PSI-1356

RECOVERY AND SEQUESTRATION OF CO_2 FROM STATIONARY COMBUSTION SYSTEMS BY PHOTOSYNTHESIS OF MICROALGAE

Quarterly Technical Progress Report for the Period Ending 30 June 2001

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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 CO_2 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 CO_2 sequestration. University of Hawaii initiated effort on system optimization of the CO_2 sequestration system.

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1. INTRODUCTION

Emissions of carbon dioxide are predicted to increase in this century[1] leading to increased concentrations of carbon dioxide in the atmosphere. While there is still much debate on the effects of increased CO_2 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 CO_2 emissions. Meeting this demand without huge increases in CO_2 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 CO_2 emissions from fossil fuel usage.

The costs of removing CO_2 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 CO_2.[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 CO_2 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 CO_2 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 CO_2 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 10-15 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 CO_2 uptake
2. Mineralization of CO_2, resulting in permanently sequestered carbon
3. Revenues from substances of high economic value
4. Use of concentrated, anthropogenic CO_2 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 CO_2 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 micro-algae is shown conceptually in Figure 1. In this figure, CO_2 from the fossil fuel combustion system and nutrients are added to a photobioreactor where microalgae photosynthetically convert the CO_2 into compounds for high commercial values or mineralized carbon for sequestration. The advantages of the proposed process include the following.

1. High purity CO_2 gas is not required for algae culture. It is possible that flue gas containing 2~5% CO_2 can be fed directly to the photobioreactor. This will simplify CO_2 separation from flue gas significantly.

2. Some combustion products such as NO_x or SO_x can be effectively used as nutrients for micoralgae. 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.

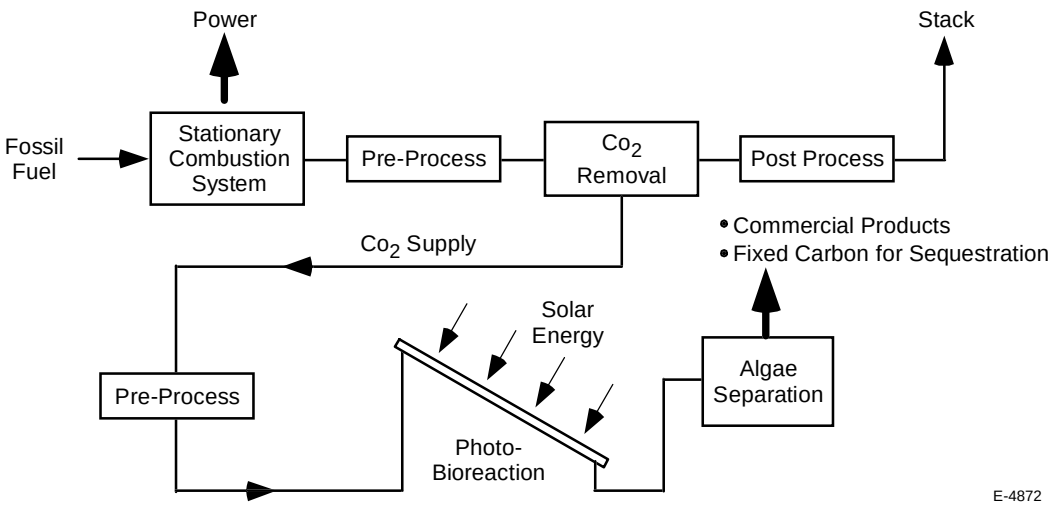

Figure 1. Recovery and sequestration of CO_2 from stationary combustion systems by photosynthesis of microalgae.

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 CO_2 into aquatic media; and (3) converting CO_2 efficiently by photosynthetic reactions to materials to be re-used or sequestered.

The work discussed in this report covers the reporting period from 1 April to 30 June 2001. Up to this point in time we have

- Characterized power plant exhaust gas
- Identified suitable CO_2 separation method and clean-up technologies
- Conducted analysis of carbon dissolution methods
- Tested 41 different species of microalgae for growth at three different temperatures (15, 20, and 25 °C)
- Analyzed 15 different species for high-value pigments
- Started testing 4 strains (3 species) for pH shift tolerance in chemostat culture
- Started testing six species at the chemostat level for carbon uptake rate
- Started testing three different species for carbon sequestration potential into carbonates for long-term storage of carbon
- Designed and purchased the components necessary for carbon sequestration potential experiments utilizing five different simulated flue gases
- Completed subcontract agreement between PSI and University of Hawaii for system analysis work

3. WORK ACOMMPLISHED

3.1 Task 1: Supply of CO_2 from Power Plant Gas to Photobiorector

Much of the work within the two subtasks (Task 1.1: Power Plant Exhaust Characterization and Task 1.2: Selection of CO_2 Separation and Cleanup technologies) has been conducted during the last reporting period. No significant activities were made during the present reporting period.

3.2 Task 2: Selection of Microalgae

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

3.2.1.1 Microalgal Culture Collection

The Aquasearch Culture Collection consists at the present time of 66 different strains of microalgae representing an estimated 62 species (Figure 2). Fifty-five strains have been isolated locally (i.e., in Hawaii) by the staff at Aquasearch and are maintained as unialgal cultures. It is expected that strains isolated in Hawaii are adapted to relatively high temperatures. Furthermore, eleven strains have been imported from established culture collections and about 10 to 20 more strains will be imported over the following months. The strains are maintained on an agar-

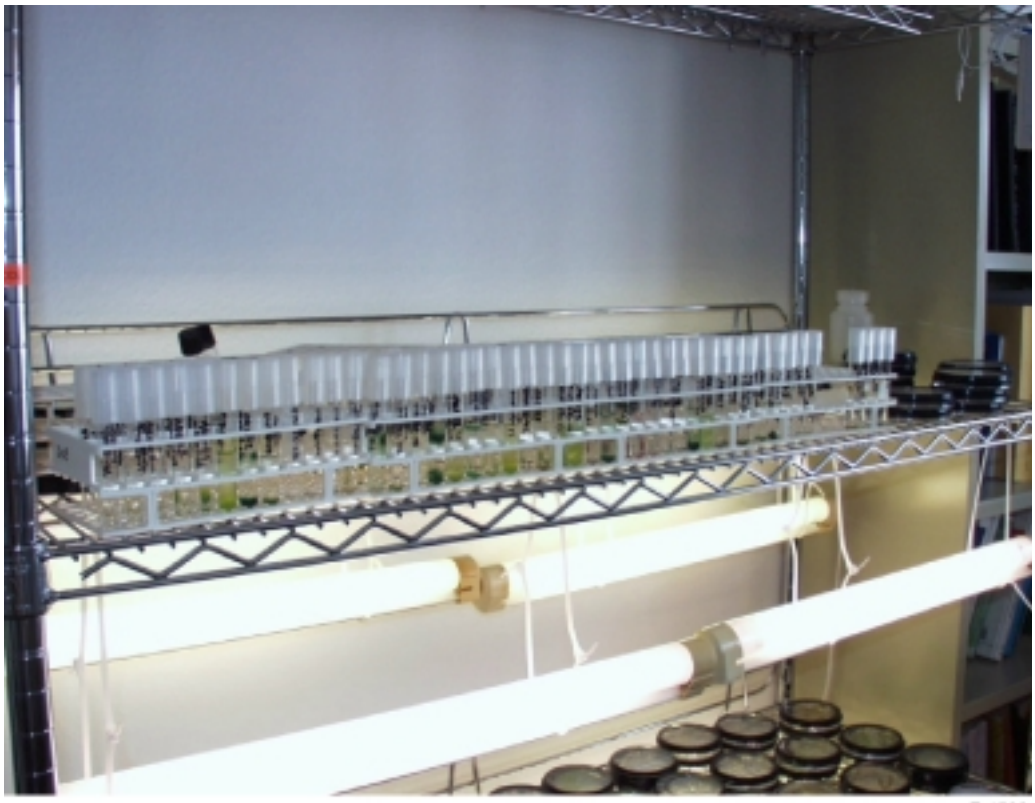

Figure 2. The Aquasearch Culture Collection. At the present time over 60 strains of microalgae are maintained. Ten more strains will be added over the next 2 months.

based nutrient medium. When needed for an experiment, cells from the agar cultures are transferred to test tubes containing liquid growth medium. After a few days of growth (may vary depending on the strain) the cultures are transferred to larger containers such as 250 ml Erlenmeyer flasks. Further scale up is performed according to the type of experiment planned.

In February 2001 scaleup of the microalgal cultures was started. Up to this point the cultures had been maintained in Petri dishes on a solid nutrient medium (agar based). The cultures were started in liquid nutrient medium in small test tubes (5 ml). The cultures were grown under a 14:10 hr light:dark cycle in a temperature-controlled room (24 °C ±2 °C) until enough biomass was produced to inoculate Erlenmeyer flasks (250 ml flasks with 100 to 150 ml of nutrient medium).

Table 1 lists the strains that have already been grown on liquid medium as well as the largest cultivation container to which they have been scaled. For proprietary considerations strains are only identified by their culture collection identifiers.

Table 1. List of strains that have already been grown on liquid medium (51 strains), as well as the largest cultivation container to which they have been scaled (Tt = test tube, E2 = 250 ml Erlenmeyer flask, CH = 3.3 liter chemostat). For proprietary considerations the strains are only identified by their culture collection identifier. The dates indicate when the different growth experiments were started.

AQ strain	Largest scale	15C	20C	25C	30C	35	chemosta	HPLC
AQ0001	E2	5/15/200	5/15/200	5/15/200				No
AQ0002	E2	5/15/200	5/15/200	5/15/200				No
AQ0003	E2	5/15/200	5/15/200	5/15/200				No
AQ0008	E2	5/15/200	5/15/200	5/15/200				No
AQ0009	E2	5/15/200	5/15/200	5/15/200				No
AQ0011	CH	3/24/200	3/24/200	3/24/200			6/11/01	No
AQ0012	CH	3/17/200	3/17/200	3/17/200			4/17/01,	Yes
AQ0013	E2	3/24/200	3/24/200	3/24/200				No
AQ0015	E2							Yes
AQ0016	E2	4/18/200	4/18/200	4/18/200				Yes
AQ0017	E2	4/18/200	4/18/200	4/18/200				No
AQ0018	E2	3/17/200	3/17/200	3/17/200				Yes
AQ0019	E2	4/18/200	4/18/200	4/18/200				No
AQ0020	E2	4/18/200	4/18/200	4/18/200				No
AQ0021	E2	4/18/200	4/18/200	4/18/200				Yes
AQ0022	CH	3/24/200	3/24/200	3/24/200			7/30/01	No
AQ0023	E2	3/17/200	3/17/200	3/17/200				Yes
AQ0024	CH	3/24/200	3/24/200	3/24/200			7/31/01	No
AQ0025	CH	3/24/200	3/24/200	3/24/200			8/01/01	No
AQ0027	E2	4/18/200	4/18/200	4/18/200				No
AQ0028	E2	3/24/200	3/24/200	3/24/200				No
AQ0029	E2	3/24/200	3/24/200	3/24/200				No
AQ0030	E2	4/18/200	4/18/200	4/18/200				Yes
AQ0031	E2	4/18/200	4/18/200	4/18/200				Yes

AQ strain	Largest scale	15C	20C	25C	30C	35	chemosta	HPLC
AQ0032	E2	3/17/200	3/17/200	3/17/200				Yes
AQ0033	CH	4/29/200	4/29/200	4/29/200			6/24/01	Yes
AQ0034	E2	4/29/200	4/29/200	4/29/200				No
AQ0035	E2	4/29/200	4/29/200	4/29/200				No
AQ0036	CH	4/29/200	4/29/200	4/29/200			6/24/01	Yes
AQ0037	E2	4/18/200	4/18/200	4/18/200				Yes
AQ0038	E2	4/24/200	4/24/200	4/24/200				No
AQ0039	E2	4/18/200	4/18/200	4/18/200				Yes
AQ0040	E2	3/24/200	3/24/200	3/24/200				No
AQ0041	E2	3/24/200	3/24/200	3/24/200				No
AQ0042	E2	3/24/200	3/24/200	3/24/200				No
AQ0043	E2	3/24/200	3/24/200	3/24/200				No
AQ0044	E2	5/29/200	5/29/200	5/29/200				No
AQ0045	E2	5/29/200	5/29/200	5/29/200				No
AQ0046	E2	5/29/200	5/29/200	5/29/200				No
AQ0048	Tt							No
AQ0049	Tt							No
AQ0050	E2	5/29/200	5/29/200	5/29/200				No
AQ0051	E2	5/29/200	5/29/200	5/29/200				No
AQ0052	CH	5/15/200	5/15/200	5/15/200			6/19/01	Yes
AQ0053	CH	5/15/200	5/15/200	5/15/200			6/19/01,	Yes
AQ0056	Tt							No
AQ0058	E2							No
AQ0059	Tt							No
AQ0060	Tt							No
AQ0062	Tt							No
AQ0063	Tt							No

3.2.1.2 Culture Growth

Culture growth in batch cultures

Estimates of culture growth are calculated from changes in culture biomass estimated once daily. Culture biomass is estimated from *in vivo* fluorescence. A Pulse Amplitude Modulated (MINI PAM, Walz, Germany) fluorometer is used to measure culture *in vivo* fluorescence. The fluorescence measured is proportional to the amount of chlorophyll, and thus biomass, of the culture. The following formula is then used to estimate growth rates:

$$\mu = \mathrm{Ln}\left(\frac{F_2 / F_1}{\Delta T}\right)$$

where μ is the growth rate (d^{-1}), F_2 is the fluorescence at time 2, F_1 is the fluorescence at time 1 and ΔT is the difference between time 2 and time 1 in days.

Culture growth in chemostat cultures

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 (see below).

Temperature experiments

To determine the growth rates of microalgae at different temperatures, cultures were incubated submerged in temperature-controlled water baths at 60 μE m^{-2} s^{-1} of PAR. The cultures were batch grown in 250 ml Erlenmeyer flasks. The flasks were agitated three times daily. Changes in biomass were estimated from changes in fluorescence.

Figure 3 summarizes the results of culture growth for 41 strains at three different temperatures. The data indicate there is a large degree of uncertainty about the mean growth rate of each culture (standard deviation > mean in all cases, not shown). This is the case for two reasons. First, these experiments were designed to quickly provide information on temperature tolerances for the different strains. As such the cultures were grown in batch mode. Cultures in batch mode show different growth rates at different stages of the cultures' growth curve. Second, a number of these cultures are of a filamentous and clumping nature. Thus the cells are not uniformly distributed throughout the growth medium. This translates into inherently noisy data. However, from the point of view of the objective of the experiment (to determine the temperature tolerances of the different microalgal strains) the results clearly show that, except for

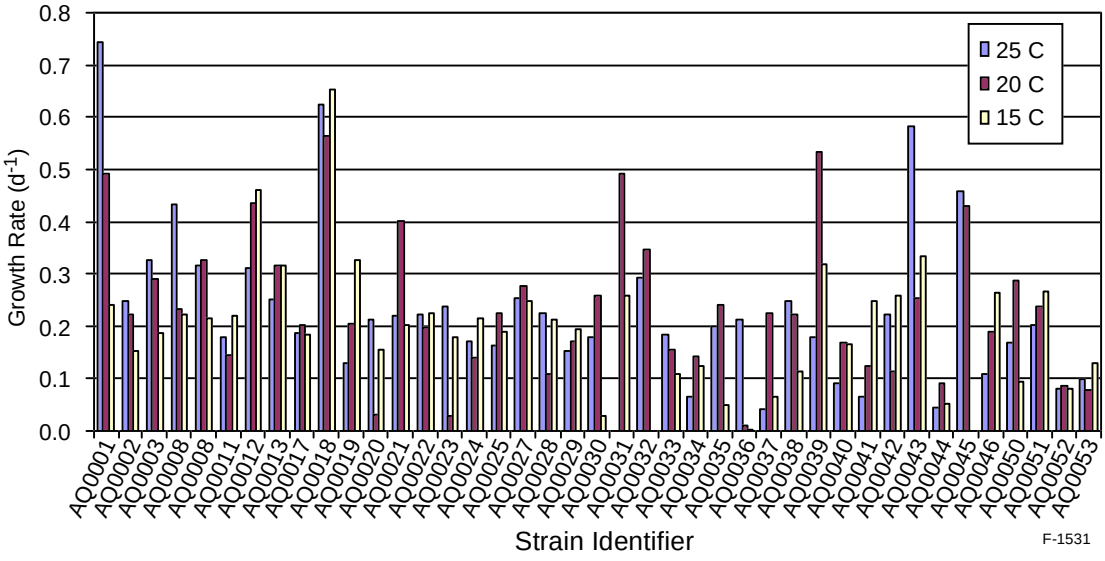

Figure 3. Results of growth rate measurements on 41 species of microalgae at three different temperatures.

one, the strains tested to date grow well at up to 25 °C. Further experiments will test growth at 30 and 35°C.

pH experiments

Cultures were grown in chemostats at a ph range of 7.4 to 7.6. pH was automatically controlled by CO_2 injections into the growth medium in response to raises in pH. 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.

Table 2 summarizes the results of the pH experiments. The table lists the fluorescence based biomass values measured in the chemostats following adjustments in the pH set points. Except for strain AQ0011, pH in the growth medium had little effect in the steady state biomass level in the chemostat cultures. 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 CO_2 limitation of the cultures. Lower biomass levels at low pH (7.5) could be interpreted as a detrimental effect on the cells due to the acidity of the medium.

Table 2. Summary table listing the fluorescence based biomass values measured in the chemostats following adjustments in the pH set points.
For growth at pH = 6.5, the pH set points were 6.4 and 6.6.
For growth at pH = 7.5, the pH set points were 7.4 and 7.6.
For growth at pH = 8.5, the pH set points were 8.4 and 8.6.

Strain/Culture ID	pH 6.5	pH 7.5	pH 8.5
AQ0036-010624	61	61	55
AQ0033-010624	15	15	17
AQ0011-010611	83	144	42
AQ0012-010612	35	41	36

3.2.2 Task 2.2 – Achievable Photosynthetic Rates and High Value Product Potential

3.2.2.1 CO_2 Utilization Efficiency

Fluorescence measurements

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.

CO_2 utilization efficiency of a commercial microalgal facility

We have estimated the CO_2 utilization efficiency of Aquasearch's commercial facility which produces a high value pigment (astaxanthin) from the microalga *Haematococcus pluvialis*. The efficiency was calculated as the ratio of the amount of carbon contained in the biomass of *H. pluvialis* produced by Aquasearch to the amount of CO_2 that Aquasearch purchases for biomass production.

The calculated CO_2 utilization efficiency for Aquasearch's commercial facility for the production of astaxanthin from *H. pluvialis* is about 12.5%. This means that 12.5% of the CO_2 purchased by Aquasearch to control the pH of, and provide carbon nutrition to, its cultures is captured in the biomass harvested.

CO_2 utilization efficiency of experimental chemostat cultures

The CO_2 utilization efficiency of the experimental chemostat cultures was calculated as the ratio of the amount of carbon taken up by the experimental culture per unit time to the amount of CO_2 that is fed into the chemostat per unit time. The uptake of CO_2 by the culture is estimated from changes in the concentration of CO_2 in the growth medium as indicated by changes in pH and from changes in the biomass. The amount of CO_2 that is fed into the chemostat is estimated from the flow rate of the CO_2 gas times the period of time during the CO_2 is flowing into the chemostat.

The chemostat cultures are temperature and pH regulated. A pH probe is immersed in the growth medium and automatically logs the pH of the culture at a resolution of 15 seconds. A computer is programmed with the high and a low pH set points. When the pH of the culture rises, caused by CO_2 uptake by the microalgal cells, above the high set point, a stream of CO_2 is automatically injected into the culture causing a drop in pH. When the pH becomes lower than the low set point, the CO_2 stream is automatically shut off. The flow rate of the CO_2 stream multiplied by the duration of the injection results in the total amount of CO_2 injected. The amount of carbon actually taken up by the cells in the culture is estimated from the biomass of the culture and its growth rate.

We estimate the amount of dissolved carbon species in the medium using a standard titration method (Clesceri et al., 1995). Following an injection of CO_2 into the medium (see above), the pH of the medium decreases, reflecting an increase in the concentration of CO_2. After the injection period, the pH increases caused by photosynthetic uptake of CO_2 by the algae. The slope of this increase results from the rate of uptake of CO_2 by the culture.

Figure 4 is made up of two photographs of the same chemostat containing a culture of microalgal strain AQ0012 but at different times (April 20 and April 27, 2001, respectively) and it shows the potential for carbon sequestration of microalgal cultures. Figure 5 shows the changes of pH in this chemostat over a 2-day and 1 night period (39 hr). The upward changes in pH are produced by photosynthetic uptake of CO_2 from the culture medium, the drops are produced by automatic injection of CO_2 into the culture medium. The data is collected by our automatic monitoring and control system and is used to estimate the amount of CO2 injected into the culture.

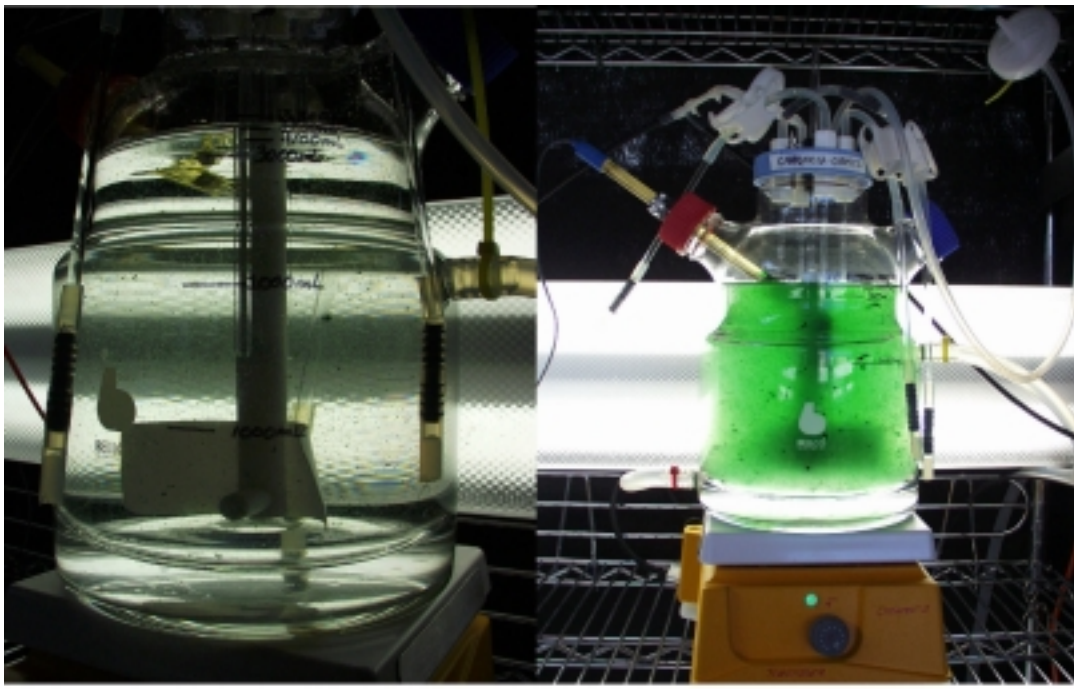

Figure 4. Photographs of the same chemostat culture (AQ0012) 7 days apart showing the large capacity for carbon sequestration of microalgal cultures. The panel on the left shows little biomass, mostly concentrated in 3 to 4 mm clumps. The panel on the right is the same culture after 7 days of photosynthetic growth.

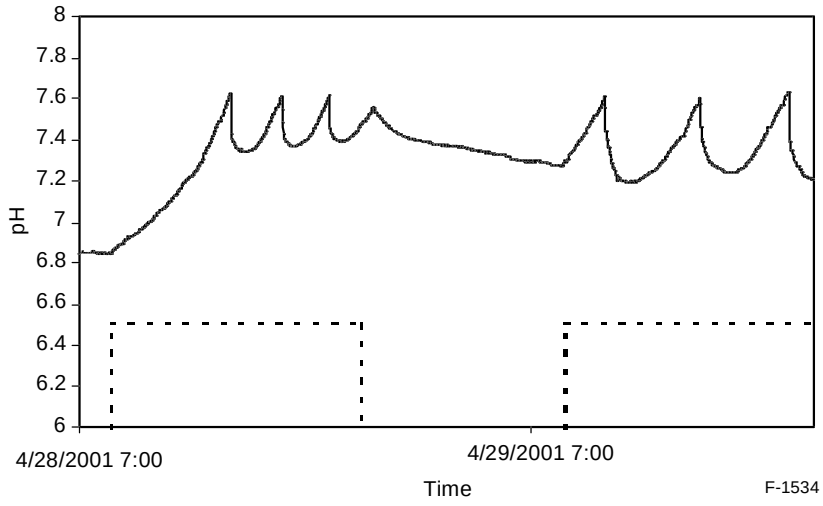

Figure 5. Computer generated trace of culture pH measured in the chemostat photographed in Figure 4. Rises in pH are caused by photosynthetic uptake of CO_2 by the algal cells. When the pH reaches the high set point (7.6 pH in this case), CO_2 is automatically injected into the culture medium and the pH drops. The broken line represents the periods during which the culture received light. The slope of the rise in pH, driven by photosynthesis, indicates carbon uptake be the culture.

The average growth rate of this chemostat culture was about 0.65 d^{-1}, which is equivalent to about one doubling of the biomass per day. At a typical biomass concentration of 0.4 g/l, in a 3 l chemostat, this is equivalent to 1.2 g of biomass produced per chemostat per day. As a first approximation we will assume that 50% of the biomass weight is carbon. Thus, the chemostat culture fixes about 0.6 g of carbon per day. This is equivalent to 2.2 g of CO_2 per day or about 1.1 l of CO_2 per day. Typical amounts of CO_2 injected into the chemostat culture are about 1.25 l of CO_2 per day. The efficiency of carbon transfer from gaseous CO_2 to algal biomass carbon is, thus, about 88% at this point. While this is well above our benchmark (12.5%). It should be noted that this is a preliminary result from our first chemostat culture and will need to be corroborated.

CO_2 utilization capacity of microalgal cultures grown in chemostats

The automated pH monitoring and control system allows us to closely follow changes in pH in all the chemostat cultures. Figure 6 shows the changes in pH over the life history of several different chemostat cultures. Changes in pH reflect changes in the concentration of dissolved CO_2 and the total dissolved inorganic carbon ($DIC = CO_2 + HCO_3^- + CO_3^{2-}$). We make the assumption that increases in DIC in the medium are produced either by respiration by the algae or by the injection of CO_2. We also make the assumption that decreases in DIC are produced by photosynthetic uptake of carbon. Figure 7 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 pH correspond to increases in DIC produced by CO_2 injections and algal respiration. Increases in pH correspond to decreases in DIC caused by photosynthetic uptake of CO_2 by the algae. Because algal respiration continues during photosynthetic carbon uptake, this represents the net uptake of carbon by the culture.

Using the changing concentrations of DIC over time we can calculate the net rate of carbon uptake (mg CO_2 l^{-1} min^{-1}). The results of this calculation, with a resolution of 5 minutes, are shown in Figure 8. Positive values indicate net uptake of CO_2 by the culture while negative values indicate injection of CO_2 into the medium and cellular respiration. If we calculate an average net CO_2 uptake rate, using the values when no CO_2 is being injected, we obtain an average net rate of 0.094 mg CO_2 l^{-1} min^{-1}. For a 14 hr day this is equivalent to 79 mg CO_2 l^{-1} d^{-1} or 21.5 mg C l^{-1} d^{-1}.

While scale-up of these systems will not occur until year 2 and 3 of this project we can compare this value with the productivity of existing commercial scale outdoor photobioreactors. Olaizola (2000) showed the productivity of such systems to be on the order of 40 to 50 mg l^{-1} d^{-1} of dry weight biomass for *Haematococcus* cultures. If we assume that about 50% percent of *Haematococcus* biomass is made up of carbon this is equivalent to about 20 to 25 mg C l^{-1} d^{-1}. Comparing this value with that obtained for AQ0036 grown in chemostats (21.5 mg C l^{-1} d^{-1}) we can expect that results obtained in our chemostat experiments are applicable to outdoor conditions and can be used to predict the behavior of our strains at full scale outdoor conditions.

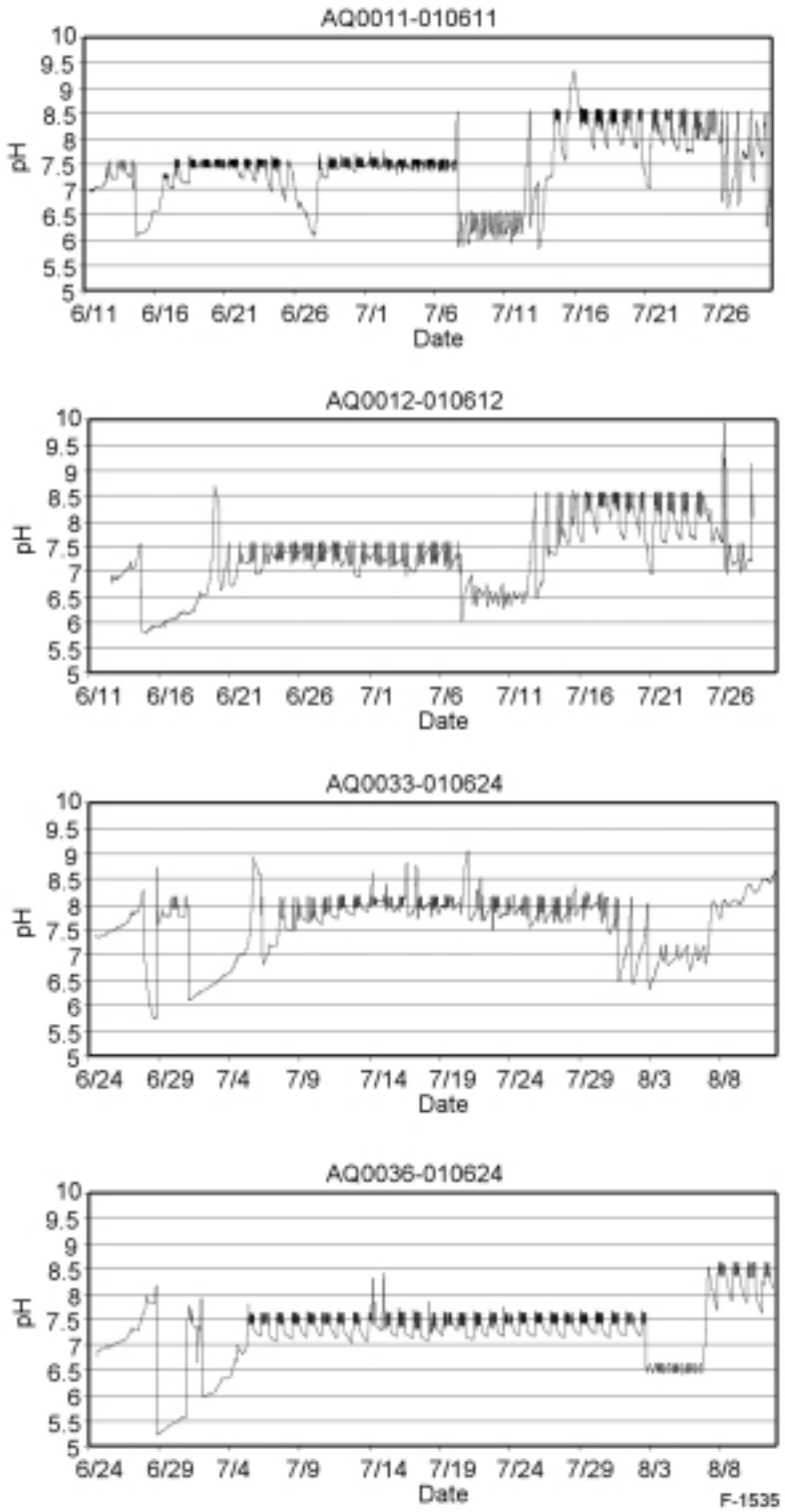

Figure 6. Computer generated trace of culture pH measured in four chemostat cultures used for the determination of differences in steady state biomass levels in response to changes in culture pH.

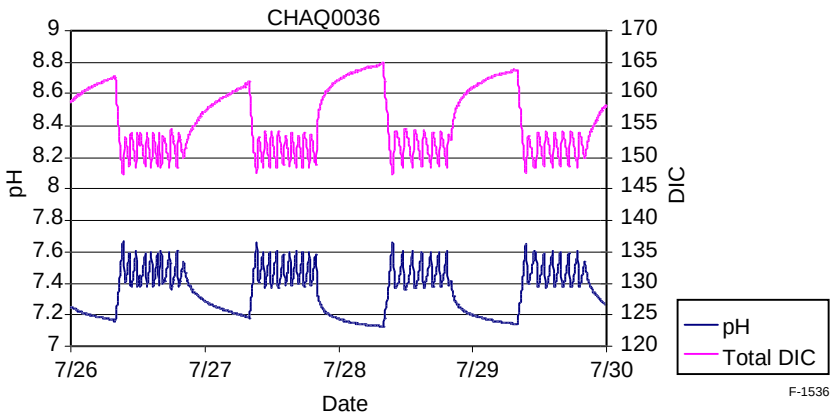

Figure 7. Changes in pH and total DIC over a 4-day period for a chemostat culture of strain AQ0036.

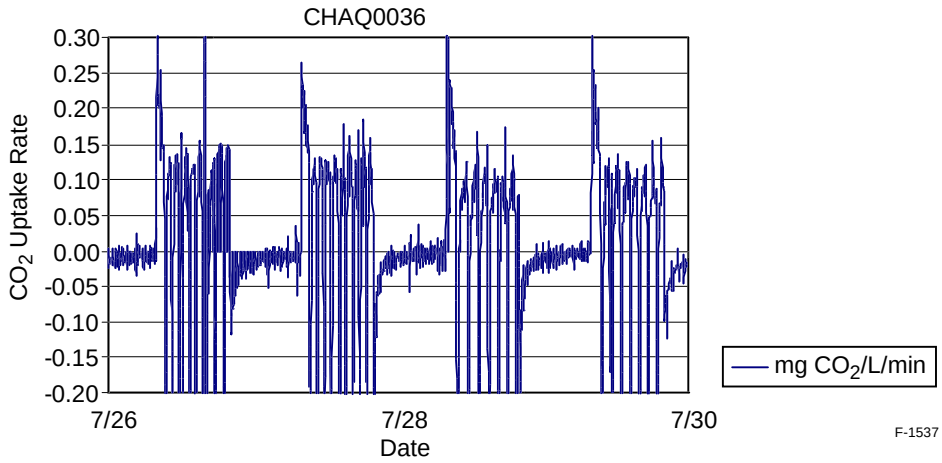

Figure 8. Changes in carbon uptake rate over a 4 day period for a chemostat culture of strain AQ0036. Positive values indicate net uptake rate of carbon. Negative values represent injection of CO_2 into the culture and respiration by the cells.

The close agreement between the results from a chemostat and an outdoor photo-bioreactor systems may seem surprising. However, it is easily explained by the fact that both systems are routinely managed as light limited systems. We have taken this approach because light utilization efficiency is higher for light limited than light saturated microalgal cultures. If one considers the possible limitations of outdoor cultures (i.e.; light, nutrients, CO_2) light is the only one that, on an area basis, cannot be increased. Thus, light is the ultimate productivity-limiting agent in outdoor cultures.

We have not finished analyzing the rest of the chemostat pH data at this point. We expect to report on those results at the next quarterly report.

3.2.2.2 Production of High Value Products from Microalgae to offset the cost of Carbon Sequestration

High value pigment analysis

Microalgae are a diverse group of over 30,000 species of microscopic plants that have a wide range of physiological and biochemical characteristics. Microalgae produce many different substances and bioactive compounds that have existing and potential applications in a variety of commercial areas, including human nutrition, pharmaceuticals, and high value commodities. Algal pigments (carotenoids and phycobiliproteins) are one such group of molecules. Examples of natural algal pigments that already been commercialized include B-carotene (food additive grade worth about $1,400 per kg, market size estimated >$500 million per year), astaxanthin (feed additive grade worth about $2,500 per kg-market size about $200 million- but up to >$100,000 per kg for nutraceutical grade-market size not know at this point).

Phycobiliprotein pigments

Presence/absence of phycobiliproteins is determined by visual inspection of microalgal biomass after extraction of chlorophylls and carotenoids using an organic solvent. Microalgal biomass is centrifuged and the overlying medium is decanted. The remaining pellet is mixed with an appropriate amount of solvent (e.g., acetone, methanol) and centrifuged a second time. The solvent is decanted removing the chlorophyll and carotenoids. The pellet is then visually inspected for color. A blue colored pellet is indicative of phycocyanin while a pink colored pellet is indicative of phycoerythrin.

Carotenoid pigments

Carotenoids are analyzed via High Performance Liquid Chromatography (HPLC). The carotenoid pigments are extracted from the algal biomass with a mixture of methanol, ammonium acetate and dimethyl sulfoxide. The extract is then injected into the HPLC system. Our system consists of a Beckman System Gold with a model 126 programmable solvent module, a model 168 diode-array detector, and a model 508 injector with a 100 uL loop. The column is a Supelco Discovery C8 column 150 x 4.6 mm, 5 µm particle size. The solvent system consisted of

A	MeOH: Acetonitrile: Acetone at 20:60:80
B	MeOH: Ammonium Acetate 0.25M: Acetonitrile at 50:25:25 using the following time program:
%B	100 → 60 over 22 minutes
%B	60 → 5 over 6 minutes
%B	5 for 10 minutes
%B	5 → 100 over 2 minutes.

The total run time is 40 minutes at a flow rate of 1.5 ml/min.

So far we have analyzed the pigment content of 15 different microalgal strains. We already reported on the pigment content of 11 cyanobacterial strains grown in batch cultures in our previous report (Figure 9). Here we report results of analysis carried out on cultures grown continuously in chemostats as well as analysis of cultures grown under stress conditions believed to be conducive to carotenogenesis.

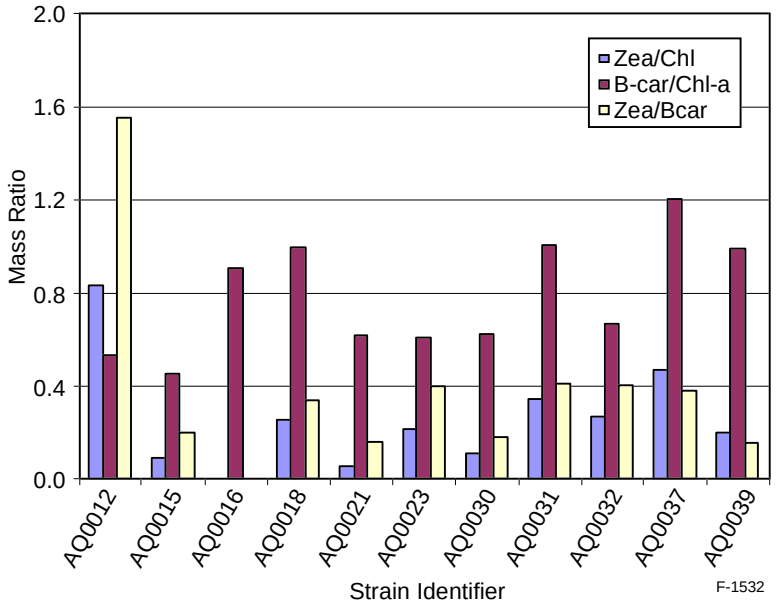

Figure 9. Summary of carotenoid pigment analysis of 11 strains of Cyanobacteria. Zea/Chl: mass ratio of zeaxanthin to chlorophyll-a, B-car/Chl: mass ratio of B-carotene to chlorophyll-a, Zea/Bcar: mass ratio of zeaxanthin to B-carotene. The values reported are the average of measured concentrations in two different cultures of each strain.

Six species of microalgae were selected for the pigmentation experiments. Aquasearch identifies strains using the letters AQ followed by a number. The chlorophyte strain AQ0011 and cyanobacterium strain AQ0012 were isolated locally in Kona, Hawaii. The Porphyridium strains AQ0033 and AQ0036 represent Rhodophyta, obtained from the University of Texas at Austin, while AQ0052 and AQ0053 are *Dunaliella* species of the division Chlorophyta, obtained from the Hawaii Culture Collection.

The cultures were grown in 3.3 L chemostats, using a 10:14 light:dark cycle, with temperature (25ºC)and pH control (7.4 to 7.6). The chemostats provided the culture material for the experimental treatments. Daily fluorescence readings with a Pulse Amplitude Modulator Fluorometer (PAM) monitored the biomass indirectly. PAM measures minimal (F_o) and maximal fluorescence (F_m) of the culture in a dark adapted state. The difference between F_o and F_m was F_v. The ratio F_v/F_m was used to estimate the photosynthetic efficiency of the cells. Initially, the chemostats were grown in batch mode. When a certain cell density was reached, the cultures were switched to continuous mode, which allowed the cells to attain a well-defined physiological state (Nyholm and Peterson, 1997).

Light Intensity Experiments

Each species of microalgae was first tested under intense light conditions (sunlight). Preliminary PAM readings and pH measurements were taken before exposure to light. Flasks with 200 ml of culture were placed in an outdoor water bath at 25°C in full sunlight for a period up to 5 or 8 hr. Light intensity was monitored by roof top solar panels. Each hour, PAM readings were first taken in ambient sunlight and again in darkness. Flasks were swirled, and the pH was monitored hourly. In addition, 45 to 50 ml samples were collected for pigment extraction from each sample and 175 ml of culture was used for dried biomass analysis of the initial and final flasks. Samples were collected in duplicate. Pigments were extracted using 5 ml of dimethyl sulfoxide (DMSO), and re-extracted with 2 ml DMSO until the extract color was very pale. The pigments were analyzed by High Pressure Liquid Chromatography (HPLC) using the method described by Zapata et. al. 2000. Spectrophotometry was also conducted on the extracts, after further dilution in DMSO.

Nitrate Deprivation Experiments

Nitrate deprivation experiments were conducted with AQ0033, AQ0036, AQ0011, and AQ0012. Two hundred ml of each culture was collected from the chemostat and inoculated in 800 ml of 413 media without nitrate. Freshwater media was prepared for AQ0011 and AQ0012, while AQ0033 and AQ0036 were grown in 9 ppt salt media. The cultures were grown in 2800 ml Fernbach flasks in a 25°C water bath on a 14:10 light:dark cycle, with lights measuring an intensity of 60 $\mu E\ m^{-2}\ s^{-1}$. The flasks were mixed by air agitation, and it is probable that ambient CO_2 contributed slightly as a source of carbon. PAM readings were taken daily and pH was monitored. Two additional light banks were added on day 6 of the experiment to increase photosynthesis and expedite the nutrient deprivation effects. The average light intensity was measured to be 175 $\mu E\ m^{-2}\ s^{-1}$. After 10 days, pigments were extracted from 50 ml of each culture and dried biomass analysis was conducted using 350 ml of culture.

Salt/Sodium Acetate Experiments

The third experiment exposed *Dunaliella* species AQ0053 to high sodium chloride and sodium acetate concentrations. Triplicate samples of 200 ml of culture were collected from the chemostat and receiver and grown in batch mode in 250 ml Erlenmeyer flasks. Initial samples were collected for dried biomass (170 ml) and pigment analysis (50 ml). Initial PAM readings were taken of each flask. Sodium chloride was then added to three flasks, creating a 10% salt solution. Sodium acetate was also added to three flasks bringing the concentration of sodium acetate to 1g/l. This additive serves as a source of organic carbon readily taken up by the cells and has been used to increase carotenoid yields. Both sodium chloride and sodium acetate were added to three additional flasks in the previously determined amounts. Flasks were grown in batch mode in a 25°C water bath on a 14:10 light:dark cycle for 3 days. Pigments were extracted from 50 ml of culture, and 150 ml samples were used for dried biomass analysis. HPLC and spectrophotometry were conducted to analyze the pigments.

Methods for Data Analysis

PAM data measured in darkness was utilized to determine the percent functional reaction centers in the photosystem of the algal cells. The dark PAM reading of each hour was divided by the initial PAM reading to determine this value for the light intensity experiments. For the nitrate deprivation and salt/sodium acetate experiments, daily PAM readings helped to monitor the health of the cells. A decline in these Fv/Fm values indicated that the cells were experiencing stress. The Fv/Fm value from each flask was plotted each day and a linear regression analysis was performed to measure the trends.

The spectrophotometric data was normalized to 750 nm, by subtracting the absorption at 750 nm from the absorption at each wavelength within a sample to eliminate the contribution of light scattering to absorption readings. The spectra were also analyzed on a volumetric basis by multiplying the normalized absorbance by the volume extracted with DMSO divided by the culture volume. In addition, differences spectra were calculated by subtracting the spectrum of each hour from the initial spectrum to show how the pigments changed and absorbed light differently through time.

The HPLC chromatograms were analyzed by identifying the peaks of zeaxanthin, lutein, β-carotene, and chlorophyll according to published spectral data (Jeffrey et. al., 1997). The concentration of the biomass was determined by centrifuging a known volume of culture and transferring the pellet to a preweighed 15 mL tube, which was placed in a 65°C drying oven for 24 hr. After drying, the tube was re-weighed and the concentration of biomass per ml of culture was calculated. Pigments were quantified based on the areas of the peaks, which were multiplied by previously determined response factors of standard pigment samples. The amount of pigment (ng) injected was divided by the volume injected into the HPLC to determine the concentration of the extract (ng/ml), and then multiplied by the volume of DMSO used for the extraction. This determined the total amount of pigment in the extract, which was divided by the original volume of culture used for extraction. Averages were taken of duplicate samples. Percent lutein, zeaxanthin, and β-carotene were determined by dividing the concentration of the pigment by the concentration of biomass in the sample. The initial and final samples were compared for change in pigment per amount biomass and change in pigment per volume culture.

Results

Light Intensity Experiments

Irradiance readings from roof-top solar panels recorded the sunlight intensity on the dates when the light intensity experiments were conducted (Figure 10). Decreases in intensity represent clouds. PAM data showed that after 1 hr in the sun, 30% of the reaction centers were functional and remained functional throughout the 5 hr period (Figure 11). The unknown chlorophyte AQ0011 did not increase in biomass during the 5 hr period (Figure 12). HPLC confirmed the presence of large amounts of lutein present throughout the experiment, as percent lutein per dried biomass increased from 0.25% (initial) to 0.28% (5 hr). Initially, no zeaxanthin was detected by HPLC, but after 5 hr, the percent zeaxanthin per ml dried biomass had increased

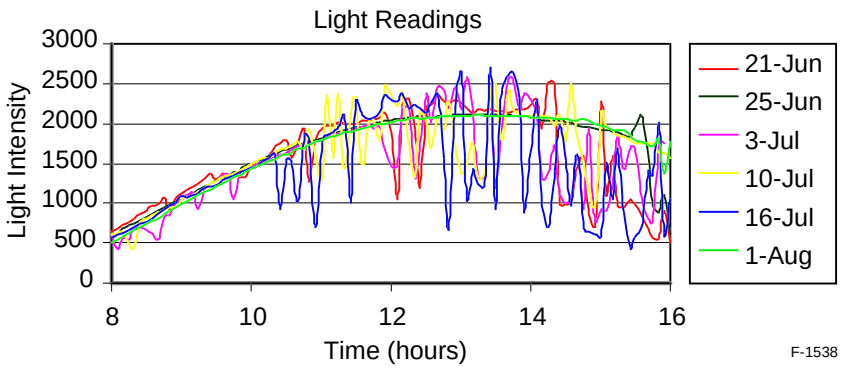

Figure 10. Light intensity (µE m^{-2} s^{-1}) measured outdoors on days when light experiments were carried out with strains AQ0011 (6/21, 7/11), AQ0012 (6/25, 7/11), AQ0052 (7/3, 7/11), AQ0053 (8/1), AQ0033 and AQ0036 (7/16).

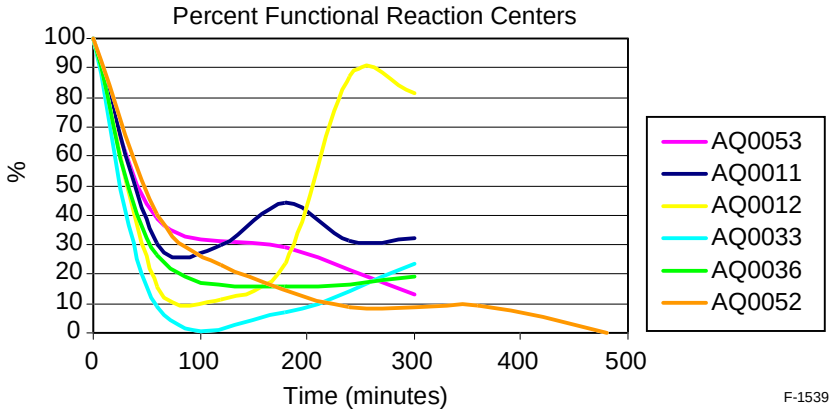

Figure 11. Percent functional reaction centers for each species from initial sample to final calculated with PAM Fv/Fm reading.

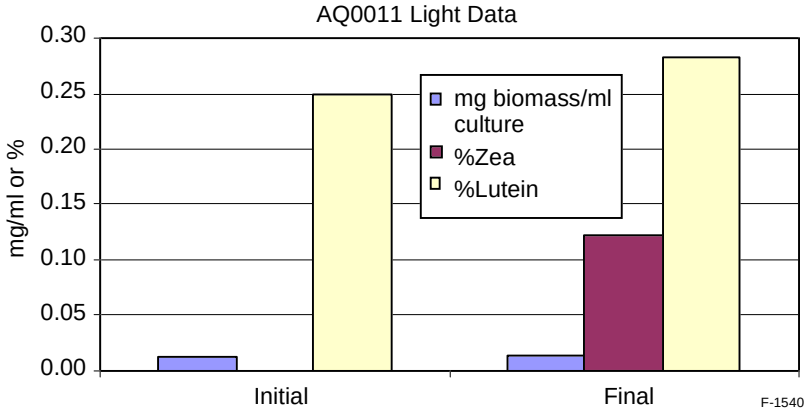

Figure 12. Biomass and % carotenoids from initial (0 hr) to final (5 hr) after intense light exposure.

to 0.12% (Figure 12). Lutein and Zeaxanthin increased per volume as well (Figure 13). In addition, a small amount of β-β carotene was present in varying levels throughout the experiment. Examples of HPLC chromatograms are shown in Figures 14 through 17. The absorbance/ml was determined volumetrically by the spectrophotometer (Figure 18). The difference spectra are shown in Figure 19.

The biomass of AQ0012, an unknown strain of cyanobacteria, increased through time. After 1 hr of light exposure, the biomass in the flask was floating at the top of the liquid in a tight clump. It is likely that this is morphological defense mechanism of the microalgae to increase shading of the cells. Data collected with the Pulse Amplitude Modulator (PAM) in the light show that after 2 hr of intense light exposure, only one-tenth of the initial reaction centers are functioning (Figure 11). Photosynthetic capacity of the cells is very low after 5 hr. HPLC analysis revealed the initial percent of zeaxanthin per dried biomass to be 0.15% and final percentage was 0.14% (Figure 20). The amount of zeaxanthin per volume increases proportionately with the increasing biomass (Figure 21). It was also noted that AQ0012 produced β-β carotene, but the amount was small and did not change significantly with time.

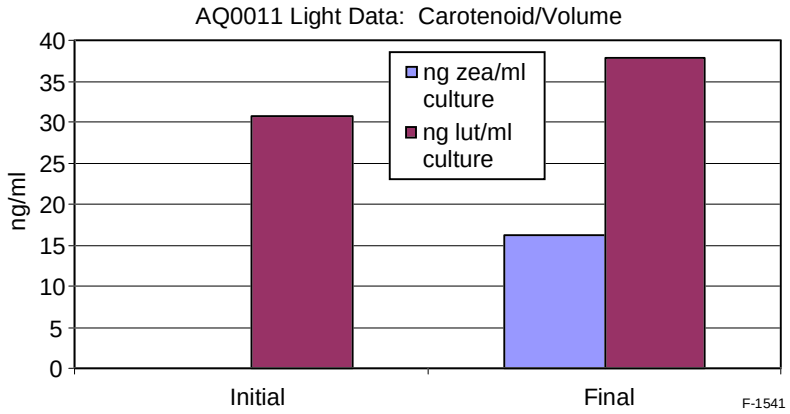

Figure 13. Carotenoid amount per culture volume initially and after 5 hr of intense sunlight.

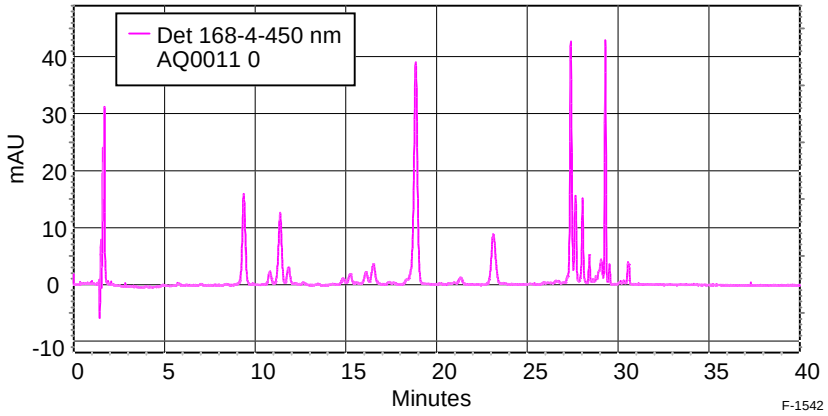

Figure 14. HPLC chromatogram for strain AQ0011, 0 hr sample.

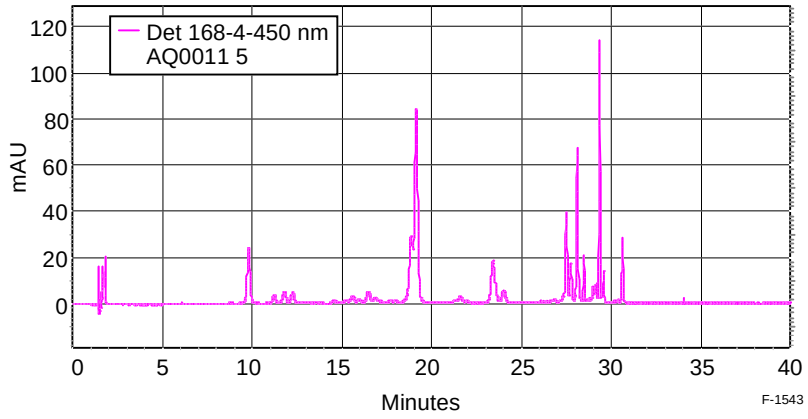

Figure 15. HPLC chromatogram for strain AQ0011, 5 hr sample.

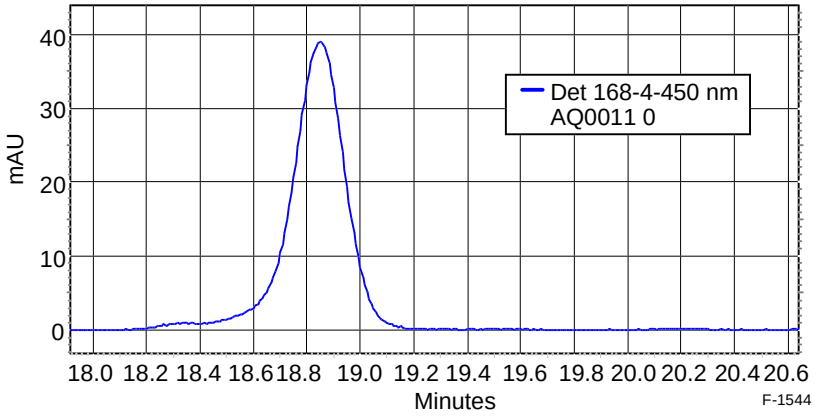

Figure 16. AQ0011 HPLC chromatogram showing the lutein peak at 0 hr. No zeaxanthin present.

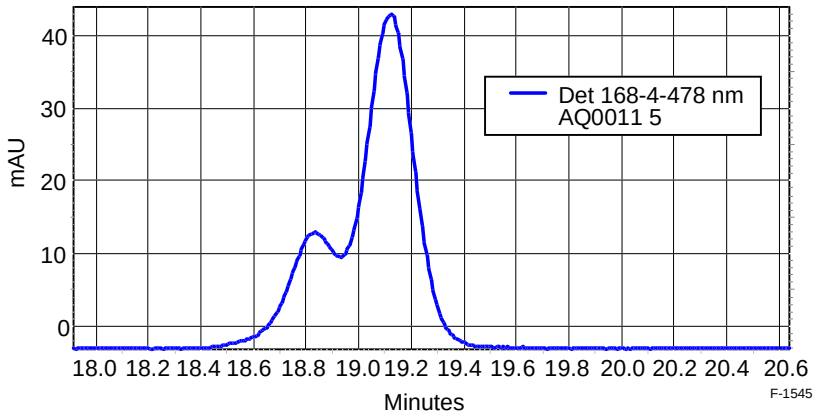

Figure 17. AQ0011 HPLC chromatogram of the 5 hr sample. Zeaxanthin peak present at base of the lutein peak.

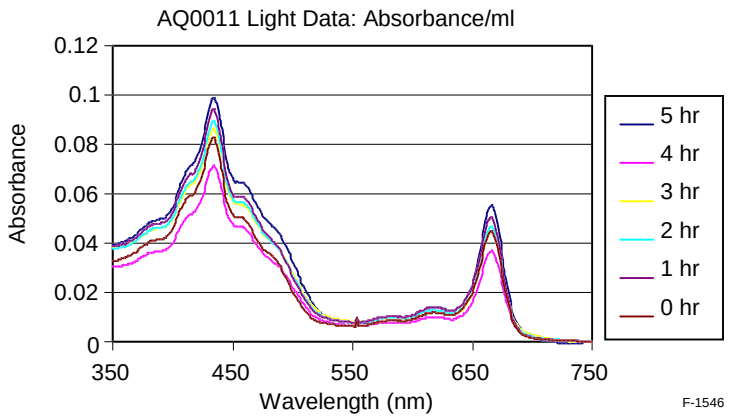

Figure 18. Absorbance per volume determined spectrophotometrically over a 5 hr period of intense sunlight.

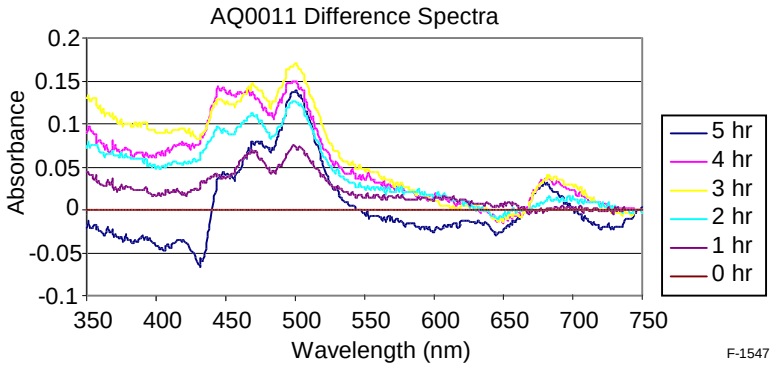

Figure 19. Spectral differences over a 5 hr period of intense sunlight.

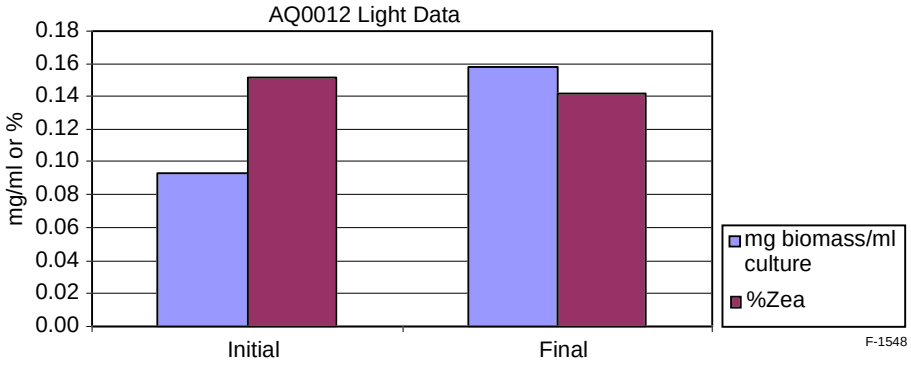

Figure 20. Biomass and % zeaxanthin from initial (0 hr) to final sample (5 hr).

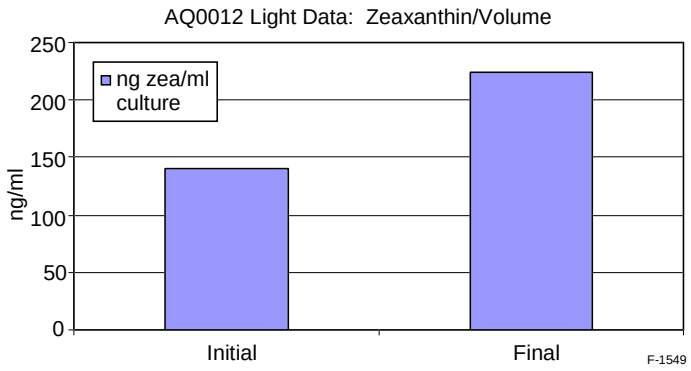

Figure 21. Zeaxanthin measured per culture volume from initial (0 hr) to final (5 hr).

Dunaliella strain AQ0052 was exposed to intense sunlight for a period of 8 hr to determine if extended light exposure would induce additional zeaxanthin production. After 2 hr of sunlight, 22% of reaction centers were operating, and this percentage continued to decrease until no reaction centers were functional at 8 hr (Figure 22). The biomass increased slightly, and the zeaxanthin increased from 0% to 0.05% per dried biomass (Figures 22 and 23). AQ0052 also contained lutein, which decreased during the light exposure from 0.2% to 0.11% per ml dried biomass. Chlorophyll a decreased dramatically throughout the experiment, and was not detectable in the 8 hr sample by HPLC.

Dunaliella strain AQ0053 was exposed to intense light conditions for 5 hr and PAM data showed that after 5 hr of intense light exposure, 12% of reaction centers were functioning (Figure 11). The biomass began to settle to the bottom center of the 1000 mL flask during the experiment, and some small clumps of cells were visible at the end of the time period. Biomass increased from initial to final sample (Figure 24). HPLC data showed that the % lutein increased from 0.31% to 0.35% per dried biomass (Figure 25). Lutein also increased on a volumetric basis.

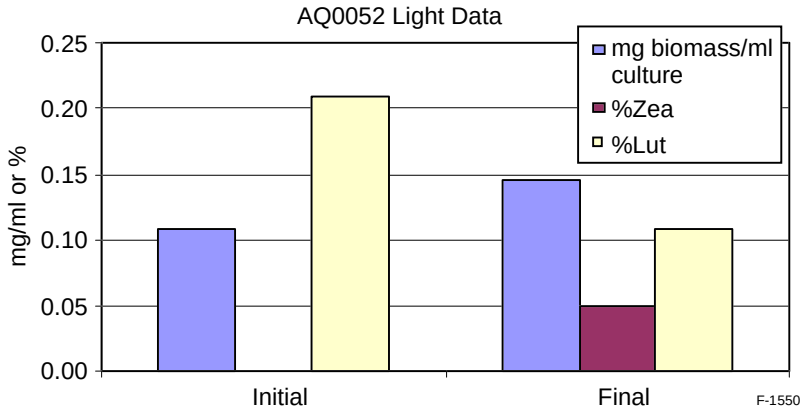

Figure 22. Biomass and % carotenoids from initial sample (0 hr) to final sample (8 hr).

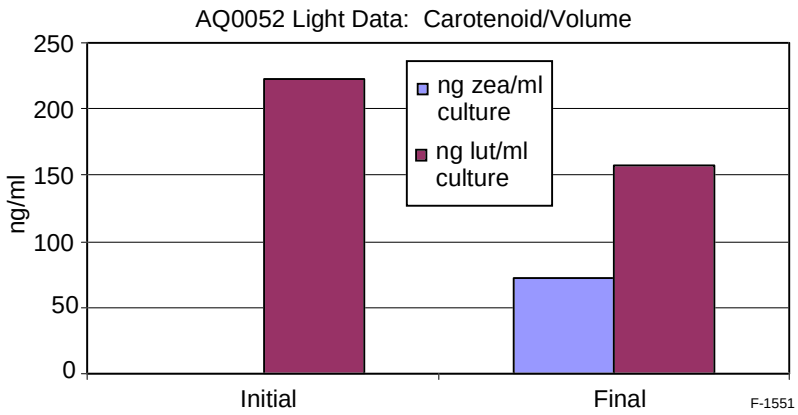

Figure 23. Carotenoid per volume of culture from initial (0 hr) to final sample (8 hr of intense sunlight).

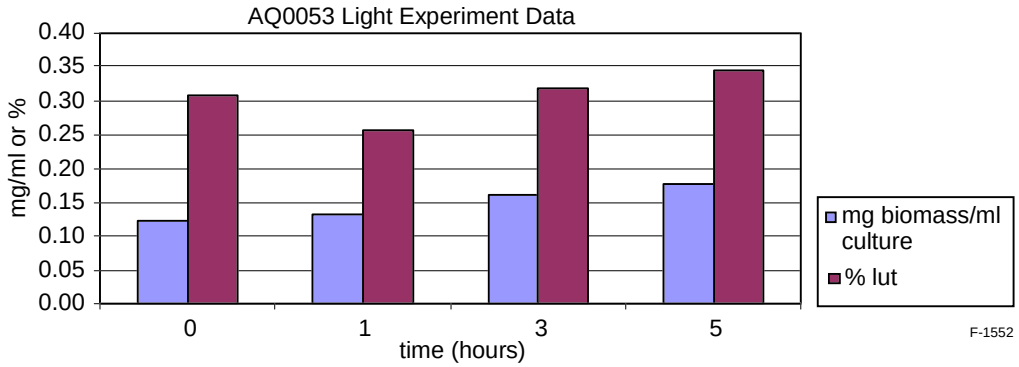

Figure 24. Biomass and % lutein after 0, 1, 3, and 5 hours of intense light.

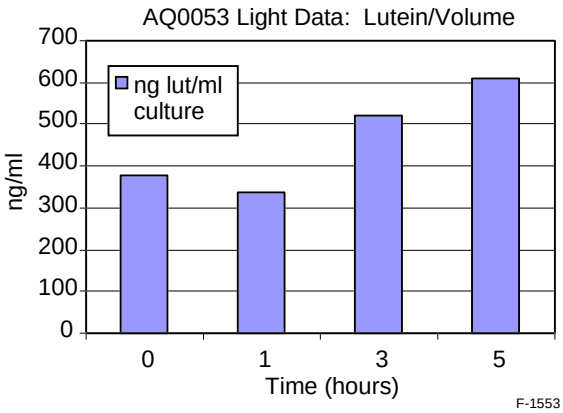

Figure 25. Lutein per culture volume over period of 5 hours.

Strain AQ0033 was exposed to intense light conditions for 5 hr, and the biomass decreased slightly. Similar to AQ0012, the biomass clustered together in the flask, but formed a loose mass rather than a tight clump. PAM data showed that only 7% of reaction centers were functional after 3 hr of sunlight (Figure 11). HPLC data confirmed that the 0 hr sample contained 0.2% zeaxanthin per dried biomass, a value higher than any percent zeaxanthin obtained (Figure 26). However, after intense light exposure, this amount decreased to 0.1%. Zeaxanthin decreased on a per volume basis as well (Figure 27). The reasons for this decrease are unknown and are still under investigation. It is possible that because *Porphyridium* was originally a soil algae (Lee, 1989), these strains do not have the ability to efficiently adapt under intense light.

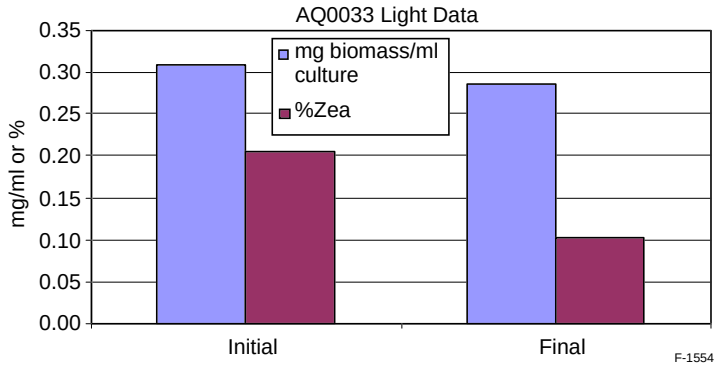

Figure 26. Biomass and zeaxanthin from initial (0 hr) to final sample (5 hr).

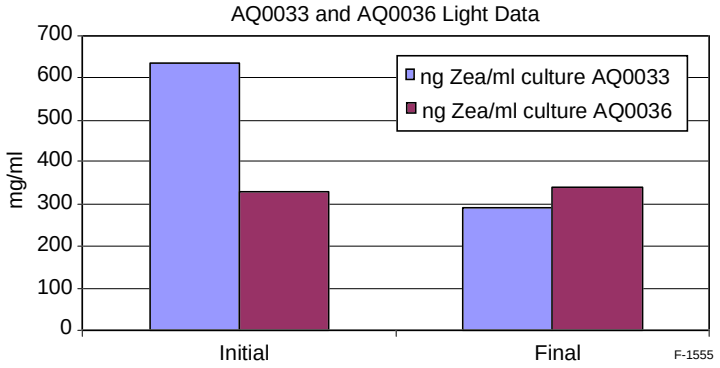

Figure 27. Zeaxanthin per culture volume from initial (0 hr) to final sample (5 hr).

The biomass of *Porphyridium* strain AQ0036 significantly increased during the 5 hr experiment. PAM data showed that after 1 hr of light exposure, 25% of the reaction centers that harvest light for photosynthesis were operating (Figure 11). This number remained fairly constant throughout the experiment. The amount of zeaxanthin per dried biomass also decreased from 0.13% to 0.05%, levels that are still higher than those of AQ0052 (Figures 22 and 28). Zeaxanthin per volume also decreased for AQ0036 (Figure 27).

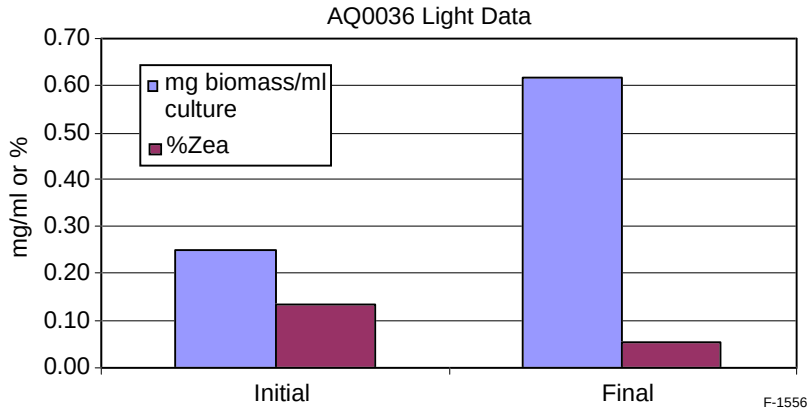

Figure 28. Biomass and % zeaxanthin from initial (0 hr) to final sample (5 hr).

Nitrate Deprivation Experiments

Daily PAM readings showed that the Fv/Fm values varied greatly for each strain tested. In Figure 29, the slope of the line for AQ0033 was –0.0218, representing the rate of decrease in Fv/Fm. A flask of AQ0033 was broken on day 9 of the experiment, but data until this point was included in the analysis. For AQ0036, the linear regression line was nearly flat, with a slope of -0.0039 (Figure 30). The slope for AQ0011 also decreased, but at a rate of –0.0167 (Figure 31). In addition, the line for AQ0012 had a negative value of -0.0019, which represents that the cells were experiencing only slight stress after 10 days of nitrate deprivation (Figure 32).

The percent carotenoid per dried biomass decreased for AQ0011, AQ0033, and AQ0036, but remained constant for AQ0012 (Figure 33). AQ0011 produced only lutein during this experiment, unlike the light intensity experiment where it showed zeaxanthin production after 1 hr. AQ0012, AQ0033, and AQ0036 all produced zeaxanthin. Both AQ0011 and AQ0012 increased in biomass per culture volume as seen in Figure 34 while the biomass of AQ0033 decreased and AQ0036 was constant. Although the pigments of AQ0011 and AQ0012 did not increase on a % dried biomass basis, the pigments did increase dramatically on a volumetric

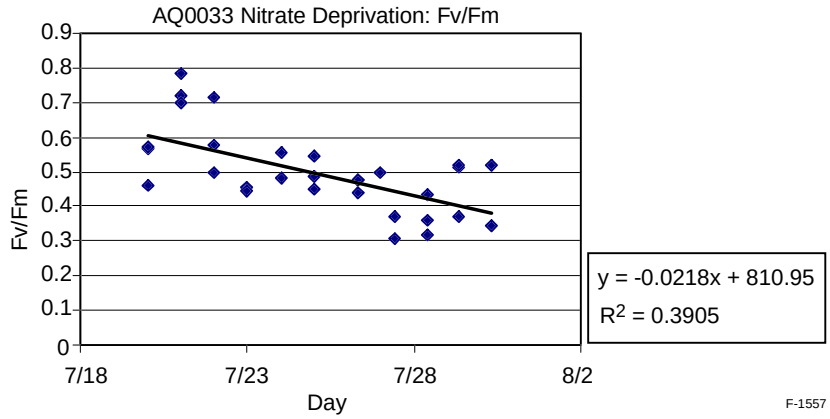

Figure 29. AQ0033 Fv/Fm readings from PAM data over 10-day nitrate deprivation experiment with linear regression analysis.

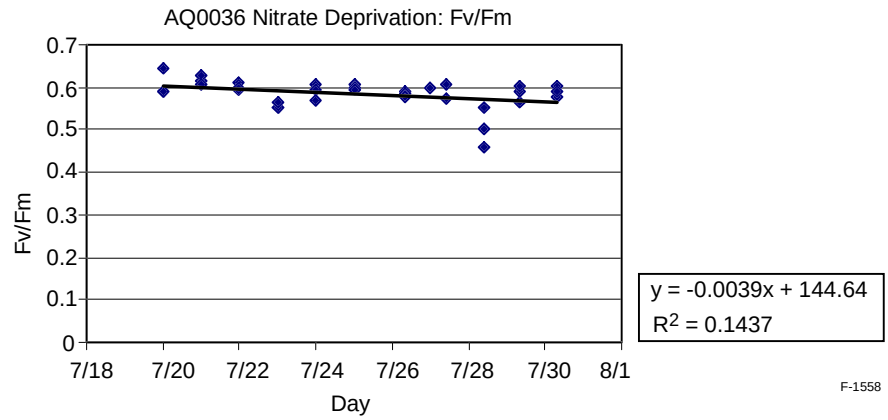

Figure 30. AQ0036 Fv/Fm readings from PAM data over 10-day nitrate deprivation experiment with linear regression analysis.

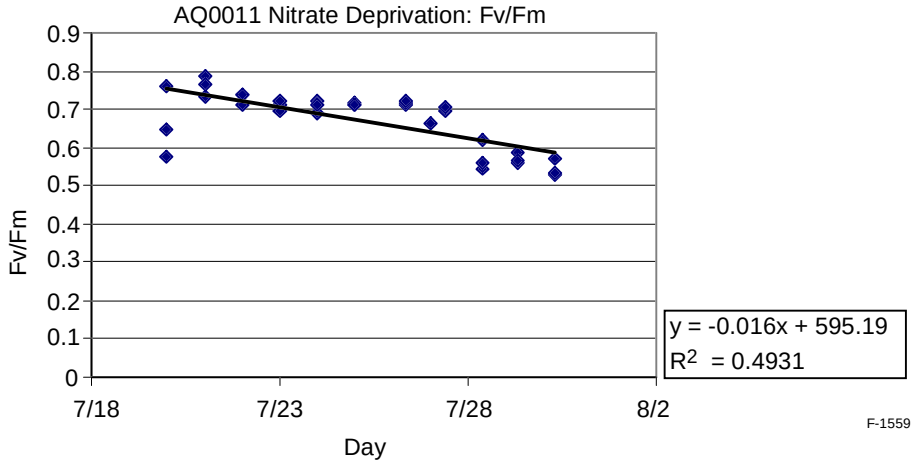

Figure 31. AQ0011 Fv/Fm readings from PAM data over 10-day nitrate deprivation experiment with linear regression analysis.

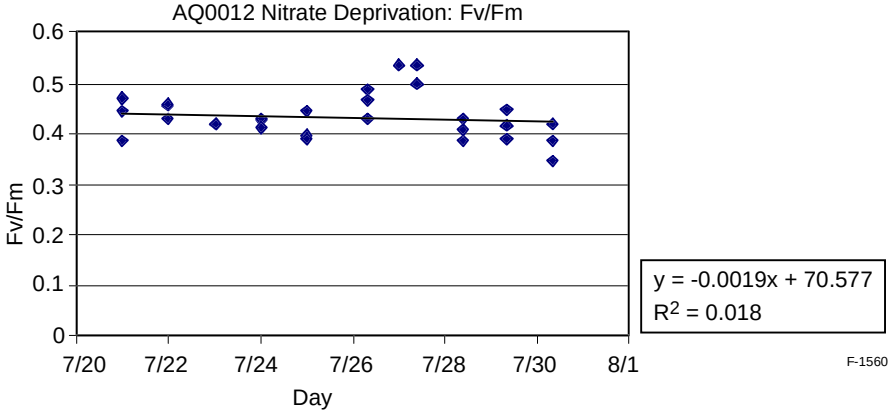

Figure 32. AQ0012 Fv/Fm readings from PAM data over 10-day nitrate deprivation experiment with linear regression analysis.

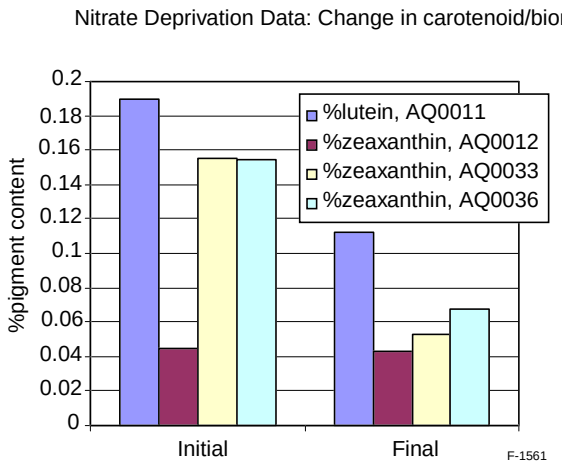

Figure 33. Carotenoid percentages per biomass over 10-day nitrate deprivation experiment.

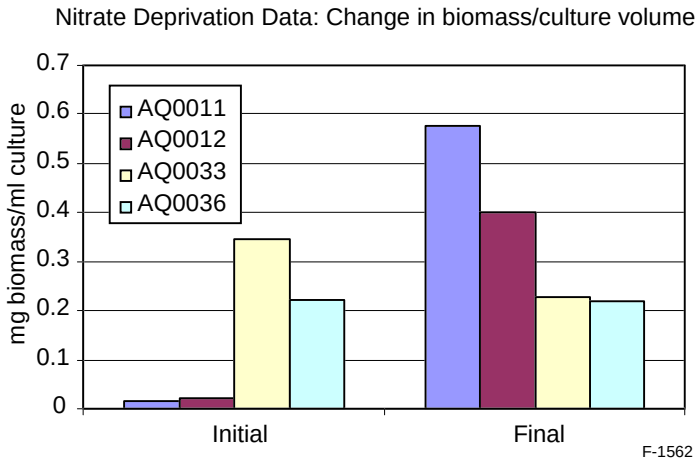

Figure 34. Biomass per culture volume calculated for initial (day 0) and final samples (day 10).

basis (Figure 35). Thus, as the culture grew over the experimental period, the absolute amount of pigments in each flask increased for AQ0011 and AQ0012 and decreased for AQ0033 and AQ0036.

Because 200 ml of culture were used to inoculate the flasks, it is possible that this volume contained enough nitrate from the original media to support the cultures for the experimental period. It is possible that using a smaller inoculum volume would cause the cells to experience stress more quickly, and perhaps alter the pigments more dramatically.

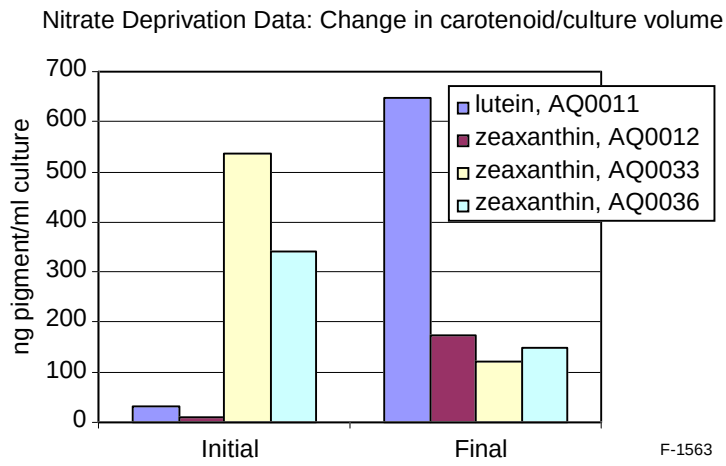

Figure 35. Carotenoid per culture volume for initial (day 0) and final samples (day 10).

Salt/Sodium Acetate Experiments

PAM data showed that the Fv/Fm values of AQ0053 were the same for the initial samples before additions of salt and/or sodium acetate were made compared to the Fv/Fm values immediately after the additions. However, the Fv/Fm ratio plummeted from 0.7 on day 1 to 0.2 to 0.3 on day 2. An example of the Fv/Fm ratios with NaCl is shown in Figure 36. The dramatic decrease was unexpected, and it is possible that the media was contaminated or that the concentrations were higher than calculated.

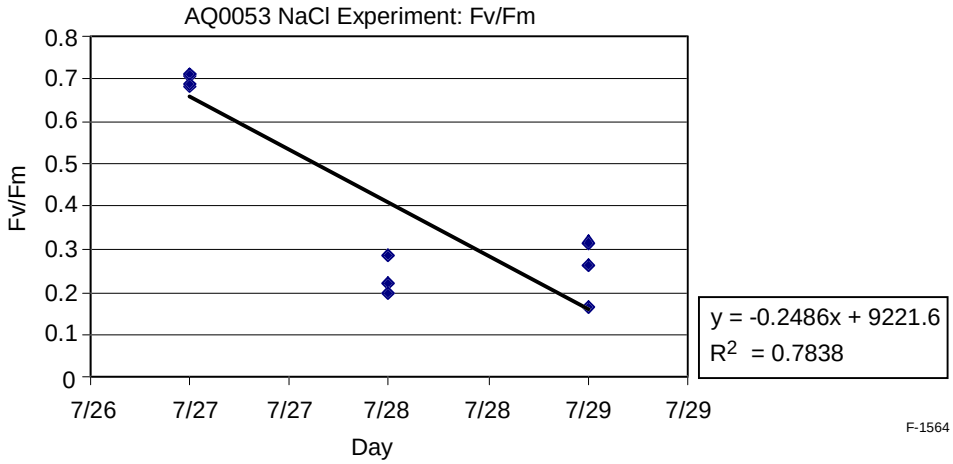

Figure 36. AQ0053 Fv/Fm values from PAM data with linear regression analysis.

The mg biomass per culture volume increased for all flasks slightly during the 3-day experiment, with the greatest increase seen in the flasks with NaCl and NaAc in combination. AQ0053 produced lutein initially at 0.34% per dried biomass, a level higher than any carotenoid for all strains. The % lut decreased for all flasks from initial to final sample, with the lowest value for the NaCl/NaAc flasks at 0.09% (Figure 37). The amount of lutein decreased for all samples volumetrically as well.

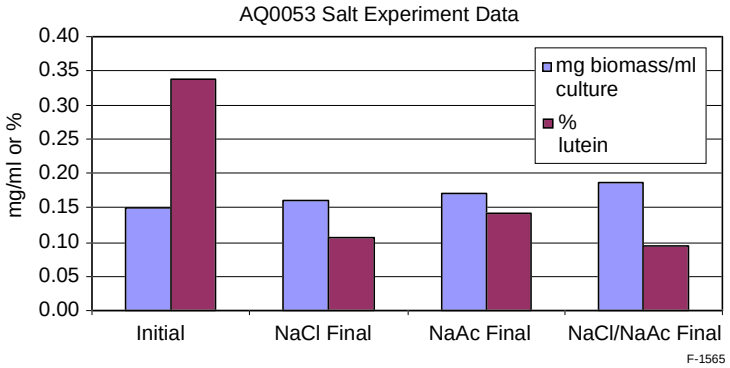

Figure 37. Biomass and % lutein for initial sample (0 day) and samples with additives after 3 days.

Potential for Commercial Production

One goal of this experiment was to determine which strains would be worthwhile to grow at a commercial level to offset the cost associated with microalgal mediated carbon sequestration. The pigment composition of each strain was analyzed before and after exposure to environmental stress to evaluate how the stress affected pigment production. This research was conducted at Aquasearch Inc., where the 25,000 L enclosed outdoor photobioreactors, known as the Aquasearch Growth Modules (AGMs), are producing *Haematococcus pluvialis* at a rate of 9 to 13 g m^{-2} d^{-1} (Olaizola, 2000). Similar growth rates or higher could be expected by growing the tested strains in the AGMs. For AQ0033, AQ0036, AQ0012, and AQ0052 the highest percent zea was found in the initial sample before treatment with environmental stress (Table 3). Thus, the most pigment per dried biomass was produced when the cultures were grown in nutrient rich media at low light intensities. On the other hand, AQ0011 and AQ0053 produced the greatest amount of pigments after 5 hr of intense light exposure. AQ0052 produced the most lutein without treatment, but produced a small amount of zeaxanthin after 8 hr of intense sunlight. The

Table 3. Highest percent carotenoids per dried biomass obtained in experiments and predicted pigment production rates at a production rate of 13 g dry biomass m^{-2} d^{-1}, a typical rate of Aquasearch Growth Modules.

Strain	Treatment which gave highest % pigment	Pigment	% Pigment	Production @ Growth rate of 13 g m^{-2} d^{-1}
AQ0011	5 hours sunlight	Lutein	0.28	0.037 g m^{-2} d^{-1}
AQ0011	5 hours sunlight	Zeaxanthin	0.12	0.016 g m^{-2} d^{-1}
AQ0012	No treatment	Zeaxanthin	0.15	0.020 g m^{-2} d^{-1}
AQ0033	No treatment	Zeaxanthin	0.21	0.027 g m^{-2} d^{-1}
AQ0036	No treatment	Zeaxanthin	0.13	0.017 g m^{-2} d^{-1}
AQ0052	No treatment	Lutein	0.21	0.027 g m^{-2} d^{-1}
AQ0052	8 hours sunlight	Zeaxanthin	0.05	0.006 g m^{-2} d^{-1}
AQ0053	5 hours sunlight	Lutein	0.35	0.049 g m^{-2} d^{-1}

ability of AQ0011 to produce both lutein and zeaxanthin makes it a highly attractive strain to grow in the AGM. AQ0053 could be expected to produce 0.04487 g m^{-2} d^{-1} lutein after 5 hr of intense light exposure, which is the highest value found of any carotenoid of any strain in this study. The values in Table 3 most likely represent the minimal amounts of carotenoids that can be obtained from these strains, as yields will increase with optimization of mass cultures. During the year 2 of this project we will begin to scale up the promising strains to commercial levels and to optimize the conditions under which these strains will produce the most pigment.

Carbon sequestration into carbonate minerals utilizing microalgae

One of the goals of this project is to identify under what conditions microalgal cultures can be induced to precipitate $CaCO_3$. This would represent a stable, long term, sink of atmospheric CO_2, a goal of the US Department of Energy.

Initially, we proposed to carry out this research by growing microalgal species known to produce cellular structures out of $CaCO_3$. While we still intend to work with such organisms we have decided to take the concept a step further. We intend to describe culture conditions that will induce the precipitation of carbon into $CaCO_3$ via photosynthetically mediated changes in medium pH. As cells photosynthetise and take up CO_2 from the culture medium, the pH of the medium raises. This change in pH produces an increase in the concentration of CO_3^{2-} ions in the medium. In the presence of sufficient amounts of Ca^{2+} $CaCO_3$ is expected to precipitate out of solution. Because the photosynthetically mediated change in pH is not specific to species that produce cellular carbonate structures, in principle, any species of microalgae can be used for this process.

These experiments were conducted using three different strains of microalgae and one species of cyanobacteria. The species of microalgae will be referred to as AQ0008, AQ0011, and AQ0053, where AQ0008 is *Haematococcus pluvialis*, AQ0053 is a *Duneliela sp*. obtained from the Hawaii Culture Collection, and AQ0011 is an unidentified locally isolated chlorophyte. AQ0012 is an unidentified species of filamentous cyanobacteria also isolated locally. All experiments were conducted using a 14:10 light:dark cycle with a light intensity of 60 μE m^{-2} s^{-1}. The source of the culture material for these experiments was our chemostat system.

Our standard growth medium (413) was enriched in Ca^{2+} by the addition of $CaSO_4*2H_2O$ (gypsum) for these experiments. Changes in the concentration of dissolved inorganic carbon species (CO2, HCO_3^-, CO_3^{2-}) were determined using a standard titration method (Clesceri et al., 1995). Production of $CaCO_3$ was determined by observing the formation of a white precipitate. The precipitate was collected by filtration or centrifugation, dried. A few drops of hydrochloric acid were mixed with the powder thus obtained. A positive reaction (bubbling caused by CO_2 effervescence) was interpreted to indicate the presence of $CaCO_3$.

The first experiment was conducted with species AQ0011. Two types of media were prepared for the experiment. 413 media was prepared for flasks 1 and 3, and 413 media without bicarbonate was prepared for flasks 2 and 4. Each 250 ml flask was filled with 200 ml of its respective media. Flasks 3 and 4 were enriched with 6.16×10^{-4} moles of Ca^{2+}. Four 50 ml samples of AQ0011 were centrifuged and the pellets were used to inoculate each flask. Initial

pH and alkalinity was measured and recorded. All flasks were placed in a 25°C water bath under the above mentioned growth conditions. The pH of each flask was monitored periodically. Fluorescence measurements were also measured throughout the 5-day experiment using a Pulse Amplitude Modulated Flourometer (PAM). After the cultures had grown for 5 days, final pH and alkalinity measurements were taken from each flask. The contents of each flask were gravity filtered using Whatman 15.0 cm filter paper. The filtrate was then tested for the presence of $CaCO_3$ by adding concentrated HCl and observing whether or not a reaction occurred. Bubbling of the filtrate indicated that $CaCO_3$ was present. This test was used throughout all of the experiments.

A second experiment was performed with microalgae species AQ0011, this time on a larger scale. A volume of 2500 ml of culture and media were removed from the receiver. The sample was divided into two 1600 ml volumes, one of which was enriched with 2.09×10^{-2} moles Ca^{2+} and stirred until dissolved. Alkalinity and pH measurements were taken from 40 ml of both the Ca^{2+} and non-Ca^{2+} enriched mediums. Four 1 L flasks were used and 780 ml of non-calcium culture was added to both flasks 1 and 2. A volume of 780 ml of Ca^{2+}-enriched culture was added to both flasks 3 and 4. All flasks were grown as previously described. After the pH in each flask reached 9.0 or higher, the contents of each was centrifuged and the pellet dried. The concentrated HCl bubble test was used on the dried pellets to determine if $CaCO_3$ precipitation had occurred.

Another experiment examined cyanobacteria species AQ0012. Approximately 800 ml of culture in medium was removed from the receiver. This volume was divided in half, and 5.60×10^{-3} moles Ca^{2+} was dissolved in to one half. It was necessary to add an additional 20 ml of deionized water while stirring the sample in order to dissolve all of the Ca^{2+}. Two flasks were filled with 200 ml of culture each, and two were filled with culture enriched with calcium. Alkalinity and pH measurements were taken initially from each flask. All flasks were then placed in a 25°C water bath and grown as mentioned. Alkalinity and pH measurements were taken periodically. After the flasks had reached a pH of 9.0 or higher, the contents of each were examined under a microscope for $CaCO_3$ precipitates. Also, the contents of each flask was filtered and tested for $CaCO_3$ precipitation using the HCl bubble test.

A second experiment was conducted with AQ0012 on a larger scale using greater volumes and more biomass. Approximately 2 L of culture was removed from the receiver. Initial pH and alkalinity measurements were taken from 50 ml of this sample. Flasks 1 and 2 were filled with 975 ml each of the culture. Again, approximately 2 L of culture was removed from the receiver and was enriched with 2.80×10^{-2} moles of Ca^{2+}. Initial pH and alkalinity measurements were taken from 50 ml of this sample. Flasks 3 and 4 were filled with 975 ml of the sample. All flasks were grown under the same conditions as the previous experiments and pH and alkalinity measurements were taken periodically. After the pH of each flask reached 9.0 or higher, the contents of each flask was centrifuged, filtered, and dried in an oven overnight. The HCl bubble test was conducted on the dried samples to determine if $CaCO_3$ was present.

A similar experiment was done with microalgae species AQ0052. One liter of culture and medium was removed from the receiver. Initial pH and alkalinity measurements were taken and 200 ml were added to flasks 1 and 2. A volume of 500 ml of the culture was enriched with

6.39 x 10^{-3} moles of Ca^{2+} and stirred until dissolved. Flasks 3 and 4 were filled with 200 ml of this solution and the remaining culture was used for initial pH and alkalinity measurements. Flasks were grown under the same conditions and pH and alkalinity measurements were taken periodically.

Next, a chemostat system was established with a culture of cyanobacteria AQ0012. The chemostat was inoculated with AQ0012 and allowed to grow in FW 413 media between a pH of 7.4 and 7.6. Media for the chemostat was made by adding 0.134 moles Ca^{2+} to 8.5 L of filtered freshwater and sterilized. Alkalinity and pH measurements were taken from the calcium enriched media, and the chemostat. Once the culture had grown for 4 days, addition of media and removal of the culture from the chemostat began at a flow rate of 1.97 ml min^{-1}. The pH of the chemostat was then set to a range of 8.4 to 8.6. No pH control was given to the receiver that accumulated the culture. Alkalinity and pH measurements were taken daily from both the chemostat and receiver cultures.

A small experiment was also done to attempt to induce the precipitation of $CaCO_3$ by exceeding the saturation point of CO_3^{-2} in a medium. A flask with 155 ml of deionized water was enriched with 2.10 x 10^{-3} moles of Ca^{2+}. The medium was then bubbled for approximately ten minutes with CO_2 and was visually examined for precipitate formation. Initial and final pH measurements were also taken.

An experiment was conducted to observe how ion concentrations change with increases in pH and with the addition of CO_2 to the medium. A solution of $NaHCO_3$ was made by adding 3.57 moles of $NaHCO_3$ to 8 L of filtered fresh water. Alkalinity and pH measurements were then taken from the solution. Also, a Ca^{2+} solution was prepared by adding 4.10x10^{-2} moles of Ca^{2+} to 3.1 L of filtered fresh water. Three large flasks were filled with 2 L of $NaHCO_3$ solution and .8 L of Ca2+ solution. A 170 ml sample was taken after the solution was well mixed. With a pH probe submerged in each flask, a solution of NaOH was added to the flasks while stirring to raise the pH to 9.0. After the pH of each flask reached 9.0, a 170 ml sample was taken. Next, CO_2 was bubbled into the solution while stirring. When the pH of the flasks reached 8.5, 8.0, and 7.5, 170 ml samples were taken. The pH of flask 1 was decreased to 7.2 and a 170 ml sample was also taken. The samples were centrifuged and the pellets dried on pre-weighed aluminum weigh boats. From this data, the weight of the solid $CaCO_3$ was determined. The supernatant of each centrifuged sample was used for pH and alkalinity measurements.

This experiment was repeated in the same manner using only two flasks. During this experiment, the visible $CaCO_3$ precipitate was removed before the addition of CO_2. Alkalinity and pH measurements were taken from samples collected at each pH (9.0, 8.5, 8.0, and 7.5). This experiment was repeated again, but standard freshwater 413 media was used instead of filtered freshwater enriched with $NaHCO_3$. Two flasks were filled with 2 L of 413 media and 0.8 L of a calcium solution made by adding 0.112 moles of Ca^{2+} to 9.0 L of freshwater 413 media. The initial pH and alkalinity of each flask was measured before and after the addition of Ca^{2+}. NaOH was added to each flask until a pH of 9.0 was reached and a 170 ml sample was taken. The precipitate was not removed from the flasks. CO_2 was bubbled into the solution while stirring and 170 ml samples were taken at pH levels of 8.5, 8.0, 7.5, and 7.0. The samples were centrifuged and the pellets were dried on pre-weighed aluminum weigh boats to determine

the amount of $CaCO_3$ precipitate. The supernatant of each centrifuged sample was used for pH and alkalinity measurements.

Another series of experiments were conducted similar to the above method using AQ0008 and AQ0012 cultures to increase the pH of the medium instead of the addition of NaOH. Two large flasks were filled with 2 L of either AQ0008 or AQ0012 culture. The AQ0008 culture was obtained from an outdoor commercial photobioreactor (Olaizola, 2000), where the AQ0012 culture was again obtained from the chemostat system. A solution of FW 413 and Ca2+ was made by adding 1.16 x 10^{-2} moles of Ca^{2+} to 1 L of freshwater 413, and 500 ml of the solution was added to each flask. Alkalinity and pH measurements were taken of each culture before and after the addition of the Ca^{2+} solution. The flasks were then exposed to light for 14 hr in order for photosynthesis to increase the pH. Samples were taken after the pH of each flask reached 9.0 or higher. CO_2 was then bubbled into the culture while stirring and two 170 ml samples were taken at a pH of 9.0, 8.5, 7.5, and 6.5. The samples were centrifuged and the pellets were dried on pre-weighed aluminum weigh boats. The supernatant of each centrifuged sample was used for pH and alkalinity measurements.

Results

Initial experimentation with species AQ0011 gave no visual indication that $CaCO_3$ precipitation via algal mediation could occur under the conditions tested with these species. Flasks lacking bicarbonate did not increase in biomass and did not produce data indicative of $CaCO_3$ formation (Figures 38 and 39). This was possibly due to the intolerance of algae to the low pH of the 413 media caused by lack of HCO_3^-. After examining the ion concentrations of the media enriched with bicarbonate, it was apparent that a portion of carbon was missing from the calcium-enriched medium (Figures 40 and 41). A greater decrease in total inorganic carbon concentration, along with a decreased amount of dissolved carbonate ions in the calcium-enriched flask with bicarbonate indicates the possible formation of $CaCO_3$ (Figure 41). However, this was not visually confirmed through identification of $CaCO_3$ particles or through a reaction of the filtrate with concentrated HCl.

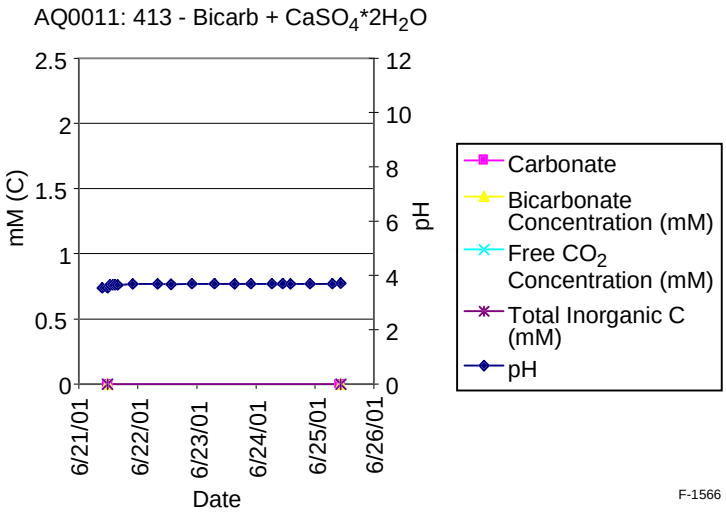

Figure 38. pH and dissolved inorganic carbon species in AQ0011 without HCO_3^- and with Ca.

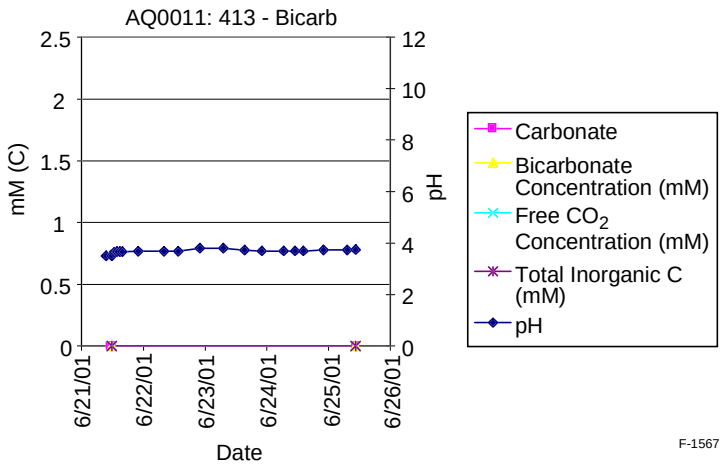

Figure 39. pH and dissolved inorganic carbon species in AQ0011 without HCO_3^-

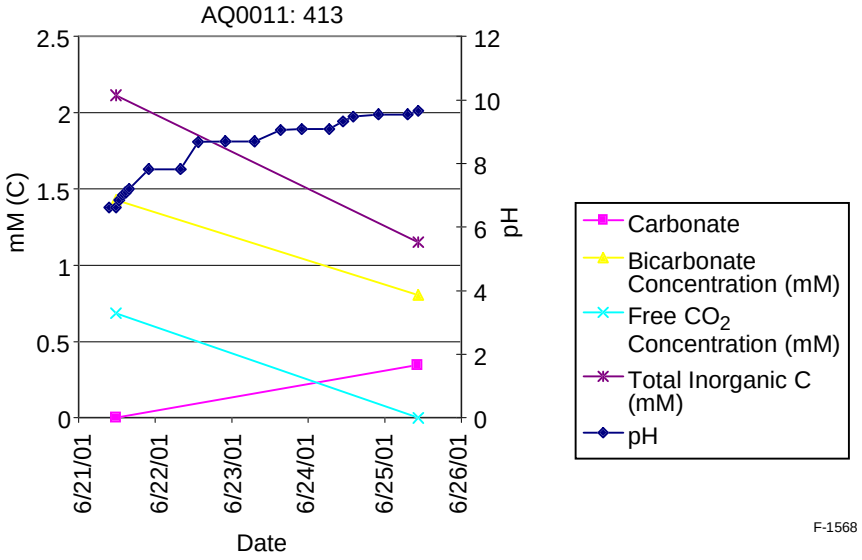

Figure 40. pH and dissolved inorganic carbon species concentrations in AQ0011 with FW413.

A second experiment with AQ0011 demonstrated similar ion concentrations, although the second experiment displays a clearer depiction of differences in ion concentrations in control and experimental flasks (Figures 42 and 43). The solid was identified as $CaCO_3$ due to the identification of a reaction occurring after the addition of concentrated HCl. Results from the second experiment with AQ0011 were more conclusive because of the method of testing for CaCO3 precipitate. In the initial experiment, the contents of each flask were filtered and exposed to HCl while still damp. Centrifuging and drying of the pellets from each flask better prepared the samples for the "bubble test" with concentrated HCl. In both experiments, it can be recognized that as the pH of the medium increases, the carbon species shift towards CO_3^{-2}. In flasks with Ca, the CO_3^{-2} concentrations are significantly lower than those not enriched with calcium, indicating the binding of CO_3^{-2} ions with the Ca^{2+} ions in solution and ultimately the

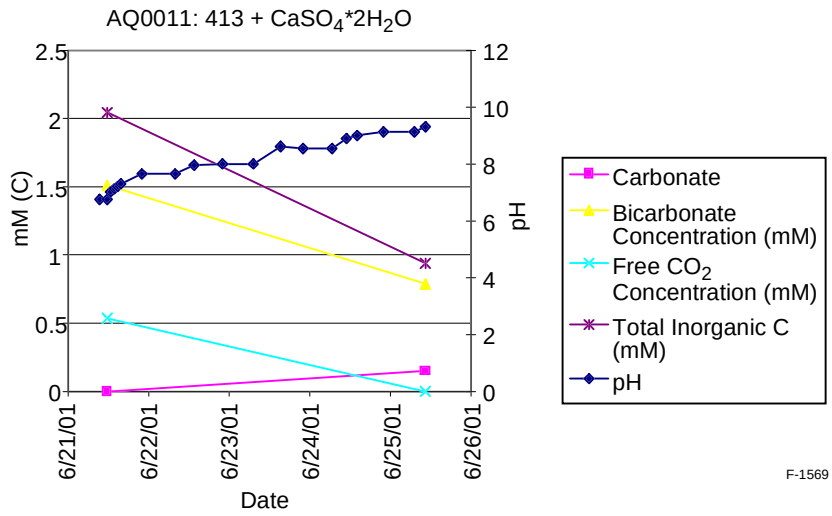

Figure 41. pH and dissolved inorganic carbon species concentrations in AQ0011 in FW 413 + Ca.

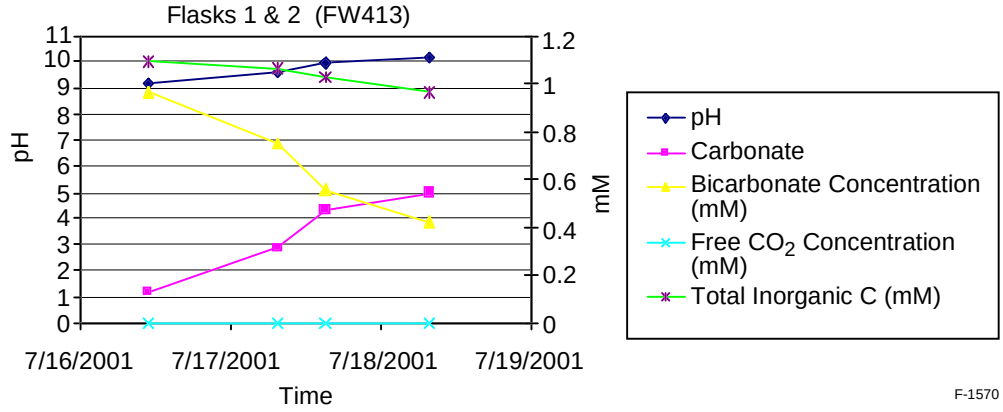

Figure 42. pH and dissolved inorganic carbon species concentrations in AQ0011 exp. 2 in FW 413, average of 2 flasks.

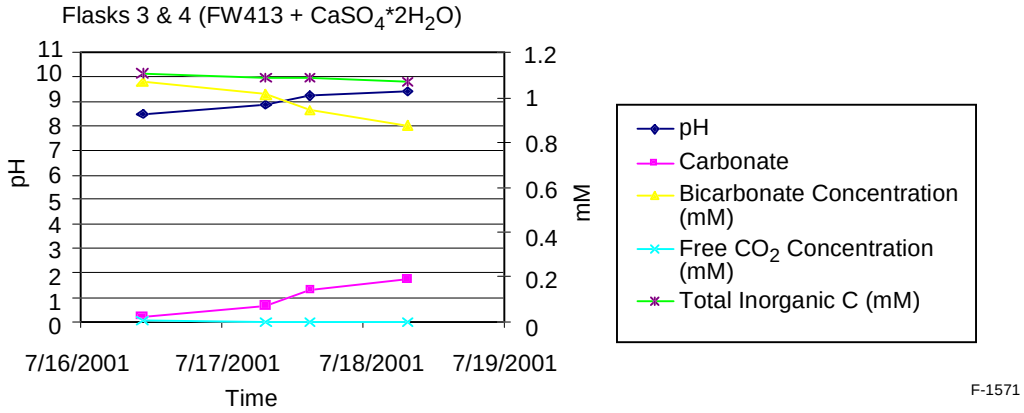

Figure 43. pH and dissolved inorganic carbon species concentrations in AQ0011 exp. 2 in FW 413 + Ca, average of 2 flasks.

formation and precipitation of $CaCO_3$. The precipitate was not visually apparent due to the biomass of algae in the flasks and the small particle size of the $CaCO_3$ crystals. This dust-like form of $CaCO_3$ is similar to that identified as the source of Bahamas whiting incidents where biologically induced precipitates cloud surface waters (Robbins and Yates 2001).

The microalgae AQ0052 did not increase significantly in biomass from the beginning to the end of experiment. The culture did not photosynthesize enough to raise the pH of the medium to a level where $CaCO_3$ could possibly precipitate. Also, the ion concentrations did not differ between experimental and control flasks (Figures 44 and 45) indicating that carbon had not been removed from the system. It has not been determined why AQ0052 did not increase in biomass, but a low initial biomass may have been the cause for the lack of rapid growth.

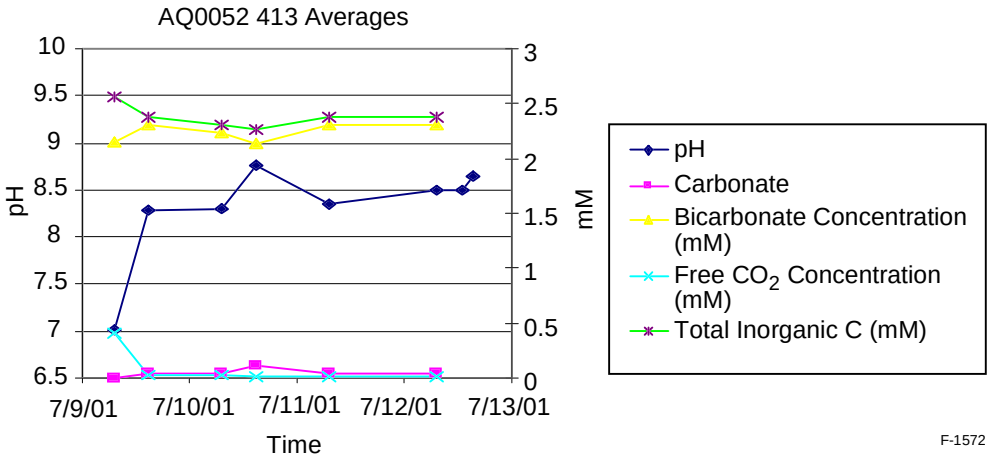

Figure 44. pH and dissolved inorganic carbon species concentrations in AQ0052 in FW 413, average of 2 flasks.

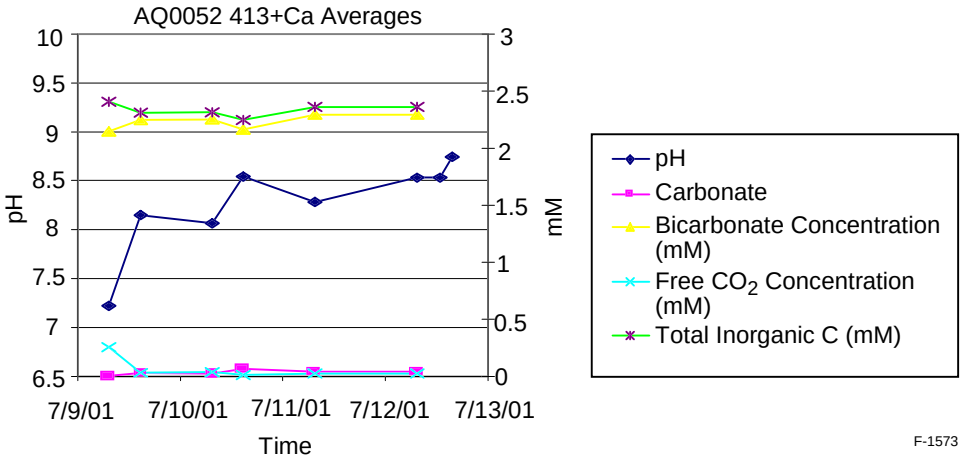

Figure 45. pH and dissolved inorganic carbon species concentrations in AQ0052 in FW 413 + Ca, average of 2 flasks.

Experimentation with cyanobacteria species AQ0012 also yielded promising results. The initial experiment resulted in white particles observed in suspension among the biomass. Dissolved carbon concentrations were found to decrease throughout the experiment in the experimental flasks containing calcium (Figures 46 and 47). In addition, the total dissolved inorganic carbon at the end of the experiment was lower in the experimental flasks (Figures 46 and 47). This indicates that carbon has been successfully removed from the system, once again suggesting the formation of solid $CaCO_3$. Data from the first experiment using species AQ0012 demonstrated a difference in initial bicarbonate ion concentration of the medium. The experiment was therefore repeated to ensure equal initial ion concentrations. A greater biomass was used to ensure a more rapid increase in pH in order to quickly induce the precipitation of $CaCO_3$. Ion concentrations from the second AQ0012 experiment demonstrate decreased total inorganic carbon, HCO_3^-, and CO_3^{-2} concentrations at the end of the experiment compared to the control flasks (Figures 49 and 49). The white amorphous particles found within the culture of both

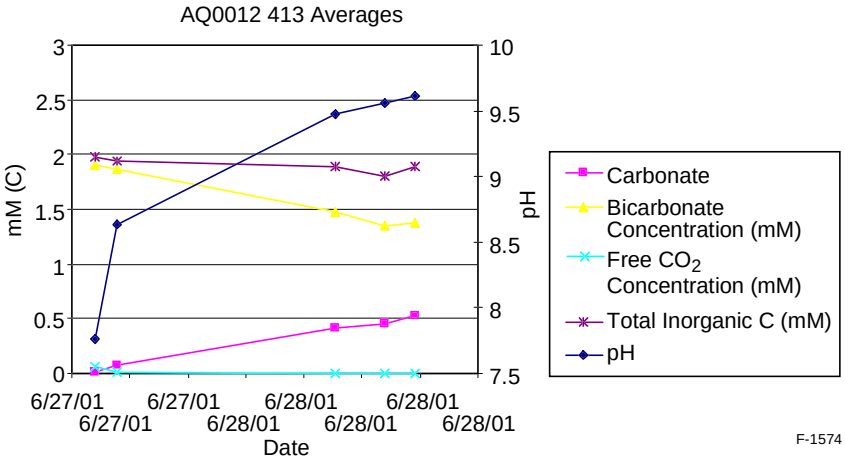

Figure 46. pH and dissolved inorganic carbon species concentrations in AQ0012 in FW 413 + Ca, average of 2 flasks.

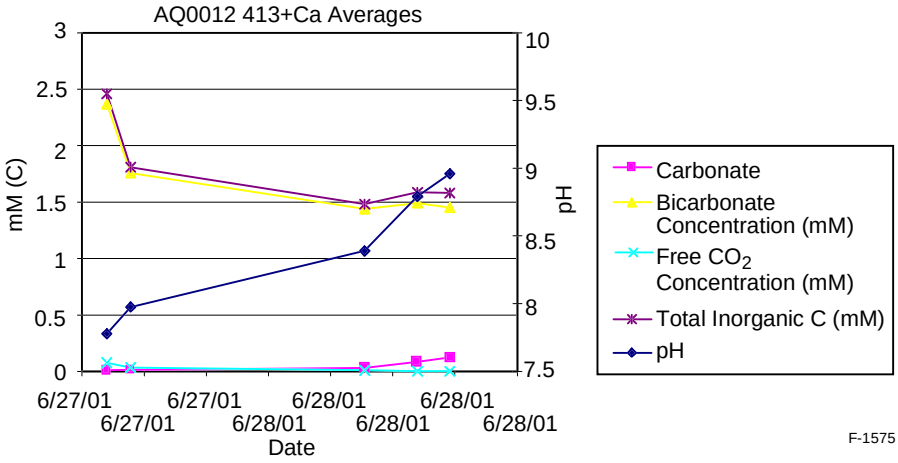

Figure 47. pH and dissolved inorganic carbon species concentrations in AQ0012 in FW 413 + Ca, average of 2 flasks.

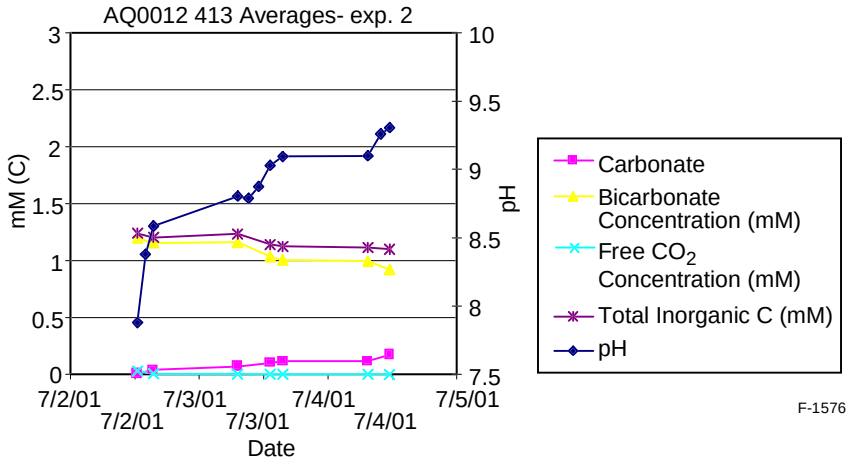

Figure 48. pH and dissolved inorganic carbon species concentrations in AQ0012 2nd exp. FW 413 + Ca, average of 2 flasks.

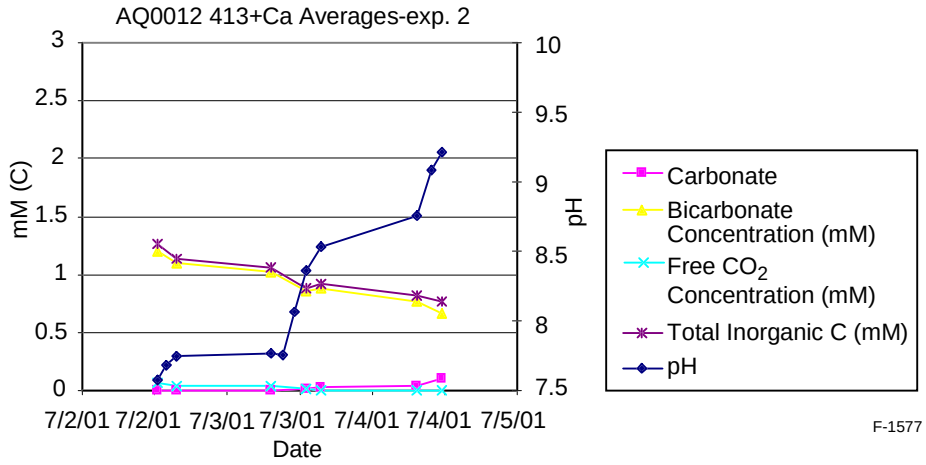

Figure 49. pH and dissolved inorganic carbon species concentrations in AQ0012 2nd exp. FW 413 + Ca, average of 2 flasks.

experiments were determined to be calcium carbonate after being tested for a reaction with concentrated HCl. It is not known why larger particulate $CaCO_3$ was formed in experiments using a cyanobacteria. Prior examination of calcification in cyanobacteria by Merz-Preiss (2000) shows that under certain conditions, filaments of the organism can become encrusted with $CaCO_3$. However, upon examination of the culture, particulate $CaCO_3$ was not encrusted on the cells of the organism (Figure 50), however were abundant in close proximity with clumps of the algal filaments.

No significant data could be obtained from the small-scale experiment where CO_2 was dissolved into a solution of deionized, calcium-enriched water. No precipitate was formed after the pH of the solution had been decreased from 6.07 to 4.17.

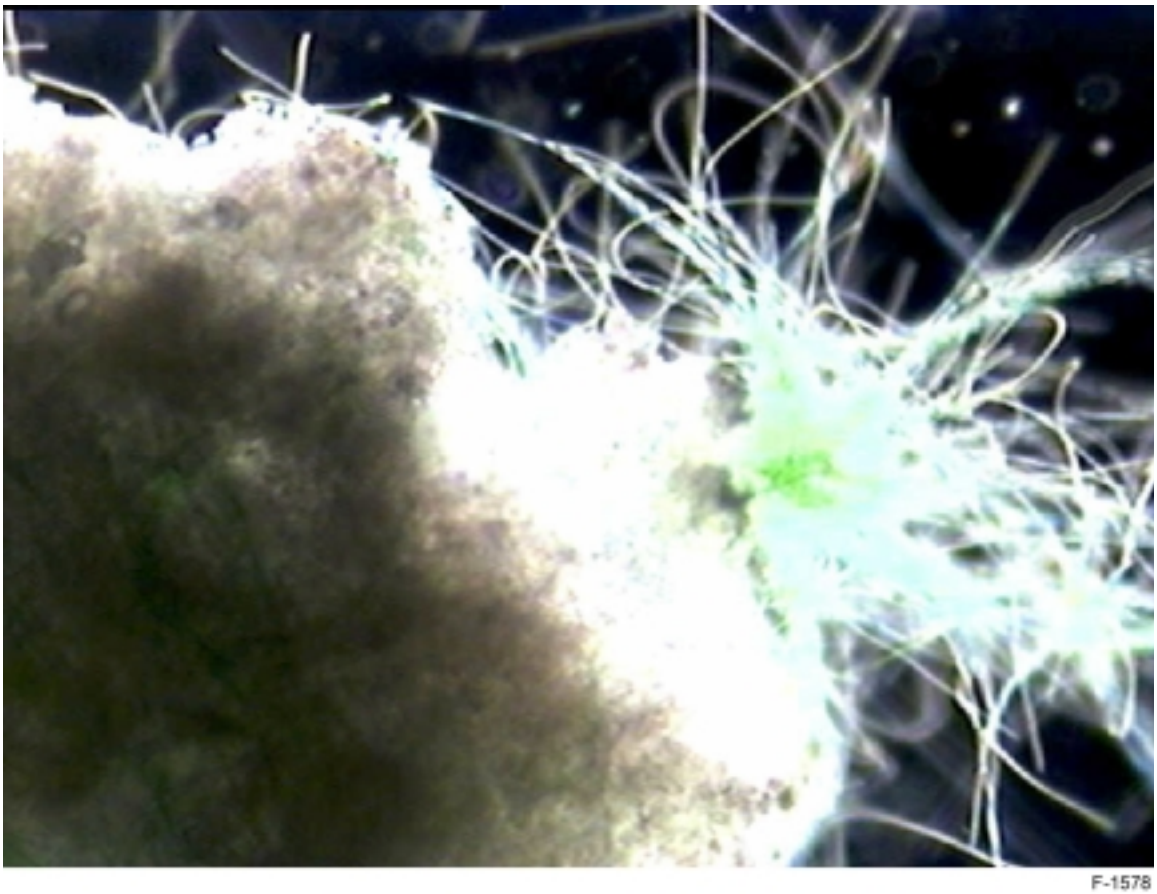

Figure 50. Photomicrograph of a clump of AQ0012 culture. The green filaments are the alga itself. The large white mass is precipitated $CaCO_3$. Although the $CaCO_3$ mass is closely associated with the algal filaments it doesn't seem to crust the filaments.

Experimentation with the chemical precipitation of $CaCO_3$ demonstrated that a medium could precipitate $CaCO_3$ after the addition of Ca to the solution, which had an initial pH above 8.0. Increasing the pH of the medium may have also increased $CaCO_3$ production however the system may have been Ca limited due to characteristics of the Ca species used. This precipitation would occur as a series of reactions are driven by the increase in pH through removal of H^+ ions. Through these reactions, HCO_3^- ions are driven to CO_3^{-2} ions (Figure 51). In the presence of high calcium concentration, $CaCO_3$ can then be produced. Also, it was determined that adding CO_2 to the system drives the reactions to produce more HCO_3^- ions, essentially replenishing the medium and allowing for the eventual production of more $CaCO_3$. These findings are significant because they demonstrate that CO_2 added to the system is captured through the formation of $CaCO_3$. By comparing the results of the experiment where solid precipitate has been removed (Figure 52) with the initial findings (Figure 51), it has been determined that the addition of CO_2 to the pH levels tested does not cause the re-dissolution of $CaCO_3$, indicating that this process can be used to continuously and permanently sequester CO_2 added to the system. In addition, the experiment using fresh water 413 media produced results similar to the first chemical precipitation experiment. However, amounts of each ion in solution were greatly decreased due to the initial concentrations of the ions in the media (Figure 53).

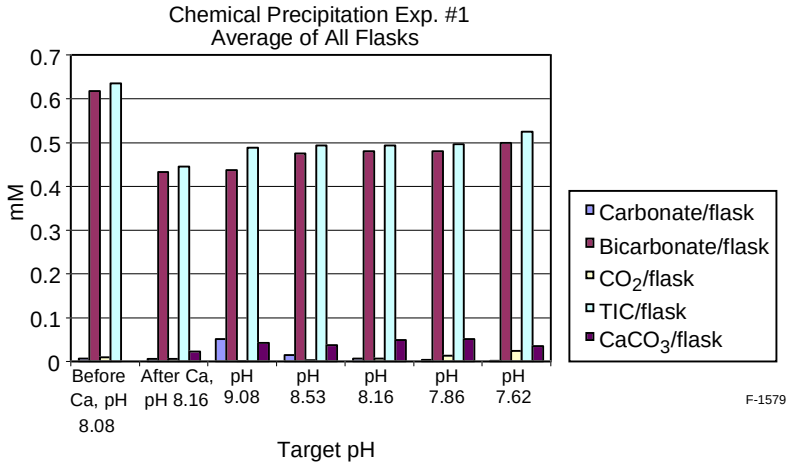

Figure 51. pH and dissolved inorganic carbon species concentrations.

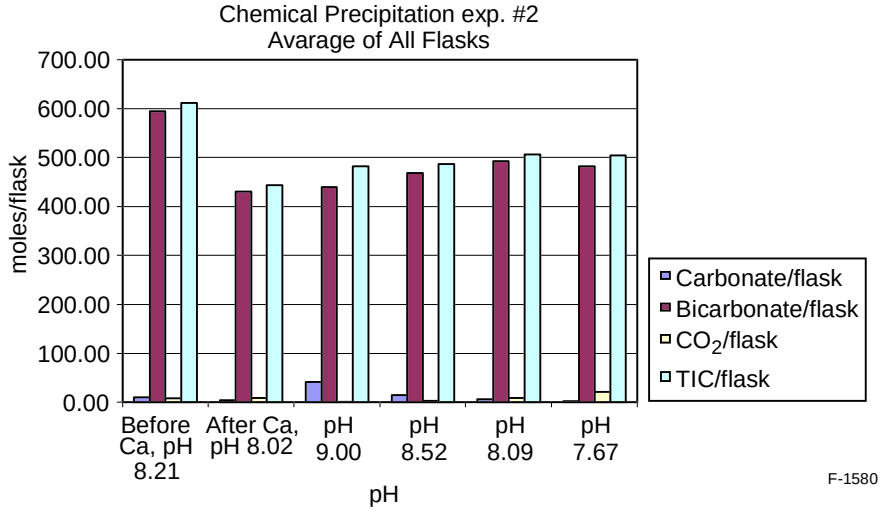

Figure 52. pH and dissolved inorganic carbon species concentrations after solid $CaCO_3$ is removed.

The experiments conducted using both AQ0012 and AQ0008 to increase the pH of the medium yielded similar trends. The initial ion and total inorganic carbon concentrations of the AQ0008 culture were greater than those found in the AQ0012 culture media. Regardless of the initial difference, however, both cultures displayed a decrease in HCO_3^- and an increase in CO_3^{-2} concentrations as the pH of the media was biologically increased (Figures 54 and 55). Likewise, both culture mediums were replenished with HCO_3^- ions after the addition of CO_2 to each flask. According to Libes (1992) HCO_3^- ions are still dominant when compared to CO_3^{-2} at a pH of approximately 9.0. It is not until an approximate pH of 10.0 is reached when the majority of HCO_3^- ions have been converted to CO_3^{-2}. Further experimentation will examine the chemistry of the medium when the pH is biologically driven to levels exceeding 9.5 to 10.0. This will determine if more CO_3^{-2} ions can be produced, resulting in an eventual increase in the quantities of $CaCO_3$ produced assuming a constant supply of calcium ions.

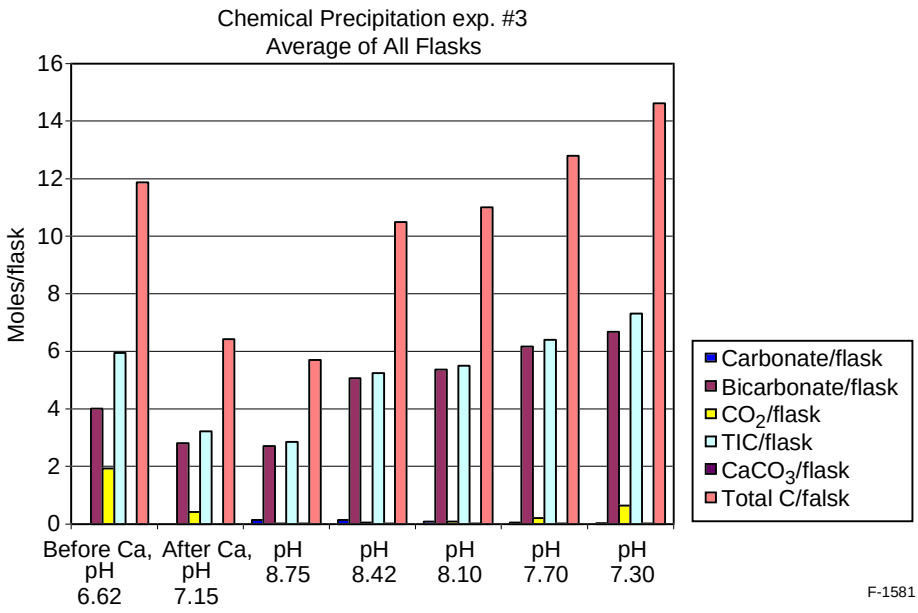

Figure 53. pH and dissolved inorganic carbon species in FW 413 media + Ca^{2+}.

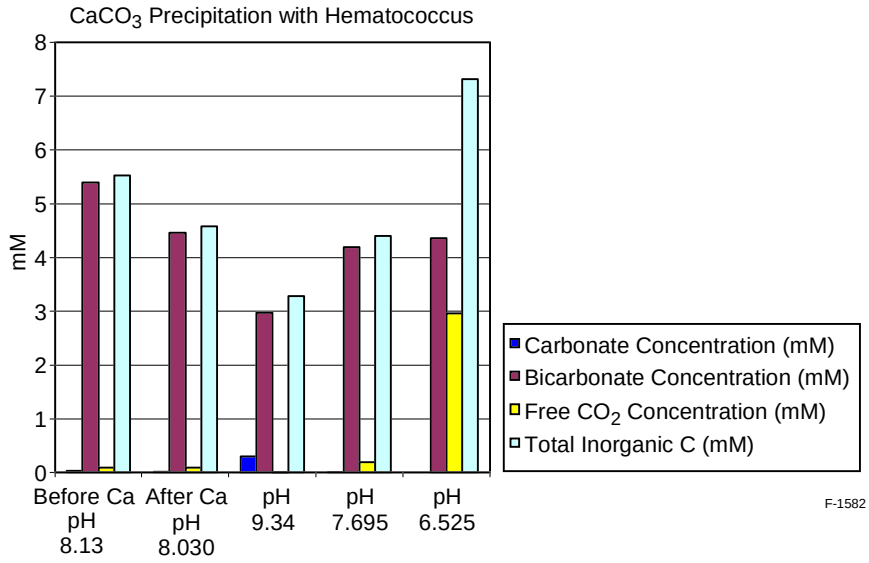

Figure 54. pH and dissolved inorganic carbon species with AQ0008 culture + Ca^{2+}.

The chemostat experiment yielded results similar to the flask experiments in that it produced visible particulate $CaCO_3$ in both the chemostat and the receiver at pH values above approximately 8.3. In addition, examination of the ion concentrations in the receiver, which received no pH control via CO_2 addition, reveals that once again HCO_3^- concentrations decreased and CO_3^{-2} increased as the pH increased (Figures 56 and 57). The increase in CO_3^{-2} ion concentration allowed for the production of $CaCO_3$ in the both the chemostat and receiver, but

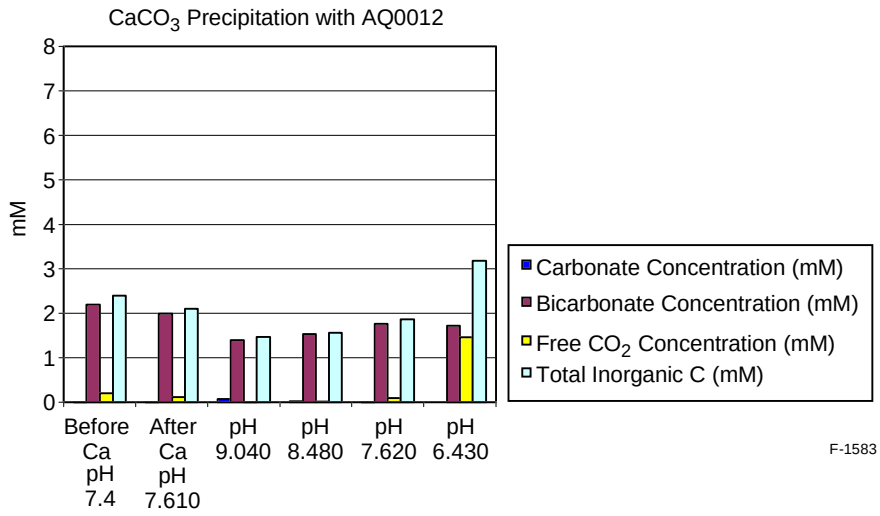

Figure 55. pH and dissolved inorganic carbon species with AQ0012 culture + Ca^{2+}.

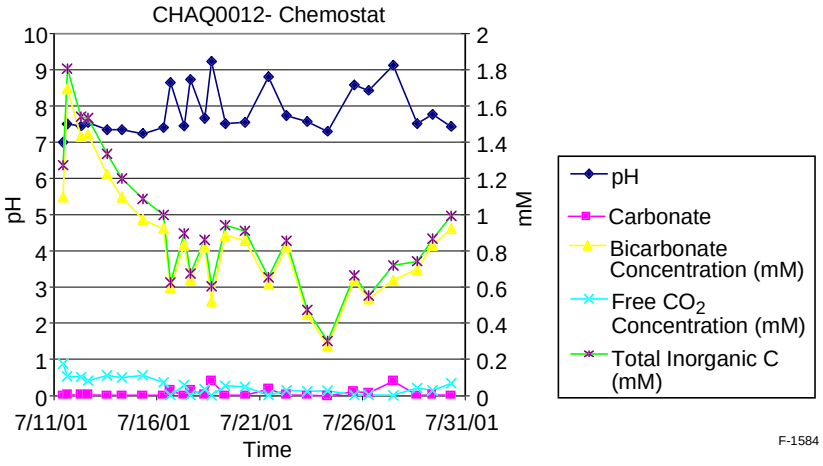

Figure 56. pH and dissolved inorganic carbon species with AQ0012 culture + Ca^{2+} in chemostat.

higher concentrations in the receiver explain why more precipitate was formed in this vessel. This data demonstrates that it is possible to have a continuous system where CO_2 is bubbled and replenishes the medium with HCO_3^- and a constant supply of Ca^{2+} is available to bind with CO_3^{-2} ions when the pH increases to a point where CO_3^{-2} is dominant.

From an industrial perspective, this process has the possibility of decreasing carbon emissions that lead to global warming. However, this process must be cost effective in order to promote the energy production industry to utilize its potential. In the past few decades, microalgae have been grown for the production of valuable byproducts of certain physiological characteristics. Some carotenoid pigments produced by algae have been identified as valuable antioxidants and present many health benefits (see section on high value products). Some are presently utilized in the nutraceutical industry. This byproduct of microalgal growth will help to

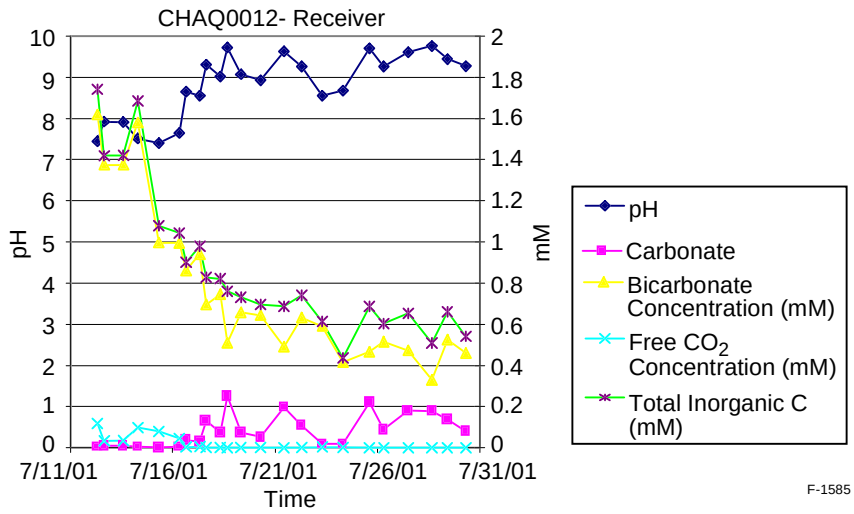

Figure 57. pH and dissolved inorganic carbon species with AQ0012 culture + Ca^{2+} in receiver.

offset the cost of implementing the algal mediated sequestration of carbon. As the sequestration method requires a calcium supply, a relatively inexpensive source is $CaSO_4*2H_2O$, or gypsum. Deposits of this mineral are abundant throughout the world and it is readily available for use in agriculture as well as other venues. The preceding experiments were all conducted using gypsum as the calcium source, and this mineral has proven successful in its ability to supply calcium to an algal medium. The use of this mineral does have limited potential, however, due to its relatively low solubility. $CaSO_4*2H_2O$ is less soluble than other species of Ca, and therefore limits the number of moles of Ca available for binding with free CO_3^{-2} ions in the experiments. Another Ca source could be used to provide more Ca^{2+} ions to a medium, however some more reactive and soluble species require energy to produce. This in essence defeats the purpose when viewed on a global perspective because CO_2 is released during energy production. Another species of Ca, more soluble than $CaSO_4*2H_2O$ that requires no energy to produce would be a better alternative, however more research must be done to determine the most suitable Ca species.

In conclusion, microalgae and cyanobacteria can be used to induce the precipitation of $CaCO_3$ from a Ca-enriched medium. This process can be used to reduce the amounts of CO_2 degassed from industrial fossil fuel combustion, reducing the large amounts of anthropogenic CO_2 contributed to the global carbon cycle each year. More information is necessary to successfully establish an industrial scale carbon sequestration system, but the research presented demonstrates the feasibility of this method. In conjunction with high value product generation, this process can prove to be affordable to industry and environmentally beneficial.

3.3 Task 4. Carbon Sequestration System Design

To evaluate the potential for application of photosynthetic sequestration of CO_2 to industrial- scale combustion systems, we will conduct a system-level design study. The purposes of this study are as follows: to identify design concepts for components and the integrated system of the proposed concept; to optimize and evaluate performance of the components and the

system; to develop deployment methodologies; and to 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.

According to the Project schedule we submitted at the kick off meeting (see Figure 58), Task 4 was to start in February of 2001. University of Hawaii, which needs preparation for the system analysis was scheduled to start their task (Task 4.2). This was to have ample time for system analysis and reflect preliminary results in the program execution. Due to paperwork it took much longer. UH was finally put under contract and is gearing up the activity. PSI has been working on similar programs using solar energy. It is ready to start working on the program.

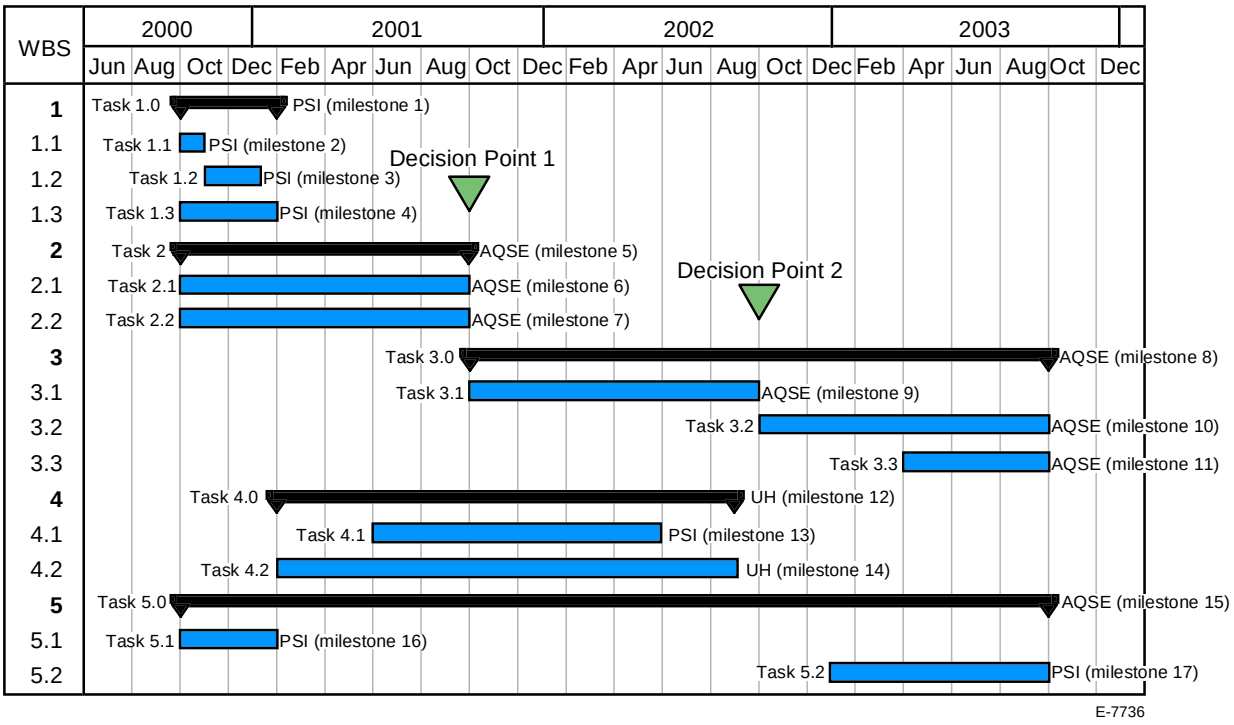

Figure 58. Project schedule submitted at the kick-off meeting.

3.3.1 Task 4.1: Component Design and Development

In this subtask we will develop design concepts for each of the key components of the industrial scale photosynthetic sequestration of CO_2. Key components to be designed include: CO_2 removal process; CO_2 injection device; photobioreactor; product algae separation process; and process control devices. As the proposed system depends on the solar energy to photo-synthetically convert CO_2 to products compounds, optimization of the photobioreactor is an important part of this task. The authors of this proposal are aware of the vast amount of work conducted in solar energy R&D programs sponsored by DOE and other agencies. Results of those programs will be utilized to optimize the design concept of the industrial scale field

deployable photobioreactor. Laboratory scale experiments will be conducted at PSI and at Aquasearch to determine the optimum design concept for the photobioreactor.

In the reporting period, initial work on Task 4.1 has begun. PSI believes that a key to the success of this program is how to make efficient use of solar energy. One option is to use hybrid solar power generation. Initial work to this direction has been made. In addition, effort was made to get access to the publications available in Japanese R&D community on the similar programs in Japan. Dr. Nakamura is planning a trip in July to visit NEDO to study the documents.

3.3.2 Task 4.2: System Integration

We will develop a process model of the integrated system and conduct parametric studies. These studies will provide quantitative insight into process performance; identify potential problems and limitations of the system; and facilitate design optimization. Results from Tasks 1 through 4.1 will be utilized to develop the operational profiles of the flue gas production module (i.e., power station combustor); the gas treatment module comprising cooling, clean-up, and CO_2 separation components; and the photobioreactor module. The modular nature of the Aquasearch photobioreactor minimizes uncertainties related to scale-up. These simulation modules and applicable property and performance databases will be integrated into a system utilizing the ASPEN PLUS process simulation software by the University of Hawaii.

Process simulations will be performed for conventional coal-fired and gas turbine power stations and natural gas boilers. These scenarios represent a broad range of gas compositions, for both CO_2 and pollutant species. Flue gas production rates (which scale with electricity generating capacity) will be varied over at least an order of magnitude. The concentrations of particulates and gas phase contaminants also will be varied within limits established by Task 1.

The parametric simulations will be analyzed to assess the suitability of photosynthetic sequestration to a range of industrial fossil fuel combustors and operating scenarios (such as variations in output in response to changes in load). System performance penalties associated with increased exhaust backpressure, parasitic loads, etc. arising from the CO_2 clean-up components will be quantified. These results will be applied to optimize component and system design.

Technical progress during the present reporting period was limited, since the subcontract agreement was executed during the final month of the quarter.

Visit to Microalgae Production Operations

In anticipation of final execution of the subject subcontract, Dr. Charles Kinoshita, HNEI, visited Aquasearch and Cyanotech, two commercial microalgae production companies at the Natural Energy Laboratory of Hawaii Authority (NELHA), Keahole, Hawaii, to view commercial microalgae production and to hold preliminary discussions on the proposed project with our collaborator at Aquasearch, Dr. Miguel Olaizola. Now that the subcontract is finally executed, we plan to return to Aquasearch to hold more detailed discussions on the project and to better define process design parameters.

Personnel Mobilization

Mr. Simon Tsang has been offered and has accepted a graduate research assistantship in the Biosystems Engineering M.S. program (effective, August 2001) with funding from the subject contract. Mr. Tsang, a recent graduate of the Biosystems Engineering undergraduate program, has formal training in bioreactor design and testing, and unit operations. He will perform engineering modeling of biological (microalgae) uptake of CO_2 from industrial-scale combustion systems and is capable of performing photobioreactor experiments, if necessary.

In preparation for ASPEN modeling of carbon dioxide sequestration, in May, 2001, HNEI renewed its site license for ASPEN Plus and Charles Kinoshita participated in advanced training on "Physical Properties in Modeling Products," at the ASPEN Training Center in Cambridge, Massachusetts. Both were undertaken using funding outside of the present contract with PSI.

CO_2 Supply, Uptake, and Sequestration System Design

Work has been initiated to lay-out a preliminary process diagram of CO_2 production, uptake, and sequestration to identify critical experimental and other data that need to be collected, alternative photobioreactor designs, and carbon sequestration options.

A search of the technical literature for publications on CO_2 uptake by microalgae was begun at the end of June 2001.

4. SUMMARY AND FUTURE PLANS

Task 2 - Selection of Microalgae

Up to this point in time we have

- tested 41 different species of microalgae for growth at three different temperatures (15, 20, and 25°C),
- analyzed 15 different species for high value pigments,
- tested 4 different strains (3 species) for pH shift tolerance in chemostat cultures,
- started testing 6 species at the chemostat level for carbon uptake rate,
- started testing 3 different species for carbon sequestration potential into carbonates for long-term storage of carbon and we have
- designed and purchased the components necessary for carbon sequestration potential experiments utilizing five different simulated flue gases.

Our preliminary results indicate that

- out of 42 species of microalgae tested so far 39 grew at 15, 20 and 25 °C (two species did not grow at 15 °C and one species did not grow at 25°C),
- out of the species analyzed so far for high value pigment content two appear to be a good source of the carotenoid zeaxanthin, two appear to be good sources of the carotenoid lutein, 7 appear to be good sources of phycocyanin and 4 appear to be good sources of phycoerythrin,
- out of 4 strain tested only one seemed to be negatively affected by changes in culture pH,
- estimates of carbon uptake based on pH changes of the culture medium for our chemostat system match very closely the estimates of carbon uptake by cultures grown in commercial outdoor photobioreactors,
- changes in culture pH caused by microalgal photosynthesis appear to be conducive to the precipitation of $CaCO_3$ from the growth medium, and
- based on preliminary analysis of our first chemostat-grown cultures, the estimated efficiency of CO_2 captured by the chemostat culture systems is several fold higher than our benchmark, a commercial microalgal facility, indicating a large potential for optimization of gas capture by industrial size outdoor photobioreactors.

Within the next quarter we expect to

- continue to carry out temperature experiments (up to 30 and 35 °C),
- carry out further carbon sequestration experiments in chemostat cultures,
- carry out simulated flue gas experiments in chemostats, and
- obtain 10 more microalgal strains known to produce high value products or mineralize carbon.

One of our sources for the microalgal strains will be the University of Hawaii. In January 2001 Aquasearch executed an agreement to develop pharmaceuticals from a unique collection of approximately 2,000 strains of microalgae held by the University of Hawaii. The agreement gives Aquasearch exclusive rights to more than 100 unique, bioactive compounds from microalgae contained in the collection. Two of the compounds are now in Phase II clinical trials for treatment of cancer. Pharmaceuticals derived from microalgae are expected to be worth 1 to 10 million per kg, which would more than offset the cost of CO_2 sequestration utilizing microalgae.

Task 4 - Carbon Sequestration System Design

In the reporting period, initial work on Task 4.1 has begun. PSI believes that a key to the success of this program is how to make efficient use of solar energy. One option is to use hybrid solar power generation. Initial work to this direction has been made. In addition, effort was made to get access to the publications available in Japanese R&D community on the similar programs in Japan. Dr. Nakamura is planning a trip in July to visit NEDO to study the documents.

In Task 4.2 area, the subcontract agreement between Physical Sciences, Inc. (PSI) and the University of Hawaii was fully executed in June 2001. During the present reporting period (04/01/01 – 06/30/01) the following technical activities were pursued:

1. Dr. Charles Kinoshita, HNEI, visited Aquasearch and Cyanotech, two commercial microalgae production companies at the Natural Energy Laboratory of Hawaii Authority (NELHA), Keahole, Hawaii, to view commercial microalgae production and to hold preliminary discussions on the proposed project with our collaborator at Aquasearch, Dr. Miguel Olaizola.

2. A Graduate Research Assistant (GRA) in the Biosystems Engineering Department has been engaged to work on this project. The GRA will perform engineering modeling of biological (microalgae) uptake of CO_2 from industrial-scale combustion systems and is capable of performing photobioreactor experiments.

3. To prepare for the process modeling, HNEI renewed its site license for ASPEN Plus and Charles Kinoshita participated in advanced training on "Physical Properties in Modeling Products," at the ASPEN Training Center in Cambridge, Massachusetts.

4. Work has been initiated to lay-out a preliminary process diagram of CO_2 production, uptake, and sequestration.

5. A search of the technical literature for publications on CO_2 uptake by microalgae was begun.

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