increased light intensity or cell density did not increase yields or efficiencies. Furthermore, while nitrogen limitation did increase lipid content (20% to 30%) per cell, the overall lipid yield decreased to 2.39 g/m<sup>2</sup> day because the overall dry weight was lower (Table 2-4). The data presented in Table 2-3 indicate that no other species gave as high a yield or efficiency as Phaeodactylum. The yield and efficiency data range from 4.0 to 21.7 g/m<sup>2</sup> day and 1.7% to 12.2%, respectively. While P. tricornutum attained the highest yields and efficiencies of the species examined, species-specific optimization of culture conditions may push other species to similar high

**Table 2-3. Yields and Photosynthetic Efficiencies of Several Microalgae at Moderate Light Intensities and Nutrient Sufficiency**

Alga	Yield (g dry wt/m <sup>2</sup> day)	Efficiency (%)	Lipid Yield (g/m <sup>2</sup> day)	Protein Yield (g/m <sup>2</sup> day)
<u>Phaeodactylum</u> (Batch culture)	21.7	12.2	5.62	13.0
<u>Phaeodactylum</u> (Continuous culture)	16.2	6.2	3.22	9.5
<u>Dunaliella</u> (Batch culture)	8.7	3.5	—	—
<u>Dunaliella</u> (Continuous culture)	12.0	3.8	2.59	8.4
<u>Monallanthus</u> (Continuous culture)	12.0	3.8	2.59	8.4
<u>Tetraselmis</u> (Batch & cont. cult.)	13.0	7.6	4.48	—
<u>Isochrysis</u> (Batch culture)	6.7	2.8	1.77	—
<u>Botryococcus</u> (Batch culture)	4.0	1.7	1.20	—

**Table 2-4. Effects of Nitrogen Deficiency on Yields and Photosynthetic Efficiencies of Several Microalgae at Moderate Light Intensity**

Alga	N Status	Yield (g dry wt/m <sup>2</sup> day)	Efficiency (%)	Lipid Yield (g/m <sup>2</sup> day)	Protein Yield (g/m <sup>2</sup> day)
<u>Phaeodactylum</u>	+N	16.2	6.2	3.22	9.5
	-N	7.8	4.1	2.39	1.7
<u>Dunaliella</u>	+N	12.0	4.2	2.59	8.4
	-N	10.2	3.8	1.40	2.7
<u>Monallanthus</u>	+N	7.1	4.0	1.42	2.8
	-N	6.6	3.7	0.79	1.3
<u>Tetraselmis</u>	+N	18.3	7.6	4.48	—
	-N	15.6	6.5	1.87	—

yields and efficiencies. Finally, while the values presented in Table 2-3 agree with values obtained by other researchers in laboratory studies using the same species, outdoor experimental systems operated by the University of Hawaii have achieved much lower yields (10.5-11.6 g/m<sup>2</sup>/day) and efficiencies (3.4%-4.4%) than achieved in the labo-

ratory studies. The degradation of the yield and efficiency of microalgae cultivation as one moves from the laboratory to outdoor cultivation systems indicates the amount of scientific and engineering research required before microalgae production systems become feasible.

The saline water resources of the southwestern United States offer a potential water supply other than seawater for the production of microalgae. The water in this region is an attractive resource because sunlight is abundant, the salt content of these waters is high, and such water is generally undesirable for conventional agricultural practices.

Scripps is the lead research subcontractor in the efforts to identify promising desert species of microalgae. This research activity consists of isolating desert algae into monoalgal cultures to assess their potential yields and efficiencies of light utilization and their chemical composition—in particular, their lipid content and yield. The collection, isolation, and analysis of individual algal species found in the desert will be conducted by a mobile field laboratory. Water samples will be collected and analyzed for inorganic constituents, and algal species will be isolated into monocultures for detailed analyses.

### 2.3.1.2 Screening and Species Development: Microalgae

Once a variety of microalgal species have been collected, identified, and stored in cultures, it is necessary to define, qualitatively and quantitatively, potential chemical and energy products from the various species. Specific objectives are to (1) examine the effects of cultivation conditions on the chemical composition of microalgal species found in saline and hypersaline environments, and (2) examine the chemical composition of the microalgal species, with particular emphasis on lipids.

Research underway at SERI involves identification and characterization of lipid-producing, salt-tolerant microalgal species. Candidate species are tested in the laboratory to obtain data on the chemical and physiological responses each strain exhibits to specific environmental conditions. These characterizations will allow researchers to evaluate and select promising microalgae based on their oil-producing potential. Currently, 24 species from 11 genera of green microalgae have been established in a culture collection. Cataloging and documenting these algae will continue during FY 1983, in addition to collecting, identifying, and isolating additional salt-tolerant species.

SERI researchers isolated a new strain, designated CX-SO3, from a local soil sample. This strain of algae exhibits fast growth characteristics, produces a high lipid concentration and tolerates up to 0.1 M salt. In addition, Chlorella SO1a has been extensively tested and characterized for environmental adaptability to salinity stress conditions. The physiological responses of Chlorella to nitrogen starvation and high salinity provide insight into the mechanisms involved in the regulation of lipid formation and metabolism.

Detailed chemical analyses of the chemical composition of various algae isolated by ASP researchers are being conducted by researchers at the Georgia Institute of Technology. Four cultures of different algal species are being maintained and subjected to extraction procedures for lipid and carbohydrate analysis. Additional algal species selected as potentially high lipid producers are being sent to Georgia Tech. as they are isolated. The four species currently in culture include Dunaliella bardawil, D. salina, Isochrysis galbana, and Botryococcus braunii. The algae are grown in artificial hypersaline media under controlled conditions of light intensity, temperature, and shaking. These cultivation experiments aid in developing baseline data for nutrient requirements, specific growth rates, and maximum cell density.

Preliminary results from the Dunaliella and Isochrysis species indicate that these algae can multiply with a doubling time of about 20 hours to a maximum cell concentration of  $2 \times 10^6$ /mL. This doubling time is not exceptional for these species; however, as a result of laboratory manipulation, the cell concentration is much higher than the  $10^6$ /mL usually exhibited. Efforts to increase the growth rate by varying environmental conditions in the culture have been initiated with particular emphasis on the impact of nutrient limitation on the biosynthesis of lipids.

After the cells are grown in the culture, samples are taken for detailed chemical analysis. These laboratory experiments require column fractionation of the algal samples using polar and nonpolar solvents. Each fraction obtained from the column is subjected to a series of tests to identify and quantify the types of hydrocarbons and lipids contained in the sample. The tests involve thin-layer chromatography, gas-liquid chromatography, and staining procedures.

Preliminary results of the Isochrysis lipid analysis have shown a marginal shift to higher total lipid production and a dramatic shift (40%) toward increased neutral lipid production. This demonstrates that significant alteration in the relative concentrations of the individual lipids is possible by environmental manipulation. This result is significant since maximum production of higher-value products like these neutral lipids is, obviously, desirable.

This project also developed and refined laboratory techniques for conducting microalgae lipid extraction and identification. These techniques were then organized into a laboratory syllabus for use by other researchers in the ASP. The syllabus was completed by Georgia Tech. researchers for a short course on laboratory procedures for lipid fractionation and identification planned for FY 1983.

### 2.3.1.3 Species Screening and Development: Macroalgae

The original concept of an energy farm based on the production of macroscopic algae (seaweed in particular) was the "open ocean farm," a suspended framework structure, buoyed and moored at depths of 700 m or more in the open ocean, to which plants like the giant kelp (Macrocystis pyrifera) would be attached. Nutrient-rich bottom water would be pumped through a pipe to the surface and through the kelp plants as their source of nourishment. Costs of pumping the deep water to the surface and other aspects of the system associated with its deep sea and open ocean location caused initial doubts concerning its economic feasibility and energy cost-effectiveness. A small test module was anchored off the coast of California in 1978 and was, from the start, beset with numerous technical problems, eventually sinking to the bottom without producing useful data.

Alternative concepts investigated through SERI funding in FY 1982 are an energy production system to be located in shallow, near-shore, and protected coastal ocean areas and a combination wastewater-treatment/energy-production facility. Work on the first concept was begun in 1979 and transferred to the University of Florida, where the work was carried out in FY 1982. This research was initiated with a screening program designed to evaluate growth and biomass production of all macroscopic algal species that could be obtained in adequate quantity in the central Florida area. A total of 42 species were assessed, including 6 green algae (Chlorophyta), 2 brown algae (Phaeophyta), and (the remainder) red algae (Rhodophyta). Of these, the most successful and suitable species was Gracilaria tikvahiae.

In FY 1982, Gracilaria was routinely maintained in suspended culture by aeration. This process was found to enhance growth better than totally unaerated, pond-bottom cultures. Aeration is, however, a major cost and energy consumer that should be minimized. To that end, research has shown that intermittent aeration, for as little as six hours per day, under two different periodicities, results in the same yields of Gracilaria as does continuous aeration.

One of the major problems and economic costs of a large-scale seaweed biomass system would be the supply of essential nutrients to the individual plants and the retention of the enriched water within the area of cultivation long enough for the nutrients to be assimilated by the seaweeds. However, research has shown that nutrient-deficient seaweed is capable of rapidly assimilating and storing inorganic nutrients which may then be drawn upon for normal growth for periods of days to weeks in the absence of an external nutrient supply. In another series of experiments, the long-term effects of rapid nutrient uptake and storage on growth of the seaweed were examined. The results of this experiment showed that soaking Gracilaria in nutrient-enriched seawater for as little as six hours every two weeks is sufficient to enable the plants to grow at the maximum possible rate under the conditions of the experiment.

The factor that has been found to date to be the most important in affecting the growth and yield of Gracilaria is the seawater exchange rate (retention time). Since pumping water is a (probably the) major cost factor in the culture system described here, clearly it would be desirable to achieve the high yields possible at very rapid exchange rates with much less water flow. First, however, it is necessary to understand the relationship between yield and water exchange.

The only essential nutrient not provided in the artificial enrichment achieved by removal and soaking is carbon dioxide, which exists in equilibrium with bicarbonate. Removal of free CO<sub>2</sub> during the photosynthetic growth of algae increases the pH; the slower the circulation of seawater through the seaweed, the higher the pH becomes. At pH 9.0, there is almost no free CO<sub>2</sub> in seawater and its rate of formation from bicarbonate to maintain the equilibrium is so slow that it may become limiting to photosynthesis and growth. Gracilaria can use little or no bicarbonate, and its photosynthesis at pH 9 is only 19% of that at pH 7.5. Other species of seaweed apparently do utilize bicarbonate readily, for they continue photosynthesis and growth at high pH. The green alga Ulva lactuca was found to produce oxygen at pH 9.1 at 72%—the same as the rate at pH 7.5 (versus 19% for Gracilaria). Ulva would thus be an ideal candidate for marine biomass production. It has grown and remained sterile in culture for a year, thereby avoiding the no-growth periods associated with reproduction.

While separate pulse feeding of seaweeds has obvious economic advantages over continuous enrichment of the seawater flowing through the cultures, an enrichment medium made from commercial fertilizers or bulk inorganic chemicals still represents a major cost of the production system. However, where seaweeds are digested anaerobically to produce methane, all of the essential nutrients remain in the digester residue or effluent and represent a potential enrichment medium that could be supplied to the production system at little or no cost. The growth of Ulva when fed this residue was found to be equal to or better than that of inorganically fed Ulva. Such was not the case for Gracilaria because of its greater resistance to digestion than Ulva.

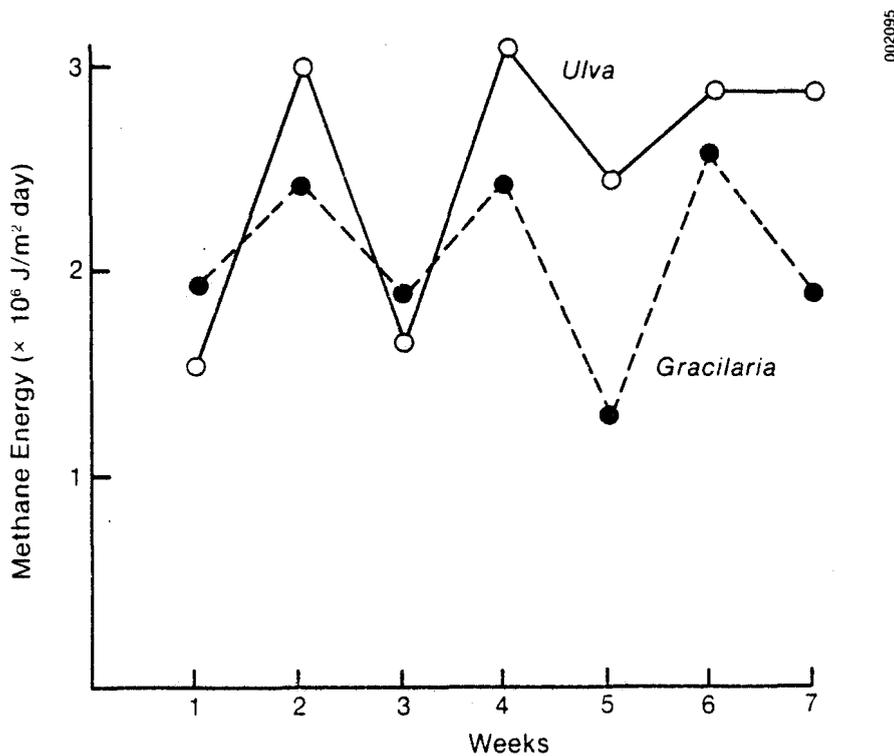
Growth of Ulva in both media declined, however, due to increasing summer temperatures that eventually proved lethal by mid-July. A more temperature-resistant species is being

sought. Figure 2-2 shows the energy produced as methane ( $J/m^2$  day) from the two residue-fed cultures. Note that although the yields of Ulva were on an average lower than those of Gracilaria, particularly during the latter part of the experiment when Ulva was losing its fight against high temperatures, the energy yield of Ulva was greater due to its greater digestibility.

**2.3.1.4 Species Screening and Development: Emergent Plants**

Research on emergent aquatics was begun by SERI in 1980 when a subcontract was let to the University of Minnesota to investigate the use of wetlands to produce biomass energy crops. Wetlands dominated by Typha (cattails) and other emergent vegetation, such as Phragmites (reeds) and Scirpus (rushes), are among the most productive systems in the temperate zone. Minnesota, with over 2 million hectares of available peatland and 1.4 million additional hectares of available wet mineral soils, appears to have considerable potential for wetland crop production. Among the attractive features of this system is the fact that wetland crops would not compete with traditional crops for prime agricultural land. The use of peatlands for the production of a renewable resource also offers an attractive alternative to peat mining.

The Minnesota research effort has demonstrated that total annual biomass yields between 20 and 30 dry tons/hectare are possible in planted stands. This compares with yields of total plant material between 9 and 16 dry tons/hectare in a typical Minnesota corn field. At least 50% of the Typha plant is composed of a below-ground rhizome system containing 40% starch and sugar, making rhizomes an attractive feedstock for alcohol production.



**Figure 2-2. Energy Yields, Expressed as Methane, Produced from Cultures Grown in the Recycled Digester Residues**

Research into *Typha* stand establishment has focused on planting material and land preparation schemes. Planting material can be in the form of seed, seedlings, or rhizome pieces. Seed is by far the least costly, but initial results indicate low first-season yields. However, it appears that densities, heights, and yields will be extremely high for the area, planted with seed in 1981, which is now being harvested and analyzed. Land preparation studies to test the feasibility of growing crops on mined peatlands involved the excavation of two areas by removing 0.6 m and 1.5 m of peat, and the simple rotovation of a third area. Excavation proved to be an effective, though costly, weed control method, and the 0.6-m excavation area required the least amount of water control. From preliminary observation of the 1982 fall harvest, *Typha* yields will be significantly greater, especially for the replanted seedling plots in the unexcavated area.

Nutrient experiments showed that phosphorous and potassium applications increased the density, and potassium application increased the below-ground dry weight. Wetland species comparisons involved *Scirpus flaviatiles* (rush), *Sparganium eurycarpum* (bur reed), *Spartina pectinata* (cordgrass), and *Phragmites australis* (reed) grown in thirty-six 1.5-m<sup>2</sup> paddies under identical conditions. *Scirpus* attained the highest first-season yields—12.8 metric tons/ha (total dry weight)—as can be seen in Table 2-5. *Phragmites* performed very poorly owing to problems with initial establishment. *Typha* rhizomes collected from five productive natural stands and a commercial nursery had yields within the range usually found in natural stands, with a mean yield of 20.6 metric tons/ha (Table 2-6). Finally, laboratory work on micropropagation resulted in the formulation of *Typha* implants in tissue culture, and infestation of the *Typha* crop with canary and reed meadow grass, the primary competitors, was effectively eliminated used 2.3 L/ha of Roundup herbicide.

Research on the wastewater-treatment/energy-production concept was done by the Reedy Creek Improvement District in Orlando, Florida. The project focused on rapid evaluations and optimizations of system parameters, optimization of biomass yields, and expansion of biomass yield capacity while maintaining acceptable wastewater treatment standards.

The system capacity has been increased by the addition of two more water hyacinth channels for testing and comparison of the effects of system variables. Despite delays due to construction and equipment-delivery problems, mechanical failures, and harvesting data variability, much information has been acquired on system operation and efficiency. The research has demonstrated that mean biomass yields of up to 132 dry

**Table 2-5. Wetland Species Comparison: Demonstration Plots**

Excavation Depth (m)	Plant Genus	Yield (metric tons dry wt/ha) <sup>a</sup>			Shoot Density (per m <sup>2</sup> )
		AG	BG	TTL	
0.0	<i>Sparganium</i>	6.7	4.0	10.6	78
0.6	<i>Sparganium</i>	2.5	3.3	5.8	50
1.5	<i>Sparganium</i>	5.2	4.9	10.1	73
0.0	<i>Scirpus</i>	7.5	5.3	12.8	105
0.6	<i>Scirpus</i>	4.7	5.3	9.9	109
1.5	<i>Scirpus</i>	5.1	3.7	8.8	94
0.0	<i>Phragmites</i>	0.2	0.2	0.4	10
0.6	<i>Phragmites</i>	0.4	0.2	0.7	17
1.5	<i>Phragmites</i>	0.7	0.4	1.1	70

<sup>a</sup>AG = above ground; BG = below ground; TTL = total.

**Table 2-6. Typha Planting Stock Comparison**

Source of Planting Stock	Yield (metric tons dry wt/ha) <sup>a</sup>				Shoot Density (per m <sup>2</sup> )
	AG	BG	TTL	Net	
Eagle Lake	11.4±0.6	22.6±0.6	11.6±1.7	23.0±1.7	51.0±5.9
Carlos Avery	9.7±0.9	11.8±2.6	21.5±3.1	20.5±3.1	53.0±7.4
Roseau	9.2±0.9	12.1±1.5	21.3±2.3	20.0±2.3	51.0±4.3
Syre	8.6±0.8	10.7±1.7	19.3±2.2	17.6±2.2	49.0±7.4
Fort Snelling	8.3±0.5	9.7±4.4	18.0±4.8	17.2±4.8	53.0±7.7
Kester	5.9±1.4	7.2±3.0	13.1 4.4	12.8±4.4	46.0±8.9

<sup>a</sup>AG = above ground; BG = below ground; TTL = total.

metric tons/acre yr are attainable and that secondary wastewater treatment standards can be met. Table 2-7 shows mean yield data for the three channels; the overall means for channels 1, 2, and 3 are 54.4, 55.1, and 95.4 dry tons/acre yr, respectively. These data indicate a dramatic improvement in yield for the longest detention times and shortest harvesting frequency. The contract has been handed over to the Gas Research Institute.

**Table 2-7. Summary of Water Hyacinth Harvest Data by Channel**

Sample Period	Total Wet Pounds	Mean Harvest Frequency (days)	Mean Yield (dry tons /acre yr)	Water Detention Time (days)
<u>Channel 1</u>				3.6
03/17/81 - 05/05/81	91,038	16.3	36.3 ± 7.2	
05/19/81 - 07/01/81	53,157	14.0	33.2 ± 8.8	
07/14/81 - 09/28/81	21,141	12.0	14.6 ± 5.9	
10/08/81 - 11/24/81	22,540	19.5	11.2 ± 2.9	
12/08/81 - 02/18/82	48,807	25.4	26.9 ± 24.6	
<u>Channel 2</u>				4.3
03/24/81 - 05/13/81	78,888	25.0	30.5 ± 9.8	
05/21/81 - 06/23/81	42,100	10.2	39.2 ± 20.3	
07/06/81 - 09/04/81	36,453	12.0	39.9 ± 25.5	
09/23/81 - 11/12/81	7,569	23.0	4.2 ± 3.9	
12/14/81 - 02/22/82	46,371	51.3	9.0 ± 10.9	
<u>Channel 3</u>				ND
05/05/81 - 06/29/81	49,457	7.8	30.1 ± 16.9	
07/09/81 - 08/27/81	37,758	7.0	52.3 ± 22.9	
09/01/81 - 10/14/81	70,295	8.1	57.6 ± 36.1	
11/09/81 - 02/02/82	91,440	22.0	30.4 ± 21.5	

Conversion Factors: lb x 4.535924 x 10<sup>-1</sup> = kg  
 ton/acre yr x 2.241693 = metric ton/ha yr

## **2.3.2 Engineering Research and Development**

### **2.3.2.1 Design Coordination**

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

Jaycor, a subcontractor to the ASP, is responsible for conducting systems analysis relating the complex interactions of a variety of physical, biological and engineering parameters, and comparing and evaluating system benefits and technical uncertainties. Experimental and theoretical data obtained from literature reviews and from SERI-sponsored experiments are used to identify technical uncertainties and to assist the development of additional experimental designs. These data and relevant technical data are being organized into a data base for use by other researchers in the Aquatic Species Program.

One of the initial tasks completed by Jaycor was an extensive patent search of microalgal technology. The report submitted by Jaycor provides a catalog of all relevant patent activity, including a listing of all the patents, statistical information, and assignees. This information provides a basis for establishing the state of the art and includes the following four appendices: (1) distribution of patenting activity by date, country, and topic; (2) alphabetical listing of assignees and unassigned patents; (3) front pages of each patent; and (4) complete copies of selected patents. In total, 107 patent filings have been identified.

As a new project initiated in May 1982, Jaycor's activities are also being directed toward providing supplemental research data to the SERI Aquatics Species Program. A literature search on microalgal research has begun to identify key biological and technical parameters associated with microalgal cultures. Additional reviews are being conducted to define the types, advantages, and costs of microalgal harvesting systems. Continued support is also being provided to SERI researchers for development of a mass culturing model for microalgae based on the equations of M. R. Droop.

The mass culturing model, which is being designed by Scientific Applications, Inc. (SAI), is to provide an analytical tool for evaluating microalgal production systems, taking into account the effects and complex interactions of the many biological, physical, chemical, and engineering factors involved in algae production, harvesting, and processing into final products. Essentially a process simulation model to describe the operations of a production facility, the SERI model is designed with sufficient flexibility to describe and evaluate alternative cultivation designs and techniques. The overall model comprises two submodels: one to describe the cultivation and harvesting of algae, and the second to describe the processing and conversion to final products. The production model has been coded and operated on the SERI computer system, and satisfactory correlations have been developed using the model to describe the algal production raceway at the University of Hawaii for a thirty-day production cycle.

### **2.3.2.2 Experimental Facilities**

Engineering research is concerned with scale-up of laboratory experiments to larger systems operating in an environment similar to that to be encountered by a commercial facility. For microalgal systems, this entails mass outdoor culturing such as that being done under SERI subcontract at the University of Hawaii. Previous laboratory-scale work at Scripps Institute of Oceanography (see Section 2.3.1) on species screening led to

the selection of the marine diatom Phaeodactylum tricornutum as a good candidate for engineering research because it tolerates a wide range of pH, salinity, and temperature, tends to dominate outdoor mass culture, can withstand buffeting, and excretes products that appear to inhibit the growth of bacteria and other algae. It was also found to exhibit anomalously high lipid contents, reaching 80% of its dry weight under appropriate conditions. The engineering design in use at the University of Hawaii is classified as a shallow covered raceway cultivation system, the basic components of which are

- An infrared exterior shield to protect the culture from radiant heat by selectively screening out and storing infrared light energy
- A CO<sub>2</sub>-enriched gaseous midlayer to help stabilize the growth environment by restricting downward heat transfer and reducing evaporative water loss
- A base algal production zone wherein a high-density culture recirculates under gravity flow.

This year's experimentation used a 50-m<sup>2</sup> flume of this design, while five 8.2-m<sup>2</sup> flumes were constructed in preparation for FY 1983 optimization studies. In the 50-m<sup>2</sup> flume, cell densities initially peaked around 10<sup>7</sup>/mL, but with addition of CO<sub>2</sub> to the medium, cell densities in excess of 2 x 10<sup>7</sup>/mL were achieved. At this point, a monad predator appeared and rapidly grazed down the population. In response to this problem, several mechanisms of predator control were developed, including sonication, sedimentation, chemical addition, and pH increase from 8 to 9.0-9.5. Using pH control and sedimentation, cell densities in the flume were consistently over 2 x 10<sup>7</sup>/mL, and the flume culture has been maintained continuously for seven months. The highest flume cell density to date has been 4.6 x 10<sup>7</sup>/mL. Production during the last three months of the study averaged over 11 g/m<sup>2</sup> day in a mass culture of P. tricornutum (see Table 2-8.)

Recent studies indicate that the low values encountered in the production efficiency can be largely eliminated by running the culture in a semicontinuous rather than batch mode. If these troughs can be eliminated, production can be expected to more than double.

During the year the idea of using foils to effect systematic vertical mixing in the flume was developed. The foils are shaped like airfoils to force the water flowing over them to spiral downward, creating a helical flow pattern downstream of the foil. The optimum angle of attack was found to be approximately 23 degrees at the flow rate and culture depth of the system, with the foil arrays placed 1.2 m apart. The 50-m<sup>2</sup> flume was run for a total of one week with the foil arrays in place, and then for an additional week with

**Table 2-8. Production of Phaeodactylum tricornutum in 50-m<sup>2</sup> Flume**

Month	Production (g/m <sup>2</sup> d) <sup>a</sup>	Light Intensity (einstein/m <sup>2</sup> day)	Efficiency (%)
Nov.	3.0	23.6	1.5
Dec.	2.4	22.5	1.3
Jan.	4.6	30.7	1.8
Feb.	10.5	37.0	3.4
Mar.	11.6	33.6	4.2
Apr.	11.3	40.2	3.4

<sup>a</sup>Grams dry weight.

the foil arrays removed. The results of these two 1-week studies are summarized in Table 2-9. In terms of cost, the foils are simple to fabricate from molds, involve no moving parts, and create negligible pressure drop through the system.

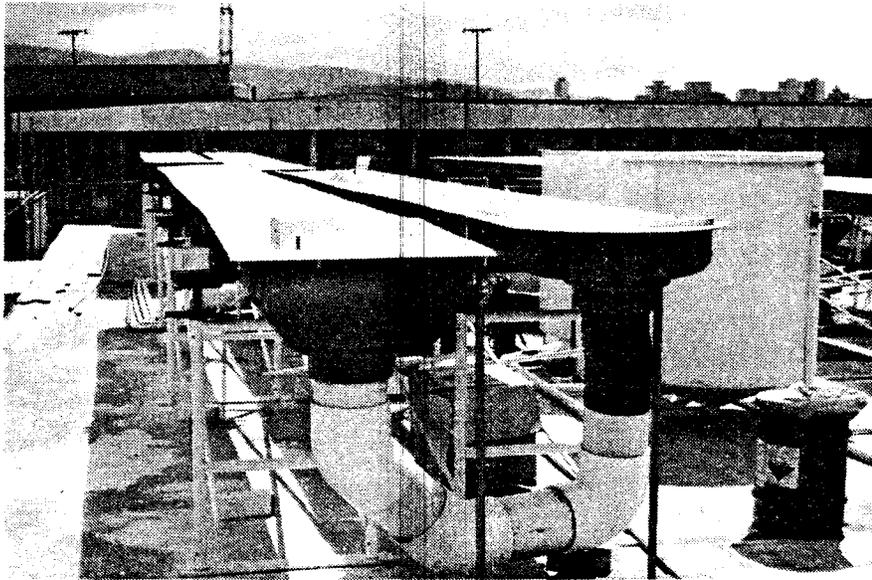
Finally, construction has been completed on the five 8.2-m<sup>2</sup> flumes (see Figure 2-3). These raceways allow the use of factorially designed experiments enabling relatively quick evaluation of a complicated array of system variables, including culture depth, cell concentration, flow rate, temperature, light quality (blue light versus ordinary sunlight), pH, salinity, and nitrogen source. Based on results of foil insert testing in the 50-m<sup>2</sup> raceway, foil design has been optimized and the foil arrays to be used in the 8.2-m<sup>2</sup> raceways have been constructed. A total of 16 different combinations of 8 parameters will be tested with the aim of reducing the number of parameters to be studied in detail. The performance of each raceway will be expressed in terms of both photosynthetic efficiency and productivity. In addition, analysis, modeling, and laboratory support studies will be initiated to provide information on variables that cannot be adequately studied in the mass outdoor cultures. The areas to be investigated include flashing light/turbulence effects, induced by the foils, on algal photosynthesis, algae harvesting strategies, and the existence of strains of Phaeodactylum that are superior in terms of temperature tolerance and light utilization.

An experimental production facility is being operated by EnBio, Inc., of California to investigate various freshwater microalgae species to determine species succession, culture stability, biomass productivity, and lipid yield based on various system operational parameters (see Figure 2-4). The research plan is to inoculate into the open ponds a number of mixed cultures to determine which species dominate after a period of time. These dominant species are subjected to various environmental changes, such as nitrogen deprivation, and data are collected to determine the physical, chemical, and biological responses. Laboratory studies of the species are conducted to determine the gross chemical content of the microalgae. Protein, carbohydrate, and lipid content are assessed.

The open pond experiments are a new research activity in the SERI ASP. At the time of this publication, subcontract negotiations are underway. Thus no research data are available.

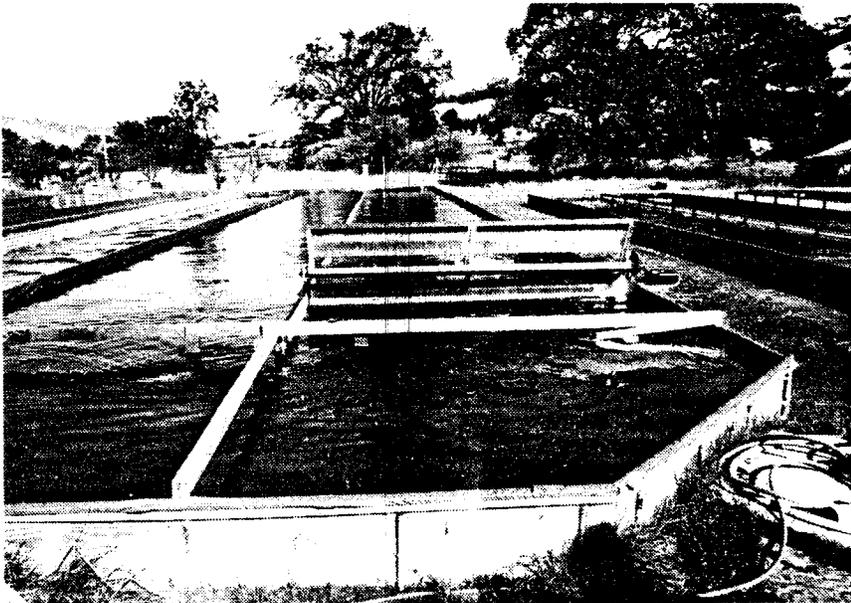
**Table 2-9. Results of One-Week Productivity Studies with and without Foil Arrays**

	With Foils	Without Foils
Average production (g dry wt/m <sup>2</sup> day)	5.35	12.57
Incident light (einstein/m <sup>2</sup> day)	40.6	42.9
Photosynthesis conversion efficiency (%)	3.3	1.5



002482

**Figure 2-3. The Two Troughs of One of the Five 4.8-m<sup>2</sup> Shallow Raceways Operating at the University of Hawaii**



002484

**Figure 2-4. One of the Deep Raceways Operating at the EnBio Facility**

### 2.3.2.3 Component Development

One of the more significant obstacles in producing microalgae-derived oils is the concentration of dilute suspensions into a slurry for subsequent processing. The extent to which present technology for harvesting is economic for microalgae production depends upon the final product value and the type of species used in the growth ponds. For example, existing microalgae facilities producing health food and protein supplements use species such as Spirulina which have a sufficiently large cell size and high enough product value to allow for a high harvesting operational expense. Because some of the microalgal species studied in the ASP are approximately the size of bacteria and fuel is not as highly valued a product, an effective yet cheaper harvesting technology is required.

The ASP has developed several research tasks designed to evaluate existing harvesting methods and to propose innovative methods for increasing the density of the product stream from the growth ponds.

### 2.3.3 Technology Analysis

The technology analysis activities provide R&D guidance for the Aquatic Species Program by analyzing and integrating the results of the biological and engineering research subprograms. This in-house work is accomplished by conducting systems studies of the various research initiatives and identifying the R&D options that offer potential gains to the overall program objectives.

#### 2.3.3.1 Resource Assessment

The objective of this subtask is to assess and evaluate the availability and location of resources required for support of microalgal oil production in order to ascertain the potential magnitude of this production.

The SERI ASP has directed considerable effort to assess the land, climate, and water resources of the southwestern United States and their potential use for producing fuels and chemicals from microalgae. Scripps recently finished one task involving the preparation of an annotated bibliography listing most of the published research on the resources and microalgal species found in the Southwest. The report, "Algae from the Arid Southwest United States: An Annotated Bibliography," has been submitted in draft form to SERI. Efforts are underway to put this bibliography on the SERI computer to aid future researchers in locating published data on specific topics.

A resource assessment of the United States Southwest is being conducted by SERI's Renewable Resource Assessment and Instrumentation Branch, assisted by researchers from Battelle Columbus Laboratories. In concert, the researchers identify, collect, and characterize critical resource data necessary for microalgal production. Available land and water resources found in the region are quantitatively and qualitatively estimated along with essential climatic data. These data are assembled in map form to aid identification of suitable sites for future production facilities. Climate, land, and water maps are overlaid in a computer geographic information system to stratify the Southwest into zones of suitability for microalgal production. The resulting composite maps will enable future researchers to identify and quantify site-specific parameters that may affect facility design and operation.

The primary resource and environmental parameters of significance are listed in Table 2-10. These parameters have been categorized as prohibitive factors, factors that eliminate sites from further consideration; factors affecting construction costs; factors affecting operating costs; and factors affecting biomass production efficiency.

Factors that will prohibit microalgal production at a specific location are those related to land and water resources. These include certain land ownerships and uses such as urban/industrial, national parks and monuments, regions exhibiting rugged topography, and locations with zero or very limited water supply. The parameters listed in Table 2-10 that will determine the cost of constructing a microalgae system include land ownership, land use/cover, topography, soil characteristics, water supply and demand, water rights, and the hydrologic systems (the geographic location and depth of the water supply). Resource parameters affecting operating costs are related almost exclusively to the cost of pumping and delivering water to the system. Therefore, evaporation rates, precipitation, the depth of the water, and the length of supply lines will all affect operating costs since they determine the quantity of water required and the energy requirements for pumping and transporting the water. Climatic factors can influence biomass production efficiency. The conversion of solar energy to bound chemical energy via the photosynthesis process is directly related to insolation and strongly influenced by temperature.

Table 2-11 lists the parameters that were mapped for the southwestern United States and shows the range of values found in this region. The region mapped includes west Texas (west of 100° W long.), the Oklahoma panhandle, southern Colorado (south of 40° N lat.), southern California (east of 120° W long.), and all of New Mexico, Arizona, Utah, and Nevada. Saline groundwater reservoirs with a salinity of >3000 ppm were mapped, as were areas having a slope of >10%. Land ownership maps were obtained from federal and state sources. Land use/cover maps were prepared by photointerpretation of some 107 Landsat images. Figure 2-5 is the land use/cover map of Utah. The legend for this map (and for the maps of all of the other states in the study) is given in Table 2-12. All of the original maps have been prepared. The process of overlaying the maps by photographic and computer methods has just begun. All of the groundwork for developing this plan has been laid by locating and characterizing the data and data sources of interest.

**Table 2-10. Resource and Environmental Parameters Affecting Microalgal Production Systems**

Climate	Water	Land
Insolation	Supply	Ownership
Temperature	Demand	Use/cover
Evaporation rate	Legal constraints	Topography
Precipitation	Quality	Soil characteristics
Severe storms	Hydrologic system	Geology

**Table 2-11. Parameters Used in Stratifying the Southwest into Zones of Suitability for Microalgal Systems**

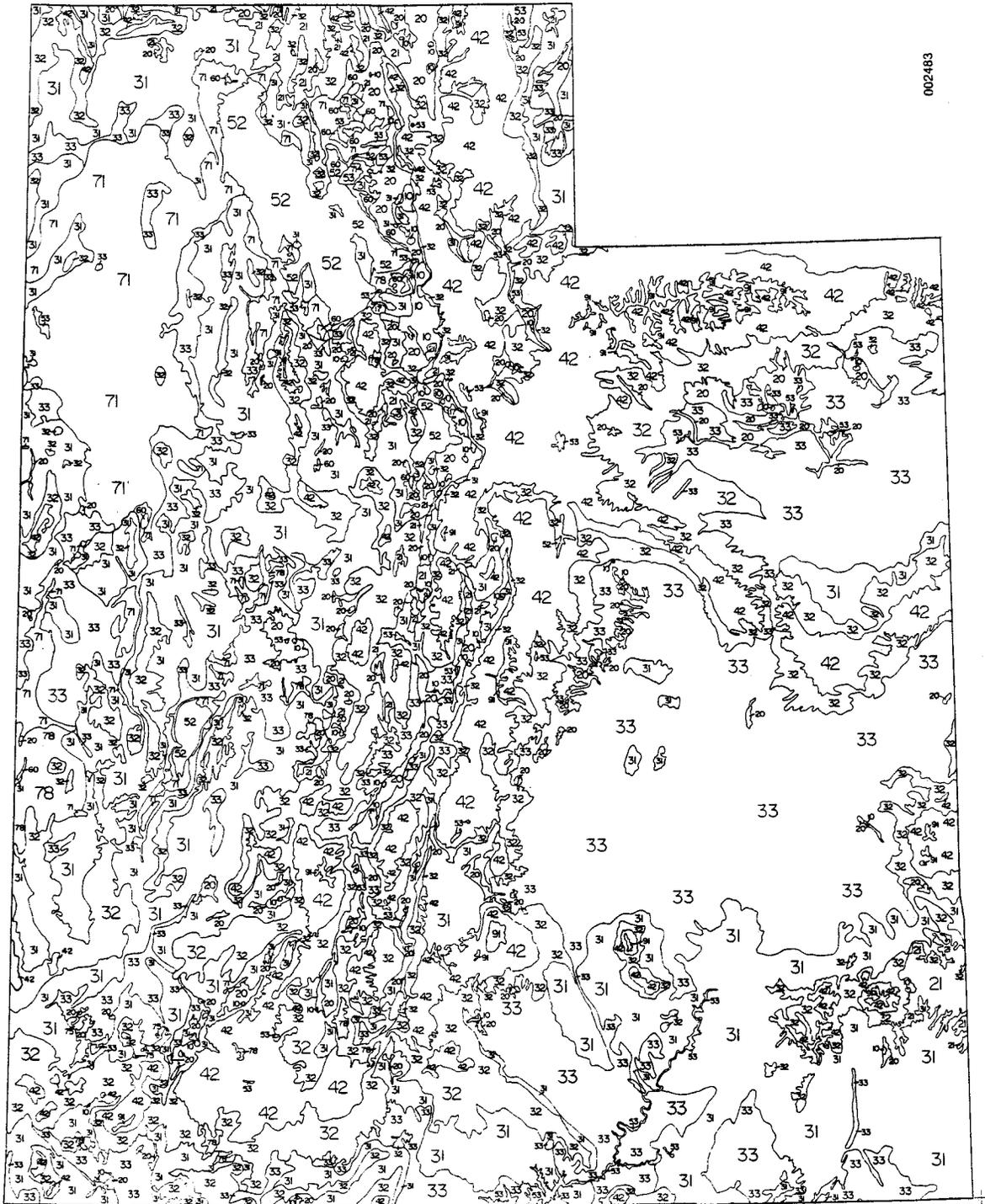
Parameter	Units	Range of Values <sup>a</sup>
Insolation (annual)	kWh/m <sup>2</sup>	5-5.75
Freeze-free period	days	120-300
Evaporation (annual)	inches	32-82
Precipitation (annual)	inches	4-48
Thunderstorm days (annual)	days	5-70
Saline groundwater	ppm	3000-35,000
Slope	%	>10%
Land ownership	—	—
Land use/cover	—	—

<sup>a</sup>These are the ranges of values found in the Southwest.

Conversion Factor: inches x 2.54 x 10<sup>-2</sup> = meters.

**Table 2-12. Land Use/Cover Categories Used for Stratifying the Southwest**

Symbol	
10	Urban
14	Transportation, military air fields
18	Oil and gas fields
20	Irrigated agriculture
21	Other cropland and pasture
30	High biomass rangeland
31	Average biomass rangeland
32	Shrub and brush rangeland or deciduous forest
33	Low biomass rangeland
42	Evergreen forest
52	Lakes
53	Reservoirs
60	Wetland
71	Dry salt flats
73	Sandy areas other than beaches
75	Strip mines, quarries, and gravel pits
78	Other barren land, sparse rangeland
91	Perennial snowfields



**Figure 2-5. Land Use/Cover Map of Utah.**  
See Table 2-12 for legend.

### 2.3.3.2 Economic Evaluation

The objective of this subtask is to analyze and evaluate microalgal production designs and techniques to help establish the potential for meeting the program element goals.

Work at SERI is currently developing an annual cash-flow model and a product pricing model. This model is being designed for use with the production model to allow financial measures of economic feasibility to be determined. The financial model will examine and evaluate the economic trade-offs between capital equipment and the production capacity of microalgae, and evaluate various facility designs based on equipment and material requirements specified in the production model. Cost histories of existing and proposed algal facilities are being developed to provide a data base for estimating capital investment requirements and annual operating expenses. This data base is supplemented, where necessary, to allow quantitative evaluation of any potential production design.

## 2.4 SUMMARY

While early microalgal research focused on understanding the photosynthetic process, current research on developing fuel and chemical products from microalgae emphasizes environmental manipulation of the chemical composition of algae to produce useful energy products. Algal research sponsored by SERI is divided into the following three tasks: biological research, engineering research, and technology analysis.

As part of the biological research, the species screening activity is involved in finding new strains of algae that may produce a high yield of lipid for conversion to petroleum replacement products. The initial research in species screening and cultivation was conducted by the Scripps Institution of Oceanography from 1980 through 1982. *Phaeodactylum* exhibited the highest biomass yield (25 g dry weight/m<sup>2</sup> day) and efficiency (12.2%). Lipid and protein yields were 5.62 and 13 g dry weight/m<sup>2</sup> day, respectively. Work on species screening is being carried out for microalgae by S. Lien at SERI, for macroalgae by J. Ryther at the Harbor Branch Foundation, and for emergent aquatic plants by D. Pratt at the University of Minnesota. This activity involves research into the response of the algae to environmental stimuli. Lien is characterizing 24 species from 11 genera of green microalgae in the laboratory. Ryther has screened two species of macroalgae (genera *Ulva* and *Gracilaria*) and is using large outdoor tanks to study their response to variations in a number of operational parameters. Pratt is concentrating his efforts on defining the optimum growth and harvesting conditions to maximize biomass yields from cattails. Research on species development is being done by T. Tornabene at the Georgia Institute of Technology to improve the analytic data for species selection and characterization, and to provide a data base from which potential chemical and energy products from microalgae can be identified. Tornabene developed and refined the laboratory techniques for conducting microalgal lipid extraction and identification. These techniques were then developed into a laboratory syllabus for use by other researchers in the ASP.

In the area of engineering research, E. Laws at the University of Hawaii is experimenting with a shallow covered raceway, microalgae cultivation system. This year's experimentation used a 50-m<sup>2</sup> flume of this design, while five 8.2-m<sup>2</sup> flumes were constructed in preparation for FY 1983 optimization studies. Production during the last three months of the study averaged over 11 g/m<sup>2</sup> day in a mass culture of *P. tricornutum*. Another experimental production facility is being operated by EnBio, Inc., of California. The project's objective is to conduct field experiments with various freshwater microalgal

species to determine species succession, culture stability, productivity, and lipid yield under various conditions of system operational parameters. One of the more significant obstacles in producing microalgae-derived oils is the concentration of dilute suspensions into a slurry for subsequent processing. In the component development part of the engineering research area, several research tasks have been developed that are designed to evaluate existing harvesting methods and to propose innovative methods to increase the density of the product stream from the growth ponds. Finally, work is being done to analyze and integrate the results of the biological and engineering research. One of the initial tasks completed by Jaycor was an extensive patent search. The report submitted by Jaycor provides a catalog of all patent activity regarding microalgal technology.

Technology analysis activities involve work on resource assessment, including a review of existing research literature describing the local environment, climate, water resources, hydrology, and biota of the southwestern United States. Also involved is the development of a mass culturing model at SERI to provide an analytical tool for evaluating microalgal production systems, taking into account the effects and complex interactions of the many biological, physical, chemical, and engineering factors involved in algae production and harvesting and in processing algae into final products.

## SECTION 3.0

### PROGRAM ELEMENT: ANAEROBIC DIGESTION

#### 3.1 BACKGROUND

Anaerobic digestion is the use of natural biological processes in the absence of oxygen for the breakdown of organic materials with the simultaneous production of methane, carbon dioxide, and ammonia. Methane, the major component of commercial natural gas, is a clean burning, easily transported fuel which is presently in short supply. The production of methane during the decomposition of organic material has been known since the 1700s. The fact that bacteria were involved in anaerobic digestion was first realized in the middle of the nineteenth century. In 1881 anaerobic digestion was reported to be a useful method for reducing the mass and offensive nature of the organic material present in sewage and wastewaters. Since that time the applications of anaerobic digestion (also called fermentation) have grown steadily, along with knowledge concerning the chemistry and microbiology involved. In the past several years, interest in anaerobic digestion has increased because of the methane by-product. Methane can serve as a useful fuel to help offset a growing demand for energy.

The emphasis of the Anaerobic Digestion Program has shifted from short-term, on-farm research and development to long-term, high-risk basic research. It is hoped that with a basic research approach and by understanding the fundamental biological processes involved in anaerobic digestion, large improvements in efficiencies and rates can be realized in the production of fuels from biomass.

The microbiological conversion of agricultural residue to methane gas has the potential of supplying a significant proportion of the current natural gas demand in the United States. Studies on the bioconversion of agricultural residue to methane have emphasized one significant problem which both adds to the cost of methane production and reduces the actual gas production to 20%-60% of the potential yield. This problem is the inability of microorganisms to completely consume all the organic matter in residues [usually measured as volatile solids (VS)].

Pfeffer (1973) has outlined the potential benefits that would result from the development of an economical means for biologically converting organic residues into methane gas. The total energy demand in the United States is rising rapidly from over  $60 \times 10^{18}$  J (60 quadrillion Btu) in 1970 to an estimate of more than  $90 \times 10^{18}$  J (90 quadrillion Btu) by 1990. Natural gas (largely methane) represented about 31% of the total energy demand in 1968. However, the natural gas reserves are quite limited and are not increasing as rapidly as the demand. Between 1947 and 1968, the reserves-to-product ratio declined from 29.5 to 14.6. It is expected that the future demand for gas will remain greater than additions to reserves. Thus, if additional sources of gas are not found, the outlook for satisfaction of demand, even in the very near future, is not good.

For years it has been recognized that a considerable portion of the organic matter that is treated by anaerobic digestion is not converted to methane gas by the microorganisms carrying out the degradation process. A nondigestible residue remains from methane fermentation of most materials. Similar findings for other natural materials have been reported by Golueke (1958) in studies of the decomposition of a number of natural products.

The cost of methane production from organic decomposition is tied closely to the percentage of the available material that can be decomposed. A report by Dynatech (1973) contained a sensitivity analysis for a proposed system for the conversion of municipal refuse into methane. Assuming refuse is 75% degradable by this process, their estimated cost per million Btu equivalents of methane gas was \$0.99. However, the estimated cost varied from \$0.24 if the refuse was assumed 100% degradable to \$3.68 if it was assumed only 40% degradable. Thus, there is a great penalty to treating waste with a large non-degradable fraction. According to Dynatech researchers, the increased cost with lower digestible fraction results from lower methane production per unit of waste treated and higher costs for residual disposal. Since many agricultural residues that have potential for methane fermentation are less than 50% biodegradable, there is clearly a need to find means to increase the degradability if the process is to produce methane economically.

Because of the sensitivity between cost for methane production and degree of biodegradability of organic materials, the determining factor affecting the economic practicability of anaerobic digestion for producing methane may be the ability to increase the biodegradable fraction. The economic benefit from a method to increase biodegradability could be immediate and substantial.

Lignocellulosics, the cell-wall materials in vascular land plants, are the most abundant renewable source of chemical energy on earth, yet their potential remains highly underutilized primarily because of their complex physical and chemical structure. Anaerobic digestion has the ability of capturing up to 90% of the heat content (heat of combustion) of organics in the form of methane gas.

Lignocellulose is comprised predominantly of three components: cellulose, hemicellulose, and lignin, all in varying proportions depending on the source (e.g., hardwoods, softwoods, grasses, corn stover). Because of its large molecular size, poor solubility, and chemical complexity, lignin is highly refractory (nonbiodegradable). Apparently, there are no known environments in which the rate of lignin destruction through microbial action is rapid. Consequently, progress in the study of its microbiological degradation has been limited, and the rates of turnover of naturally occurring lignins (forest, agricultural) remains incompletely understood. Even less is known concerning the rates of decomposition of chemically altered lignins such as kraft lignins, generated during the alkaline pulping of wood for paper production.

Physical and chemical processing of lignocellulose to separate constituents is not a new science. In fact, the primary goal of chemical pulp processing for over 100 years has been quite similar to our objectives: to separate carbohydrate and noncarbohydrate constituents while minimizing chemical degradation of the polysaccharides. Furthermore, the forest-products industries are continuously investigating new techniques for separating and concentrating the components of lignocellulose with the intention of better utilizing the vast quantities of wood-processing residues. Thus, it appears that the groundwork for lignocellulose pretreatment has been laid, yet application is still relatively limited.

Anaerobic digestion for the recovery of methane has two significant advantages over other chemical and biological means for extracting useful products from lignocellulose: (1) It employs a mixed bacterial population that can adapt or acclimate to most of the organic products that are formed by the harsh reactions required for separation of constituents. Also, the mixed bacterial system permits the use of sewage sludge, wastewater effluents, or other complex wastes as a source of nutrients for bacterial growth instead of the more expensive nutrient additions that would be necessary for a pure

microbial system or in application as an animal-feed supplement. (2) The usable product, methane gas, is readily extractable from the main processing stream.

These important considerations indicate that a thorough evaluation of all pretreatment alternatives is necessary to properly assess the potential of bioconversion systems for recovery of energy from lignocellulosics. The following is a listing of pretreatments which require further research:

- Physical methods
  - size reduction
  - irradiation
  - thermal stress
- Swelling
  - sodium hydroxide
  - aqueous ammonia
  - steaming at moderate temperatures ( 160° C)
- Polysaccharide hydrolysis
  - mineral acids
  - oxidative hydrolysis (wet oxidation)
- Delignification
  - alkaline (soda or kraft processing)
  - acid (sulfite)
  - neutral (sulfite)
  - gaseous sulfur dioxide
  - microbiological (enzymic or selective delignification by fungal systems).

Under acid conditions, heat treatment results in the solubilization of carbohydrates (cellulose and hemicelluloses), leaving lignin as an insoluble and nonbiodegradable residue. Alkaline heat treatment results in the solubilization of lignins, leaving insoluble polysaccharides as the digestible products. This process is termed delignification. In addition to making carbohydrates available for biodecomposition, up to 26% of the solubilized lignin products have been found to be fermentable. It remains to be understood, then, what the potential is for methanogenic degradation of the more complex molecules that are present following heat treatment of lignin. The data in the literature suggest that the molecular size of the lignin fractions affects its degradability. There is conflicting data, however, as to whether increasing molecular size is a deterrent to decomposition.

Work must be done to determine the best and least expensive pretreatment for a given feedstock. Acids, alkalis, and enzymes are all expensive and their use must be minimized in order to minimize the cost of methane and maximize the conversion of the biomass to methane.

The microbiology involved in anaerobic digestion has been poorly studied due to the great difficulties encountered when working with extreme obligate anaerobes. It has only been in the last 10 years that the techniques required for handling strict anaerobes have become available. Fewer than 20 laboratories in the United States are equipped for the competent manipulation of methane-producing bacteria (methanogens).

Effective biological conversion of organic matter in anaerobic digesters depends on a diverse, yet stable microbial population. Present understanding of bacterial populations in anaerobic digesters is rather limited and mainly based on analysis of bacteria routinely isolated from sewage sludge and animal manure digesters or from the rumen of cud-chewing animals. Research is still urgently needed on enumeration, isolation, and characterization of the predominant microbial populations in anaerobic digesters that process a specific waste (i.e., agricultural crop residues). Essentially nothing is known about how the bacterial population varies in digesters that receive different wastes (e.g., municipal sewage sludge, animal manure, crop residue, food processing wastes, and agricultural wastes).

The anaerobic degradation of complex organic matter to methane is a result of the combined and coordinated metabolic activity of the digester population. The population is comprised of several major physiological groups that carry out different functions. At present, four different physiological groups are recognizable and include: hydrolytic bacteria (Group I) that break down saccharides (sugars), proteins, lipids, and other minor chemical constituents of biomass; hydrogen-producing acetogenic (acetate-producing) bacteria (Group II) that break down certain fatty acids and neutral end products; homo-acetogenic bacteria (Group III) that utilize single-carbon compounds (e.g.,  $H_2/CO_2$  or formic acid) or hydrolyze multicarbon compounds to form acetic acid; and the methanogenic bacteria (Group IV) that convert acetate and one-carbon compounds to methane. The coordinated activity of these physiological groups as a whole ensures process stability during anaerobic digestion. Environmental parameters that directly influence the catabolic activity of only one group can indirectly influence the entire bacterial population.

Metabolic interactions between methanogenic and nonmethanogenic species is of tremendous importance to the anaerobic digestion process. Very little information is available concerning the physiology and nutritional requirements of the four different physiological groups involved with anaerobic digestion. Biological characterization of anaerobic digestion is a difficult task not only because the microorganisms are sensitive to oxygen but also because they are interdependent. Organisms behave differently when isolated than when they are a member of a microbial association. These associations need to be studied and thoroughly understood before a complete understanding of the biological processes involved in anaerobic digestion can be obtained.

The Anaerobic Digestion Program is working on the physiology and biochemistry of methanogens and fatty acid (e.g., acetic acid) utilizers and producers. Also, the nutritional requirements of the methanogens involved in converting acetic acid to methane are being studied and defined.

Finally, through the study of physiological mutants, the genetics of methanogens are being studied in order to better understand the physiology and biochemistry of methanogenesis. A future possibility is to be able to either genetically engineer or induce a methanogenic mutant that grows faster, produces more methane, and improves the rates and efficiencies of anaerobic digestion.

Although pretreatment and microbiology are extremely important research areas, the practical aspects of anaerobic digestion must not be overlooked. The engineering involved in the development of a cost-effective anaerobic digestion system must be developed concurrently with other research areas. New and innovative concepts need to be explored. New designs for digesters need to be developed and studied. Operating parameters need to be improved to maximize rates of digestion and methane production. The reactor volume is an inverse function of the feed concentration; thus, an increase in

feed concentration would proportionately reduce the required volume. For example, if the liquid and solids are mixed prior to feeding, the fluidity of the mixture limits the maximum concentration to about 10%. However, if solids and liquids are fed separately, the only limit on feed concentration is the fluidity of the reactor contents. With a conversion of 66.6% and a 30% feed concentration, the reactor concentration is 10%, still a fluid mixture. Such a system would result in a 66% reduction in reactor volume.

The kinetics (i.e., reaction rate) of continuous anaerobic digestion is limited by the microorganism concentration. Cells are continually discharged with the reactor effluent. The liquid portion of the effluent contains a large fraction of the discharged microorganisms. Separation and recycle of the liquid fraction would substantially increase the cell population and consequently should enhance the reaction rate. Also, less substrate should be required to maintain cell growth so that more substrate will be available for methane production.

Feed concentration, cell recycle, digester design, and digester control are just some of the parameters that need to be studied. Engineering research must be maintained so that the information developed through the study of pretreatment and biological processes may be incorporated into operating systems.

### 3.2 OBJECTIVES

The Anaerobic Digestion Program strives to develop the technology base for anaerobic digestion concepts using cellulosic residues which could provide a cost-competitive fuel on a site-specific basis. As discussed in Section 3.1, this is approached through the study of pretreatment processes, biological processes, and the refining and development of innovative engineering parameters. Table 3-1 presents a summary of the tasks being carried out under the Anaerobic Digestion Program.

**Table 3-1. Anaerobic Digestion Program Tasks and Projects**

---

#### Task I: Pretreatment

Autohydrolysis of Organic Residues - Stanford University  
Anaerobic Digestion of Manure and Residues - USDA/MARC  
Conversion of Agricultural Residues to Diesel Fuel - JPL  
Biotechnology of Anaerobic Digestion - SERI

#### Task II: Biological Processes

Physiology and Biochemistry of Methanogens and Fatty Acid Utilizers and Producers -  
University of Georgia  
Nutrition of Methanogens - Drexel University  
Genetics of Methanogens - JPL

#### Task III: Engineering Parameters

Continuous Culture Biomass Fermentation Studies - University of Arkansas  
Dry Fermentation - Cornell University  
Anaerobic Baffled Reactor - Stanford University  
Anaerobic Digestion of Manure and Residues - USDA/MARC

---

### 3.3 STATUS

#### 3.3.1 Pretreatment

The pretreatment of feedstock is being studied at Stanford University, the USDA/MARC, the Jet Propulsion Laboratory, and SERI. Pretreatment is being studied in order to increase the biodegradability of cellulosic/lignocellulosic feedstocks so that the feedstock can be more efficiently converted to biogas methane.

At Stanford University, the researchers are in the second year of a proposed three-year project. The autohydrolysis process being developed involves the heating of the feedstock in an aqueous solution to 175°-225° C in the absence of oxygen. Some of the cellulose and hemicellulose fractions are solubilized and converted to organic acids. This causes the pH to drop to about 2.5 and brings about the rapid hydrolysis of the remaining polysaccharides in the feedstock. The organic acids can then be extracted from the reactor and fed to an anaerobic digester for conversion to methane and CO<sub>2</sub>.

This year, small batch reactors (Figure 3-1) have been developed for evaluating the kinetics of transformation of cellulose and its autohydrolysis products. The solutions to be studied are placed in the reactors, buffered for pH, and then immersed in a hot oil bath to heat the solution to the desired temperature within one or two minutes. The reactors are then taken one at a time from the bath, cooled by immersion in water, and analyzed for products.

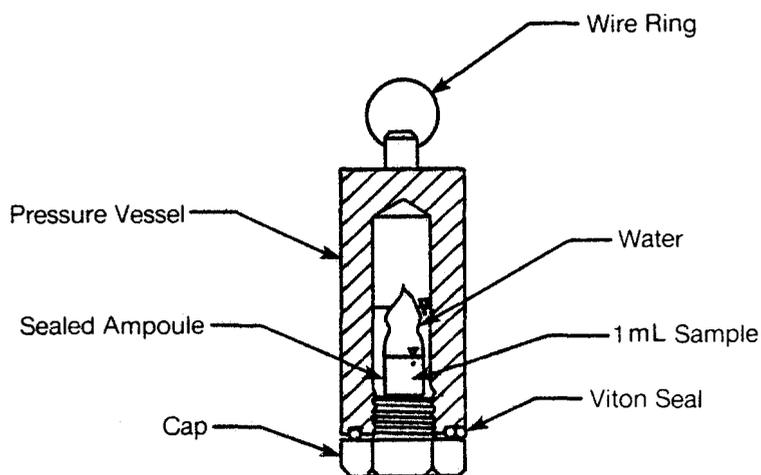


Figure 3-1. Cross Section of a Reaction Vessel for Determining Autohydrolysis Decomposition Kinetics of Biomass Products

Initial results from the autohydrolysis optimization study have indicated that the pH of treatment has a significant effect on the fraction of solubilized material that is converted to refractory (nonbiodegradable) material. The higher the pH, the greater the portion converted to refractory material.

An example of the formation of products resulting with time from glucose decomposition is presented in Figure 3-2. In general, humic solids and 5-hydroxymethyl-2-furfural (HMF) were the primary products formed as a result of glucose decomposition. The formation of levulinic acid from HMF decomposition occurred only at low pH levels.

At the USDA, mechanical and chemical pretreatments of straw are being investigated. This project was initiated in late July. As a result, most of the effort has been in the performance of experiments and collection of the initial data. The effect of pretreatment on the fermentation of straw was examined. Straw was used cut, uncut, hammermilled, and ball-milled. The straw was also subjected to alkaline treatment. Experiments were completed on the batch acidogenic and subsequent methanogenic fermentation of ball-milled straw. The pretreatments employed are shown in Table 3-2.

The material from these pretreatments was fermented to fatty acids, then fermented to methane. These experiments were just completed and the data are being analyzed in terms of fatty acid production and methane yield.

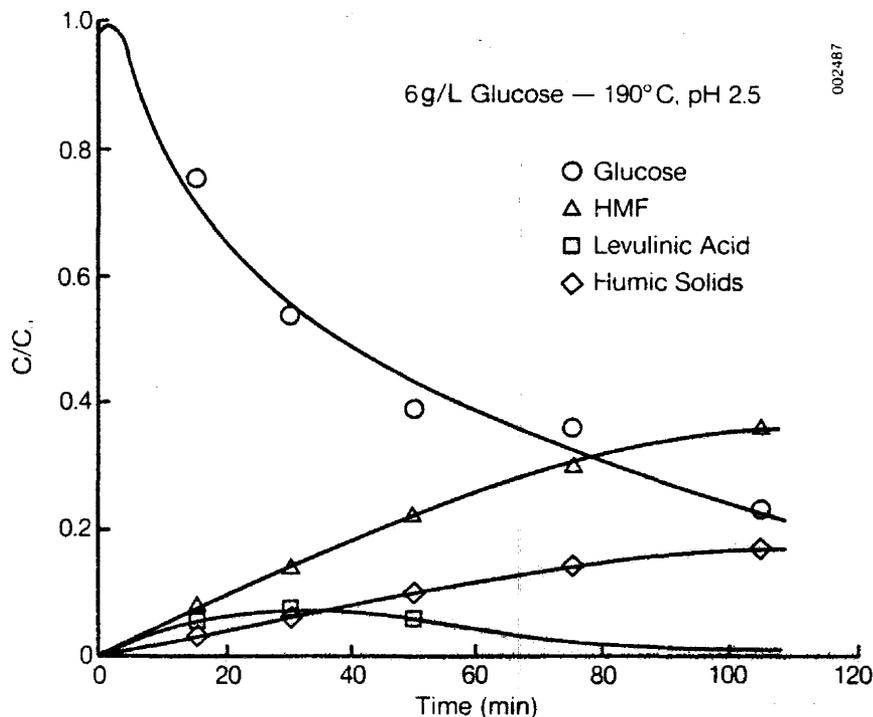


Figure 3-2. Effect of Time on Glucose Decomposition and Formation of Decomposition Products

Table 3-2. Alkaline Pretreatments of Straw

Experiment	Temperature (°C)	Time (h)	NaOH Dose (g/kg VS)	Straw Concentration (kg VS/m <sup>3</sup> )
1	50	24	80	100
	50	24	80	60
	50	24	80	40
	50	24	80	20
2	30	24	80	80
	50	24	80	80
	70	24	80	80
	80	24	80	80
3	80	24	60	80
	80	24	40	80
	80	24	20	80
	80	24	0	80

Experimentation on biomethanation of ammonia-treated, ball-milled straw has been initiated. These are batch fermentations at 55° C and straw concentrations of 80 kg VS/m<sup>3</sup>. Treatments (two fermenters per treatment) are:

1. Control
2. BMS
3. BMS + 70 g NH<sub>4</sub>OH/kg VS
4. BMS with 35 g NH<sub>4</sub>OH/kg VS at 80° C, 24 h
5. BMS with 52.5 g NH<sub>4</sub>OH/kg VS at 80° C, 24 h
6. BMS with 70 g NH<sub>4</sub>OH/kg VS at 80° C, 24 h.

(BMS is ball-milled straw and NH<sub>4</sub>OH is ammonium hydroxide.)

Promising pretreatments identified in these batch fermentations will be used to treat straw fed daily to fermenters. Data from these daily fed fermentations will be used to determine the overall kinetics of pretreatment and biomethanation of straw manure mixtures.

A new one-year project is being carried out at JPL. The objective of this project is to provide bench-scale testing of the JPL-developed agricultural residue hydrolysis process designed to produce aliphatic compounds (e.g., acetone, butanol) and fuel substitutes. The research is designed to verify the processing concept and to improve operating parameters.

A reactor will be designed and constructed to run on representative sugarcane bagasse agricultural residue. The residue will undergo two acid/heat extractions. The first heat extraction with dilute acid at approximately 150° C will result in the production of furfural. The resulting residue will be rehydrolyzed with concentrated acid at 275°-300° C to decompose the cellulose component. The resulting sugar solution will be neutralized

and concentrated. The sugar solutions will be fermented using specific strains of Clostridium thermocellum or Clostridium acetobutylicum (two nonmethanogenic fermentative microorganisms) to yield desirable aliphatic compounds. The process will be analyzed at each step to determine yields and optimization requirements.

The hydrolysis portion of the process is being studied. The results obtained so far with the biological portion are discussed in Section 3.3.2 under the relevant program element task.

Finally, at SERI a project has been underway with the objective to increase the rate and yield of biological methane production from the hemicellulosic portion of biomass by combined chemical/enzymatic treatment. This project has concentrated initially on isolation and identification of thermophilic anaerobic bacteria hydrolyzing sugar (xylan) polymers.

Ten strains of thermophilic anaerobic bacteria producing exocellular xylanase have been isolated from enrichment cultures obtained from hot springs located in Yellowstone National Park. Four strains were selected for further study on the basis of high exocellular xylanase production as determined by xylan degradation assays on agar plates. A complete taxonomic study has been carried out on one of the four selected strains. The microorganism has been tentatively identified as Thermobacteroides saccharolyticum sp. nov. The microorganisms grow at a temperature range of 40° to 67° C with the optimum at 55° C, and at a pH range of 4.0 to 8.0, within a broad optimum of pH 5.0 to 7.0 (see Figures 3-3 and 3-4). The strain ferments a wide range of substrates, including xylan, starch, xylose, cellobiose, and eleven other saccharides. The main fermentation products are lactate, ethanol, acetate, hydrogen, and carbon dioxide. Preliminary data on induction of xylanase produced by this microorganism have been obtained and purification of the enzyme has been initiated.

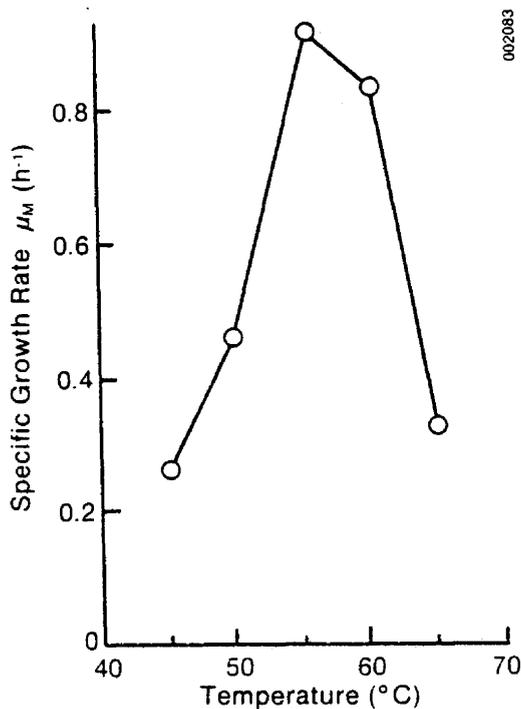


Figure 3-3. Temperature Profile for Growth of Thermobacteroides saccharolyticum sp. nov.

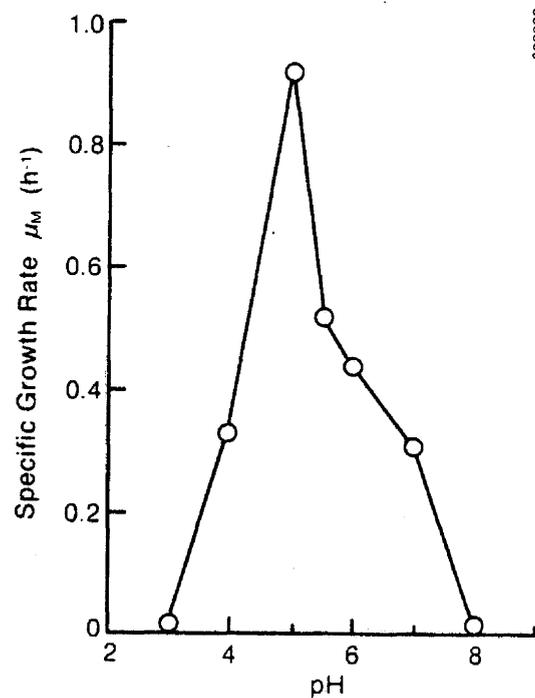


Figure 3-4. pH Profile for Growth of Thermobacteroides saccharolyticum sp. nov.

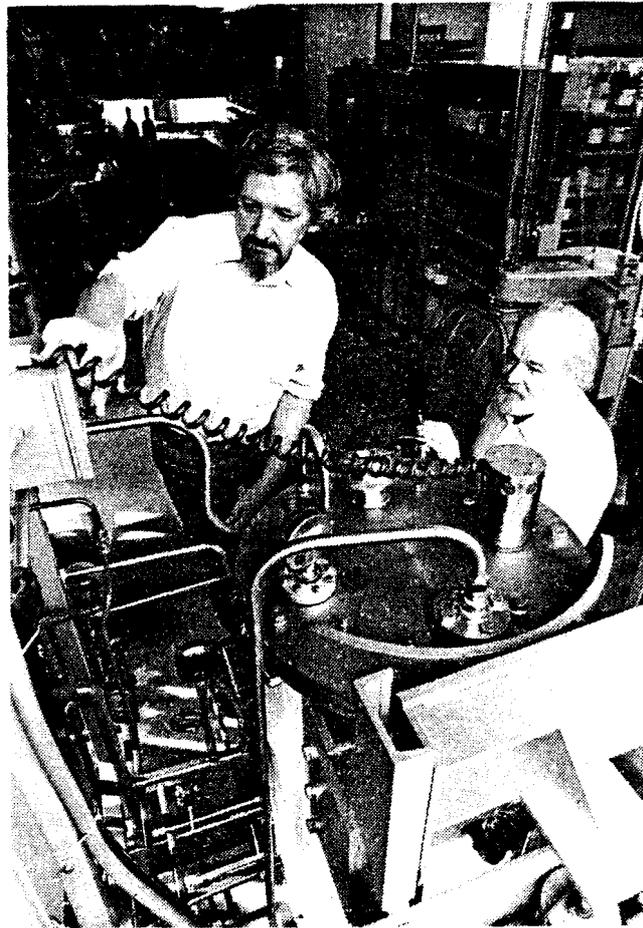
In summary, a broad range of pretreatment techniques are under investigation. These include autohydrolysis with no additives, acid and alkaline hydrolysis at high and low temperatures, and enzymatic hydrolysis of the hemicellulosic components of biomass. These several pretreatments are being investigated not only to find the best pretreatment at the lowest cost but also to determine the pretreatment that is best suited for a given feedstock (e.g., wood, straw, corn stover).

### **3.3.2 Biological Processes**

To better understand the processes involved in anaerobic digestion, a better understanding of the microbiological processes involved is required. The microbiology involved in anaerobic digestion has been poorly studied due to the great difficulty encountered when working with strict anaerobes.

Studies of the physiology of the anaerobic bacteria will result in a better understanding of the biological processes leading to the formation of methane. With this better understanding, anaerobic digestion may be more effectively engineered and become more reliable and efficient. The enzymes and trace nutritional requirements (iron, sulfur, vitamins, etc.) need to be characterized to optimize the anaerobic digestion process.

At the University of Georgia (Figure 3-5) a project is underway to increase the rate and yield of methanogenesis by supplementing biomass fermentations with various microbio-



**Figure 3-5. Researchers Operating a 400-L Fermenter for the Cultivation of Large Quantities of Methanogenic Bacteria**

logical, nutritional, and chemical additives. This work is a three-year project and is starting its second year. Throughout the contract period the interactions of methanogenic bacteria with fatty acid producers and utilizers will be investigated and biomass/methane enrichments will be established and maintained. New microorganisms will be isolated and their biochemistry and physiology determined. Nutritional supplements, microbiological supplements, and combined nutritional/microbiological supplements will be investigated to increase rate and yield.

During the first year of the project, stimulation of methanogenesis was achieved by the addition of pyrophosphate in cultures of *Methanobacterium formicicum* grown on formate and in cultures of *Methanosarcina barkeri* grown on methanol,  $H_2/CO_2$ , and acetate. The results are presented in Figures 3-6 and 3-7. Although there is little increase in the cell mass, the amounts of methane produced in the presence of pyrophosphate is increased severalfold. A transient accumulation of acetate was observed. These results show that methane production can be increased without a concomitant loss of carbon, since no increase in cell mass was observed.

To obtain a better understanding of the growth conditions and requirements of the bacteria that are linked in the anaerobic food chain via hydrogen transfer and fatty acid production and/or utilization, studies of growth requirements and their physiological roles were undertaken.

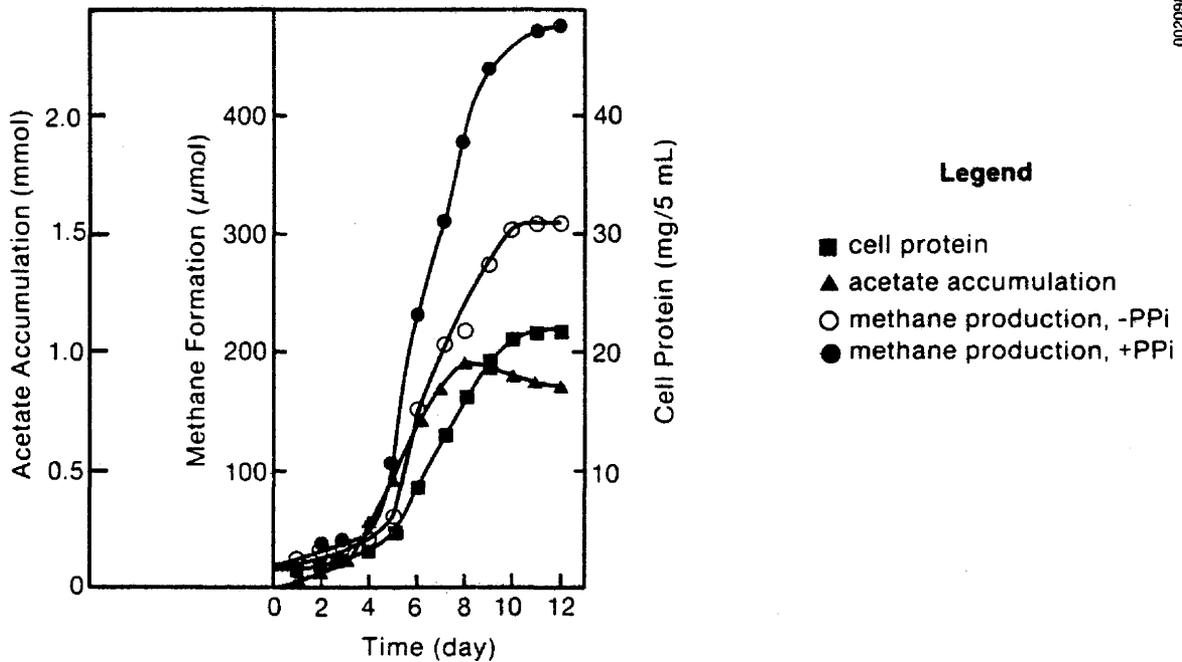


Figure 3-6. Growth of *M. barkeri* on  $H_2/CO_2$  in the Presence of Pyrophosphate

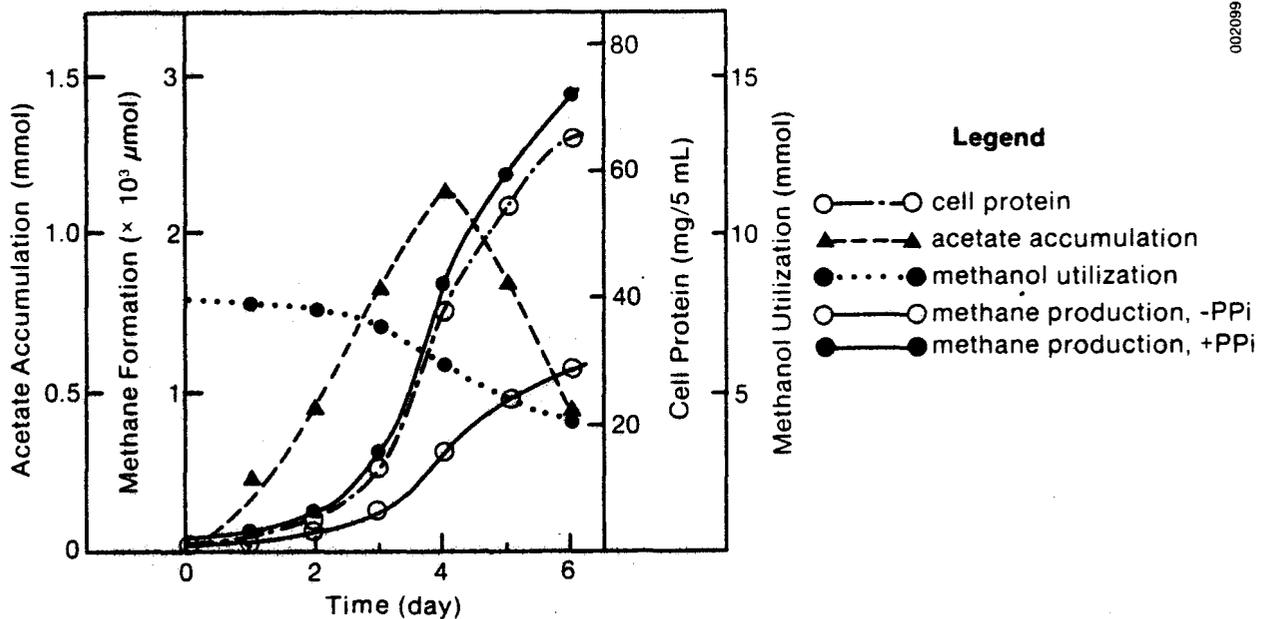


Figure 3-7. Growth of *M. barkeri* on Methanol in the Presence of Pyrophosphate

Iron is needed in important quantities for the biosynthesis of hemes (ATP production) and the various iron-sulfur clusters. These clusters are particularly important because they are present in the key enzyme hydrogenase, which contains 11 to 12 atoms of iron per molecular weight (MW) of 90,000 or 60,000. The growth of the acetate-forming and hydrogen-producing *Desulfovibrio* is stimulated by an iron chelator which is found in the commercial sodium lactate used for the growth of this organism. Nickel is a constituent of the hydrogenases from the hydrogen producer *Desulfovibrio* or from *Methanobacterium thermoautotrophicum* and *Methanosarcina barkeri*. Results with *Methanobacterium* and *Methanosarcina*, which possess very different metabolisms, show that they both contain similar  $F_{430}$  proteins. Since these proteins are most probably part of the Coenzyme M reductase complex, and since  $F_{430}$  contains nickel, it appears that this metal is compulsory for methane production, although it is not necessary for hydrogen evolution or activation.

It was discovered that the methyl transferase of *M. barkeri* is a  $B_{12}$ -containing enzyme, and this discovery led to the study of the content of vitamin  $B_{12}$  in *M. barkeri* cells. First results show that strain DSM 800 accumulates 300 mg of vitamin  $B_{12}$  per 200 g wet weight. A study of all known strains of *M. barkeri* and their growth on different media has been started to try to increase  $B_{12}$  production.

A sulfite reductase was isolated from *M. barkeri*. The role this enzyme plays in the sulfur metabolism of the organism is still undetermined. Growth experiments using sulfite as a terminal electron acceptor have been negative. However, an active reduction of sulfite to sulfide occurs with resting cells in the presence of molecular hydrogen. This shows that sulfite will inhibit methanogenesis in complex fermentations when methanogenic bacteria having physiological profiles similar to *M. barkeri* are present. The possibility remains that the sulfite reductase is used in "reverse" either to obtain energy from

some reduced form of sulfur, in a way similar to Thiobacillus denitrificans, or to oxidize sulfur for the biosynthesis of Coenzyme M, which contains a sulfonyl group. Recently, one of the two sulfite reductases present in Desulfovibrio vulgaris was crystallized. These crystals will be studied in collaboration with L. Sieker of the University of Washington. This study can lead to the establishment of the structure of this important enzyme.

At Drexel University, the nutritional requirements of a methane-producing consortia are being studied. Acetate is reported to be the precursor of approximately 65% of the methane produced during anaerobic digestion. Therefore, the nutrients that stimulate the conversion of acetate to methane are being studied. The objective of this project is to investigate the stimulatory effects of phosphate, nickel, iron, cobalt, and yeast extract, both individually and in combination, on acetate-grown methane bacteria and to sustain rapid methane formation for an extended period of time.

This is the second year of a two-year proposal to study the nutritional stimulation of methanogenesis. The project has been successful and stimulatory nutrients have been identified. Results have demonstrated the possibility of enhancing the maximum specific utilization rate (grams acetate/gram VS day) by a factor of 5 to 10. Further research is required to determine how these very high reaction rates may be maintained.

The experimental setup consists of two-liter continuously stirred tank reactors (CSTR) with pH-stat controls. Acetate concentrations are held constant and factorially designed experiments are conducted with variable concentrations of phosphate, nickel, iron, cobalt, and yeast extract in the feed medium. Culture acetate utilization as a function of nutrients is monitored.

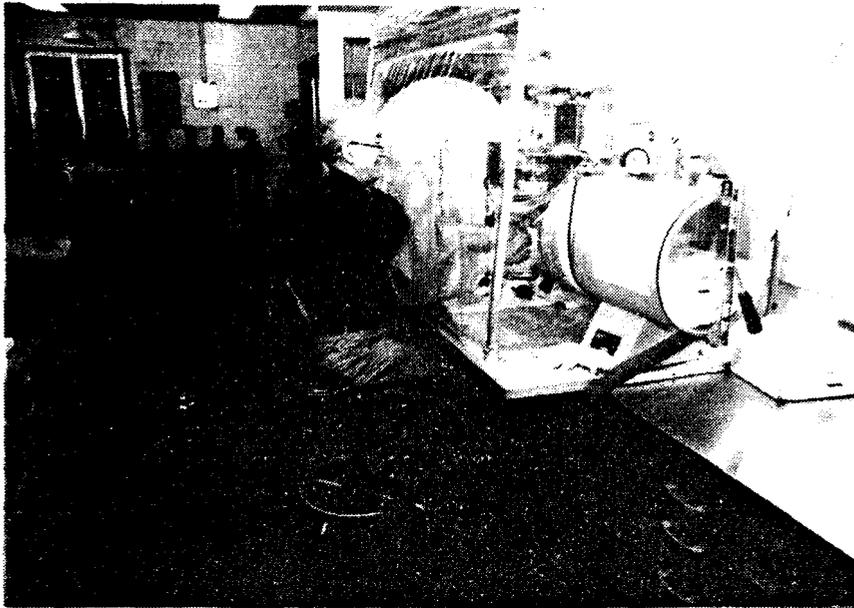
Since this project began only recently, most research activities have involved setting up reactors and initiating the experiments. The pH-stats have been activated to study the effect of nutrients on acetate utilization. Previous work has indicated that for short periods an acetate utilization rate of 52 g/L day—a 17-fold rate increase—was possible. This rate could not be maintained, perhaps because of a lack of specific nutrients. If the nutritional factors that support accelerated acetate utilization can be identified, this knowledge can be applied to actual digestions to substantially increase methane production.

At JPL (conversion of agricultural residue to diesel fuel) the residue from the acid/heat hydrolysis is being biologically processed using different strains of clostridia as mentioned in Section 3.3.1. So far, a tube reactor system has been reactivated for the initial stages of the process and a second reactor is being designed for the cellulose decomposition and sugar production reactions. In preliminary experiments, the secondary hydrolysate from a bagasse feedstock was tested for compatibility with three strains of clostridia (Cl. butylicum, Cl. acetobutylicum, and Cl. pasteurianum). The basic medium was BBL thioglycollate broth without dextrose. The hydrolysate contained between 0.5% and 1% reducing sugar. The hydrolysate was added to the medium prior to inoculation, and dextrose was added to the control. All three strains grew on dextrose and the hydrolysate. Growth rates  $\mu$  ( $\text{h}^{-1}$ ) for cells grown on dextrose were 0.99, 0.87, and 0.67  $\text{h}^{-1}$  for Cl. pasteurianum, Cl. acetobutylicum, and Cl. butylicum, respectively. When grown on approximately equivalent amounts of reducing sugar from the hydrolysate, Cl. pasteurianum grew as well as it did when grown on dextrose. Growth was reduced for Cl. butylicum and Cl. acetobutylicum to about one-third the rate for dextrose (0.24 and 0.27  $\text{h}^{-1}$ , respectively). Maximum growth on hydrolysate was always less than on dextrose media and was always followed by a decrease in absorbance. More work is required to optimize

the fermentations by clostridia. These experiments could identify biological cost-effective processes effective in converting agricultural residues into aliphatic compounds and valuable chemicals.

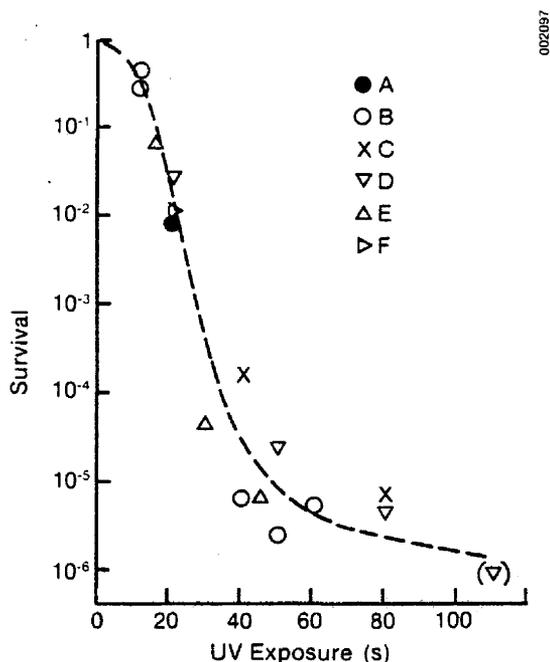
Also at JPL (Figure 3-8), a project is underway to characterize the genetic system of one or two strains of methanogenic bacteria. This project is in its second year. The first year's effort was successful in developing methods for the induction and isolation of methanogenic mutants. Very little information exists concerning the genetics of methanogens due to their extreme sensitivity to oxygen and their generally slow growth rates. It is believed that significant progress can be made in the exploitation of methanogenesis for technical purposes when a fairly detailed knowledge of the genes involved, their products, and their regulation is obtained. The isolation of mutants is a necessary first step for any project in genetics and complements future experimentation in molecular genetics. Once mutants have been isolated, gene-transfer mechanisms in methanogens may be tested.

Both ultraviolet exposure and chemical screening are used to isolate mutant species. These mutant species are tested for genetic recombination. Bacteriophage and plasmids are being sought. Two species, Methanococcus voltae and M. thermoautotrophicum, are being subjected to extensive screening and manipulation. Nutritional mutants of these two strains will be studied to determine uptake rates. Once a set of satisfactory mutants is obtained, two types of genetic recombination experiments (conjugation and DNA transformation) will be carried out.

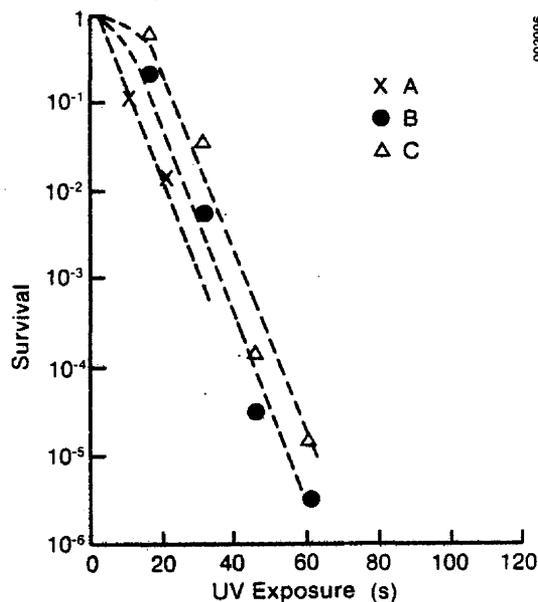


**Figure 3-8. An Anaerobic Chamber for the Transfer and Manipulation of Methanogens under an Oxygen-Free Atmosphere**

This year temperature-sensitive nutritional mutants (i.e., failure to grow at 38°C on defined medium) and BES\* mutants of *M. voltae* and a BES\* mutant of *M. thermoautotrophicum* were isolated. These mutants provide a starting point for the initiation of research into gene-transfer mechanisms in methanogens. The characteristics of these mutants may provide genetic markers that can be used in gene-transfer experiments. Ultraviolet (UV) irradiation survival curves and antibiotic sensitivities were also determined. UV survival curves for *M. voltae* and *M. thermoautotrophicum* are shown in Figures 3-9 and 3-10, respectively. The curve for *M. voltae* shows a peculiar flattening at doses greater than 200,000 erg/cm<sup>2</sup>. A colony which survived 110 s of irradiation was grown up and tested for its sensitivity to a 20-s dose of ultraviolet. Its survival was normal. The flattening of the curve does not seem to be due then to the presence of a subfraction of the population that is genetically resistant to UV. A very interesting alternative would be the possibility that at these high doses, where relatively high concentrations of bacteria have to be plated in order to obtain reasonable numbers of colonies, cell-to-cell interactions occur involving exchange of genetic material and thus genetic reactivation of damaged DNA.



**Figure 3-9. Survival of *Methanococcus voltae* after Ultraviolet Irradiation\*\***



**Figure 3-10. Survival of *Methanobacterium thermoautotrophicum* after Ultraviolet Irradiation\*\***

\*BES is bromo-ethane sulfonate, a structural analog for Coenzyme M, a coenzyme required for methanogenesis.

\*\*The different symbols represent different trials.

The survival curve for *M. thermoautotrophicum* does not show the presence of a highly resistant fraction of cells and does show that *M. thermoautotrophicum* is more sensitive to UV. *M. voltae* was tested for sensitivity to the following antibiotics: tetracycline, bacitracin, gramicidin, gentamicin, and lasalocid. This was done by spotting small amounts of antibiotic (powder) on an agar plate inoculated with about  $10^6$  bacteria to form a confluent lawn. Bacitracin gave a very strong inhibition, tetracycline and gentamicin a very small inhibition, and lasalocid a barely detectable inhibition. Bacitracin is being considered as a candidate to replace penicillin in the classical procedure of enrichment for auxotrophic mutants as applied to methanogens.

This work is continuing and existing mutants are being characterized and evaluated further in order to determine their suitability for genetic exchange experiments. Additional mutants are being sought and a bacteriophage screening program is being carried out in hopes of finding a natural method of transduction (genetic transfer mediated by a virus).

### 3.3.3 Engineering Parameters

Although the biology of anaerobic digestion is a critical research area, the engineering and practical aspects of anaerobic digestion cannot be overlooked. Therefore, research into novel digester designs, important engineering parameters, and new low-cost methods of running anaerobic digestion processes are being investigated.

At Stanford University, during the course of research concerning autohydrolysis (as discussed in Section 3.3.1), a new type of anaerobic reactor was developed. The reactor, termed an anaerobic baffled reactor (ABR), is shown in Figure 3-11. This reactor demonstrates excellent kinetics. The system has been operated at a 6-h detention time with a

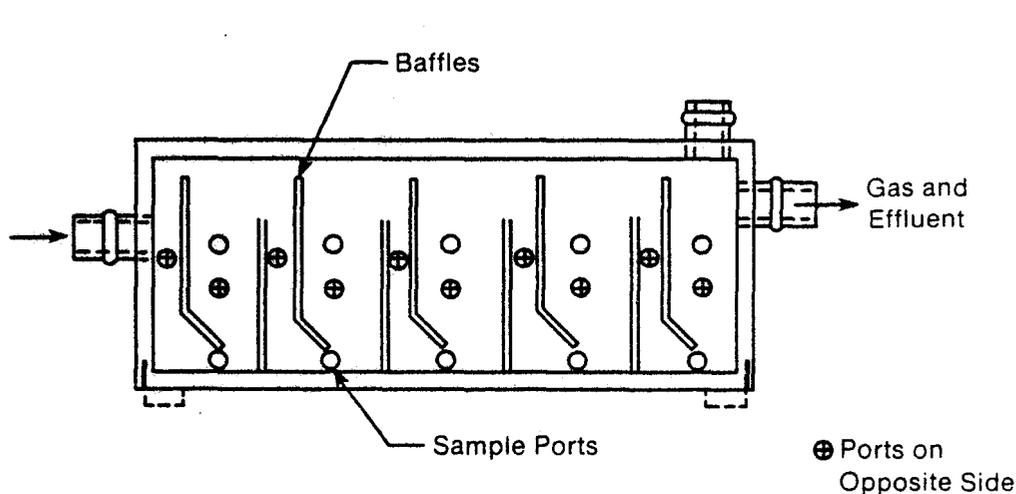


Figure 3-11. Diagram of a 6.3-L Anaerobic Baffled Reactor

feed COD (chemical oxygen demand) of about  $8 \text{ kg/m}^3$ . The COD reduction was 70% and the gas production was  $11 \text{ m}^3/\text{m}^3$  of reactor, with 45% being methane. The system was stressed at these loading rates. Because of the growing interest of various companies in the commercialization of the reactor, including a company in Australia, Stanford University has decided to apply for a patent on it. This reactor could be extremely useful for the anaerobic digestion of dilute wastestreams due to the high reaction rates that can be obtained. A kinetic model is being developed to evaluate the performance of the baffled reactor based on fundamental concepts of liquid-layer mass transport, Monod kinetics, and molecular diffusion.

At the University of Arkansas, basic engineering parameters are being studied using conventional CSTRs shown in Figure 3-12. The objectives of this research are threefold: first, using corn stover, to determine the maximum solids concentration that can be fed to the reactor; second, to determine the effect of high solids concentration on reactor kinetics; and third, to determine the effect of high solids concentration on the power required for variables such as agitation and pH.

The maximum permissible solids concentration within the anaerobic digestion reactor is determined by the need to maintain fluidity of the reaction mixture. For corn stover this limit is 10% solids, and previous studies have not exceeded this loading rate. If liquids and solids are fed separately, however, the only limit on feed concentration is the fluidity of the reactor contents. Thus, a 30% solids feed fraction with a 66% conversion rate could still result in a fluid reaction mixture.

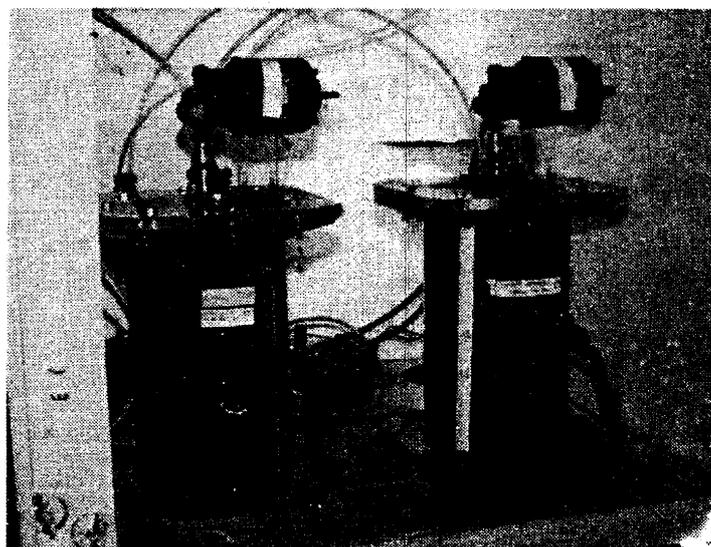


Figure 3-12. Photograph of 3-L Continuously Stirred Anaerobic Reactors for the Study of High Solids Loading of Corn Stover

The feed concentration in two reactors has been increased from 10% to 20%. This increase was brought about gradually over a period of several months, and no problems with pH or nutrient deficiency have been noted. Steady state has been achieved at a 60-day retention time. A corn stover conversion of 60% has been obtained, and the same first-order kinetic model holds as with 10% feed. The gas production rate at steady state is 0.54 L/g day. Since the required volume of a reactor is inversely proportional to the solids loading concentration, an increase in loading from 10% to 30% would result in a two-thirds reduction in reactor size. A commensurate reduction in reactor cost also would be expected. Thus, this research is expected to increase the economic feasibility of anaerobic digesters using agricultural waste as feedstock.

Recycle experiments have shown that nearly 80% of the liquid can be recovered from the effluent using a vacuum recovery system. This type of system is a considerable improvement over direct settling, and requires only a few seconds each day for the recovery operation.

Essentially, researchers are trying to make conventional anaerobic digestion systems more cost-effective and more efficient through the use of high loading concentrations and culture recycling.

At Cornell University a low-cost approach to anaerobic digestion termed "dry fermentation," which requires minimal additives, was pursued. The general approach of this proposed three-year study was to further develop the understanding of low-cost anaerobic fermentation design for animal and crop residues. Specific objectives of this study included the development of long-term, cold-weather operational reliability of dairy manure digestion in full-scale, completely mixed, and plug-flow reactors; the development of information on biogas utilization systems for the dairy manure fermenters; a feasibility analysis of crop residue digestion to confirm the potential for anaerobic fermentation of untreated dry crop residues; the use of pilot plant analysis to determine the kinetics of dry fermentation of three major crop residues, and the influence of temperature on operation, nutrient availability, and start-up requirements in terms of bacterial seed inoculum and alkalinity requirements; the development of a minimum-cost, full-scale, dry-crop-residue fermenter design; and the construction and operation of a full-scale dry reactor system of a size that will enable rapid scale-up to community-size systems [this will involve a gas production rate design goal between 280 to 3000 m<sup>3</sup>/day (10,000 to 100,000 ft<sup>3</sup>/d)] Funding for this project lapsed after two years. Crop residue fermentation using the new concept of dry fermentation was about 75% defined, and an initial scale-up experiment to a prototype unit (110 m<sup>3</sup>) (Figure 3-13) was successful.

Dry fermentation was successfully demonstrated with corn stover, wheat straw, and grass last year. This project is a close-out of a previous contract to allow the collection of final data points, to evaluate the dry digestion process, and to shut down the reactors. Thus far, 90% of the operation has been shut down, large-scale prototype testing has been completed, and the mass balance analysis has been finished.



**Figure 3-13. Photograph of a 110-m<sup>3</sup> Dry Fermentation Reactor Showing the Distribution Grid through Which Leachate Was Recycled for Moisture and Temperature Control**

At the USDA/MARC a project is underway to investigate the potential of a pilot-scale, two-stage fermentation system designed to take advantage of the varying rates of conversion of manure and crop residues. Since funds available from USDA are not sufficient to conduct a thorough laboratory-scale investigation of the digestion kinetics of manure/crop residue mixtures, and because the results of this research are beneficial to the Anaerobic Digestion Program, a co-funding arrangement exists between SERI and USDA, with SERI funding the laboratory-scale work.

The objective of this research is to assess the technical and economic feasibility of recovering methane and high protein biomass from the anaerobic digestion of beef cattle manure and crop residues. A two-stage digestion system is being developed and quantitatively evaluated. The first stage of the system converts organic matter into fermentable sugars and/or volatile fatty acids; the second stage is an alkaline pretreatment process that breaks down first-stage solid residues. The kinetics of methane fermentation in a reactor receiving output from the second stage of the digestion system will also be evaluated.

### **3.4 SUMMARY**

The Anaerobic Digestion Program is making significant progress toward its goal of providing a cost-competitive fuel on a site-specific basis.

The development of a cost-effective pretreatment process for cellulosic residues could have a large impact on the overall cost of anaerobic digestion. The proper pretreatment would increase the percentage of cellulosic residue that could be converted to a fuel and

increase the rate at which the feedstock could be digested. Work will continue on the development of pretreatment systems. However, new innovative ideas need to be encouraged and developed to improve the probability of developing a low-cost pretreatment for anaerobic digestion.

The microbiology has been a neglected aspect of anaerobic digestion. It is projected that significant improvements can be attained through the knowledgeable manipulation of the microorganisms involved in anaerobic digestion. Therefore, although a significant biological effort is presently supported by the Anaerobic Digestion Program, a larger effort concerning the earlier stages of digestion is projected for next year. The microbiology of the hydrolysis of feedstock polymers and the ensuing acidogenic and acetogenic steps need careful examination. This work would greatly complement ongoing research on the methanogenic and fatty-acid-utilizing organisms. So far, excellent results have been obtained concerning the microbiology of anaerobic digestion. The identification of stimulating nutrients and an expanded understanding of the physiology and ecology of the organisms involved in the terminal steps of anaerobic digestion have a great number of applications to future research. This work will continue, and it is expected that an even better understanding of anaerobic digestion will result.

Research into new digester designs, new dry fermentation techniques, and important engineering parameters, such as loading rate and cell recycle, will continue. This type of research will help define the physical parameters of digestion and place the biological developments in the proper perspective. Engineering research will continue emphasizing the development of new digester designs and the study of basic digestion parameters in order to establish the practical and theoretical limits of anaerobic digestion.

### 3.5 REFERENCES

- Golueke, C. G. 1958. "Temperature Effects on Anaerobic Digestion of Raw Sewage Sludge." Sewage and Industrial Wastes. Vol. 30: pp. 1225-32.
- Pfeffer, J. T. 1973. Reclamation of Energy from Organic Refuse. Report prepared for EPA, Grant No. EPA-R-800776, by the Civil Engineering Department of the University of Illinois.

## SECTION 4.0

### PROGRAM ELEMENT: PHOTO/BIOLOGICAL HYDROGEN

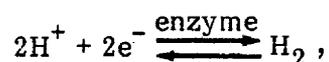
#### 4.1 BACKGROUND

Research in the Photo/Biological Hydrogen Program (P/BHP) has traditionally focused on development of direct solar technologies for producing hydrogen from renewable resources. When the program was transferred to SERI for management, research was being conducted in the following areas: generation of hydrogen (1) using microorganisms, (2) through direct photoelectrolysis of water, and (3) via chemical treatment of biomass. Recently the scope of the program was narrowed to include only the potential technologies based on the use of cells and/or cell fragments, and the program name was changed to its present form.

The P/BHP is responsible for developing new solar biotechnologies that produce hydrogen from renewable resources, such as water and waste streams. One of the avenues being explored is exploitation of the ability of photosynthetic microorganisms such as photosynthetic bacteria (PSB), cyanobacteria, and algae to evolve hydrogen under specialized culture conditions. It is also possible to remove the components of the photosynthetic system found in these organisms, reassemble these outside the cell, and form a water-splitting system which will generate hydrogen and oxygen when exposed to light. Such "cell-free" water-splitting systems form the basis for the second major developmental thrust of the P/BHP.

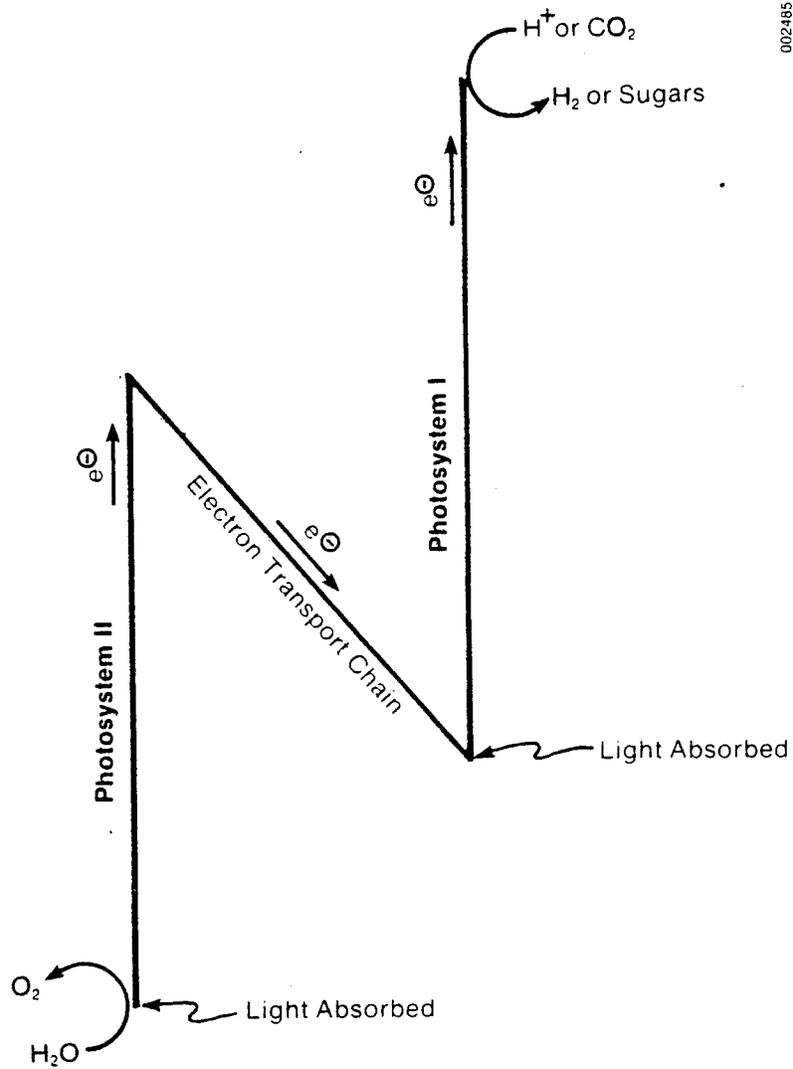
##### 4.1.1 Whole-Cell Hydrogen Technologies

Biological hydrogen production is based on the following reaction:



where an enzyme (nitrogenase or hydrogenase) supplies the two electrons required by two protons to form hydrogen. In photosynthetic organisms the electrons necessary to perform this reduction are usually generated by light. The manner in which the light performs this function is shown in Figure 4-1. Photosynthetic organisms usually contain two photosystems, which when connected by an electron transport chain are referred to as the "Z-scheme" of photosynthesis. Photosystem II splits water to evolve oxygen. Photosystem I provides the reducing power to fix carbon dioxide as sugars. Hydrogen evolution is associated with photosystem I.

In photosynthetic bacteria only photosystem I is present. Normally PSB grow by absorbing fixed carbon compounds from their environment though they are capable of fixing carbon dioxide as sugars in light under a hydrogen atmosphere. The ammonia which is also necessary for growth is formed by the cell itself from atmospheric nitrogen. This reduction is carried out by the enzyme nitrogenase. When there is no nitrogen available and air is excluded from the culture, PSB will continue to absorb organics from the environment and use these compounds to support hydrogen evolution via nitrogenase. PSB are relatively efficient at recycling cellular nitrogen compounds and will continue to remain viable and able to evolve hydrogen for long periods of time in the absence of a nitrogen supply.



002485

Figure 4-1. Z-Scheme of Photosynthesis Showing the Flow of Electrons from Water to Hydrogen Evolution or Carbon Fixation under the Influence of Light

Cyanobacteria (blue-green algae), which evolve hydrogen, normally grow in long filaments. In these filaments 1 out of every 6 to 10 cells is morphologically and functionally very different from the others. These specialized cells, called heterocysts, contain nitrogenase and supply the ammonia which the remaining vegetative cells in the filament need for growth. In turn, the vegetative cells supply the heterocysts with reduced carbon compounds to support nitrogen fixation. When no atmospheric nitrogen is available for fixation, the heterocysts evolve hydrogen. However, the ammonia normally generated by the heterocysts is necessary to maintain viability of the vegetative cells. Thus, a balance between ammonia production and hydrogen generation must be struck in cyanobacteria.

Green algae are reported to produce hydrogen from water. The enzyme involved in this hydrogen evolution reaction is hydrogenase. In green algae both the oxygen-evolving and carbon-fixing functions of photosynthesis are present (as they are in cyanobacteria, but green algae have no specialized means, such as heterocysts, for protecting their hydrogen enzyme from oxygen inactivation). Since all hydrogen-evolving enzymes are sensitive to oxygen, as soon as photosynthetic production of hydrogen commences in algae, it is shut off by the concurrent oxygen production. Therefore, in order for hydrogen production to continue, oxygen must either be removed from the environment or hydrogenase must be rendered insensitive to oxygen.

The theoretical limit for the photosynthetic efficiency of production of any chemical in the living cell is thought to range between 12% to 15%. In the laboratory it is now possible to generate hydrogen at 5% to 6% conversion efficiency using PSB, while the efficiency attainable using cyanobacteria and algae ranges between 1% and 2%. Thus, hydrogen production using PSB is a good deal more advanced than that using algae.

#### **4.1.2 Cell-Free Hydrogen Technologies**

The use of light to produce hydrogen in cell-free hydrogen technologies is based on the isolation of stable, photochemically active portions of the biological hydrogen-producing system. The best-known source organism can thus be used to produce each component. The objective is then to recombine these components outside the cell to form a system for splitting water into hydrogen and oxygen.

There are several technical challenges associated with developing a practical cell-free hydrogen technology. For instance, once removed from the cell, biological components cannot be maintained by the cell. Hence, a methodology for stabilizing components is an important research thrust in the P/BHP. In addition, the efficiency with which light-generated electrons are produced by the components must be maintained. Moreover, the manner in which the components are recombined must be such as to ensure that the electrons generated flow to the production of useful products, e.g., hydrogen.

The great advantage of the cell-free approach to producing hydrogen is that it is independent of the energy-consuming reactions of the living cell. Hence, solar conversion efficiencies achievable in cell-free technologies are not limited by cell metabolism and should approach the theoretical limit for photochemical systems, i.e., greater than 30%. In the laboratory such systems are now operating at 1% to 2% solar conversion efficiency.

### 4.1.3 Engineering Research

The P/BHP also contains an engineering research component which functions to support system concept development based on information generated from research on whole cells and cell fractions. In this arm of the program, concepts for design of system components as well as the overall system are proposed, tested, modeled, and evaluated. Engineering research is initiated when laboratory experiments begin to show promise of leading to a practical system. At this time, only hydrogen production based on photosynthetic bacteria has reached this stage.

## 4.2 OBJECTIVES

The overall goal of the P/BHP is to reduce the risks attendant with the introduction of new solar biotechnologies for producing hydrogen to a level acceptable to the private sector. Certain research objectives have been identified for the various program tasks which should make the stated goal attainable. The P/BHP is, as described previously, divided into three major research thrusts: (1) whole-cell research, (2) cell-free research, and (3) engineering research. Each of these tasks is divided into several subtasks. A summary of the tasks, subtasks, and specific projects in each task can be found in Table 4-1.

For whole-cell technologies the research objective is to achieve a 10% or better solar conversion efficiency for hydrogen production. This is to be accomplished through (1) isolation of the best hydrogen-producing microorganisms from natural habitats, (2) study of the biochemistry and biophysics of hydrogen evolution in these organisms, (3) establishment of the conditions necessary to sustain maximum rates of hydrogen production over long periods, and (4) manipulation of the genetic control of hydrogen production. The figure of 10% conversion of light to hydrogen energy was chosen as the research objective because a preliminary evaluation of the technology indicated that such a system should produce hydrogen at competitive prices.

The research objective for cell-free hydrogen technologies is to achieve a solar conversion efficiency for hydrogen production of 25% or better. At this solar conversion efficiency, roughly  $0.06 \text{ m}^3$  (2 standard cubic feet) of hydrogen would be produced per square meter of surface per hour. A solar conversion efficiency of 25% or better was chosen for two reasons: (1) Since the theoretical limit for such a technology is 33% solar conversion efficiency, 25% should be an achievable performance goal. (2) 25% solar conversion efficiency allows hydrogen to be generated at 40% of the price of electrolytic hydrogen, which is a very competitive price.

The objective of the engineering research task is to identify possible technical hurdles in these new biotechnologies for hydrogen production and to determine solutions for the problems while staying within the technical performance and cost goals for each technology.

**Table 4-1. Photo/Biological Hydrogen Program Tasks, Subtasks, and Projects**

---

**Task I: Whole-Cell Research**

**Species Isolation**

- SERI - Hydrogen Production by PSB
- ORNL - Photosynthetic Watersplitting
- University of Miami - Hydrogen Production by Tropical Marine PSB

**Biochemical/Biophysical Studies**

- SERI - Hydrogen Production by PSB
- ORNL, University of Missouri, University of Florida - support of primary project objective

**Genetic Engineering**

- University of Missouri - Photobiological Production of Hydrogen
- University of Florida - Genetic Engineering of Hydrogen Evolution in Blue-Green Algae

**Optimum Culture Conditions**

- SERI - Hydrogen Production by PSB
- ORNL - Photosynthetic Water-Splitting

**Task II: Cell-Free Research**

**Novel Processes**

- SERI - Biomass Electrochemistry

**Improved Materials/Efficiencies**

- SERI - Algal Hydrogenase
- Battelle - Feasibility of Hydrogen Production by Solar Photoelectrolysis of Biomass Waste Streams

**Molecular Architecture**

- SERI - Primary Photosynthesis Studies
- BNL - Photoproduction of Hydrogen Using Membrane Systems

**Task III: Engineering Research**

**Modeling and Simulation**

- SERI - Bacterial Hydrogen System Analysis and Design
  - SERI - Hydrogen Container Materials Research
-

### 4.3 STATUS

#### 4.3.1 Whole-Cell Research

The first step toward development of a hydrogen production biotechnology based on whole cells is the isolation of organisms from the natural environment that produce hydrogen at rapid rates. Several investigators in P/BHP participate full- or part-time in this activity. At the University of Miami over 350 new strains of tropical marine PSB have been isolated and screened for hydrogen production capacity. In FY 1982, ten new Rhodospseudomonas species of tropical marine photosynthetic bacteria were isolated, seven of which were able to grow and produce hydrogen from cellulose (see Table 4-2). Of these seven, Rhodospseudomonas miami PBE 227(1) produced hydrogen at high rates from cellulose. The ability to convert cellulose to hydrogen is expected to lead to increases of 50% in the overall hydrogen yield from PSB hydrogen production systems.

Researchers at SERI (see Figure 4-2) isolated 15 new strains of photosynthetic bacteria (several of which tolerate low pH) from Coors waste beer. Since many waste streams that will be used to support hydrogen production are expected to be acidic, it is important to have strains of PSB that will tolerate low pH while continuing to produce hydrogen.

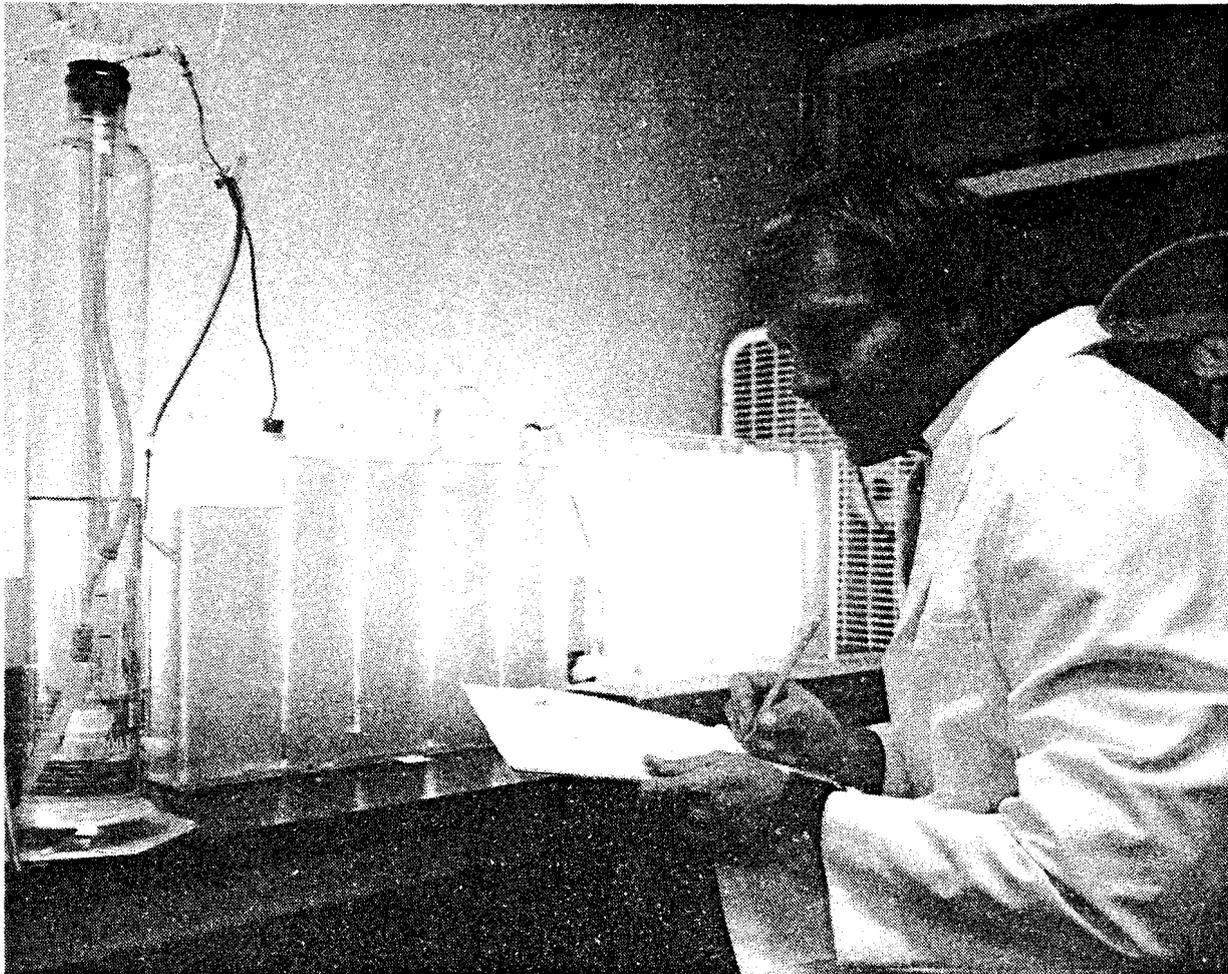


Figure 4-2. Paul Weaver of SERI Following Hydrogen Evolution Rates from Cultures of Photosynthetic Bacteria

Table 4-2. Growth of New Rhodopseudomonas Species on Various Substrates<sup>a</sup>

Substrate	<u>Rhodopseudomonas</u> Species									
	115	136	217	227	258	263	266	285	301	338
<u>Organic Acids</u>										
Formate	++	-	-	-	-	+	-	-	-	++
Acetate	++	+	++	++	+	++	++	++	++	++
Propionate	-	-	+	+	-	++	++	++	-	+
Butyrate	+	-	-	+	++	++	++	++	++	++
Valerate	++	+	=	+	++	++	++	++	+	++
Caproate	+	-	+	-	+	++	++	+	+	++
Caprylate	-	-	-	-	-	-	-	-	-	-
Pyruvate	+	+	+	+	+	+	++	+	+	++
Lactate	-	-	-	-	+	+	+	-	-	-
Citrate	-	-	-	+	-	+	++	-	-	++
Malate	-	-	++	++	-	-	++	-	-	+
Fumarate	-	+	++	-	-	-	+	-	-	+
Succinate	-	-	++	-	-	-	++	-	-	+
Tartrate	-	+	-	-	+	-	+	+	-	-
B-glycerophosphate	+	+	-	-	+	-	+	-	-	+
Malonate	+	-	+	+	-	-	+	+	-	++
Glyconate	+	-	++	++	++	-	+	+	-	++
<u>Alcohols</u>										
Methanol	+	-	+	+	-	+	+	+	-	+
Ethanol	-	-	++	+	-	+	+	+	+	+
Glycerol	-	-	+	-	-	-	++	+	+	-
<u>Sugars</u>										
Arabinose	+	-	+	-	-	-	+	++	+	+
Dextrin	++	+	++	++	+	+	++	++	++	++
Glucose	++	+	++	++	++	+	++	++	++	+
Fructose	+	+	++	++	+	-	++	++	++	+
Lactose	+	+	++	-	+	+	+	+	+	+
Maltose	++	+	++	++	+	+	+	+	++	++
Mannitol	+	-	++	++	++	+	++	++	+	++
Xylose	++	+	++	+	+	-	+	-	+	++
Cellulose	+	++	++	++	+	++	++	++	++	++
Mannose	++	++	+	+	+	++	++	++	+	++
Sorbitol	++	++	++	++	+	++	++	+	+	+
Sucrose	++	++	+	++	++	++	++	++	+	++
<u>Organic Polymers</u>										
Cellulose	+	+	+	+	-	+	+	-	-	+
Soluble starch	+	+	++	++	-	++	+	++	+	-
Pectin	+	+	++	++	++	+	++	++	+	++
<u>Amino Acids</u>										
Aspartate	-	-	+	++	-	-	-	+	+	-
Arginine	-	-	+	+	-	-	-	+	-	-
Glutamate	-	-	-	-	-	-	+	-	-	-
Casamino acid	++	+++	+++	++	+++	++	+++	+++	+	++
<u>Aromatics</u>										
Phenol	-	-	-	-	-	-	-	-	-	-
Benzoate	-	-	-	-	-	-	+	-	-	-
<u>Inorganic Compounds</u>										
Thiosulfate	++	++	++	++	+	++	++	+	++	+

<sup>a</sup> - Growth not better than in control without added carbon source  
 + Light growth (OD<sub>650</sub> up to 0.1)  
 ++ Moderate growth (OD<sub>650</sub> 0.1 to 0.5)  
 +++ Heavy growth (OD<sub>650</sub> more than 0.5)

At Oak Ridge National Laboratory, a multicellular green alga (Volvox globator) that produces hydrogen was identified. This represents a naturally occurring cell immobilization system which may prove useful in producing larger volumes of hydrogen from smaller volumes of algae. Also, a strain of the green alga Chlorella, isolated at SERI, was found to contain very high levels of active hydrogenase. This is encouraging since high hydrogenase activity leads to enhanced hydrogen evolution in algae.

The biochemistry and biophysics that support hydrogen production in naturally occurring organisms must be understood in order to devise intelligent means of genetically engineering hydrogen-producing "superbugs." Therefore, studies along these lines are carried out by investigators in the P/BHP.

In FY 1982 at SERI, hydrogen production by photosynthetic bacteria under anaerobic conditions in the dark was demonstrated. Surprisingly, the rate of hydrogen production was high—approximately 40% of that produced under saturating light. Tests showed that the enzyme hydrogenase was responsible for this dark production of hydrogen. (Light-driven hydrogen evolution in these organisms is dependent on the enzyme nitrogenase.) However, this hydrogenase-based hydrogen generation is sensitive to hydrogen pressure: at approximately 20 kPa (0.2 atm), net hydrogen production ceases. [Light-driven, nitrogenase-dependent hydrogen evolution continues at pressures in excess of 5000 kPa (50 atm).] The SERI research group has been able to partially reconstitute\* this membrane-associated biochemical system for anaerobic (without oxygen) dark growth and hydrogen production outside the living cell—an accomplishment that has eluded researchers in the field for 40 years.

This unexpected dark hydrogen evolution mode could impact the overall yield from a PSB hydrogen production system significantly. Preliminary calculations indicate that the average daily hydrogen yield could be as much as 160% of that originally anticipated. An additional possible benefit from this discovery could be increased methane yields from sewage treatment. The high ammonia content of sewage makes light-driven hydrogen generation from this source problematic because ammonia prevents nitrogenase-related hydrogen production. Hydrogenase-based production of hydrogen, however, is unaffected by ammonia. Methanogens use hydrogen, carbon dioxide, and/or acetate to generate methane. PSB produce these compounds in dark anaerobic fermentation. It is expected that the methanogens would take up the hydrogen supplied by the PSB sufficiently rapidly to prevent hydrogen pressure buildup. The overall result could be increased methane yields from sewage treatment.

Genetic engineering of a hydrogen-producing "superbug" depends on the availability of a strong gene pool in the form of the culture collections supported by culture isolation efforts and on the information generated by the biophysical and biochemical studies. The steps leading to generation of a hydrogen "superbug" will include manipulating the microorganisms genetically (1) to either reverse or override biochemical control of hydrogen production enzymes and (2) to enhance hydrogen evolution by adding biochemical functions to the cell that it normally does not possess.

---

\*Reconstitution of a system involves first removing it from the living cell and then manipulating the system in such a way as to restore its normal function in vitro ("in glass").

One of the most straightforward genetic manipulations that can be used to isolate mutants is called selection pressure. This technique consists of applying an environmental stress, such as high salt concentration or high temperature, to a population of cells. The stress causes the portion of the population that can tolerate it to take over the culture; all other cells are killed. At SERI this year a 600% increase has been achieved in the tolerance to alcohol of the best hydrogen producer discovered to date. This is important because many candidate waste streams for hydrogen production using PSB contain alcohol.

Another method for creating mutants involves treating the cells with chemicals and/or ultraviolet light. This treatment chemically modifies the basic genetic material (DNA) and is very effective in generating mutants. Unfortunately, the mutations of interest must then be identified (screened) from the millions created. At the University of Missouri a successful screening procedure for nitrogenase-derepressed mutants was developed as a result of a 2-year effort to design tight, unambiguous screening procedures (see Figure 4-3). The development of these procedures—a difficult task due to the ability of PSB to grow and maintain cells in at least 11 metabolic modes—is important because such mutants are expected to have higher hydrogen-evolution capacity than wild-type organisms. Also, in PSB, mutations removing the ability to assimilate nitrogen have been shown to be linked (i.e., to be located in the same region of the chromosome) to the regulatory region for nitrogenase (hydrogen-producing enzyme). This is an important discovery because it locates the genes for uptake of ammonia and amino acids which regulate nitrogenase expression and, hence, hydrogen production.

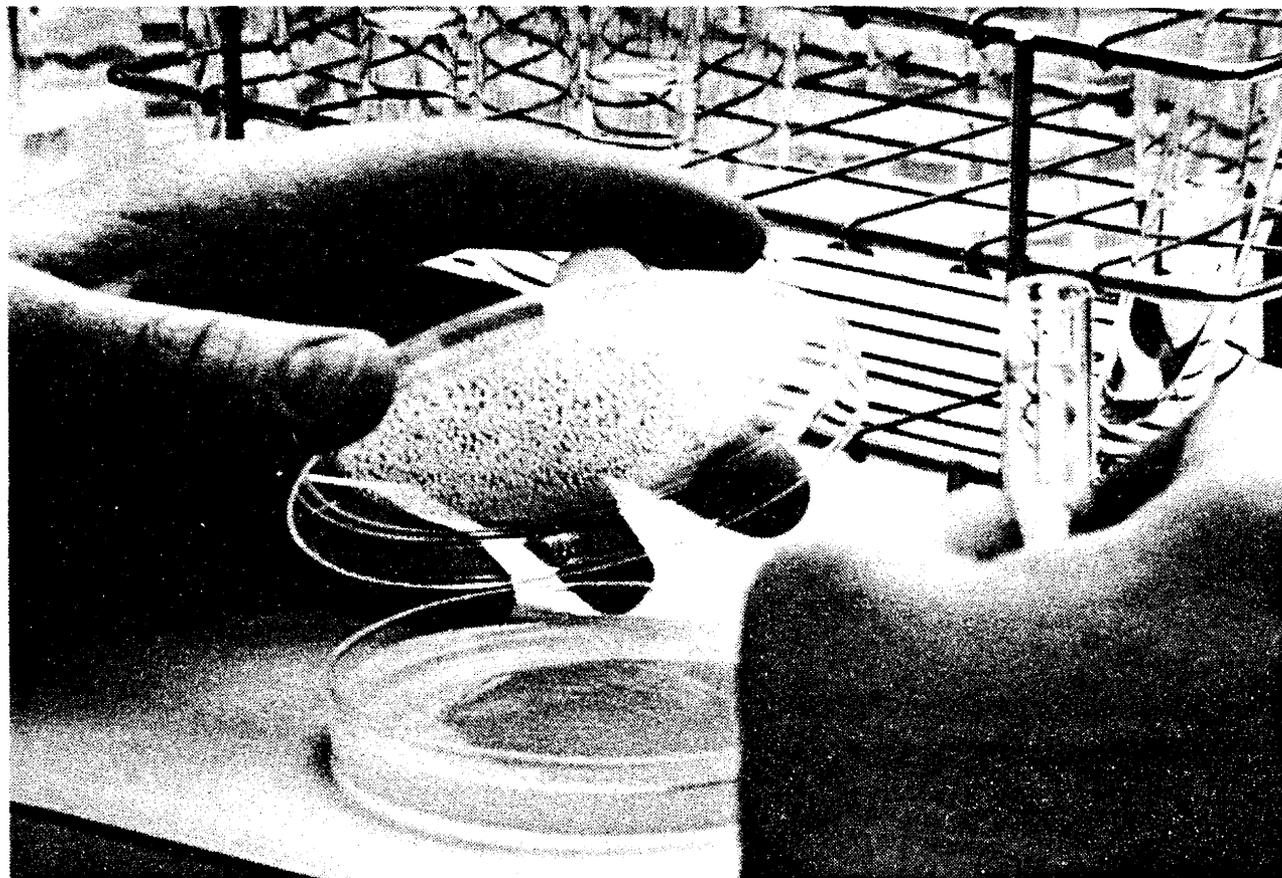


Figure 4-3. Lawn of Mutants on Screening Plate (Petri Dish)

Genetic engineering involves the transfer of one or more genes from one organism to another. To do this the gene must normally be cloned. In this process genes of interest are incorporated into a plasmid, virus, etc., which act as carriers. Before cloning, the genes of interest must be mapped; i.e., located on the chromosome.

The positions of the genes responsible for regulation of hydrogen metabolism in *E. coli* have been mapped on the *E. coli* chromosome by researchers at the University of Florida (see Figure 4-4). Since no differences in hydrogen metabolism between *E. coli* and blue-green algae have been observed, and since a great deal of genetic information is available for *E. coli*, this organism is ideal for establishing a biochemical and genetic model for manipulation of hydrogenase-related hydrogen metabolism in blue-green algae (cyanobacteria).

The enzyme nitrogenase is also present in cyanobacteria. Normally ammonia shuts off nitrogenase expression, thus causing hydrogen production to cease. Researchers at the University of Florida have isolated the first *gln<sup>-</sup>* (a mutation conferring the inability to synthesize glutamine) mutant in blue-green algae. This mutation frequently leads to derepressed expression of nitrogenase in the presence of ammonia. The ability to synthesize nitrogenase in the presence of ammonia would lead to increased, long-term stability of hydrogen production using blue-green algae.

The establishment of a successful hydrogen-producing biotechnology also depends on determining the culture conditions required to support maximum hydrogen evolution rates, first in the laboratory, then in the field. In studies performed at SERI in FY 1982, natural substrates, including orange, peach, potato, lemon, pear, cantelope, grapefruit, banana, pineapple, plum, and apple, all provided abundant usable carbon for growth and hydrogen production by photosynthetic bacteria. Cheese whey, spent beers, and thin stillage from milo and grape alcohol distilleries also support vigorous growth and hydrogen production. The types of compounds found in these natural substrates and the relative abilities of these compounds to support growth and hydrogen production are listed in Table 4-3.

Stabilization of long-term hydrogen production in PSB has been achieved at SERI through the addition of small amounts of ammonium ion. This allows the PSB to make repairs to its photophosphorylation mechanism which produces ATP (adenosine triphosphate), a substance necessary for hydrogen evolution in PSB.

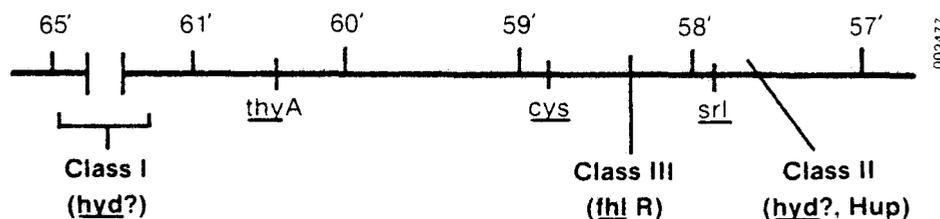


Figure 4-4. Genetic Map Locations of the Genes Involved in Hydrogen Metabolism in *Escherichia coli*

**Table 4-3. Growth and H<sub>2</sub> Photoproduction by Rhodospseudomonas sphaeroides SCJ on Defined Substrates**

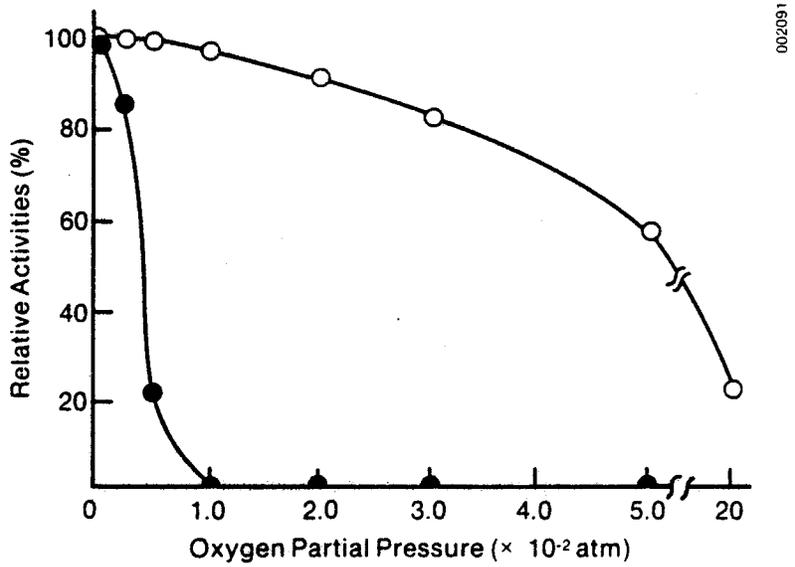
Substrate Class	Function	Substrate Effectiveness <sup>a</sup>
Sugar derivatives	H <sub>2</sub> photoproduction	malate, pyruvate, lactate, succinate, butyrate, isobutyrate, tartrate, valerate, acetate, caproate, propionate, malonate, formate, oxalate, glycolate, citrate, caprylate, nonanate (no growth)
Organic acids	H <sub>2</sub> photoproduction	lactate, acetate, pyruvate, tartrate, propionate, butyrate, isobutyrate, valerate, glycolate, succinate, malate, fumarate, caproate, malonate, formate
Sugars and sugar derivatives	cell growth	gluconate, mannitol, sorbitol, ribose, D-xylose, mannose, fructose, glucuronate, cellobiose, sucrose, galactose
Sugars and sugar derivatives	H <sub>2</sub> photoproduction	xylose, ribose, fructose, glucuronate, mannose, mannitol, gluconate, sucrose, galactose, sorbitol, cellobiose

<sup>a</sup>Substrates are listed in order of decreasing ability to support growth and hydrogen production.

Another approach to stabilizing long-term hydrogen production is immobilization. The cells are concentrated (usually by dewatering) and then imbedded in some biologically innocuous polymer. Immobilization of tropical marine PSB in agar sheets by researchers at the University of Miami protected the cells and permitted hydrogen production to continue over long periods of time [see Figures 4-5(a) and 4-5(b)].

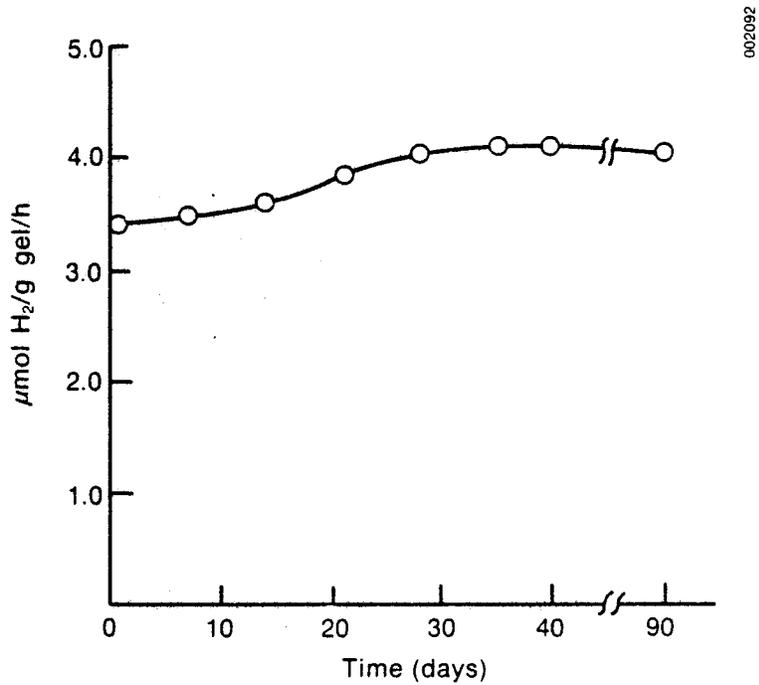
At Oak Ridge National Laboratory the ability of green algae to sustain simultaneous production of hydrogen and oxygen for approximately 10 days was demonstrated (see Figure 4-6). It is important to know how long it is possible to continue producing hydrogen from algae while maintaining viability.

Investigators at the University of Missouri developed a stable coculture of a cellulose-digesting bacterium (Cellulomonas) and a hydrogen-producing PSB. Conditions for maximum hydrogen production by the coculture were determined. A mutant PSB (Rhodopsuedomonas capsulata ST410) that lacks the ability to take up hydrogen for recycle produced the largest amount of hydrogen for the longest period of time (see Figure 4-7). The ability to digest cellulose in organic waste streams leads to an increased overall yield of hydrogen. The increased yield is expected to be approximately 150% of the original yield without cellulose digestion.



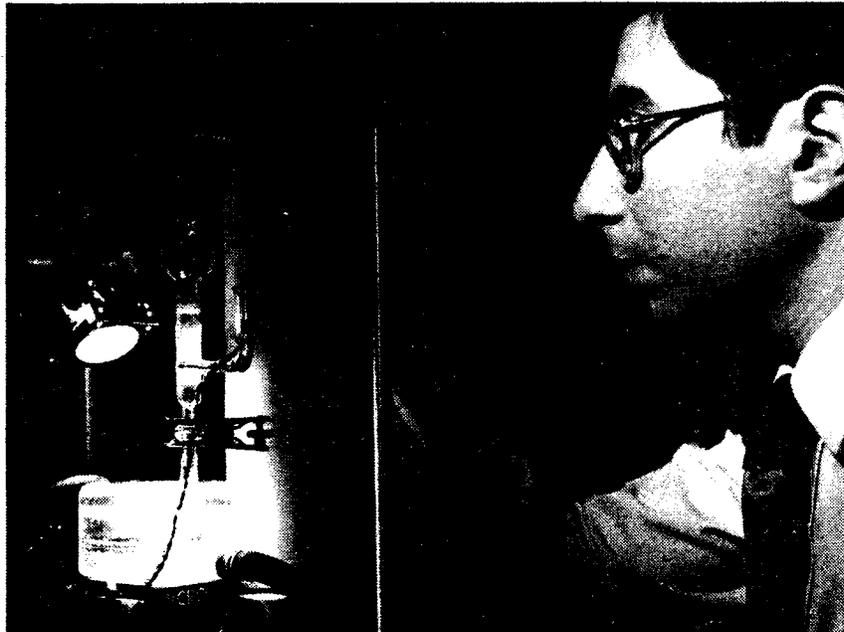
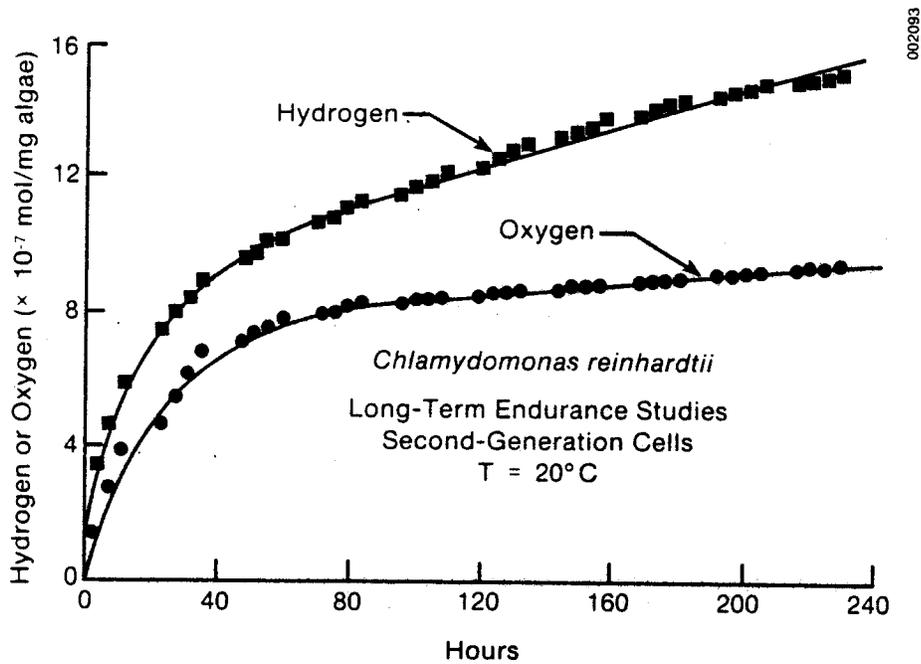
002091

**Figure 4-5(a).** Effect of Oxygen Partial Pressure on Hydrogen Production in Cell Suspension (●) and Immobilized Cells (○). After the atmosphere of the flasks was replaced with argon, oxygen was injected to give the oxygen partial pressure.

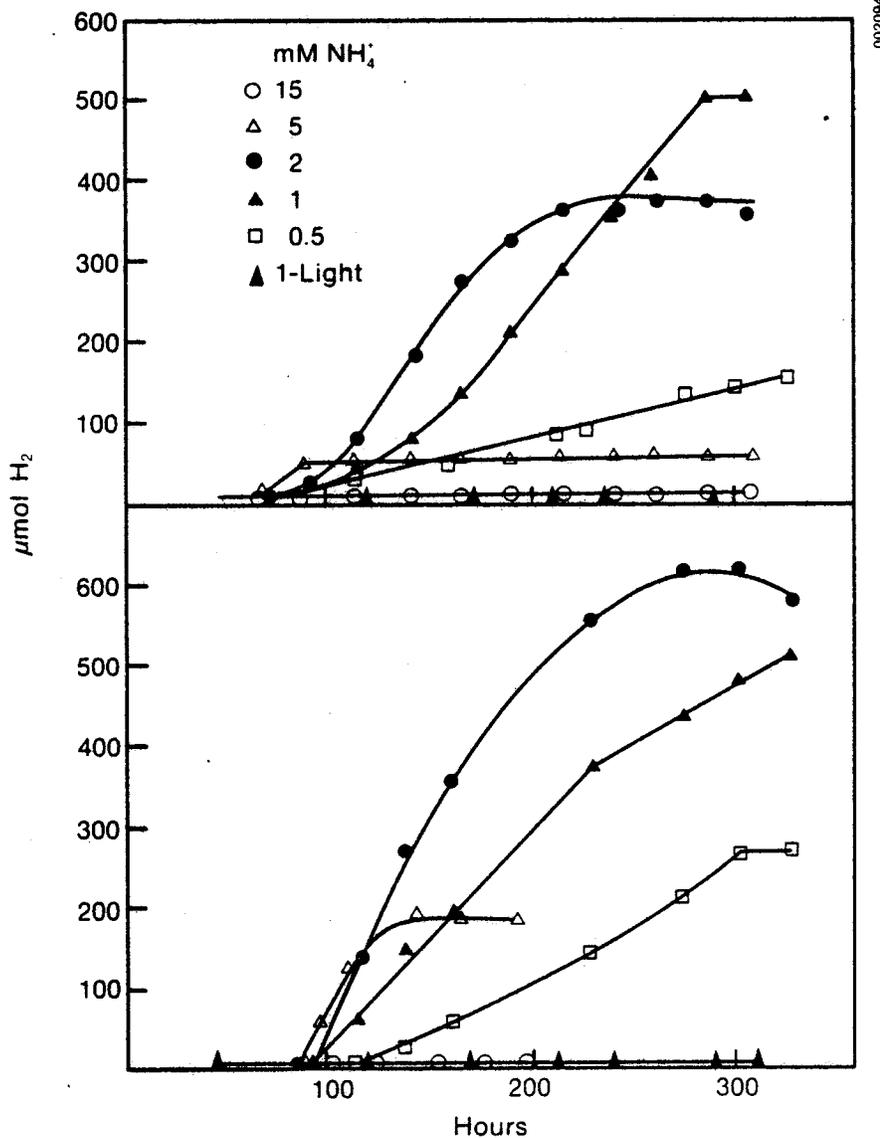


002092

**Figure 4-5(b).** Storage Stability of Immobilized Cells in Seawater



**Figure 4-6.** The Cumulative Yields of the Simultaneous Photoproduction of Hydrogen and Oxygen in Anaerobically Adapted *Chlamydomonas reinhardtii*. The algae were illuminated using a cycle of 3 hours on and 1 hour off. The light-off period was used to reestablish the baseline. No additional nutrients were added during the course of the experiment. The measuring technique is shown in the photo.



**Figure 4-7.** Photoproduction of H<sub>2</sub> by *R. capsulata/Cellulomonads* sp. Cocultures at Initial NH<sub>4</sub><sup>+</sup> Concentrations from 0.5 to 15 mM, Incubated in Darkness. Top graph: B100 cocultures; bottom graph: ST410 cocultures. B100 is a wild-type organism; ST410 is a hydrogen-uptake-minus mutant.

#### 4.3.2 Cell-Free Research

Potentially practical cell-free technologies for producing hydrogen are identified through the information generated in biochemical and biophysical studies on whole cells. The successful cell-free hydrogen production technology will probably be composed of a combination of biologically derived catalysts, inorganic catalysts, and organic materials. These elements will be combined to produce a system that funnels light-generated electrons to the production of useful products, including hydrogen.

Cell-free technologies for hydrogen production are expected to be made up of, in part, photoactive water-splitting components taken from the cell, and means must be found for protecting these components from degradation. In addition, inorganic components of a potential cell-free system undergo complex electrochemical reactions which must be understood before these components can be protected against dissolution. Research performed in FY 1982, aimed at improving both the materials available for use in cell-free systems and the efficiency of conversion of light energy to products, is described below.

As mentioned previously, hydrogenase is the hydrogen production enzyme in green algae. At SERI this year (Figure 4-8) the first algal hydrogenase ever isolated has been purified 80-fold over the activity of the enzyme found in the whole cell (see Table 4-4).

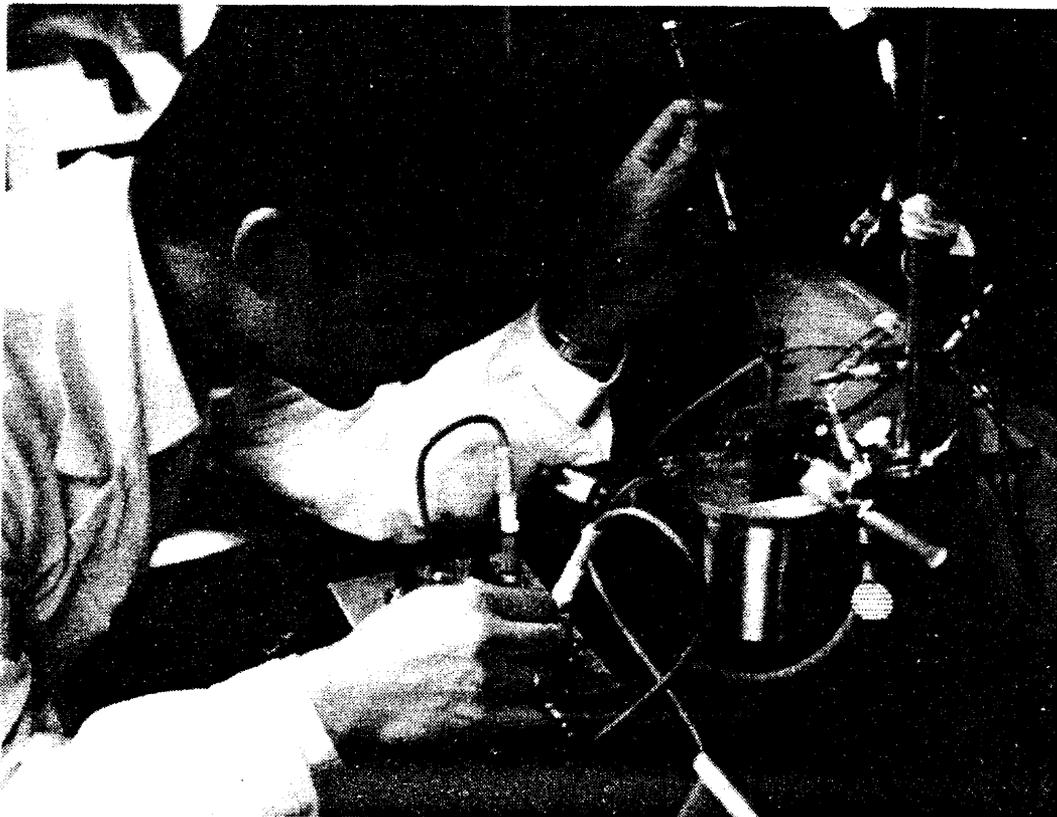


Figure 4-8. Steve Lien of SERI Assaying Hydrogenase Activity

**Table 4-4. Purification of Hydrogenase from *Chlamydomonas reinhardtii***

Step	Activity (units/mL) <sup>a</sup>	Total Volume (mL)	Total Activity (units)	% Yield	Protein (mg/mL)	Specific Activity (units/mg protein)	Purification
Clarified crude preparation	438	760.0	332,880	100.0	2.54	160.6	1.0x
1st DEAE column	3224	42.0	135,408	41.0	11.3	285.3	1.78x
DEAE gradient	2964	26.0	77,064	23.0	1.98	1,497.0	9.32x
Sephadex G-100 SF	3915	14.1	55,202	16.6	0.305	12,834.6	79.92x

<sup>a</sup>A unit of hydrogenase is defined as the amount of enzyme that catalyzes the production of 1 nmole H<sub>2</sub>/min in the presence of 1 M ferredoxin and 10 mM sodium dithionite at pH 6.8 and 25° C.

Normally hydrogenase requires another molecule—a cofactor—that provides the reducing power for hydrogen production. This cofactor is a molecule called ferredoxin. Algal ferredoxin is present in algae in low quantities and is difficult to isolate and purify. Therefore, the ability of algal hydrogenase to interact with spinach ferredoxin (more easily purified in quantity) was tested. The interaction was found to be strong, and good rates of hydrogen evolution were observed. Surprisingly, a synthetic coenzyme for hydrogenase (an isopoly-tungsten complex) gave twice the hydrogen evolution rate observed with the natural cofactor. Perhaps this synthetic complex will prove to be the cofactor of choice for use in experimental cell-free technologies for hydrogen production based on hydrogenase.

For a cell-free technology to be complete, hydrogen evolution must be balanced by oxygen evolution or some other process. If a biomass waste stream, rather than water, is used as a feedstock for a cell-free system, the possibility exists for producing fuel mixtures of hydrocarbon and hydrogen as products. This is achieved via a photo-Kolbe reaction. At Battelle in FY 1982 the product yields under various conditions for the photo-Kolbe reaction were determined (see Table 4-5). Results show that the type of hydrocarbon products generated depends strongly on the pH of the reactant solution. This is an important fundamental contribution to the knowledge in this field.

A large variety of photoactive semiconductors were tested in powder form at Battelle for their ability to carry out photo-Kolbe reactions (see Table 4-6). This kind of screening effort gives essential information regarding those semiconductors which show sufficient promise for further investigation.

Table 4-5. Product Yields in the Photo-Kolbe Reaction

Semiconductor	Solution Composition	Current Efficiency for Various Products <sup>a</sup>				
		O <sub>2</sub>	CO <sub>2</sub>	CH <sub>4</sub>	C <sub>2</sub> H <sub>6</sub>	H <sub>2</sub> C=O
TiO <sub>2</sub>	18:1 acetate buffer <sup>b</sup> + 1.0 M NaClO <sub>4</sub>	0.30	0.19	0.01	0.00	0.14
TiO <sub>2</sub>	18:1 acetate buffer <sup>b</sup> + 1.0 M KCl	0.32	0.22	0.01	0.00	0.11
TiO <sub>2</sub>	1.0 M KOAc in glacial HOAc	0.00	0.10	0.03	0.12	0.08
SrTiO <sub>3</sub>	1.0 M KOAc in glacial HOAc	0.00	0.06	0.02	0.01	0.00
SrTiO <sub>3</sub>	1.0 M NaOAc + 3.0 M NaOH	0.55	(not measured)			0.06

<sup>a</sup>Current efficiency defined as  $Q_{\text{product}}/Q_{\text{total}}$ , where  $Q_{\text{product}} = nFN$  ( $n$  = electron equivalents per molecule of product,  $F$  = Faraday constant,  $N$  = moles of given product), and  $Q_{\text{total}} = I_{\text{total}} dt$ ; based on electron stoichiometry as follows: O<sub>2</sub> ( $n = 4$ ), CO<sub>2</sub> ( $n = 1$ ), CH<sub>4</sub> ( $n = 1$ ), C<sub>2</sub>H<sub>6</sub> ( $n = 2$ ), H<sub>2</sub>C=O ( $n = 3$ ); H<sub>2</sub>C=O concentrations measured by chromatographic acid test procedure; other products analyzed by mass spectroscopy.  $Q$  = yield;  $I$  = current.

<sup>b</sup>18:1 HOAc/NaOAc; total acetate concentration = 1.0 M; pH = 3.4.

Table 4-6. Relative Rates of Gas Production with Photocatalytic Semiconductor Powders<sup>a</sup>

Catalyst	UV Cutoff Filter Used	Gas Analysis Performed <sup>b</sup>	Rate of Total Gas Generation (mL/min)
Ti <sub>50</sub> O <sub>99</sub>	No	Yes	1.86
Fe <sub>2</sub> TiO <sub>5</sub>	No	Yes	1.45
FeTiO	No	No	1.06
Ti <sub>15</sub> O <sub>29</sub>	No	No	1.00
TiO <sub>1.998</sub>	No	No	0.91
V <sub>2</sub> O <sub>5</sub>	No	No	0.63
GaP	No	Yes	0.54
Ti <sub>2</sub> O <sub>3</sub>	No	No	0.44
Fe <sub>3</sub> O <sub>4</sub>	No	Yes	0.45
Fe <sub>2</sub> O <sub>3</sub>	No	Yes	0.35
Ti <sub>40</sub> O <sub>99</sub>	Yes	No	0.00
FeTiO <sub>3</sub>	Yes	No	0.00
Fe <sub>2</sub> TiO <sub>5</sub>	Yes	Yes	0.00
GaP	Yes	Yes	0.00
Al <sub>2</sub> O <sub>3</sub>	No	No	0.00

<sup>a</sup>Solution Composition: 5.6 M HOAc containing 70 mM KCl in H<sub>2</sub>O; 32° ± 4°C.

<sup>b</sup>Gas analysis was by mass spectrometry. The gas typically contained methane and carbon dioxide in a 3:1 ratio, with the balance of the carbon dioxide assumed to be dissolved in the electrolyte solution.

Photosynthetic organisms contain an electron transport system consisting of two photosystems which are linked to one another. Photosystem II (PSII) is responsible for evolving oxygen from water. Photosystem I (PSI), normally associated with carbon fixation, can be linked to hydrogen production.

At SERI a rapid method for isolating and purifying photosystem I reaction-center complexes (the site of the primary events of bacterial photosynthesis) was established. This procedure permits isolation of reaction centers in seven hours (as opposed to 2-8 days) and results in a two- to threefold increase in yield. This new preparation method will greatly accelerate research progress with respect to the basic mechanisms of photosynthesis.

Reaction centers isolated using the new SERI procedure were oriented in a monolayer, and the area occupied per reaction center was determined (see Figure 4-9). This work was a prelude to studying the optical and electrochemical properties of reaction-center monolayers and multilayers. Such studies will lead to an increased understanding of the biological electrochemistry of hydrogen evolution under cell-free conditions.

An active PSII oxygen-enzyme particle was isolated at SERI, providing the base material for studying the electrochemistry of oxygen evolution from water. The mechanism by which oxygen is evolved in photosynthetic water-splitting was studied in these active PSII particles. Results from these studies will lead to a better understanding of the potential for producing hydrogen from algae because the electrons required for hydrogen evolution in algae originally come from PSII.

Investigators at BNL, using a PSII membrane preparation from a thermophilic blue-green alga (*Phormidium laminosum*), obtained a conversion efficiency of monochromatic light

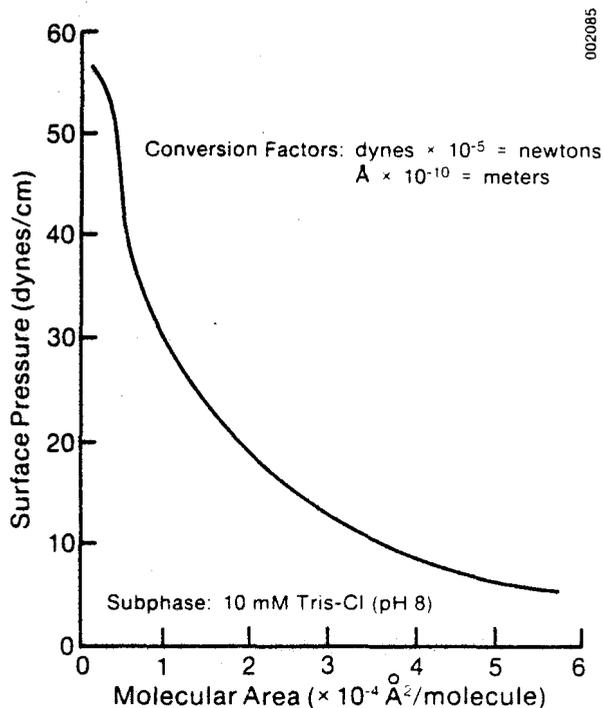


Figure 4-9. Surface Pressure/Area Curve for Isolated Bacterial Reaction-Center Complexes Obtained on the Air/Water Interface of Langmuir Trough

for producing hydrogen from algae because the electrons required for hydrogen evolution in algae originally come from PSII.

Investigators at BNL, using a PSII membrane preparation from a thermophilic blue-green alga (*Phormidium laminosum*), obtained a conversion efficiency of monochromatic light to oxygen of 12%. Since the object of cell-free, water-splitting systems is to recombine oxygen- and hydrogen-forming biological systems outside the cell to split water into hydrogen and oxygen, the above result is favorable.

Investigators at BNL also found that the pattern of proteins associated with green sulfur photosynthetic bacterial (PSB) reaction-center particles (hydrogen-producing systems) resembled that of purple nonsulfur PSB more than that of cyanobacteria (blue-green algae) or chloroplasts. This indicates that the pathway for electron transport to hydrogen production using hydrogenase may differ in the two types of photosynthetic organisms.

### **4.3.3 Engineering Research**

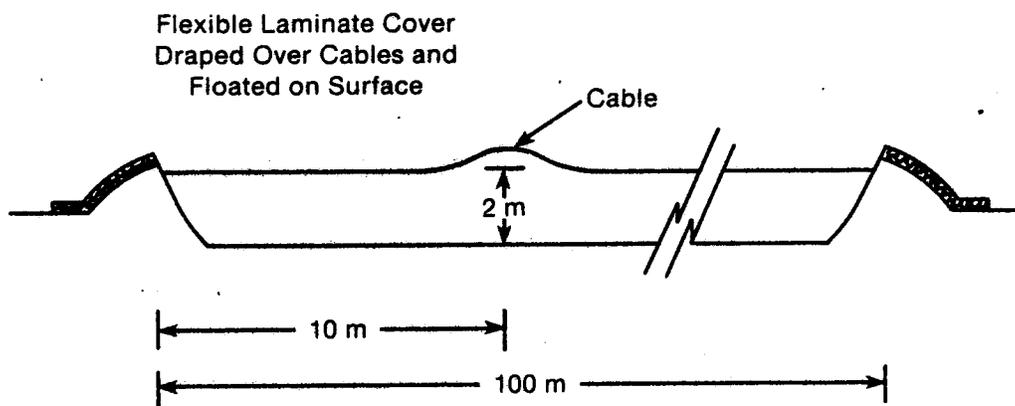
Totally new engineering ground will need to be broken before the practicality of hydrogen production biotechnologies can be established. Much research will therefore be needed both at the laboratory scale and in the field. Engineering research is begun for a developing solar biotechnology when laboratory experiments show sufficient progress to suggest that the system will eventually be practical. Currently, only PSB hydrogen production has reached this phase. However, information and materials generated by research performed on this system should be applicable to other hydrogen production systems under development in this program element.

The first activity initiated under the engineering research task is modeling and simulation. This involves gathering information from literature and industry to assess the state of the art of engineering expertise. From this information a hypothetical system is devised, components designed or selected, and the hypothetical system costed. Additional modeling efforts involve writing computer programs to simulate component and system performance. All the research currently performed in this task is carried out at SERI.

An analysis of a hypothetical PSB hydrogen production system was performed which included identification of four conceptual reactor designs and a systems cost analysis based on these designs. Two potentially practical reactor concepts were identified in this effort [see Figures 4-10(a) and 4-10(b)]. The projected costs of hydrogen produced using the proposed system are given in Tables 4-7(a) and 4-7(b). These prices appear to be competitive with the bulk hydrogen gas market prices.

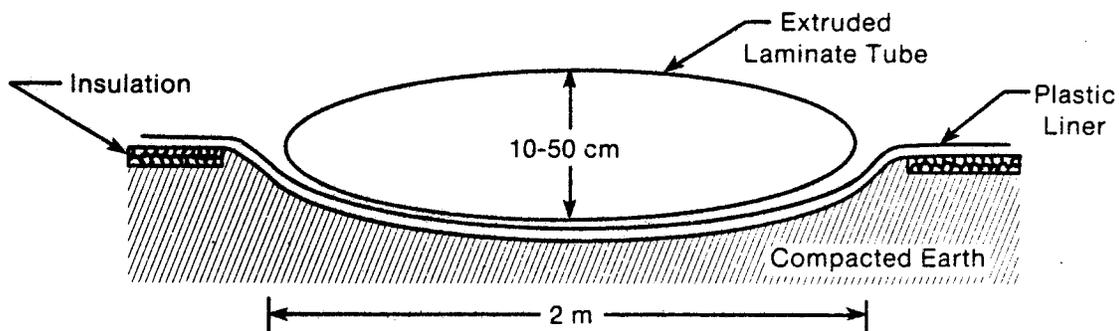
Development of a computer program named SOLBUG which models the thermal, biological, and convective behavior of the proposed PSB reactor designs has been initiated. The thermal and biological portions of the computer simulation are operational. Results from sample runs are shown in Figures 4-11(a) and 4-11(b). These computer programs allow evaluation and optimization of proposed reactor designs before commitment of funds in actual test modules.

In support of the modeling work described above, a literature survey was conducted to identify materials that might be suitable for use in proposed PSB reactors. Twelve polymers were identified as potentially suitable for use as covers on photosynthetic



002086

Figure 4-10(a). Uninsulated Channel Reactor (end view)



002087

Figure 4-10(b). Deep Pond Reactor (side view)

**Table 4-7(a). Conceptual Near-Term Plant Capital and Operating Costs**

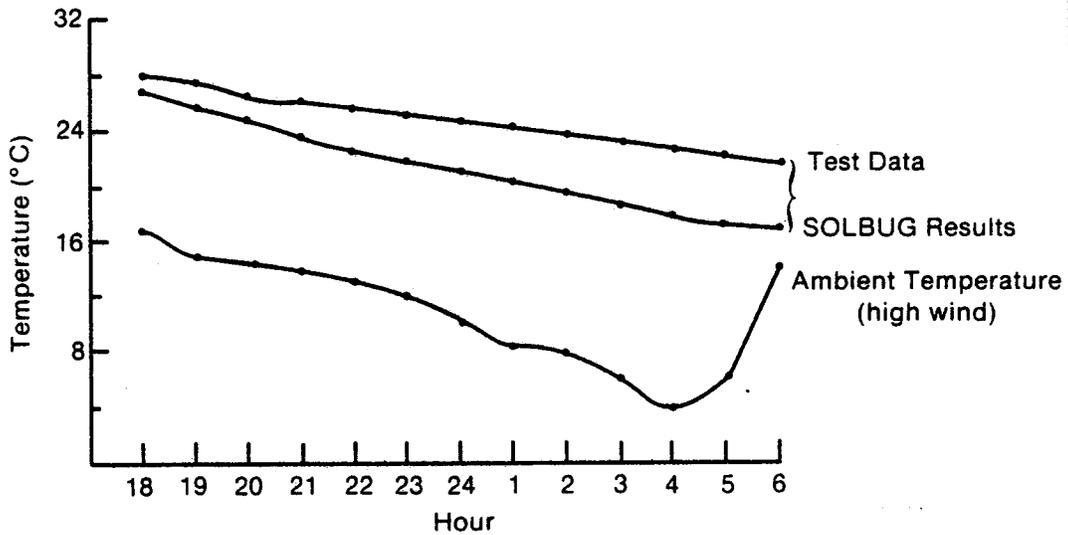
Item	Capital (\$)	O&M (\$/yr)
Solar bacterial reactor <sup>a</sup>	\$5,961,000	\$ 119,000
Substrate storage	302,000	6,000
Effluent treatment	30,000	3,000
H <sub>2</sub> compression	400,000	166,000
H <sub>2</sub> purification	987,000	57,000
H <sub>2</sub> storage	—	—
Land	500,000	—
	<u>\$8,180,000</u>	<u>\$ 401,000</u>
Cost of capital (0.25 fixed charge rate)		<u>\$2,045,000</u>
Annual operation cost		<u>\$2,446,000</u>
First-year cost of hydrogen		\$83.30/MWh <sub>t</sub> (\$24.40/10 <sup>6</sup> Btu)

<sup>a</sup>Based on deep pond reactor design.

**Table 4-7(b). Research Goals for Plant Capital and Operating Costs**

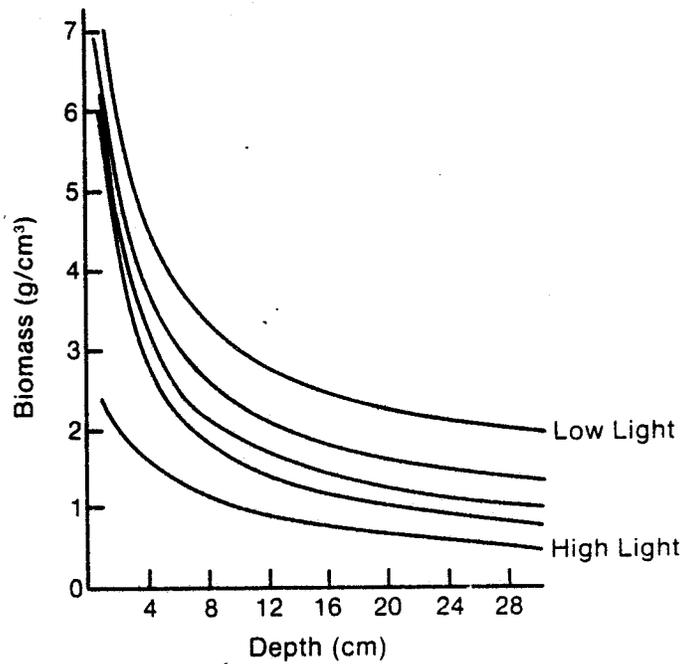
Item	Capital (\$)	O&M (\$/yr)
Solar bacterial reactor <sup>a</sup>	\$ 2,980,000	\$ 60,000
Substrate storage	302,000	6,000
Effluent treatment	30,000	3,000
H <sub>2</sub> compression	400,000	166,000
H <sub>2</sub> purification	987,000	51,000
H <sub>2</sub> storage	—	50,000
Land	250,000	—
	<u>\$4,949,000</u>	<u>\$ 336,000</u>
Cost of capital (0.25 fixed charge rate)		<u>\$1,273,000</u>
Annual operation cost		<u>\$1,573,000</u>
First-year cost of hydrogen		\$53.60/MWh <sub>t</sub> (\$15.70/10 <sup>6</sup> Btu)

<sup>a</sup>Based on deep pond reactor design.



002089

Figure 4-11(a). Comparison of SOLBUG Results with Test Site Data: Nighttime Temperature Profile



002090

Figure 4-11(b). Cell Concentration as a Function of Depth at Various Light Intensities

Table 4-8. Thin Polymer Sheeting for Photosynthetic Bacterial Hydrogen Reactors

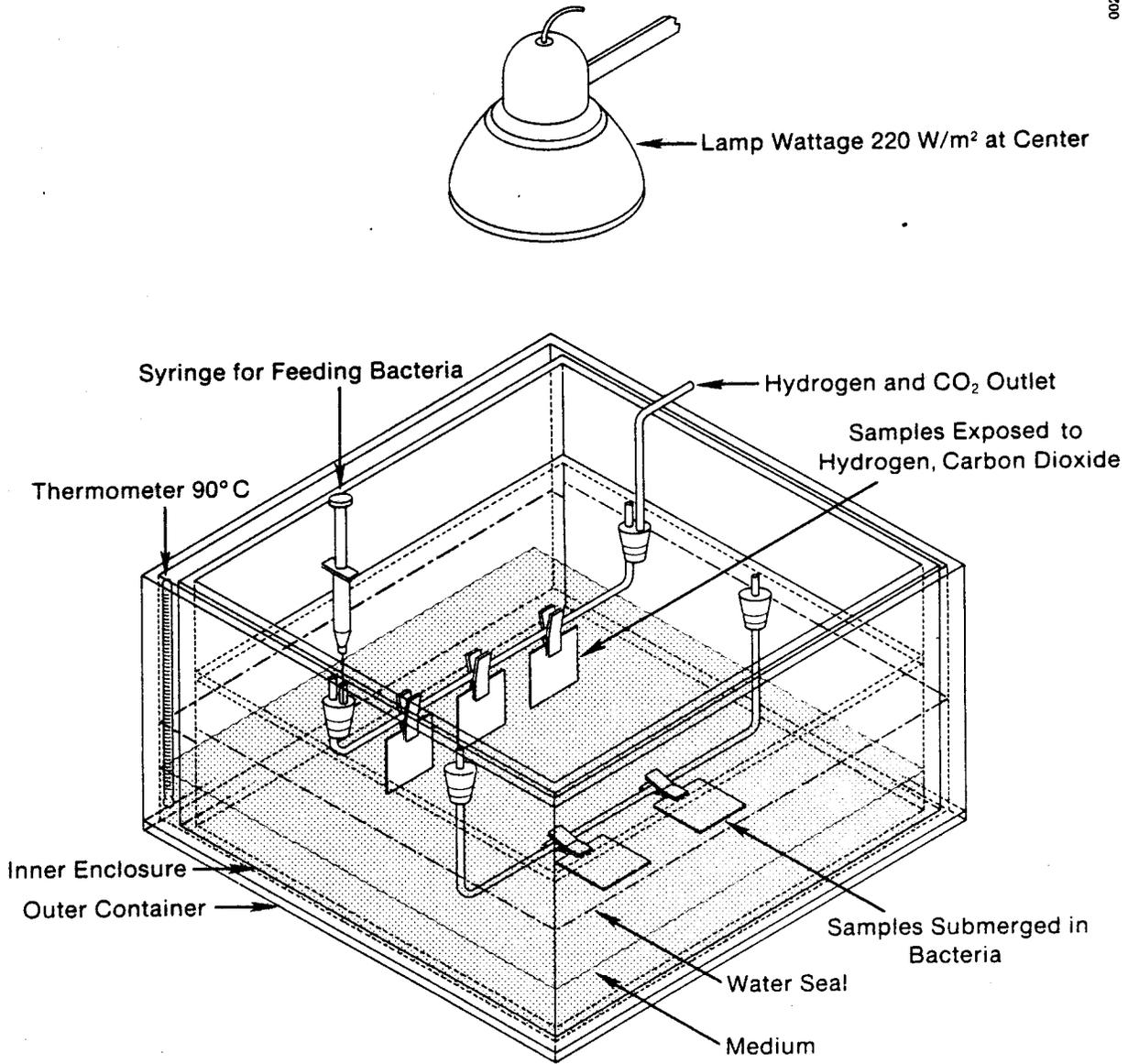
Generic Name	Manufacturer	Thickness (mils)		Solar Transmittance (%)	UV Resistance	Continuous Temp. (°C)
Polymethylmethacrylate (PMMA)	3-M	2	23.63	92	very good	100
Allyl ester	PPG Ind.	3	16.70	90	unknown	100
Ethylene-Chlorotrifluoroethylene	Applied Chemical	2	52.75	81	good	200
Fluorinated ethylene-propylene (FEP)	DuPont	2	51.30	94.6	good	200
Polyvinylidene fluoride	Penwalt	4	16.54	90	good	107
Polyvinyl fluoride	DuPont	3	33.08	93	excellent	135
Nylon 6	Allied Chemical	6	5.51	92.5	fair	93
Polyester	Martin Proc.	5	29.29	88	stabilized	177
Silicone elastomers	Dow Corning	2	28.91	75	good	115
Polyimide	DuPont	2	23.00	85	good	110
Polysulfone	Union Carbide	2	13.75	90	good	143
Fluoropolymer	DuPont	2	73.00	90	good	150

Conversion Factor: mils  $\times 2.54 \times 10^{-5}$  = meters.

bacterial hydrogen reactors (see Table 4-8). A screening test was devised for narrowing the selection of polymers. A small photosynthetic bacterial hydrogen reactor was constructed in which test coupons of polymer could be exposed for long periods of time to the aggressive environment of Rhodospseudomonas sphaeroides growing on a nonsterile medium of lactic acid (see Figure 4-12). Periodic measurements of samples removed from this environment will indicate the extent and rate of degradation caused by chemical, biochemical, or biofouling attack.

A preliminary set of measurements was performed on six different polymers after an exposure period of 14 days. Test results predictably showed little degradation but confirmed the practicality of the procedures. Exposure periods of several months may be necessary before significant degradation is measurable.

002086



**Figure 4-12. Exposure of Various Polymer Films in a Photosynthetic Bacterial Hydrogen Reactor**

The permeability of these polymers to hydrogen and oxygen are not known well enough to predict the performance of large reactor covers. Important issues of safety and economics require careful evaluation of gas permeation rates and changes in gas permeation rates resulting from degradation.

#### 4.4 SUMMARY AND FORECAST

The Photo/Biological Hydrogen Program (P/BHP) Element of the Biomass Program sponsored by the U.S. DOE Biomass Energy Technology Division has as its goal to make possible the introduction of new solar biotechnologies that produce large volumes of hydrogen from renewable resources at reasonable prices. There are two bases for developing these biotechnologies: (1) the use of the biochemical/biophysical system which exists in living cells, and (2) the use of a quasi-synthetic system based on components of naturally occurring biological hydrogen production systems.

Hydrogen production systems based on living cells, usually microorganisms, take advantage of biochemical and biophysical systems already in place in the cell for evolving hydrogen. However, these naturally occurring systems need to be enhanced in order for them to provide a practical basis for commercial hydrogen production. Research in the P/BHP in this task has been aimed, therefore, at improving the efficiency of conversion of light to hydrogen energy. In the course of this research hundreds of microorganisms have been screened for hydrogen production capacity. A number of very promising hydrogen-evolving organisms have been selected. The biochemistry and biophysics of hydrogen production by these selected organisms is being studied. Detailed information concerning the mechanisms used by the organism to produce hydrogen is being used to plan and execute genetic engineering aimed at producing hydrogen-evolving "superbugs." The culture requirements for maintaining maximum hydrogen production rates are being determined both in the laboratory and in the field.

The progress toward achieving the research goal of at least 10% conversion of light to hydrogen energy in whole-cell systems has been so encouraging (laboratory-scale experiments yielded 5%-6% conversion for photosynthetic bacteria) that research toward devising a conceptual engineering system for producing hydrogen has been undertaken. A preliminary system flow has been developed. Components for the system have been identified or conceptualized. The proposed system has been costed and the projected cost goals for hydrogen are such that the system appears to have real promise. In addition, development of a computer program to model system behavior has been initiated and is two-thirds completed.

It is possible to devise cell-free technologies for producing hydrogen based on information gleaned from studying the biochemistry and biophysics of hydrogen generation in living cells. Living cells use hydrogen production as a means of dumping excess reducing power. The source of the hydrogen produced is generally water. In cell-free technologies the aim is to mimic the mechanisms that whole cells use to produce hydrogen. The advantage of cell-free technologies is that the potential exists for producing hydrogen at higher rates and with less maintenance than is considered possible for whole-cell systems. To realize the potential of cell-free hydrogen production technologies, much research needs to be performed in identifying promising systems, stabilizing the components of the system, improving the efficiency of light-to-hydrogen conversions, and learning how to construct these systems for long life and proper function. To date most of the research in this task of the P/BHP has focused on improvement of the isolation techniques for removing hydrogen-producing components from the cell and complete characterization of these components so that they can be successfully manipulated.

Progress has been encouraging, with high conversion efficiencies of light to hydrogen energy being demonstrated.

In future years in the P/BHP, work will continue along essentially the same lines as in FY 1982. However, for whole-cell systems, species isolation is expected to continue only at a maintenance level after FY 1983 unless a need arises for a particular type of micro-organism that is not available in any of the culture collections.

There is a need to greatly expand research in the cell-free area. A more systematic approach toward identifying promising system concepts and stabilizing and enhancing the hydrogen production efficiency of the components is especially needed.

## SECTION 5.0

## PUBLICATIONS

This section contains references to all publications related to work supported during the period covered by this report.

## 5.1 AQUATIC PUBLICATIONS

Anderson, D. B., et al. 1982 (Jan.). Assessment of Blue-Green Algae in Substantially Reducing Nitrogen Fertilizer Requirements for Biomass Fuel Crops. Prepared by Battelle Pacific Northwest Laboratories for the Solar Energy Research Institute. Draft Report.

Bonnewell, V., W. L., Koukkari, and D. C. Pratt. 1982 (in press). "Light, Oxygen, and Temperature Requirements for Typhalatifolia L. Seed Germination." Canadian Journal of Botany.

Dubbe, D. R., N. J. Andrews, and D. C. Pratt. 1982 (in press). "Bio-Energy Production and Peatland Development." Proceedings of "Peat as an Energy Alternative II" Conference, sponsored by Institute for Gas Technology.

Habig, C., T. A. Debush, and J. H. Ryther. In press. "Effect of Nitrogen Content on Methane Production by the Marine Algal Gracilaria tikvahial and Ulva Species." Applied and Environmental Microbiology.

Habig, C. and J. H. Ryther. In press. "Methane Production from Anaerobic Digestion of Some Marine Macrophytes." Resource and Conservation.

Hill, Andrew. "Cost Budgeting for Microalgae Systems."

Laws, E. A., et al. 1982. "Preliminary Results from a Single Algal Production System Designed to Utilize the Flashing Light Effect." Submitted to Biotechnology and Bio-engineering.

Levine, L. S. "Biological and Engineering Parameters of Algal Mass Culture."

Lien, Stephen. "Studies on the Production and Accumulation of Oil and Lipids by Microalgae."

Lien, Stephen. "Microalgal Production of Oils and Lipids."

Lien, Stephen. "From Water to Oil via Microalgae."

Pratt, D. C. 1982 (in press). "University of Minnesota Studies Wetland Energy Crop Production." Midwest Energy Newsletter. Published by Midwest Universities Energy Consortium.

Pratt, D. C., N. J. Andrews, D. R. Dubbe, E. G. Garver, M. Penko, P. E. Read, and E. S. Zimmerman. 1982. Emergent Aquatics: Stand Establishment, Management and Species Screening. Report to the Solar Energy Research Institute. 55 pp.

Pratt, D. C., D. R. Dubbe, and N. J. Andrews. 1982 (in press). "The Development of Wetland Energy Crops in Minnesota, U.S.A." Proceedings of the Second International Conference on "Energy from Biomass," Commission of the European Communities, Berlin, Germany; 1982.

Thomas, W. H. and S. R. Gaines. 1982 (Sept.). Algae from the Arid Southwestern United States: An Annotated Bibliography. Prepared by Institute of Marine Resources, Scripps Institute of Oceanography, for the Solar Energy Research Institute under subcontract XK-09111-1. Draft report.

Tornabene, T. G. "Chemical Profiles of Microalgae with Emphasis on Lipids."

Vigon, B. W., et al. 1982 (Sept.). Resource Assessment for Microalgal/Emergent Aquatic Biomass Systems in the Arid Southwest. Prepared by Battelle Columbus Laboratories for the Solar Energy Research Institute under subcontract XK-2-02116-01. Draft report.

## 5.2 ANAEROBIC DIGESTION PUBLICATIONS

Bachmann, A., V. L. Beard, and P. L. McCarty. 1982. "Comparison of Fixed-Film Reactors with a Modified Sludge Blanket Reactor." First International Conference on Fixed-Film Processes, Proceedings. Submitted April 1982.

Bertrani, G. and L. Baresi. Study of Methanogens by Genetic Techniques. Grant Renewal and Final Report. Submitted by G. R. Petersen.

Chen, Y. R. and A. G. Hashimoto. 1982. "A Microcomputer Program for Design of Anaerobic Digestion Systems." ASAE Paper No. 82-4021. St. Joseph, MI: ASAE.

Clausen, E. C. and J. L. Gaddy. 1982. "Methane Production from Agricultural Residues by Anaerobic Digestion in Batch and Continuous Culture." Fuels and Organic Chemicals from Biomass. CRC Press.

Clausen, E. C. and J. L. Gaddy. 1982. "Production of Methane by Anaerobic Digestion in Large and Small Scale Facilities." Prog. in Solar Energy.

Clausen, E. C. and J. L. Gaddy. 1982. "Methane Production by Fermentation of Agricultural Residues." Adv. Biotech Processes. Vol. 1.

Colberg, P. J. and L. Y. Young. 1982. "Biodegradation of Lignin-Derived Molecules under Anaerobic Conditions." Canadian Journal of Microbiology. Vol. 28: pp. 886-889.

Colberg, P. J. and L. Y. Young. 1982. "Methanogenic Degradation of Lignin Catabolites." Abstracts 1982 Annual Meeting of American Society of Microbiology; p. 199.

Doyle, O. P., G. C. Magruder, E. C. Clausen, and J. L. Gaddy. 1982. "Large-Scale Production of Methane from Agricultural Residues." Proceedings of 12th Biochemical Engineering Symposium; Manhattan, KS.

- Gossett, J. M., D. C. Stucky, W. F. Owen, and P. L. McCarty. 1982. "Heat Treatment and Anaerobic Digestion of Refuse." EES. Jour. Environmental Engineering Division, Amer. Soc. of Civil Engineers. pp. 437-454.
- Hashimoto, A. G. 1982. "Microbial Hydrolysis of Thermochemically Treated and Untreated Manure-Straw Mixtures." Agricultural Wastes. Vol. 4(No. 5): pp. 345-364.
- Hashimoto, A. G., Y. R. Chen, V. H. Varel, and R. L. Prior. 1982 (Apr.). "Methane and Protein from Beef Cattle Manure." Roman L. Hruska U.S. Meat Animal Research Center, Beef Research Program. Progress Report No. 1. Triannual Progress Report. pp. 42-43.
- Hashimoto, A. G. 1982. "Methane Production from Beef Cattle Manure: Effects of Temperature, Hydraulic Retention Time and Influent Substrate Concentration." ASAE Paper No. 82-4020. St. Joseph, MI: ASAE.
- Hashimoto, A. G., Y. R. Chen, V. H. Varel, and S. A. Robinson. 1981 (Jun.). Anaerobic Fermentation of Beef Cattle Manure and Crop Residues. SERI/TR-98372-1. Annual report. Golden, CO: Solar Energy Research Institute; 90 pp.
- Hashimoto, A. G. 1982. "Methane from Cattle Waste: Effects of Temperature, Hydraulic Retention Time and Influent Substrate Concentration on Kinetic Parameter (K)." Biotechnology and Bioengineering. Vol. 24(No. 9): pp. 2039-2052.
- Hashimoto, A. G. 1982. "Conversion of Straw-Manure Mixtures to Methane at Mesophilic and Thermophilic Temperatures." Biotechnology and Bioengineering. Accepted 4 August 1982.
- Hashimoto, A. G. 1982. "Thermophilic and Mesophilic Anaerobic Fermentation of Swine Manure." Agricultural Wastes. Accepted 24 June 1982.
- Hashimoto, A. G. 1982. Book review of Biomass Energy Systems and the Environment by H.M. Braunstein et al., Pergamon Press, 1981. Resources and Conservation. (Invited.)
- Jewell, W. J. 1982. "New Approaches in Anaerobic Digester Design." Proceedings of the International Gas Research Conference; Los Angeles, CA: 30 September 1981. pp. 796-808.
- Jewell, W. J. and J. W. Morris. 1982. "Influence of Varying Temperature, Flow Rate and Substrate Concentration on the Anaerobic Attached Film Expanded Bed Process." Proceedings of the 36th Annual Purdue Industrial Waste Conference; Purdue University, West Lafayette, IN; 12-14 May 1981. pp. 655-664.
- LeGall, J. and H. D. Peck, Jr. "Hydrogenases: Physiology Location and Relevance for Sulfate Reducing and Methane Forming Bacteria." NATO Symposium on Non-Heme Iron Proteins. Edited by H. B. Dunford, D. Dolphin, K. N. Raymond, and L. Sieker. Boston, MA: D. Reidel Pub. Comp. pp. 207-222.
- McCarty, P. L., K. Baugh, A. Bachmann, W. Owen, and T. Everhart. 1982. "Auto-hydrolysis for Increasing Methane Yields from Lignocellulosic Materials." Fuels and Organic Chemicals from Biomass. Edited by D. Wise. CRC Publishing Co. Submitted August 1982.

McCarty, P. L., L. Y. Young, P. J. Colberg, K. Baugh, A. Bachmann, V. Beard, and D. Heim. 1982. "Autohydrolysis of Organic Residues to Increase Biodegradability to Methane." Quarterly Progress Report for Period 15 Oct. 1981 to 31 May 1982. Submitted June 1982.

Moura, I., Moura, J. J. G., Santos, H., Xavier, A. V., Burch, G., Peck, H. D., Jr., and LeGall, J. In press. "Proteins Containing the Factor F<sub>430</sub> from Methanosarcina barkeri and Methanobacterium thermoautotrophicum: Isolation and Properties." Biophys. Biochim. Acta.

Moura, J. J. G., Moura, I., Santos, M. H., Xavier, A. V., Scandellari, M., and LeGall, J. 1982. "Isolation of P<sub>590</sub> from Methanosarcina barkeri: Evidence for the Presence of Sulfite Reductase Activity." Biochem. Biophys. Res. Comm. Vol. 108: pp. 1002-1009.

Peck, H. D., Jr. and LeGall, J. 1982. "Biochemistry of Respiratory Sulfate Reduction." Philosophical Transactions of the Royal Society of London. Vol. 198: pp. 443-466.

Prior, R. L., R. A. Britton, and A. G. Hashimoto. 1982. "Nutritional Value of Anaerobically Fermented Beef Cattle Wastes as a Feed Ingredient for Livestock. I. Chemical Composition and In Vitro Digestibility of Fermentor Biomass." J. Animal Science. Submitted 9 March 1982.

Prior, R. L., R. A. Britton, and A. G. Hashimoto. 1982. "Nutritional Value of Anaerobically Fermented Beef Cattle Wastes as a Feed Ingredient for Livestock. II. In Vitro Metabolism Studies in Beef Cattle and Sheep." J. Animal Science. Submitted 9 March 1982.

Prior, R. L., A. G. Hashimoto, J. D. Crouse, and M. E. Dykeman. 1982. "Nutritional Value of Anaerobically Fermented Beef Cattle Wastes as a Feed Ingredient for Livestock. III. Growth and Carcass Traits of Beef Cattle and Sheep Fed Fermentor Biomass." J. Animal Science. Submitted 9 March 1982.

Prior, R. L. and A. G. Hashimoto. 1981. Book review: "Potential for Fermented Cattle Residue as a Feed Ingredient for Livestock." In Fuel Gas Production From Biomass, Chapter 5. Edited by D. L. Wise. CRC Press, Inc. pp. 215-238.

Varel, V. H. and A. G. Hashimoto. 1982. "Methane Production from Fermentor Cultures Acclimated to Waste from Cattle Fed Monensin, Lasalocid, Salinomycin and Avoparcin." Applied and Environmental Microbiology. Vol. 44(No. 6).

### 5.3 PHOTO/BIOLOGICAL HYDROGEN PROGRAM

Chum, H. L., M. Ratcliff, F. L. Posey, J. A. Turner, and A. J. Nozik. "Photo-electrochemistry of Levulinic Acid on Undoped Platinized n-TiO<sub>2</sub> Powders." Manuscript submitted for publication.

Chum, H. L., M. Ratcliff, H. A. Schroeder, and D. A. Sopher. "Characterization, Fractionation, and Electrochemical Reduction Reactions of Ethanol-Extracted Explosively-Depressurized Aspen (Populus tremuloides) Lignin." Manuscript in preparation.

- Chum, H. L., D. W. Sopher, K. Oh, and M. Himmel. "The Molecular Weight Distribution of Hydrothermally Degraded Aspen (Populus tremuloides) and Mixed Hardwood Lignins by High-Pressure GPC." Manuscript in preparation.
- Chum, H. L. and D. W. Sopher. 1982. "Membrane Separation of Carboxylic Acids." Patent application written; to be filed Nov. 1982 with DOE Patent Office (IR#81-29).
- Eisenmeier, S., H. Spiller, and K. T. Shanmugam. 1982. "Enhancement of Hydrogen Production by Fructose in Anabaena variabilis: Differential Effect of O<sub>2</sub> and Iron C<sub>2</sub>H<sub>2</sub> Reduction and H<sub>2</sub> Evolution." Env. Microbiol. Submitted for publication.
- Greenbaum, E. 1982. "Biosolar Hydrogen and Oxygen Production." Hydrogen Energy Progress IV. Edited by T. N. Vezirogov, W. D. Van Port, and J. H. Kelley. pp. 763-769.
- Greenbaum, E. and J. Ramus. 1982 (in press). "Survey of Selected Seaweed for Simultaneous Production of Hydrogen and Oxygen." J. of Phycol.
- Greenbaum, E., R. L. Guillard, and W. G. Sunda. 1982. "Hydrogen and Oxygen Photo-production by Marine Algae." Photochem. and Photobiol. Submitted for publication.
- Greenbaum, E. 1982. "Application of Intact Algae to the Biophotolysis Problem." Bio-technol. and Bioeng. Submitted for publication.
- Kendall-Tobias, M. and M. Seibert. 1982. "A Rapid Procedure for the Isolation and Purification of Photosynthetic Reaction Centers from Rps. sphaeroides R-26." Arch. Biochem. Biophys. Vol. 216(No. 1): p. 255.
- Kendall-Tobias, M. 1982 (in press). "A Horizontally Rotating Light-Beam Chopper." Rev. Sci. Inst.
- Lavorel, J. and M. Seibert. 1982. "Patterns of Oxygen Emission from Active Oxygen-Evolving Photosystem II Particles Subjected to Sequences of Flashes." FEBS Lett., Vol. 144(No. 1): p. 101.
- Lien, S. 1982. "Studies on Algal Hydrogenase and Hydrogen Metabolism." Submitted to SERI Biomass Program Principal Investigators' Review Meeting.
- Odom, J. M. and J. Wall. 1982. "Photoproduction of Hydrogen from Cellulose by an Anaerobic Bacterial Co-Culture." Appl. and Env. Microbiol. Submitted for publication.
- Ratcliff, M., F. L. Posey, and H. L. Chum. 1982. "Photoelectrochemistry of Levulinic Acid on Undoped Platinized n-TiO<sub>2</sub> Powders." Appl. and Env. Microbiol. pp. 13-26.
- Schwerzel, R. E., H. J. Byker, D. G. Vobetakis, and V. E. Wood. 1982. "The Photo-Kolbe Reaction: A Promise Wrapped in a Puzzle." Photoelectrochemistry: Fundamental Processes and Measurement Techniques (Proceedings). Edited by W. L. Wallace, A. J. Nozik, S. K. Deb, and R. H. Wilson. Pennington, NJ: Electrochemical Society Inc. Vol. 82-3: pp. 681-696.
- Seibert, M. and M. Kendall-Tobias. 1982 (in press). "Photoelectrochemical Properties of Electrodes Coated with Photoactive-Membrane Vesicles Isolated from Photosynthetic Bacteria." Biochim. Biophys. Acta.

- Seibert, M., G. Folger, and T. Milne. 1982. "Alcohol Co-Production from Tree Crops." Progress in Solar Energy. Edited by K. Haggart and C. Franta. Boulder, CO: ASES Press. Submitted for publication.
- Seibert, M. and J. Lavorel. 1982. "Oxygen-Evolution Patterns from Oxygen-Evolving Photosystem II Particles." Submitted to SERI Biomass Program Principal Investigators' Review Meeting.
- Sopher, D. W., M. Ratcliff, A. Hauser, and H. L. Chum. 1982. "Electrochemistry of Levulinic Acid and Selected Derived Compounds." Submitted to SERI Biomass Program Principal Investigators' Renew Meeting. pp. 27-32.
- Sopher, D. W., H. A. Schroeder, and H. L. Chum. 1982. "Electrochemistry and Chemical Characterization of Steam-Exploded Aspen (Populus tremuloides) Lignin." Electrochemistry Applied to Biomass. SERI/PR-622-1321. Golden, CO: Solar Energy Research Institute; pp. 3-12.
- Vobetakis, D. G., H. J. Byker, V. E. Wood, and R. E. Schwerzel. 1982. "Alternative Photo-Kolbe Reaction Pathway: Photoelectrochemical Production of Formaldehyde from Acetic Acid." J. Amer. Chem. Soc. Submitted for publication.
- Vobetakis, D. G., H. J. Byker, V. E. Wood, and R. E. Schwerzel. 1982. "Surface Analysis of Photocorrosion in n-SrTiO<sub>3</sub> Photoanodes." J. of Electrochem. Soc. Submitted for publication.
- Wall, J. and S. Takakuwa. 1982 (in press). "Hydrogen Production and Nitrogen Fixation in Rhodospira rubra Mutant Blocked in Hydrogen Uptake Activity." Proceedings of the Inaugural Symposium of Plant Biochemistry and Physiology.
- Weaver, P. F., J. S. Schultz, and P. C. Maaness. 1982. "Enhancement of H<sub>2</sub> Production by Photosynthetic Bacteria." Submitted to SERI Biomass Program Principal Investigators' Review Meeting.

## SECTION 6.0

### MEETINGS AND PRESENTATIONS

This section lists all meetings attended or planned during the period covered by this report (asterisks indicate planned meetings).

#### 6.1 AQUATIC SPECIES PROGRAM

SERI Biomass Program Principal Investigators' Review Meeting, Washington, DC, 23-25 June 1982.

Southeast Section of the American Society of Microbiology, 27 September 1982. Ryther presented paper entitled "Marine Biomass Production."

Florida Soil and Crop Science Society, 26 October 1982. Ryther to present paper entitled "Marine Biomass Production in Florida."\*

World Mariculture Society, 2 January 1983. Ryther to present paper entitled "Marine Biomass Production."\*

Second EC Conference on Energy from Biomass, International Congress Center, Berlin, Germany, 20-23 September 1982. D. C. Pratt presented paper entitled "The Development of Wetland Energy Crops in Minnesota," by D. C. Pratt and N. J. Andrews.

Minnesota Energy Policy Development Council, "Biomass Potential In Minnesota," St. Paul, MN, April 1982. D. C. Pratt presented paper entitled "The Development of Wetland Energy Crops in Minnesota" by D. C. Pratt and N. J. Andrews.

Biomass Lecture Series at University of Minnesota, "Producing Biomass on Minnesota Peatlands," St. Paul, MN, March 1982. D. C. Pratt presented paper entitled "The Development of Wetland Energy Crops in Minnesota," by D. C. Pratt and N. J. Andrews.

SERI Semiannual Contractor Review Meeting, San Diego, CA, 4 March 1983.\*

1983 Institute of Gas Technology (IGT) Conference on Energy from Biomass Wastes. S. Lien to present paper entitled "Microalgal Production of Oils and Lipids."\*

Photobiological Energy Conversion Symposium at the Electrochemical Society Annual Meeting, Montreal, Canada, 10 May 1982. S. Lien presented paper entitled "From Water to Oil via Microalgae."

#### 6.2 ANAEROBIC DIGESTION PROGRAM

1982 Annual Meeting of the American Society for Microbiology, Atlanta, GA, 7-12 March 1982. P. J. Colberg (Stanford, CA) presented paper on "Methanogenic Degradation of Lignin Catabolites."

First International Conference on Fixed-Film Biological Processes, Kings Island, OH, 20-23 April 1982. A. Bachmann (Stanford, CA) presented paper on "Comparison of Fixed-Film Reactors with a Modified Sludge Blanket Reactor."

SERI Biomass Program Principal Investigators' Review Meeting, Washington, DC, 22-25 June 1982. All subcontractors were present or represented.

Solar and Biomass Workshop, Atlanta, GA, 13-15 April 1982. Hashimoto presented a research progress report.

Summer meeting of the American Society of Agricultural Engineers, Madison, WI, 27-30 June 1982. Hashimoto presented a paper entitled "Methane Production from Beef Cattle Manure: Effects of Temperature, Hydraulic Retention Time, and Substrate Concentration."

Stanford University Seminar, April 1982. Speece presented a paper.

Copenhagen Anaerobic Attached Film Conference (discussion), June 1982, Speece attended.

International Workshop, "Anaerobic Treatment in Fixed-Film Reactors," Copenhagen, Denmark, June 1982. Jewell was organizer and keynote speaker.

Cornell University Faculty Seminar, "Use of Crop Residues for Energy Production," February 1982. W. J. Jewell presented a paper.

First International Conference on Fixed-Film Biological Processes, Kings Island, OH, 20-23 April 1982. W. J. Jewell presented paper entitled "Anaerobic Attached Film Expanded Bed Fundamentals."

Anaerobic Genetics Meeting, Banbury Center, Cold Spring Harbor Laboratories, NY, 16-17 August 1982. L. Baresi and G. R. Peterson presented a paper entitled "Some Mutants of Methanococcus Voltae," authored by G. Bertani and L. Baresi.

Biotechnology in Energy Symposium, Oak Ridge National Laboratory, Gatlinburg, TN, May 1982. G. C. Magruder, E. C. Clausen, and J. L. Gaddy presented a paper entitled "Methane Production by Anerobic Digestion of Agricultural Residues in Industrial and Small Scale Facilities."

Biotechnology Session of International Solar Energy Society, June 1982. E. C. Clausen and J. L. Gaddy presented a paper entitled "The Production of Methane by Anaerobic Digestion in Large and Small Scale Facilities."

AICHE Tri-Sectional Meeting, Tulsa, OK, March 1982. J. L. Gaddy presented lecture entitled "Biomass as Source of Chemicals."

UCLA Chemical Engineering Department, Los Angeles, CA, June 1982. J. L. Gaddy presented seminar entitled "Energy and Chemicals from Biomass."

FEMS Symposium on the Physiology of Sulfur Bacteria, Freiburg, Germany, 1982. Y. Berlier, G. Fauque, J. LeGall, A. Guitton, and P. A. Lespinat presented a paper entitled "Relationship between Nitrogenase and Hydrogenase Activities in Desulfovibrio desulfuricans Strain Berre Sol."

12th Biochemical Engr. Symp., Manhattan, KS, April 1982. O. P. Doyle, G. C. Magruder, E. C. Clausen, and J. L. Gaddy presented a paper entitled "Large-Scale Production of Methane from Agriculture Residues."

FEMS Symposium on the Physiology of Sulfur Bacteria, Freiburg, Germany, 1982. G. Fauque, Y. Berlier, J. LeGall, and P. A. Lespinat presented a paper entitled "Activation of the Periplasmic Hydrogenase from D. gigas."

American Chemical Society, Las Vegas, NV, 1982. G. Fauque, J. LeGall, P. A. Lespinat, and P. O. Ljungdahl presented a paper entitled "The Stabilization of Hydrogenase and the Mechanism of Its Activation."

Societe Francaise de Photobiologie, Meeting on Solar Biotechnology, Paris, France, 1982. J. LeGall presented a paper entitled "Hydrogenases and Mechanisms of H<sub>2</sub> and CH<sub>4</sub> Production."

Annual Meeting of the American Society of Microbiology, 7-12 March 1982 (Gaddy).

SERI Semiannual Contractor Review Meeting, Golden, CO, 6 December 1982.\*

SERI Semiannual Contractor Review Meeting, Washington, DC, 5-6 May 1983.\*

Meeting of the Royal Society of London on Sulfur Bacteria, February 1982 (LeGall).

American Society for Microbiology, Atlanta, GA, March 1982 (LeGall).

American Chemical Society, Las Vegas, NV, April 1982 (LeGall).

Fed. of Am. Soc. for Exp. Biol., New Orleans, LA, May 1982 (LeGall).

Soc. Franc. Photobiologie, Paris, France, May 1982 (LeGall).

FEMS Meeting, Freiburg, Germany, June 1982 (LeGall).

Southeastern Magnetic Resonance Conference, October 1982 (LeGall).

### **6.3 PHOTO/BIOLOGICAL HYDROGEN PROGRAM**

1982 American Society for Microbiology Meeting, Atlanta, GA, April 1982. Weaver attended and presented poster.

1982 American Solar Energy Society Meeting, Houston, TX, June 1982. Seibert attended and presented two papers.

1982 SERI Biomass Program Principal Investigators' Meeting, Washington, DC, June 1982. All program participants attended.

10th Annual American Society for Photobiology Meeting, Vancouver, BC, June 1982. Seibert attended and presented paper.

1982 World Hydrogen Energy Conference IV, Pasadena, CA, June 1982. Herlevich, Karpuk, Weaver, and Lindsey attended and presented poster and exhibit.

1982 American Society for Plant Physiology Meeting, Champaign-Urbana, IL, June 1982. Lien attended and presented paper.

- 1982 Annual American Chemical Society Meeting, Kansas City, KS, September 1982. Herlevich and Lindsey attended and presented paper.
- 3rd Engineering Foundation Conference on Biochemical Engineering, Santa Barbara, CA, September 1982. Herlevich and Lindsey attended and presented poster.
- 1982 Inaugural Symposium of Plant Biochemistry and Physiology, Columbia, MO, April 1982. Wall presented posters and papers.
- 4th International Symposium on Photosynthetic Prokaryotes, Bombannes, France, September 1982. Wall attended and presented poster.
- 3rd Engineering Foundation Conference on Biochemical Engineering, Santa Barbara, CA, September 1982. Mitsui attended and presented poster.
- 1982 Solar Technology Conference and International Exposition, Houston, TX, June 1982. Olson attended and presented paper.
- 1982 World Hydrogen Energy Conference IV, Pasadena, CA, June 1982. Greenbaum attended and presented paper.
- 1982 Biotechnology for Fuels and Chemicals Production, Gatlinburg, TN, May 1982. Greenbaum attended and presented paper.
- 6th DOE Solar Photochemistry Research Conference, Boulder, CO, June 1982. Greenbaum attended and presented paper.
- 1982 DOE/NRC Sigma Xi, Rockville, MD, May 1982. Greenbaum attended.
- Los Alamos National Laboratory, Los Alamos, NM, April 1982. H. L. Chum presented a paper entitled "Electrochemistry Applied to Biomass and to Thermal Conversion."
- Extension course on "Organic Electrochemical Synthesis" at the University of California, Los Angeles, CA, February 1982. H. L. Chum presented a lecture entitled "Electrochemistry of Biomass and Derived Materials."
- Workshop on the Status of Industrial Organic Electrochemistry. H. L. Chum presented a paper entitled "Non-Petroleum Derived Feedstocks."
- Fundamentals of Thermochemical Biomass Conversion: An International Conference, Estes Park, CO, October 1982. H. L. Chum, D. W. Sopher, and H. A. Schroder presented a paper entitled "Electrochemistry of Lignin Materials and Derived Compounds."
- 161st Electrochemical Society Meeting, Montreal, Canada, May 1982. N. L. Weinberg and H. L. Chum presented a paper entitled "Electroterminated Polymerization of Formaldehyde."

<b>Document Control Page</b>	1. SERI Report No. SERI/TR-231-1918	2. NTIS Accession No.	3. Recipient's Accession No.
4. Title and Subtitle SERI Biomass Program Annual Technical Report: 1982		5. Publication Date February 1983	
7. Author(s) Paul W. Bergeron, Robert E. Corder, Andrew M. Hill, Hilde Lindsey, and Michael Z. Lowenstein		6.	
9. Performing Organization Name and Address Solar Energy Research Institute 1617 Cole Boulevard Golden, Colorado 80401		8. Performing Organization Rept. No.	
		10. Project/Task/Work Unit No. 1356.10	
		11. Contract (C) or Grant (G) No. (C)  (G)	
12. Sponsoring Organization Name and Address		13. Type of Report & Period Covered Technical Report	
		14.	
15. Supplementary Notes			
16. Abstract (Limit: 200 words) Most products derived from petroleum and natural gas can be produced directly from biomass. The "biomass" with which this report is concerned includes aquatic plants, which can be converted into liquid fuels and chemicals; organic wastes (crop residues as well as animal and municipal wastes), from which biogas can be produced via anaerobic digestion; and organic or inorganic waste streams, from which hydrogen can be produced by photobiological processes. The challenge is to develop technology that will be competitive with existing processes using nonrenewable resources. The Biomass Program Office supports research in three areas which, although distinct, all use living organisms to create the desired products. The Aquatic Species Program (ASP) supports research on organisms that are themselves processed into the final products, while the Anaerobic Digestion (ADP) and Photo/Biological Hydrogen Program (P/BHP) deals with organisms that transform waste streams into energy products. The P/BHP is also investigating systems using water as a feedstock and cell-free systems which do not utilize living organisms. This report summarizes the progress and research accomplishments of the SERI Biomass Program during FY 1982.			
17. Document Analysis			
a. Descriptors Algae; Anaerobic digestion; Bioconversion; Biomass; Hydrogen production; Methanogenic bacteria; Photosynthetic bacteria; Program management; Research programs; Solar Energy Research Institute; Synthetic fuels			
b. Identifiers/Open-Ended Terms Aquatic plants; Photobiology			
c. UC Categories 61a,b			
18. Availability Statement National Technical Information Service U.S. Department of Commerce 5285 Port Royal Road Springfield, Virginia 22161		19. No. of Pages 95	
		20. Price \$11.50	