**RECOVERY AND SEQUESTRATION OF CO₂ FROM STATIONARY COMBUSTION
SYSTEMS BY PHOTOSYNTHESIS OF MICROALGAE**

Quarterly Technical Progress Report for the Period Ending 31 March 2002

(Quarterly Report #6)

Reporting Period Start Date: 1 January 2002

Reporting Period End Date: 31 March 2002

Prepared by

Dr. T. Nakamura
Physical Sciences Inc.
20 New England Business Center
Andover, MA 01810-1077

in collaboration with

Dr. Miguel Olaizola
Aquasearch Inc.
73-4460 Queen Kaahamanu Highway
Suite 110, Kailua-Kona, HI 96740

and

Dr. Stephen M. Masutani
Hawaii Natural Energy Institute
School of Ocean & Earth Science and Technology
University of Hawaii at Manoa
2540 Dole Street, Holmes Hall 246
Honolulu, HI 96822

DOE Contract No. DE-FC26-00NT40934
U.S. DEPARTMENT OF ENERGY
National Energy Technology Laboratory
P.O. Box 10940
Pittsburgh, PA 15236

July 2002

Government Purpose License Rights (SBIR Program)

Contract No.: DE-FC26-00NT40934
Contractor: Physical Sciences Inc.

For a period of 4 years after delivery and acceptance of the last deliverable item under the above contract, this technical data shall be subject to the restrictions contained in the definition of "Limited Rights" in DFARS clause at 252.227-7013. After the 4-year period, the data shall be subject to the restrictions contained in the definition of "Government Purpose License Rights" in DFARS clause at 252.227-7013. The Government assumes no liability for unauthorized use or disclosure by others. This legend, together with the indications of the portions of the data which are subject to such limitations, shall be included on any reproduction hereof which contains any portions subject to such limitations and shall be honored only as long as the data continues to meet the definition on Government Purpose License Rights.

DISCLAIMER:

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency thereof, nor any of 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. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

TABLE OF CONTENTS

<u>Section</u>	<u>Page</u>
ABSTRACT	vi
1. INTRODUCTION	1
2. EXECUTIVE SUMMARY	3
3. WORK ACCOMPLISHED	4
3.1 Task 1: Supply of CO ₂ from Power Plant Gas to Photobioreactor	4
3.2 Task 2 - Selection of Microalgae	4
3.2.1 Task 2.1 - Characterization of Physiology, Metabolism and Requirements of Microalgae	6
3.2.2 Task 2.2 – Achievable Photosynthetic Rates, High Value Product Potential and Sequestration of Carbon into Carbonates	10
3.3. Task 3 - Optimization and Demonstration of Industrial Scale Photobioreactor	15
3.3.1 Task 3.1 - Pilot Evaluation	15
3.3.2 Task 3.2 - Full Scale Production Runs	16
3.3.3 Task 3.3 - Algae Separation and Final Product	16
3.4 Task 4 - Carbon Sequestration System Design	16
3.4.1 Task 4.1 - Component Design and Development	16
3.4.2 Task 4.2 - System Integration	17
4. SUMMARY AND FUTURE PLANS	18
4.1 Task 2 - Selection of Microalgae	18
4.2 Task 4 - Carbon Sequestration System Design	19
5. REFERENCES	21

LIST OF FIGURES

<u>Figure No.</u>	<u>Page</u>
1. Recovery and sequestration of CO ₂ from stationary combustion systems by photosynthesis of microalgae.....	3
2. Average fluorescence based estimate of biomass measured at steady-state in chemostat cultures at different pH.....	7
3. The average F_v/F_m value (a measure of the photosynthetic efficiency) measured in steady-state chemostat cultures grown at different pH.....	8
4. Average fluorescence based estimate of biomass measured at steady-state in chemostat cultures under different simulated flue gases	9
5. The average F_v/F_m value (a measure of the photosynthetic efficiency) measured in steady-state chemostat cultures grown under different simulated flue gases	9
6. Example of fluorescence-based biomass estimates during the initial growth phase of a chemostat cultures (i.e., before nutrient additions were started and steady state was reached) used to estimate a maximal growth rate of the culture.....	10
7. Growth rate of different microalgal strains during the initial phase (the ramp-up phase) of the chemostat cultures	11
8. Computer generated trace of culture pH in a chemostat culture (strain AQ0022)	11
9. Changes in pH and total DIC over a four day period for a chemostat culture of strain AQ0036.....	12
10. Changes in carbon uptake rate over a 4 day period for a chemostat culture	12
11. Rates of CO ₂ uptake and/or degassing for a chemostat culture	13
12. Difference between rates of CO ₂ uptake and degassing light and dark periods for 14 microalgal strains	14
13. CO ₂ uptake as percentage of total CO ₂ lost from the growth medium during the light period	14
14. Relationship between photosynthetic CO ₂ uptake rate and %of available carbon taken up photosynthetically for the three pH conditions	15

LIST OF TABLES

<u>Table No.</u>		<u>Page</u>
1.	List of Strains and Experiments Carried Out.....	5
2.	Typical Composition of Industrial Flue Gases as per the Type of Fuel Combusted	8
3.	High-Value Products from Microalgae.....	18

ABSTRACT

Most of the anthropogenic emissions of carbon dioxide result from the combustion of fossil fuels for energy production. Photosynthesis has long been recognized as a means, at least in theory, to sequester anthropogenic carbon dioxide. Aquatic microalgae have been identified as fast growing species whose carbon fixing rates are higher than those of land-based plants by one order of magnitude. Physical Sciences Inc. (PSI), Aquasearch, and the Hawaii Natural Energy Institute at the University of Hawaii are jointly developing technologies for recovery and sequestration of CO₂ from stationary combustion systems by photosynthesis of microalgae. The research is aimed primarily at demonstrating the ability of selected species of microalgae to effectively fix carbon from typical power plant exhaust gases. This report covers the reporting period 1 April to 30 June 2001 in which PSI, Aquasearch and University of Hawaii conducted their tasks. Based on the work conducted during the previous reporting period, PSI initiated work on the component optimization work. Aquasearch continued their effort on selection of microalgae suitable for CO₂ sequestration. University of Hawaii initiated effort on system optimization of the CO₂ sequestration system.

1. INTRODUCTION

Emissions of carbon dioxide are predicted to increase in this century¹ leading to increased concentrations of carbon dioxide in the atmosphere. While there is still much debate on the effects of increased CO₂ levels on global climate, many scientists agree that the projected increases could have a profound effect on the environment. Most of the anthropogenic emissions of carbon dioxide result from the combustion of fossil fuels for energy production. It is the increased demand for energy, particularly in the developing world, which underlies the projected increase in CO₂ emissions. Meeting this demand without huge increases in CO₂ emissions requires more than merely increasing the efficiency of energy production. Carbon sequestration, capturing and storing carbon emitted from the global energy system, could be a major tool for reducing atmospheric CO₂ emissions from fossil fuel usage.

The costs of removing CO₂ from a conventional coal-fired power plant with flue gas desulfurization were estimated to be in the range of \$35 to \$264 per ton of CO₂.² The cost of power was projected to increase by anywhere from 25 to 130 mills/kWh. DOE's goal is to reduce the cost of carbon sequestration to below \$10/ton of avoided net cost.

Photosynthesis has long been recognized as a means, at least in theory, to sequester anthropogenic carbon dioxide. There has been relatively little research aimed at developing the technology to produce a gaseous combustion effluent that can be used for photosynthetic carbon sequestration. However, the photosynthetic reaction process by plants is too slow to significantly offset the point source emissions of CO₂ within a localized area. Aquatic microalgae have been identified as fast growing species whose carbon fixing rates are higher than those of land-based plants by one order of magnitude.

The Department of Energy has been sponsoring development of large-scale photovoltaic power systems for electricity generation. By this analogy, a large-scale microalgae plantation may be viewed as one form of renewable energy utilization. While the PV array converts solar energy to electricity, the microalgae plant converts CO₂ from fossil combustion systems to stable carbon compounds for sequestration and high commercial value products to offset the carbon sequestration cost. The solar utilization efficiency of some microalgae is ~ 5%, as compared to ~ 0.2% for typical land based plants. Furthermore, a dedicated photobioreactor for growth of microalgae may be optimized for high efficiency utilization of solar energy, comparable to those of some photovoltaic cells. It is logical, therefore, that photosynthetic reaction of microalgae be considered as a mean for recovery and sequestration of CO₂ emitted from fossil fuel combustion systems.

Stationary combustion sources, particularly electric utility plants, represent 35% of the carbon dioxide emissions from end-use of energy in the United States.¹ The proposed process addresses this goal through the production of high value products from carbon dioxide emissions. Microalgae can produce high-value pharmaceuticals, fine chemicals, and commodities. In these markets, microalgal carbon can produce revenues of order \$100,000 per kg C. These markets are currently estimated at >\$5 billion per year, and projected to grow to >\$50 billion per year within the next 10 to 15 years. Revenues can offset carbon sequestration costs.

An ideal methodology for photosynthetic sequestration of anthropogenic carbon dioxide has the following attributes:

1. Highest possible rates of CO₂ uptake
2. Mineralization of CO₂, resulting in permanently sequestered carbon
3. Revenues from substances of high economic value
4. Use of concentrated, anthropogenic CO₂ before it is allowed to enter the atmosphere.

In this research program, Physical Sciences Inc. (PSI), Aquasearch, and the Hawaii Natural Energy Institute at the University of Hawaii are jointly developing technologies for recovery and sequestration of CO₂ from stationary combustion systems by photosynthesis of microalgae. The research we propose is aimed primarily at quantifying the efficacy of microalgae-based carbon sequestration at industrial scale. Our principal research activities will be focused on demonstrating the ability of selected species of microalgae to effectively fix carbon from typical power plant exhaust gases. Our final results will be used as the basis to evaluate the technical efficacy and associated economic performance of large-scale carbon sequestration facilities.

Our vision of a viable strategy for carbon sequestration based on photosynthetic microalgae is shown conceptually in Figure 1. In this figure, CO₂ from the fossil fuel combustion system and nutrients are added to a photobioreactor where microalgae photosynthetically convert the CO₂ into compounds for high commercial values or mineralized carbon for sequestration. The advantages of the proposed process include the following.

1. High purity CO₂ gas is not required for algae culture. It is possible that flue gas containing 2~5% CO₂ can be fed directly to the photobioreactor. This will simplify CO₂ separation from flue gas significantly.
2. Some combustion products such as NO_x or SO_x can be effectively used as nutrients for microalgae. This could simplify flue gas scrubbing for the combustion system.
3. Microalgae culturing yields high value commercial products that could offset the capital and the operation costs of the process. Products of the proposed process are:
(a) mineralized carbon for stable sequestration; and (b) compounds of high commercial value. By selecting algae species, either one or combination or two can be produced.
4. The proposed process is a renewable cycle with minimal negative impacts on environment.

The research and experimentation we propose will examine and quantify the critical underlying processes. To our knowledge, the research we propose represents a radical departure from the large body of science and engineering in the area of gas separation. We believe the proposed research has significant potential to create scientific and engineering breakthroughs in controlled, high-throughput, photosynthetic carbon sequestration systems.

Figure 1. Recovery and sequestration of CO₂ from stationary combustion systems by photosynthesis of microalgae.

2. EXECUTIVE SUMMARY

The proposed program calls for development of key technologies pertaining to: (1) treatment of effluent gases from the fossil fuel combustion systems; (2) transferring the recovered CO₂ into aquatic media; and (3) converting CO₂ efficiently by photosynthetic reactions to materials to be re-used or sequestered.

The work discussed in this report covers the reporting period from 1 January to 31 March 2002. Up to this point in time we have:

- tested 50 different strains of microalgae for growth at different temperatures
- analyzed 34 different strains for high value pigments,
- tested 21 different strains for tolerance to simulated flue gases,
- tested 28 strains at the chemostat level for growth and carbon uptake rate, and
- tested 3 different strains for carbon sequestration potential into carbonates for long-term storage of carbon.
- conducted work on designing key components including: CO₂ removal process; CO₂ injection device; photobioreactor; product algae separation process; and process control devices.
- are working on design concept for photobioreactor incorporating the method for full utilization of solar energy.
- shared the ASPEN model has been with UH, PSI and Aquasearch for review and discussion.

- planning an extended visit to the Aquasearch facility by the UH graduate research assistant has been tentatively scheduled for late June 2002.
- Discussions were conducted with Dr. Winston Su, an expert on photobioreactors, to obtain advice and information on modeling this component.
- A review of the technical literature on tubular photobioreactors was initiated.

3. WORK ACCOMPLISHED

3.1 Task 1: Supply of CO₂ from Power Plant Gas to Photobioreactor

Much of the work within the two subtasks (Task 1.1: Power Plant Exhaust Characterization and Task 1.2: Selection of CO₂ Separation and Cleanup technologies) has been conducted during the previous reporting periods. No significant activities were made during the present reporting period.

3.2 Task 2 - Selection of Microalgae

As part of Task 2 we have continued to scale up cultures of microalgae to the 3.3 l chemostat scale to determine their tolerance to changes in pH and simulated flue gas streams following the methods indicated below and in previous reports. Table 1 lists the experiments already carried out. We have now analyzed the growth and photosynthetic response to changes in pH and gas mixtures for over 20 strains (Table 1). We have also analyzed the carbon sequestration potential of those strains by measuring their growth rate during the exponential phase of growth and measured their actual photosynthetic carbon uptake in chemostat cultures under different pH conditions.

Thus, we consider this task nearly finished and we are now shifting our focus to the scaleup of cultures to the outdoor photobioreactor scale. We will continue, however, to carry out small scale work on a few strains that we consider promising on a non-priority basis. These strains will originate from the University of Hawaii Cyanobacterial Collection with whom Aquasearch has negotiated access for drug discovery. A number of strains in this collection have already been identified as producers of molecules with potential therapeutic applications in human health.

Table 1. List of Strains and Experiments Carried Out

AQ strain	Temperature Tolerance					pH Tolerance			Flue Gas Tolerance				
	15C	20C	25C	30C	35C	6.5 pH	7.5 pH	8.5 pH	A	B	C	D	E
AQ0001	X	X	X										
AQ0002	X	X	X										
AQ0003	X	X	X										
AQ0008	X	X	X						X	X	X	X	X
AQ0009	X	X	X	X	X								
AQ0011	X	X	X	X	X	X	X	X	X	X	X	X	X
AQ0012	X	X	X	X	X	X	X	X	X	X	X	X	X
AQ0013	X	X	X	X	X	X	X	X	X	X	X	X	X
AQ0016	X	X	X	X	X								
AQ0017	X	X	X	X	X				X	X	X	X	X
AQ0018	X	X	X	X	X								
AQ0019	X	X	X	X	X	X	X	X					
AQ0020	X	X	X	X	X	X	X	X					
AQ0021	X	X	X	X	X								
AQ0022	X	X	X	X	X	X	X	X	X	X	X	X	X
AQ0023	X	X	X	X	X								
AQ0024	X	X	X	X	X	X	X	X	X	X	X	X	X
AQ0025	X	X	X	X	X	X	X	X	X	X	X	X	X
AQ0027	X	X	X	X	X								
AQ0028	X	X	X	X	X	X	X	X	X	X	X	X	X
AQ0029	X	X	X	X	X	X	X	X					
AQ0030	X	X	X	X	X								
AQ0031	X	X	X	X	X								
AQ0032	X	X	X	X	X								
AQ0033	X	X	X	X	X	X	X	X	X	X	X	X	X
AQ0034	X	X	X	X	X				X	X	X	X	X
AQ0035	X	X	X	X	X	X	X	X	X	X	X	X	X
AQ0036	X	X	X	X	X	X	X	X	X	X	X	X	X
AQ0037	X	X	X	X	X				x				
AQ0038	X	X	X	X	X	X	X	X	X	X	X	X	X
AQ0039	X	X	X	X	X								
AQ0040	X	X	X	X	X	X	X	X	X	X	X	X	X
AQ0041	X	X	X	X	X	X	X	X	X	X	X	X	X
AQ0042	X	X	X	X	X	X	X	X	X	X	X	X	X
AQ0043	X	X	X	X	X								
AQ0044	X	X	X	X	X	X	X	X	x				
AQ0045	X	X	X	X	X								
AQ0046	X	X	X	X	X	X	X	X					
AQ0048	X	X	X	X	X								
AQ0050	X	X	X	X	X								
AQ0051	X	X	X	X	X								
AQ0052	X	X	X	X	X	X	X	X	X	X	X	X	X
AQ0053	X	X	X	X	X	X	X	X	X	X	X	X	X
AQ0054	X	X	X	X	X				x				

	Temperature Tolerance					pH Tolerance			Flue Gas Tolerance				
AQ0055	X	X	X	X	X								
AQ0056	X	X	X	X	X								
AQ0058	X	X	X	X	X								
AQ0059	X	X	X	X	X				x				
AQ0062	X	X	X	X	X								
AQ0064	X	X	X	X	X								
AQ0067									X	X	X	X	X
AQ0073	X	X	X	X	X								
AQ0074	X	X	X	X	X								
AQ0077	X	X	X	X	X								
AQ0079	X	X	X	X	X								

3.2.1 Task 2.1 - Characterization of Physiology, Metabolism and Requirements of Microalgae

Chemostat cultures, as opposed to batch cultures, receive a continuous supply of nutrient medium. Our cultures are grown under light limitation. As the cells are diluted by the continuous medium addition more light/cell is available, permitting cell growth. Thus, the growth rate is dependent on the rate of medium addition. At steady state (no change in cell concentration in the chemostat culture) the growth rate is equivalent to the dilution rate. Also at steady state, the growth conditions stay constant, allowing for better characterization of the physiological state of the cells. The physiological state of the cells was determined daily using fluorescence techniques based on the techniques developed by Schreiber et al.³ Briefly, fluorescence measurements were carried out on the experimental cultures using the MiniPAM system (Walz, Germany). The MiniPAM can be used to estimate the so-called F_v/F_m , an estimate of the fraction of open reaction centers in photosystem II of photosynthetic organisms. The fraction of open reaction centers is directly proportional to the probability that the energy of an absorbed photon will participate in photosynthesis. Thus, it is a measure of the photosynthetic efficiency of the cells and of their physiological state.

While at steady state the dilution rate (or rate of nutrient addition to the culture) determines the growth rate, during the initial ramp up phase the chemostat culture is managed as a batch culture. A 3.3 liter chemostat vessel is inoculated with a starter culture and allowed to grow. Once the culture reaches a sufficiently high biomass (the culture starts to reach light limitation), nutrients are continuously added using a pump. The pH tolerance and flue gas tolerance experiments were conducted on cultures grown in chemostats.

3.2.1.1 pH Tolerance Experiments

To test the pH tolerance of the different microalgal strains, the cultures were grown in chemostats and exposed to different pH conditions. Initially, the cultures were grown in chemostats at a pH range of 7.4 to 7.6. The pH of the cultures was automatically controlled by CO₂ injections into the growth medium in response to raises in pH. Fluorescence-based biomass estimates during the initial growth phase of the chemostat cultures (i.e., before nutrient additions were started and steady state was reached) were used to estimate a maximal growth rate of the

culture. These growth rates represent a near-maximum attainable since both nutrients and light are not limiting during this period of culture growth (see Subsection 1.2.1).

Once the chemostats were at steady state (no change in biomass from day to day) the pH set points were changed to either 6.4 to 6.5 or 8.4 to 8.6. A decrease in biomass in response to the pH manipulations would indicate that the culture was being washed out, that the growth rate did not keep up with the dilution rate. An increase in biomass in response to pH manipulations would indicate the growth rate of the culture was higher following changes in pH.

Of the 21 strains tested so far, three strains (AQ0020, AQ0052 and AQ0053) were not successfully grown for a long enough period of time to test the different pH levels. The data for four more strains has yet to be analyzed. We report here results obtained from the response of other eighteen strains to pH changes.

Figure 2 summarizes the results of the pH experiments. The figure shows the fluorescence based biomass values measured in the chemostats following adjustments in the pH set points. A lower biomass level versus that at pH 7.5 indicates that the culture's growth is not keeping up with the chemostat's dilution rate and, thus, the growth rate is lower than under the standard condition (pH = 7.5). Lower biomass levels at high pH (8.5) could be interpreted as CO₂ limitation of the cultures. Lower biomass levels at low pH (6.5) could be interpreted as a detrimental effect on the cells due to the acidity of the medium.

Figure 2. Average fluorescence based estimate of biomass measured at steady-state in chemostat cultures at different pH.

Of the 18 strains so far characterized, 5 maintained a higher biomass level at pH 6.5 than 7.5 possibly indicating carbon limitation at 7.5 pH. Similarly, 13 strains maintained higher biomass level at pH 7.5 than 8.5 indicating carbon limitation at 8.5 pH. This will become important in the final phase of this project, during the design of a microalgal facility to sequester smoke stack CO₂. An oversized facility would translate into lower CO₂ available for the cultures, decreasing its efficiency.

Figure 3 shows the results of the fluorescence based photosynthetic efficiency measurements (F_v/F_m), averaged over several days, for the chemostat cultures grown at different pH. The lowest F_v/F_m values at all pH conditions are those of AQ0012, and AQ0038. These strains belong to the Cyanophyceae or blue-green algae. Higher are the values measured in the cultures of AQ0033, AQ0035 and AQ0036 which belong to the Rhodophyceae, or red algae. These strains show a lower F_v/F_m than the green algae and diatoms due to differences in photosystem architecture. However, note that for the most part there are no discernible changes in F_v/F_m within these strains in response to changes in culture pH. AQ0012 and AQ0038, representatives of the Cyanophyceae, are the only strains that show a reduction in photosynthetic efficiency at the higher pH values (equivalent to lower levels of dissolved CO_2 in the culture medium).

Figure 3. The average F_v/F_m value (a measure of the photosynthetic efficiency) measured in steady-state chemostat cultures grown at different pH.

3.2.1.2 Flue Gas Tolerance Experiments

To test the flue gas tolerance of the different microalgal strains, the cultures are grown in chemostats and exposed to different gas mixtures selected to mimic the flue gas from power plants utilizing different fuels (Table 2).

Table 2. Typical Composition of Industrial Flue Gases as per the Type of Fuel Combusted

	Utility Boilers Bituminous Coal	Utility Boilers Sub-Bituminous Coal	Utility Boilers Natural Gas	GTCC Natural Gas	Diesel Fuel Oil
	A	B	C	D	E
CO_2	12	15	7.5	3.5	4
H_2O	7	12	15	7	3.5
O_2	6	6	6	13.5	15
N_2	74.8	66.94	71.49	76	77.475
SO_2	0.16	0.04	0	0	0.005
NO	0.036	0.018	0.009	0.00225	0.018
NO_2	0.004	0.002	0.001	0.00025	0.002

Initially, the cultures are grown in chemostats at a pH range of 7.4 to 7.6. The pH of the cultures is automatically controlled by pure CO₂ injections into the growth medium in response to raises in pH. Once the cultures have reached steady state, the stream of pure CO₂ for pH control is substituted for the mixtures of gases specific to mimic different flue gas compositions. As in the pH tolerance experiments (above) changes in the fluorescence-based estimates of biomass and photosynthetic efficiency in response to exposure to different gas mixtures will indicate whether the cells are negatively affected.

The fluorescence-based biomass indicates that out of the 21 strains reported on in Figure 4 none have responded negatively to flue gas exposure. We did find one strain (AQ0059) that has proved impossible to grow when exposed to the gas mixtures (data not shown).

Figure 4. Average fluorescence based estimate of biomass measured at steady-state in chemostat cultures under different simulated flue gases.

Similarly, the estimates of photosynthetic efficiency indicate no deleterious effect caused by the exposure to flue gases for the 21 strains tested (Figure 5).

Figure 5. The average F_v/F_m value (a measure of the photosynthetic efficiency) measured in steady-state chemostat cultures grown under different simulated flue gases.

3.2.2 Task 2.2 – Achievable Photosynthetic Rates, High Value Product Potential and Sequestration of Carbon into Carbonates

3.2.2.1 CO₂ Utilization Capacity

Exponential Phase Growth Rates for Carbon Sequestration Potential

Over the first few days following inoculation of a chemostat system we can estimate a maximal growth rate from changes in daily biomass estimated from fluorescence measurements (Figure 6). This estimated growth rate is considered ‘maximal’ since during that period in the cultures life light is not yet limiting (i.e., the culture is sufficiently dilute still).

Figure 6. Example of fluorescence-based biomass estimates during the initial growth phase of a chemostat cultures (i.e., before nutrient additions were started and steady state was reached) used to estimate a maximal growth rate of the culture.

The results of growth measurements during the exponential phase of growth are shown in Figure 7. We have grown 28 different strains at the chemostat scale for pH tolerance, flue gas tolerance or both. The biomass data obtained during the ramp up phase of the culture was used to estimate the cultures maximum growth potential. Our results indicate growth rates ranging from as low as 0.13 d^{-1} to as high as 0.84 d^{-1} depending on the strain. The average growth rate for the strains tested is 0.55 d^{-1} . The slowest growing strain so far tested is AQ0034. AQ0034 is a strain of *Porphyridium*, a red microalga, which accumulates the high value carotenoid zeaxanthin. The fastest growing strain so far tested is AQ0011. AQ0011 is a locally isolated Chlorophyta strain which accumulates the high value carotenoids lutein and zeaxanthin. The maximal growth rates will provide a benchmark against which we will compare the results obtained during the next phase of the project; the scaleup of the cultures to outdoor photo-bioreactors (2000 liter).

Figure 7. Growth rate of different microalgal strains during the initial phase (the ramp-up phase) of the chemostat cultures.

Microalgal CO₂ Utilization Capacity

The automated pH monitoring and control system allows us to closely follow changes in pH in all the chemostat cultures. As an example, Figure 8 shows the changes in pH over the life history of a chemostat culture of strain AQ0022. Changes in pH reflect changes in the concentration of dissolved CO₂ and the total dissolved inorganic carbon (DIC = CO₂ + HCO₃⁻ + CO₃²⁻). We make the assumption that increases in DIC in the medium are produced either by respiration by the algae or by the injection of CO₂. We also make the assumption that decreases in DIC are produced by photosynthetic uptake of carbon and degassing from the culture medium. Figure 9 shows an example of such changes. The figure shows two traces. The first trace is the pH of the culture medium over 4 days and nights for a culture of strain AQ0036. Decreases in

Figure 8. Computer generated trace of culture pH in a chemostat culture (strain AQ0022). Showing the periods of time during which the culture's pH was maintained at 6.5, 7.5, and 8.5.

Figure 9. Changes in pH and total DIC over a four day period for a chemostat culture of strain AQ0036.

pH correspond to increases in DIC produced by CO₂ injections and algal respiration. Increases in pH correspond to decreases in DIC caused by photosynthetic uptake of CO₂ by the algae or degassing.

Using the changing concentrations of DIC over time we can calculate the net rate of carbon uptake ($\text{mg CO}_2 \text{ l}^{-1} \text{ min}^{-1}$). The results of this calculation, with a resolution of 5 minutes, are shown in Figure 10. Positive values indicate net uptake of CO₂ by the culture and degassing of CO₂ from the culture medium while negative values indicate injection of CO₂ into the medium and cellular respiration. Note that, in this case, degassing during the night periods (which would

Figure 10. Changes in carbon uptake rate over a 4 day period for a chemostat culture.

be evidenced by a rise in pH) is negligible. If we calculate an average net CO₂ uptake rate, using the values when no CO₂ is being injected, we obtain an average net rate of 0.094 mg CO₂ l⁻¹ min⁻¹. For a 14 hr day this is equivalent to 79 mg CO₂ l⁻¹ d⁻¹ or 21.5 mg C l⁻¹ d⁻¹.

We have now started to analyze the data obtained in these experiments to understand not only how the physiology and the growth of the microalgal cells may change when grown at different pH but also how the different medium pH affects the degassing of CO₂ from the medium and the uptake of CO₂ by the microalgae. For example, if we consider the changes in pH during the light and dark period (i.e., no photosynthetic carbon uptake) for the AQ0011 culture grown at different pH, both the rate of uptake and the rate of degassing are inversely proportional to the medium pH (Figure 11). At lower pH the concentration of CO₂ is higher in the medium so diffusion of CO₂ out of the medium is expected to be faster (Figure 11). If we compare the calculated rates between the light and dark periods, the difference should be that due to photosynthetic uptake of CO₂. The result indicates that the photosynthetic uptake of CO₂ is also pH dependent.

Figure 11. Rates of CO₂ uptake and/or degassing for a chemostat culture.

The photosynthetic carbon uptake estimates thus calculated are summarized in Figure 12. As for strain AQ0011, the results indicate a pH dependency on the uptake/degassing rates calculated. This is expected since at lower pH the degassing of CO₂ from the liquid medium is expected to be faster due to the larger partial pressure of CO₂ in the medium.

We can take this analysis one step further and calculate what percentage of the total CO₂ that is lost from the medium is taken up by the microalgae photosynthetically (i.e., the data in Figure 12). The results are shown in Figure 13 and indicate that a larger fraction of the available carbon is taken up by the microalgae at the higher pH settings as opposed as being lost back to the atmosphere.

Figure 12. Difference between rates of CO₂ uptake and degassing light and dark periods for 14 microalgal strains.

Figure 13. CO₂ uptake as percentage of total CO₂ lost from the growth medium during the light period.

Finally, we can consider the relationship between CO₂ uptake rate by the culture (Figure 12) and %CO₂ uptake from the medium (Figure 13). The relationships at the three pH conditions are positive but have different slopes (Figure 14). The changes in slope indicate that at higher pH the relationship is steeper, thus the efficiency of CO₂ uptake is higher. This data will be used in the final phases of the project, when we undertake the design of a commercial facility for carbon sequestration, since the pH at which that plant is managed will dictate the efficiency of the photosynthetic carbon uptake.

Figure 14. Relationship between photosynthetic CO₂ uptake rate and % of available carbon taken up photosynthetically for the three pH conditions.

3.2.2.2 Production of High Value Products from Microalgae to Offset the Cost of Carbon Sequestration

We are in the process of analyzing samples from several more microalgal strains but have no new data to report at this time. As the data become available the potential for commercial production and carbon sequestration cost mitigation will be analyzed.

3.2.2.3 Carbon Sequestration into Carbonate Minerals Utilizing Microalgae

We have not conducted further experiments related to microalgal mediated carbonate mineral production during this quarter. The next set of microalgal carbonate mineral production will be carried out at the outdoor photobioreactor scale (2000 l).

3.3 Task 3 - Optimization and Demonstration of Industrial Scale Photobioreactor

The goal of years 2 and 3 of this research program is to optimize carbon sequestration, high value component production and CO₂ carbonation utilizing microalgal cultures at a commercially significant scale. This will be done in two phases. During year 2 we will conduct a pilot evaluation and during year 3 we will conduct full scale production runs (below).

3.3.1 Task 3.1 - Pilot Evaluation

During this quarter we have started to scale up the first candidate species to the 2,000 l photobioreactor scale. We have chosen strain AQ0011 as our first candidate. AQ0011 is a locally isolated green algal strain. In previous reports we indicated that AQ0011 contains commercially significant amounts of lutein and zeaxanthin, two high value carotenoids with applications in human health. The second chosen candidate is AQ0012, a Cyanobacterium that

we have shown accumulates zeaxanthin. We will report on these experiments in the 7th quarterly report.

3.3.2 Task 3.2 - Full Scale Production Runs

This task will be taken up during year 3 of the project. Species determined to be outstanding performers during Task 3.1 will be selected and scaled up to 25,000 l outdoor photobioreactors.

3.3.3 Task 3.3 - Algae Separation and Final Product

We expect to carry out some preliminary work on this task during year 2 as biomass produced during the pilot evaluations becomes available.

3.4 Task 4 - Carbon Sequestration System Design

To evaluate the potential for application of photosynthetic sequestration of CO₂ to industrial-scale combustion systems, we will conduct a system-level design study. The purposes of this study are as follows:

- (1) Identify design concepts for components and the integrated system of the proposed concept
- (2) Optimize and evaluate performance of the components and the system
- (3) Develop deployment methodologies
- (4) Identify key technology issues for further development.

This task consists of two sub-tasks: Task 4.1-Component Design and Development, and Task 4.2-System Integration. Process simulations will be performed for conventional coal-fired and gas turbine power stations and natural gas boilers.

3.4.1 Task 4.1 - Component Design and Development

The purpose of this subtask is to develop design concepts for each of the key components of the industrial scale photosynthetic sequestration of CO₂. Key components to be designed include: CO₂ removal process; CO₂ injection device; photobioreactor; product algae separation process; and process control devices. As the proposed system depends on the solar energy to photosynthetically convert CO₂ to products compounds, optimization of the photobioreactor is an important part of this task.

In the reporting period, we have conducted analysis of engineering aspects of solar utilization by microalgae. As reported in the last progress report, PSI believes that a key to successful CO₂ sequestration is efficient use of solar energy. The area required for photosynthetic process by microalgae is a key cost driving factor. Therefore, we need to develop a

photobioreactor design which makes efficient use of solar light. Progress in this area is under way.

3.4.2 Task 4.2 - System Integration

3.4.2.1 Carbon Dioxide Supply, Separation, and Biological Uptake System

Work continued on the development of the process model. PSI and Aquasearch were again requested to review the model to confirm that the modules were consistent with the subsystems they were developing and to provide necessary empirical and other process parameters. The modeling task cannot proceed without this critical input.

In order to secure essential information on the operation of the Aquasearch microalgae production system to incorporate into the model, discussions were initiated with Dr. Miguel Olaizola of Aquasearch to send Mr. Simon Tsang, the UH Graduate Research Assistant on this project, to their facility in Kona, Hawaii for a period of about 1 week. While there, Mr. Tsang will observe and participate to some non-intrusive degree in the microalgae production activities. Insight and information obtained by this hands-on means will be applied to the UH modeling task. The visit to Aquasearch has been tentatively scheduled for late June 2002.

Photobioreactors

A primary component of systems under investigation to capture and sequester CO₂ by photosynthesis of microalgae is a photobioreactor employed to grow microalgae. There are several photobioreactor experts on the faculty of UH. One of these experts is Dr. Winston Su, an Associate Professor in the Molecular Biosciences and Bioengineering Department. Dr. Su has visited Aquasearch and observed their operations. Dr. Su was consulted to obtain information and insight on general photobioreactor design and characteristics that will be applied to the UH modeling task. Highlights of those discussions are summarized below.

Aquasearch utilizes pond and tubular photobioreactors. The pond photobioreactor is an open-air system and the tubular photobioreactor is a closed-air system. The primary advantages of an open-air system is the simplicity of its operation and low cost; however, open-air systems can easily be contaminated by microorganisms or other substances. Contamination may preclude the growth of a microalgal mass culture. A closed-air system reduces the potential for contamination. Depending on how they are configured, tubular photobioreactors can have additional favorable features. If, as in the Aquasearch facility, air is used to circulate the cells, then cell damage associated with mechanical pumping can be avoided. The air also acts to minimize photosynthesis inhibition due to dissolved oxygen in the fluid by removing oxygen produced by photosynthesis.⁴

Tubular photobioreactors have been used in large-scale production of high-value products from microalgae.⁵ Table 3 lists a number of these products.

Table 3. High-Value Products from Microalgae⁵

Product	Source Organism	Current or Potential Use
Amphidinolides and amphidinins	<i>Amphidinium</i> sp.	Antitumor agent
Astaxanthin	<i>Haematococcus pluvialis</i> , <i>Chlorella</i> sp.	Pigment
β -Carotene	<i>Dunaliella</i>	Colorant, food supplement
Docosahexaenoic Acid	<i>Isochrysis galbana</i>	Essential fatty acid
γ -Linolenic acid	<i>Spirulina</i> sp.	Essential fatty acid
Other polyunsaturated fatty acids	<i>Phaeodactylum tricornutum</i> , <i>Isochrysis galbana</i>	Health care, food supplement
Fucoxanthin	<i>Phaeodactylum tricornutum</i>	Antioxidant
Goniodominas	<i>Alexandrium hiranoi</i>	Antifungal agent
Oscillapeptin	<i>Oscillatoria agardhii</i>	Elastase inhibitor
Phycobiliproteins	Red algae, cyanobacteria	Colorants
Phycocyanin	<i>Spirulina platensis</i>	Colorant

Successful tubular photobioreactor generally consist of an airlift system and a solar receiver. The airlift system circulated the microalgal culture through the solar receiver tubing where most of the photosynthesis occurs.⁴ Oxygen produced by photosynthesis accumulates in the fluid until the fluid returned to the airlift system where it is stripped by air.⁴

Parameters in Modeling the Tubular Photobioreactor

When modeling the tubular photobioreactor, radiant loading and characteristics; nutrient, carbon dioxide and oxygen transport and generation/consumption, are primary considerations. Biomass productivity also depends on the effluent cell concentration and the dilution rate.⁵ At steady state, dilution rate should equal the specific growth rate, which depends on the average irradiance,⁵ the amount of light experienced by a single cell randomly moving inside the culture. Specific growth rate increases with average irradiance until a maximum is attained.

4. SUMMARY AND FUTURE PLANS

4.1 Task 2 - Selection of Microalgae

Up to this point in time we have

- tested 50 different strains of microalgae for growth at five different temperatures (15, 20, 25, 30 and 35°C) and 4 strains at three temperatures (15, 20, and 25°C),
- tested 21 different strains for pH shift tolerance in chemostat cultures,
- analyzed 34 different strains for high value pigments,
- tested 21 different strains for tolerance to simulated flue gases,
- tested 28 strains at the chemostat level for growth and carbon uptake rate, and

- tested 3 different strains for carbon sequestration potential into carbonates for long-term storage of carbon.

Our preliminary results indicate that:

- out of 54 strains tested two did not grow at 15°C, one did not grow at 15 and 20°C, nine did not grow at 35°C, and one did not grow at 30 and 35°C,
- out of 18 strains tested so far and successfully grown at the chemostat scale for pH tolerance only two strains, both Cyanobacteria, appear to lower their ability to photosynthesize as the pH increases,
- out of 18 strains tested at chemostat scale five maintained a higher biomass level at 6.5 pH than 7.5 pH and 13 strains maintained lower biomass at 8.5 pH than 7.5 pH,
- out of 34 strains analyzed for high value pigment content we have identified possible good sources of zeaxanthin, lutein, fucoxanthin, phycocyanin, and phycoerythrin,
- changes in culture pH caused by microalgal photosynthesis appear to be conducive to the precipitation of CaCO_3 from the growth medium,
- out of 21 strains tested so far and successfully grown at the chemostat scale for flue gas tolerance we have not found any deleterious effects on the strains' ability to photosynthesize, and
- for the 14 strains for which the data is already analyzed, on average, while the rate of photosynthetic carbon uptake is higher at lower pH, the percent of the available CO_2 in the medium that is photosynthetically taken up is higher at higher pH.

Within the next quarter we expect to

- scaleup of promising strains to the 2000 L outdoor photobioreactor scale.

4.2 Task 4 - Carbon Sequestration System Design

In the reporting period, work on Task 4 continued. Summary of the results in this task is as follows:

- We have conducted work on designing key components including: CO_2 removal process; CO_2 injection device; photobioreactor; product algae separation process; and process control devices.

- The test results obtained during PSI experiments showed that full utilizations of solar spectra is possible for two distinct purposes: CO₂ sequestration; and electric power generation.
- We are working on design concept for photobioreactor incorporating the method for full utilization of solar energy.
- The process model for CO₂ supply, separation, and biological uptake using microalgae has been studied. A skeleton ASPEN flowsheet for the preliminary model is presently being simulation tested.
- The ASPEN model incorporates:
 - the CO₂ separation subsystems including an alkaline salt solution CO₂ separation process
 - the algae production subsystem utilizing closed, open, or both types of photobioreactors
 - the product processing subsystems.
- The model has the ability to perform parametric studies to identify critical experimental and other data that need to be collected, and to evaluate alternative photobioreactor designs and carbon sequestration options.
- The ASPEN model has been shared with PSI and Aquasearch for their review and discussion.
- A presentation on the UH research tasks for a review meeting that was held at NETL in Pittsburgh, PA, was prepared and submitted to PSI. Dr. Stephen Masutani, the UH P.I., participated in that meeting.
- An extended visit to the Aquasearch facility by the UH graduate research assistant has been tentatively scheduled for late June 2002. The objective of this visit is for the research assistant to gain additional insight and information on the microalgae production process that will be applied to the UH modeling task.
- Discussions were conducted with Dr. Winston Su, an expert on photobioreactors, to obtain advice and information on modeling this component.
- A review of the technical literature on tubular photobioreactors was initiated.

5. REFERENCES

1. U.S. Department of Energy, Energy Information Agency, *Emissions of Greenhouse Gases in the United States 1996*, DOE/EIA-0573(96), October 1997.
2. IEA (International Energy Agency), *Carbon Dioxide Capture from Power Stations*, 1998. [available at <http://www.ieagreen.org.uk>]
3. Schreiber, U., Schliwa, U., Bilger, W., "Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer," *Photosynth. Res.* **10**, 51-62, 1986.
4. Molina, E., Fernández, J., Ación, F.G., and Christi, Y., "Tubular Photobioreactor Design for Algal Cultures," *Journal of Biotechnology*, Vol. 92, pp. 113-131, 2001.
5. Molina Grima, E., Ación Fernández, F.G., García Camacho, F., and Christi, Y., "Photobioreactors: Light Regime, Mass Transfer, and Scaleup," *Journal of Biotechnology*, Vol. 70, pp. 231-247, 1999.