Recovery and Sequestration of CO₂ from Stationary Combustion Systems by Photosynthesis of Microalgae

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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 CO₂ from stationary combustion systems by photosynthesis of microalgae. The research is aimed primarily at demonstrating the ability of selected species of microalgae to effectively fix carbon from typical power plant exhaust gases.

This report covers the reporting period 1 October to 31 December 2002 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 feasibility demonstration of direct feeding of coal combustion gas to microalgae. Aquasearch continued their effort on selection and characterization of microalgae suitable for CO₂ sequestration. University of Hawaii continued effort on system optimization of the CO₂ sequestration system.

Table of Contents

<u>Section</u>	<u>Page</u>
Abstract	1
1. Introduction	6
2. Executive Summary	8
3. Experimental	11
4. Results and Discussion	12
4.1 Task 1: Supply of CO ₂ from Power Plant Gas to Photobioreactor	13
4.2 Task 2: Selection of Microalgae	13
4.3 Task 3: Optimization and Demonstration of Industrial Scale Photobioreactor	13
4.4 Task 4: Carbon Sequestration System Design	24
4.5 Task 5: Economic Analysis	29
5. Conclusion and Future Plans	32
5.1 Task 3: Optimization and Demonstration of Industrial Scale Photobioreactor	32
5.2 Task 4: Carbon Sequestration System Design	33
5.3 Task 5: Economic Analysis	33
6. References	34

LIST OF APPENDICES

A	- Abstracts of Presentations Supported, in Part, by this Research Project	A-1
B	- Published Manuscript by Mazzone, Guentzel and Olaizola, 2002, in MarSci	B-1
C	- Published Manuscript by Olaizola, 2003, in Biomolecular Bioengineering	C-1

List of Figures

<u>Figure</u>	<u>Page</u>
1. Recovery and sequestration of CO ₂ from stationary combustion systems by photosynthesis of microalgae.....	8
2. Coal combustion gas for photobioreactor	14
3. Initial biomass levels for three species in pilot scale MGMs used to estimate the average maximal growth rate	15
4. Changes in pH in a 20 liter carboy <i>Haematococcus</i> culture showing rapid raise in pH during daylight hours during four consecutive days.....	16
5. Concentration of the different forms of inorganic carbon in the culture, medium, and particulates	17
6. Changes in pH in a 20 liter carboy <i>Haematococcus</i> culture showing rapid rise in pH during daylight hours during three consecutive days	18
7. Changes in TAlk over 3 days of growth for a culture of <i>Haematococcus</i> not supplemented with CO ₂	18
8. Changes in bicarbonate ion concentration over 3 days of growth as it relates to the change in pH for a culture of <i>Haematococcus</i> not supplemented with CO ₂	18
9. Changes in carbonate ion concentration over 3 days of growth as it relates to the change in pH for a culture of <i>Haematococcus</i> not supplemented with CO ₂	19
10. Results obtained in pilot scale outdoor pond experiments showing that addition of CaSO ₄ *2H ₂ O to the medium did not result in a lowering of productivity	19
11. Schematic of the PSI coal reactor system	20
12. Coal reactor system at PSI coal laboratory	21
13. Coal combustion gas diagnostics	22
14. IMR 400 gas dryer main box	22
15. IMR5000 gas analyzer main box	23
16. Carbon dioxide injected into the media versus average daytime pH.....	25
17. Standing biomass versus carbon dioxide injected into the media.....	26

List of Figures (Continued)

<u>Figure</u>	<u>Page</u>
18. Biomass productivity versus standing biomass	26
19. Total organic carbon of MGM samples	28
20 Mass flows of the different materials necessary to run Mera's present production plant in Kona, Hawaii, for the production of astaxanthin from <i>Haematococcus</i>	32
21 Conceptual design of an expanded microalgal facility for the production of astaxanthin from <i>Haematococcus</i>	33

List of Tables

<u>Table</u>	<u>Page</u>
1 Current Status of Each Work Scope	10
2 Specifications of the PSI Coal Reactor System	20
3 Typical Flue Gas Compositions for Coal Combustion Systems and the Range of the Diatnotica Instruments Procured	21
4 <i>Haematococcus Pluvialis</i> Samples	27
5 Total Organic Carbon Content.....	28
6 Surface Area for the Plant.....	30
7 Plant Parameters.....	30

1. Introduction

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

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

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

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

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

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

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

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

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

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

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

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.

2. Executive Summary

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

- Completed characterization of power plant exhaust gas;
- Identified a number of CO₂ separation processes;
- Analyzed 34 different strains for high value pigments;
- Determined the productivity parameters for over 20 different algae with 5 different simulated flue gases;
- Tested the compatibility of over 20 microalgal species with 5 different simulated flue gases;

- Tested three different strains for carbon sequestration potential into carbonates for long-term storage of carbon;
- Successfully carried out scale up of three microalgal strains to the 2000 liter outdoor photobioreactors;
- Carried out preliminary work on biomass separation for two microalgal strains grown in 2000 liter outdoor photobioreactors;
- Conducted work on designing key components including: CO₂ removal process; CO₂ injection device; photobioreactor; product algae separation process; and process control devices;
- Identified a design concept for photobioreactor incorporating the method for full utilization of solar energy;
- Conducted preparation of the PSI coal reactor to be used with the Aquasearch 2000 liter outdoor photobioreactor for direct feeding of coal combustion gas to microalgae;
- Prepared and shipped the diagnostic instrumentation for characterization of coal combustion gas to Aquasearch Inc.;
- Shared the ASPEN model has been with UH, PSI and Aquasearch for review and discussion;
- UH research staff visited Aquasearch and worked on-site for one week to gather information on the performance of the photobioreactor;
- Photobioreactor data from Aquasearch were analyzed and simple linear relationships for biomass productivity as a function of solar irradiance and CO₂ were developed using multiple regression;
- A review of the technical literature on tubular photobioreactors progressed;
- A literature study progressed to develop the CO₂ flue gas separation subsystem model for both Aspen Plus and Excel models;
- Conducted CO₂ mineralization study for Haematococcus in laboratory and later in open-pond experiment;
- Initiated economic analysis for photobioreactor carbon fixation process.

In Table 1, current status of each work scope is summarized.

Table 1. Current Status of Each Work Scope

Tasks	Title	% Complete	Milestone/Status Description
Task 1.0	Supply of CO ₂ from Power Plant Flue Gas	85%	Overall status for Tasks 1.1 through 1.3.
Task 1.1	Power Plant Exhaust Characterization	100%	Most of pertinent exhaust gases were analyzed.
Task 1.2	Selection of CO ₂ Separation and Clean-up Technologies	95%	MEA method identified. Direct injection of exhaust gas into water may be an option.
Task 1.3	Carbon Dissolution Method	75%	Analytical study completed. Direct exhaust gas injection may be studied per our Task 3 outcome.
Task 2.0	Selection of Microalgae	100%	Selection of 6 species out of initial 20.
Subtask 2.1	Characterization of Physiology, Metabolism and Requirements of Microalgae	100%	Test compatibility of 20 species with 5 flue gases.
Subtask 2.2	Achievable Photosynthetic Rates	100%	Productivity parameters of 20 species with 5 flue gases.
Task 3.0	Optimization and Demonstration of Industrial Scale Photobioreactor	15%	Demonstrate viability of CO ₂ with algae at industrial scale.
Subtask 3.1	Pilot Evaluation	15%	Evaluation at 2000 L pilot scale. Experimental work with coal reactor to be made.
Subtask 3.2	Full Scale Production Runs	0%	Evaluation at 24,000 L industrial scale.
Subtask 3.3	Algae Separation and Final Product	15%	Evaluation of biomass separation.
Task 4.0	Carbon Sequestration System Design	50%	Incorporating new system concept.
Task 4.1	Component Design and Development	50%	New concept being incorporated.
Task 4.2	System Integration and Simulation Analysis	50%	Analyses of new system concept to be made.
Task 5.0	Economic Analysis	5%	Economic analysis of commercial microalgal CO ₂ sequestration
Task 5.1	Gas Separation Process	85%	Direct exhaust gas injection option to be assessed.
Subtask 5.2	Photobioreactor Carbon Fixation Process	5%	Economic analysis of photobioreactor CO ₂ fixation
Subtask 5.3	Product Processing	0%	Economic analysis of product processing

The work discussed in this report covers the reporting period from 1 October to 31 December 2002.

3. Experimental

Description of the experimental methods employed for the work conducted during the reporting period is given below.

Fluorescence-based Biomass and Growth Estimates in Photobioreactors

Microalgae cultures were grown in Mera's enclosed photobioreactors (Quarterly Report #1) as per Mera's standard operating procedures (Olaizola 2000). 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:

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

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.

Dissolved Inorganic Carbon Analysis

A standard titration method was used to estimate alkalinity throughout all experiments (Clesceri et al. 1995). In short, acid (HCl) is added to a sample of known volume with a pH probe submerged until an endpoint of pH 4.3 was reached. Normality of the acid, volume of acid used, volume of the sample, and initial pH of the sample were noted and used in a series of equations (Clesceri et al. 1995) in order to determine total alkalinity, CO_3^{2-} ion, HCO_3^- ion, free CO_2 , and total dissolved inorganic carbon concentrations (DIC). The samples were analyzed before and after centrifugation. Centrifugation of the samples eliminates all particulates from the sample including any precipitated calcium carbonate. The difference in DIC values (before and after centrifugation) thus provides an estimate of the amount of carbon that may have precipitated.

Pond Experiments

In this quarterly period we report on a set of experiments carried out in open photobioreactors (open ponds as opposed to enclosed MGMs). These experiments were designed to test whether addition of $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$, which we have shown in previous reports to enhance calcium carbonate precipitation might have deleterious effect on the productivity of the microalgal cultures. Open ponds were used simply for convenience. The ponds were managed as per Mera's standard operating procedures (Olaizola 2000). Three open ponds were used for this experiment. All three were inoculated with 0.7 kg dry weight biomass into a total volume of 23,000 liters. The first pond was treated as the control, the pond had 5 kg of NaHCO_3 added for pH stability as per our standard operating procedures in the production of astaxanthin from *Haematococcus*. A

second pond had 4.82 kg of $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ added (plus 5 kg of NaHCO_3) to help induce precipitation of CaCO_3 and a third pond had 4.82 kg of $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ (but no NaHCO_3) to test the effects of this mineral, if any, on the productivity of the system. The ponds were allowed to grow for 6 days. The pH was automatically controlled (at 7.5) by additions of CO_2 in response to changes in pH. At the end of the growth period, the ponds were harvested and the particulates (biomass and any precipitated carbonates) were centrifuged, dried and weighted.

Algae Separation

A significant cost of microalgal production is the separation of the produced biomass (and captured carbon) from the growth medium. For each microalgal strain, we intent to determine the level of effort necessary to effect the separation of the biomass.

For this work we used a pilot-scale industrial centrifuge (Clinton Separators, Model # 9021). The centrifugation of the algal biomass is carried out by placing a volume of culture in a feed tank about two feet above the centrifuge. The material is fed by gravity into the centrifuge and the amount of feed (liters per minute) adjusted via the feed tank's drain valve. The optimum rate of flow into the centrifuge is arrived to empirically. It is expected that cells of different physical characteristics (density, size, shape) will present different settling rates and, thus, will affect the capacity of the centrifuge (and, thus, the cost of the separation). We adjust, and note, the flow rate into the centrifuge to the point where less than 10% (estimated optically) of the feed biomass appears in the outflow (i.e., >90% capture of biomass).

4. Results and Discussion

The work accomplished during this reporting period reflects the directives we received from the DOE technical contract representative (COTR) as a result of our first annual progress review meeting in February 2002. The DOE directives, as communicated to PSI in a letter dated May 2, 2002, are summarized as follows:

- An adequate amount of screening for the most promising algal species to be used in CO_2 biofixation/sequestration and in the production of value-added products has been accomplished;
- A concentration of effort on a few of the most promising species of microalgae shall be made;
- Test the most promising algal species with simulated flue gas in bioreactors while varying the appropriate parameters such as pH, temperature, etc.;
- Testing actual flue gas from coal-fired power plants on the most promising algal species should follow this effort, as synthetic flue gas tends not to reflect all of the conditions encountered in actual flue gas from power plants fired with various types of fuels;

- Because of NETL's interest in biofixing/sequestering CO₂ from coal-fired electrical power generating plants, it is imperative that this project demonstrate the effectiveness of various microalgae for removing CO₂ from flue gas from coal-fired power plants and not from oil or natural gas fired power plants; and
- Flue gas from coal-fired power plants should be used on the most promising microalgae in a type of photobioreactor that would allow testing realistically the maximum amount of algal biomass for CO₂ removal.

Our work during this reporting period is to prepare to achieve objectives in compliance with those clear directives. Work accomplished in this reporting period is summarized according to the task structure of the program.

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

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

4.2 Task 2: Selection of Microalgae

Almost all work in this task was completed in the last reporting period.

4.3 Task 3: Optimization and Demonstration of Industrial Scale Photobioreactor

The main goal of this task is to demonstrate the feasibility and to quantify the performance of microalgae for biofixation/sequestration of CO₂ at a commercially significant scale. This will be done in two phases. First, we will conduct a pilot evaluation using 2,000 liter enclosed photobioreactors (pilot scale MGM, Task 3.1) and, second, we will conduct full scale production runs using 24,000 liter enclosed photobioreactors (full scale MGM, Task 3.2). Concurrently, research into the appropriate technologies for harvesting and processing the produced biomass will be conducted (Task 3.3).

We recognize that it is imperative that this project demonstrate the effectiveness of various microalgae for removing CO₂ from the flue gas from coal-fired electrical power generating power plants. To fully implement this objective, it is necessary to conduct a series of tests using actual coal combustion gas. Synthetic flue gas tends not to reflect all of the conditions encountered in actual flue gas from power plants fired with various types of fuels. We will accomplish our objectives by means of the following scheme:

1. Employ a coal combustor which can operate with different types of pulverized coal.
2. Use diagnostic instruments to monitor and quantify chemical constituents (CO₂, NO_x, SO_x) of the combustion gas.

3. Feed the coal combustion gas directly to the Aquasearch photobioreactor.

Figure 2 shows the scheme of the project.

Figure 2. Coal combustion gas for photobioreactor.

4.3.1 Task 3.1: Pilot Evaluation

4.3.1.1 Initial Algae Growth in Photobioreactor

In the last quarterly report, we reported initial results on pilot scale cultures of two locally isolated strains (AQ0011 and AQ0012). In this quarter we have carried the first scale-up culture in a third strain, AQ0015, a Cyanobacterium tentatively identified as *Lingbya sp.* Cultures used to inoculate MGMs (Mera Growth Modules, photobioreactors) are grown in chemostats. The biomass produced in the chemostats is then used to inoculate 20 liter carboys. Once the cultures in the carboys reach appropriate density, the biomass is transferred to the MGMs for grow out.

Initial Growth Rates in MGMs

Initially, following inoculation of the MGMs, we can estimate a maximal growth rate from changes in daily biomass estimated from fluorescence measurements (as described in previous reports). This estimated growth rate is considered 'maximal' since during that period in the cultures' life neither light nor nutrients are limiting. As opposed to the previous two microalgal strains AQ0015 grew much slower (Figure 3). The average maximal growth rates over the ramp-up period are 0.88, 0.40 and 0.45 d⁻¹ for strain AQ0011 and 0.57 and 0.31 d⁻¹ for strain AQ0012 cultures respectively which are comparable to the growth rates obtained during the initial ramp-up in the chemostat cultures for these strains (as reported in the 6th quarterly report). However, the initial measured growth rate for AQ0015 was only 0.14 d⁻¹. We do not have accurate growth rates of this strain at small scale with which to compare. AQ0015 presents a filamentous morphology and grows in large clumps (up to several centimeters across). Because of this morphology it was essentially impossible to obtain accurate estimates of biomass using the fluorescence technique at small scale.

Figure 3. Initial biomass levels for three species in pilot scale MGMs used to estimate the average maximal growth rate.

Potential for Carbon Sequestration in MGMs

As reported in the previous report (8th quarterly), our calculations of CO₂ consumption by the MGM cultures indicate consumption rates (i.e., photosynthetic rates) similar to those obtained in chemostat cultures. However, from the limited data available thus far it is clear that the capturing efficiency of dissolved CO₂ in the MGMs is lower than the capturing efficiency in chemostat cultures kept at the same pH. In this quarter we have conducted a further pilot experiment utilizing the strain AQ0015. Unfortunately, a glitch in our database management software caused us to lose the historical pH data for that culture which made it impossible for us to estimate the rate of consumption of CO₂. We intend to repeat this pilot scale experiment in the near future.

Previously, we have argued that the differences found in CO₂ capturing efficiency between chemostat and MGM cultures is caused by the design of the system since the MGMs are dependent on airlifts to provide turbulence. The large amount of air used in the airlift is expected to strip dissolved CO₂ from the culture medium. It is expected that changes in the design of the MGMs may result in increased capturing efficiency of dissolved CO₂. These results suggest that changes in cultivation strategy (e.g., raising the pH of the culture) would increase the efficiency of the MGMs. These questions will be taken up during the last phase of the project as they will impact the final design of a microalgal-based CO₂-sequestration facility (Task 5).

CO₂ Mineralization

The objectives of this project is develop microalgal cultures that not only sequester CO₂ into the biomass and produce high value products but also are able to mineralize CO₂ into carbon solids, such as carbonates, that can be disposed of for long term storage. Previously, we reported that altering cultures conditions (raising pH via photosynthetic CO₂ uptake by the microalgae) and addition of calcium sulfate (gypsum) to the culture medium induced the precipitation of CaCO₃, a stable form of carbon that could be used for long term sequestration, apparently

independently of the microalga used. These results were presented in the 4th Quarterly report and have also been published (Mazzone et al. 2002, Appendix B).

In this quarter, we have continued this work including scale up of the cultures to 20 to 20,000 liter scale. The first experiment was designed to test what pH a 20-liter *Haematococcus* culture would reach by allowing the cells to photosynthesize without CO₂ supplementation. A culture was placed in a 20 liter carboy under natural light conditions (sun light) and allow to grow over 4 days. The culture was mixed by continuously bubbling air through the culture. The results are summarized in Figure 4. It can be seen that the pH of the culture rose rapidly within the first few hours (between 10:30 and 15:00 on the first day of culture) from 7.2 to 10.5, assumedly caused by photosynthetic CO₂ uptake. Overnight, the pH decreased to 8.4 due to respiration and possibly to the small amount of CO₂ present in atmospheric air used for mixing in the carboy. The second day, the pH of the culture rose to 10.5 by 12:30. On the third and fourth days, the pH rose only to 10.1 and 9.8 respectively. The smaller increases in pH can be attributed to loss of culture vitality probably caused by carbon limitation.

Figure 4. Changes in pH in a 20 liter carboy *Haematococcus* culture showing rapid rise in pH during daylight hours during four consecutive days. Also, cell counts on the same culture showing increasing biomass during the second and third day but a decrease in the fourth day.

On the last day of the experiment, measurements of alkalinity were also conducted (Clesceri et al., 1995) to determine the concentrations of dissolved inorganic carbon in the culture. The culture was tested before and after centrifugation. By centrifuging the culture we are able to eliminate all particulates, including cells and any possible carbon that may have precipitated as carbonates. Thus, the values measured before centrifugation provide us with total inorganic carbon content in the culture, the values measured after centrifugation provide us with inorganic carbon content in the dissolved fraction, and the difference between the two is assumed to represent the amount of inorganic carbon in the particulate fraction. The results are presented in Figure 5 and show that a significant fraction of the carbon had apparently precipitated (i.e., present in the particulate fraction).

Figure 5. Concentration of the different forms of inorganic carbon in the culture, medium, and particulates (= culture-medium). Note that at the high pH reached (> 9.5) there is virtually no free CO₂.

A second experiment was conducted on a 20 liter carboy culture which was also not supplemented with CO₂ for 3 days. In this experiment, inorganic carbon species were measured several times each day. The pH in this culture also rose caused by photosynthetic CO₂ uptake by the microalgal cells (Figure 6) as was the case in the previous experiment (Figure 4). The measurements of alkalinity of the culture indicated that as the pH of the culture rose the total alkalinity (TAlk) changed little for the culture but the value of TAlk in the dissolved fraction decreased while the value in the particulate fraction increased (Figure 7). Similarly, analysis of the inorganic carbon species indicates that as the pH of the culture rose, a larger fraction of the inorganic carbon was in the form of carbonate (at the expense of bicarbonate) and a larger fraction of carbonate was present in the particulate fraction (Figures 8 and 9).

The objectives of this research effort are to develop microalgal cultures that not only sequester CO₂ into the biomass and produce high value products but also are able to mineralize CO₂ into carbon solids that can be disposed of for long term such as carbonates. A possible concern of such an approach is that, as culture conditions are manipulated to induce carbonate precipitation, productivity of the desired high value product may be negatively affected. To test this, a third experiment was conducted at pilot scale in outdoor reactors. For simplicity, this experiment was conducted in open ponds of 23,000 liter capacity. Open pond systems are used at Mera to induce carotenogenesis and astaxanthin accumulation in *Haematococcus*.

Three open ponds were used for this experiment. All three were inoculated with 0.7 kg dry weight biomass into a total volume of 23,000 liters. The first pond was treated as the control, the pond had 5 kg of NaHCO₃ added for pH stability as per our standard operating procedures in the production of astaxanthin from *Haematococcus*. A second pond had 4.82 kg of CaSO₄ * 2H₂O added (plus 5 kg of NaHCO₃) to help induce precipitation of CaCO₃ and a third pond had 4.82 kg of CaSO₄ * 2H₂O (but no NaHCO₃) to test the effects of this mineral, if any, on the productivity of the system. The ponds were allowed to grow for 6 days. The pH was automatically controlled (at 7.5) by additions of CO₂ in response to changes in pH. At the end of

Figure 6. Changes in pH in a 20 liter carboy *Haematococcus* culture showing rapid rise in pH during daylight hours during three consecutive days.

Figure 7. Changes in TAlk (total alkalinity) over 3 days of growth for a culture of *Haematococcus* not supplemented with CO₂. Note that although the alkalinity in the culture (medium plus particulate fraction) is relatively stable, the proportion of TAlk in the medium and particulate fractions are inversely proportional.

Figure 8. Changes in bicarbonate ion concentration over 3 days of growth as it relates to the change in pH for a culture of *Haematococcus* not supplemented with CO₂. Note that as the pH increases, the concentration of bicarbonate decrease in the culture and medium fraction but not in the particulate fraction.

Figure 9. Changes in carbonate ion concentration over 3 days of growth as it relates to the change in pH for a culture of *Haematococcus* not supplemented with CO₂. Note that as the pH increases, the concentration of carbonate increases in the culture and particulate fraction.

the growth period, the ponds were harvested and the particulates (biomass and any precipitated carbonates) were centrifuged, dried and weighted.

The results of this experiment are summarized in Figure 10 and show that the ponds receiving CaSO₄*2H₂O actually performed better (more astaxanthin produced) than the control pond. This means that, at least for this process, addition of CaSO₄*2H₂O does not have any deleterious effects on high value chemical productivity. Also, the total mass of particulates harvested (Figure 10) was higher in the ponds receiving the CaSO₄*2H₂O treatments indicating, possibly, higher content of precipitated carbonates. We will be analyzing the particulates harvested and testing them for presence of carbonates in the next quarter.

Figure 10. Results obtained in pilot scale outdoor pond experiments showing that addition of CaSO₄*2H₂O to the medium did not result in a lowering of productivity (astaxanthin or total weight of mass harvested).

4.3.1.2 Preparation for Coal Combustion Gas Generator

In this reporting period we have continued preparation for PSI coal reactor as discussed in the previous quarterly reports. Schematic representation of the coal reactor is given in Figure 11. Specification of the reactor is given in Table 2.

Figure 11. Schematic of the PSI coal reactor system.

Table 2. Specifications of the PSI Coal Reactor System

Total gas flow rate:	~ 1 scfm
Primary air:	~ 0.8 scfm
Feeder air:	~ 0.2 scfm
Preheat temperature:	up to 550°C
Coal feed:	1 ~ 10 gram/min; 4 gram/min recommended

To date all key components were tested and integrated into the system. Several new components have been ordered. Figure 12 shows the coal reactor being tested at PSI coal laboratory. We have completed all the tests and the system is ready for shipping to Aquasearch.

Figure 12. Coal reactor system at PSI coal laboratory.

4.3.1.3 Coal Combustion Gas Diagnostics

PSI procured instruments to measure the composition of the coal combustion gas: CO₂, NO_x; and SO_x. Table 3 below shows the measurement range for the diagnostic instruments.

Table 3. Typical Flue Gas Compositions for Coal Combustion Systems and the Range of the Diagnostics Instruments Procured

	Bituminous Coal	Sub-Bituminous Coal	Combustion Gas Diagnostics Measurement Range
CO ₂	12.7%	15.1%	0 ~ 100%
H ₂ O	5.0%	12.2%	
O ₂	6.0%	6.0%	
N ₂	76.9%	71.0%	
SO ₂ [ppm]	50-500	300-500	0 ~ 4000
NO _x [ppm]	50-500	50-500	0 ~ 500

The locations of the gas composition measurement are shown in Figure 13.

Figure 13. Coal combustion gas diagnostics.

The diagnostic instruments for determining concentration of CO₂, NO_x and SO₂ (IMR 400 Gas Dryer and IMR5000 Gas Analyzer in Figures 14 and 15) were tested at PSI and shipped to Aquasearch in November 2002

Figure 14. IMR 400 gas dryer main box.

Figure 15. IMR5000 gas analyzer main box.

4.3.2 Task 3.2: Full Scale Production Run

This task is scheduled after Task 3.1.

4.3.3 Task 3.3: Algae Separation and Final Product

During this quarter we have carried out a third separation of algal biomass produced in 2000 L MGMs. We have harvested a 2000 L volume of a pilot scale culture of strain AQ0015 utilizing pilot scale industrial centrifuges (Clinton Separators, Model # 9021). The centrifugation of the algal biomass is carried out by placing the volume of culture in a feed tank about two feet above the centrifuge. The material is fed by gravity and the amount of feed (liters per minute) adjusted via the feed tank's drain valve.

The optimum rate of flow into the centrifuge is arrived to empirically. It is expected that cells of different physical characteristics (density, size, shape) will present different settling rates and, thus, will affect the capacity of the centrifuge. We adjust the flow into the centrifuge to the point where less than 10% (estimated optically) of the feed biomass appears in the outflow (i.e., >90% capture of biomass). Our previous results with strain AQ0011 a small (about 4 to 6 μm diameter unialgal strain) and AQ0012 (multicellular, filamentous strain) resulted in flow rates of 1.5 and 1.8 liters per minute respectively. Strain AQ0015 is a filamentous Cyanobacterium that flocculates into large particles (several mm to cm in size) which makes it very easy to harvest by centrifugation. We found that the flow rate of the product into the centrifuge was as high as 7 liters per minute which would translate into lower costs than the previously tested strains.

We will continue conducting harvest experiments on the 2000 L MGM pilot scale cultures during the following quarters.

4.4 Task 4: Carbon Sequestration System Design

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

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

This task consists of two sub-tasks: Task 4.1: Component Design and Development, and Task 4.2: System Integration.

4.4.1 Task 4.1: Component Design and Development

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

In the reporting period, no significant progress was made in this task as PSI focused its effort on the coal reactor and the diagnostic system.

4.4.2 Task 4.2: System Integration

4.4.2.1 Analyses of Aquasearch Data

The integrated process model being developed by UH requires submodels that accurately represent the behavior of key components, notably, the photobioreactor and the CO₂ flue gas separation system. In order to obtain data on the performance characteristics of the Aquasearch photobioreactor, UH personnel visited the Aquasearch facility in July 2002. The work continued on the data obtained since the visit. A description of technical activities conducted by UH during this reporting period is provided below.

Analysis of Photobioreactor Operational Data

Mera Pharmaceuticals, Inc. (formerly Aquasearch, Inc.) provided operational data on pH levels, CO₂ injection, and *Haematococcus pluvialis* sampling and harvesting, for three of its 25,000 L photobioreactors identified as M13A-020317, M13A-020421, and M13A-020603. M13A-020317, M13A-020421, and M13A-020603 were operated respectively from March 17, 2002 to April 12, 2002; from April 21, 2002 to May 25, 2002; and from June 3, 2002 to June 25, 2002. Data were collected during these periods; however, data for the first day of operation were not included in the data sets.

The pH data comprised pH levels in the media that were measured at 5 min intervals. The CO₂ data included the durations and the flow rate of the periodic CO₂ injections. Sampling times and the *Haematococcus pluvialis* cell counts for three replications were recorded, as well as harvest times and the corresponding volumes of microalgae and media.

The volume of CO₂ injected into the media versus average daytime pH; standing biomass as a function of the amount of CO₂ injected into the media; and biomass productivity versus standing biomass are shown in Figures 16, 17, and 18, respectively. These data correspond to the periods prior to the first harvesting of the *Haematococcus pluvialis*.

Figure 16 shows that the relationship between the average daytime pH of the media and the amount of injected CO₂. Photosynthetic activity during the day consumes CO₂ and results in increasing levels of media pH. The CO₂ injection system is designed to respond to rising pH: more CO₂ is injected into the media when average daytime pH is high.

Figure 17 shows the increase in cell count in the photobioreactors as a function of the amount of injected CO₂. The CO₂ is metabolized to produce new cells via photosynthesis. Figure 18 indicates the observed scaling between biomass productivity and the number of cells in the population.

Figure 16. Carbon dioxide injected into the media versus average daytime pH.

Figure 17. Standing biomass versus carbon dioxide injected into the media.

Figure 18. Biomass productivity versus standing biomass.

Carbon Mass Balance

A carbon mass balance for the Mera Growth Module (MGM) was performed to determine the inputs and outputs of the system. Inputs to the system were CO₂ gas injected into the media and the sodium bicarbonate that was in the media. The outputs of the system were carbon contained in the cell biomass and sodium bicarbonate in the media. Sodium bicarbonate

is a solution buffer and is not consumed. Sodium bicarbonate levels in the media are not anticipated to change over time. Hence, injected CO₂ comprised the MGM carbon input stream and the output was the carbon in cell biomass and outgassed CO₂.

Carbon Sequestration Efficiency

Inputs and outputs from the carbon mass balance were used to determine the carbon sequestration efficiency of the MGM. Carbon sequestration efficiency was defined as the percentage of carbon injected into the media as CO₂ that was metabolized and bound as carbon in the cell biomass pool. The mass of carbon in the biomass was estimated by multiplying the dry weight of cells that were produced between two sampling dates and the percentage of carbon in a cell on a dry weight basis. The total mass of carbon injected as CO₂ was determined from operational data on the volumes of CO₂ injected between the two sampling dates.

In order to estimate the carbon sequestration efficiency, dry samples of *Haematococcus pluvialis* from the MGM collected at different times were requested from Mera Pharmaceuticals, Inc. for analysis. These samples were analyzed for total organic carbon to determine if any significant variations existed in the carbon content of the cells.

Samples of *Haematococcus Pluvialis*

Mera Pharmaceuticals, Inc. provided seven dry samples of *Haematococcus pluvialis* to the University of Hawaii at Manoa that were collected from bioreactors (MGM) and ponds. Samples collected from the MGMs are identified by the prefix “M.” Pond samples are identified by the prefix “P.” Table 4 summarizes the data provided with these samples, including sample identities, sampling dates and times, ages of the MGM and ponds, and dry weights. *Haematococcus pluvialis* cells from the MGM were sampled at different times over 2 days. These samples were analyzed to determine the carbon content of the cells in the MGM to estimate carbon sequestration efficiency. Samples also were provided from four different ponds, that were collected at different times over 2 days.

Table 4. *Haematococcus Pluvialis* Samples

Sample Identity	Date	Time	Age (days)	Dry Weight (g)
M13A-021105	11/12/02	9:00	7	0.4955
M13A-021105	11/12/02	13:00	7	0.7358
M13A-021105	11/13/02	20:00	8	0.9661
P01-021106	11/12/02	10:00	7	0.6634
P03-021110	11/12/02	16:25	3	0.3105
P05-021107	11/13/02	8:00	7	0.5912
P04-021109	11/13/02	11:40	5	0.4896

Sample Analysis and Results

The Agricultural Diagnostic Service Center of the University of Hawaii at Manoa analyzed the seven samples for total organic carbon content on a dry weight basis. Results of the analyses are provided in Table 5. Data for the samples from the MGM that were used to estimate carbon sequestration efficiency are plotted in Figure 19. The figure indicates that the %total organic carbon (TOC) on a dry weight basis of the cells varied only slightly from 47.4% to 48.7%. These values compare well with data in the literature that indicates typical dry weight %TOC of approximately 50% (Becker, 1994). Additional samples from the MGM will be analyzed during the upcoming quarter to obtain a more complete documentation of the variation in %TOC during the day and night cycle. This data set will then be applied to estimate carbon sequestration efficiency. Results will be employed in our ongoing systems modeling effort.

Table 5. Total Organic Carbon Content

Sample Identity	Date	Time	Age (days)	Total Organic Carbon (%)
M13A-021105	11/12/02	9:00	7	48.71
M13A-021105	11/12/02	13:00	7	47.41
M13A-021105	11/13/02	20:00	8	47.48
P01-021106	11/12/02	10:00	7	52.11
P03-021110	11/12/02	16:25	3	46.87
P05-021107	11/13/02	8:00	7	50.88
P04-021109	11/13/02	11:40	5	49.13

Figure 19. Total organic carbon of MGM samples.

4.5 Task 5: Economic Analysis

Our aim in the economic analysis is to identify those components of the carbon sequestration process that have the greatest associated costs, given the design based on current data. Subsequent modeling will explore alternative technologies and procedures that might enable significant reduction in both capital and operating costs.

4.5.1 Task 5.1: Gas Separation Process

Much of work pertaining to this subtasks has been completed in the previous reporting period. We will address this issues again after we complete Tasks 3 and 4.

4.5.2 Task 5.2: Photobioreactor Carbon Fixation Process

During this quarter we have started to put together the economic modeling effort that will result in predicted costs for a microalgal-based carbon sequestration plant. The economic models are driven by scientific/technical variables (e.g., microalgal growth rate) and can be applied to a variety of product scenarios. At present, the models are designed for facility sizes of 5 to 50 ha, and may be changed for application to larger facilities as contemplated in this research effort.

The models also treat a detailed breakdown of operating expenses, capital costs, and human resources, each of which is analyzed with regard to functional subsystems (e.g., water pretreatment, media formulation, photobioreactor operation, product processing, quality control). Finally, the models also include detailed analysis of area requirements, utility usage, and product flows within the production system.

Costs in the Mera/Aquasearch economic models are currently based on historical data for actual costs incurred. One of our key activities in this project will be to research the costs of equipment and supplies at significantly larger scales. All model assumptions will be clearly stated in detail and, where applicable, all model results will comply with international GAAP (Generally Accepted Accounting Principles) standards.

4.5.2.1 Microalgal Plant Design

Our first activity under this subtask includes the design of a microalgal facility of commercially significant scale that would produce a high value product, such as astaxanthin. The design parameters are based on Mera's experience in commercial production and historical data. Thus, this first design effort assumes no optimization concerning efficiency of CO₂ utilization, including gas dissolution into the growth medium or losses due to degassing from the medium.

The design parameters for this plant are shown in Table 6 where the support systems' area includes areas needed for laboratories, nutrient storage, biomass harvesting and processing, utilities, maintenance, CO₂ storage, drive access, pump stations, pipe runs, and office space.

Table 6. Surface area for the plant

Total plant surface area	12 ha
Culture surface area	7 ha
Support systems	5 ha

Assuming no optimization, as stated above, we can also assume the following parameters as shown in Table 7.

Table 7. Plant parameters

Productivity	8 g C m ⁻² d ⁻¹
Efficiency of CO ₂ utilization	12%
Percent of culture area under cultivation	81.6%

Based on historical production data collected at Mera's microalgal facility in Kona, Hawaii, we can estimate that this plant would capture up to about 1.6 tons of CO₂ per day. Again, assuming no optimization in CO₂ utilization efficiency (12% based on our previous reports), the plant would need to be fed by a combustion source generating about 13 tons CO₂ d⁻¹. This is approximately the amount of CO₂ generated when producing 1.7 MW of thermal power by burning bituminous coal. The same plant can be expected to produce about 8 kg of nutraceutical grade astaxanthin d⁻¹, which at a wholesale price of US\$10,000 would generate about US\$2.5 million month⁻¹.

As a first step in the design process, we have specified the mass flows of the different materials necessary to run Mera's actual plant in Kona Hawaii (Figure 20). The plant under consideration is, however, significantly larger (about 30x more capacity) and an updated mass flow diagram will be generated and presented in future reports. Such a plant is depicted in Figure 21. It should be noted that this plant's characteristics are specific to the production of a high value product, astaxanthin, from *Haematococcus*. Thus, the plant utilizes both enclosed photobioreactors (MGM), depicted green in the figure, and open pond systems, depicted red in the figure. As we continue work on this design, we will also generate alternate designs for similar sized plants to produce other types of materials which might utilize only open pond reactors (e.g., *Spirulina*) or only enclosed photobioreactors (e.g., *Nanochloropsis* for lutein production).

Figure 20. Mass flows of the different materials necessary to run Mera's present production plant in Kona, Hawaii, for the production of astaxanthin from *Haematococcus*.

Figure 21. Conceptual design of an expanded microalgal facility (about 30x capacity of Mera's facility) for the production of astaxanthin from *Haematococcus*. Green areas represent cultivation units made up of enclosed photobioreactors. Red areas represent cultivation units made up of open pond systems.

4.5.2.2 Capital and Recurring Costs of Microalgal Plant

Over the next quarter, we will start to put together the capital and recurring costs of a microalgal plant producing astaxanthin from *Haematococcus pluvialis* such as the one depicted in Figure 21. Furthermore, we will carry out similar cost analysis for microalgal plants that produce other products such as *Spirulina* biomass and lutein from *Nannochloropsis*.

5. Conclusion and Future Plans

5.1 Task 3: Optimization and Demonstration of Industrial Scale Photobioreactor

In this quarter we have continued with our scale up efforts, including describing conditions that favor formation of carbonates at larger scale.

Within the next quarter we expect to

- Continue scaling up of promising strains to the 2000 L outdoor photobioreactor scale,

- Test and install gas measuring equipment,
- Install a coal combusting unit (provided by Physical Sciences Inc.) to provide coal combustion waste gases to the MGM cultures, and
- Design and install a propane combustion system to provide propane combustion gases to the MGM cultures.

Preparation for pilot scale experiment using PSI coal reactor has progressed. To date all key components were tested and integrated into the system. Several new components have been ordered. At the writing of this report we are finalizing the performance test. Diagnostic system to measure chemical components of the coal combustion gas: CO₂, NO_x, and SO_x have been developed. The diagnostic instruments were shipped to Aquasearch, Inc. in November. The coal reactor will be shipped to Aquasearch, Inc. when we receive authorization from DOE. Within the next quarter we expect to

- Continue scaling up of promising strains to the 2000 liter outdoor photobioreactor scale,
- Test and install gas measuring diagnostic equipment at Aquasearch, and
- Prepare shipping the PSI combusting.

5.2 Task 4: Carbon Sequestration System Design

During the present reporting period (10/01/02 through 12/31/02) the following technical activities were pursued:

1. An analysis of data collected by Mera Pharmaceuticals, Inc. was conducted to understand the operational characteristics of the growth modules and the population dynamics of *Haematococcus pluvialis*.
2. Dried *Haematococcus pluvialis* cells collected by Mera Pharmaceuticals, Inc. were analyzed for total organic carbon content; these data will be employed via a carbon mass balance to estimate carbon sequestration efficiency of the Mera microalgae growth modules.

5.3 Task 5: Economic Analysis

We have also started work on Task 5 to prepare an economic analysis of microalgal-based carbon sequestration.

6. References

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Appendix A

Abstracts of Presentations Supported, in part, by this Research Project

*Abstract of Presentation at the Workshop on Formation of an International Network on
Biofixation of CO₂ and Greenhouse Gas Abatement With Microalgae
EniTecnologie, Monterotondo, Italy on January 22 - 23, 2001*

The issue of cost of biological sequestration of CO₂: closed systems offer a solution

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Microalgal sequestration of CO₂ consists in growing microalgae photoautotrophically utilizing anthropogenic CO₂ as the source of carbon for biomass production. To grow microalgae photoautotrophically the following components need to be provided:

- Water,
- Mineral salts,
- CO₂, and
- Light.

Providing water, mineral salts and CO₂ does not represent a large problem. However, if one desires to provide large quantities of cheap/free light to the algal cultures the cultures need to be taken outdoors. Successful (i.e., commercial) outdoor culture systems, up until now, have consisted of large ponds, tanks and raceways open to the elements. Such open systems have only been successful in culturing species that by virtue of their weed-like behavior (e.g., *Chlorella*) or their ability to withstand adverse growth conditions (e.g., *Spirulina* and *Dunaliella*) can outcompete contaminating organisms. Otherwise, outdoor open systems collapse under pressure from contaminating organisms. The answer to this problem is large (>10,000 liter) enclosed photobioreactors.

While enclosed photobioreactors are somewhat more capital intensive than open systems they have significant advantages:

- Contaminants are kept out
- More species of microalgae can be successfully grown
- Tighter growth conditions can be maintained
- Higher productivities are attainable
- Running costs can be lower than open systems
- More markets/products can be reached.

The cost of microalgal carbon sequestration has to be offset by the value of the biomass being produced. With fully controlled enclosed outdoor systems the possibilities to produce high value products to offset the cost of CO₂ sequestration are nearly endless. With funding from the United States Department of Energy we are now embarking on a three-year R&D program with the long-term goal of developing a microalgal based CO₂ sequestration process that will decrease the cost of CO₂ sequestration to about US\$10/ton.

Carbon sequestration using microalgae in large outdoor photobioreactors: from CO₂ to high value products

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The objective of this work is to determine under what conditions carbon sequestration by microalgal photosynthesis is economically attractive. Microalgal sequestration of CO₂ consists in growing microalgae photoautotrophically utilizing anthropogenic CO₂ as the source of carbon for biomass production. Emissions of carbon dioxide are predicted to increase in this century leading to increased concentrations of carbon dioxide in the atmosphere. Most of the anthropogenic emissions of carbon dioxide result from the combustion of fossil fuels for energy production. As demand for energy, particularly in the developing world, is expected to increase so will CO₂ emissions. Carbon sequestration, capturing and storing carbon emitted from the global energy system, could be a major tool for reducing atmospheric CO₂ emissions from fossil fuel usage.

In a collaborative effort, Physical Sciences Inc., the Hawaii Natural Energy Institute at the University of Hawaii and Aquasearch Inc. are working together to develop technologies for recovery and sequestration of CO₂ from stationary combustion systems via microalgal photosynthesis. The research is aimed primarily at quantifying the efficacy of microalgae-based carbon sequestration at industrial scale. Our principal research activities are focused on demonstrating the ability of selected species of microalgae to effectively fix carbon from typical power plant exhaust gases. Our final results will be used as the basis to evaluate the technical efficacy and associated economic performance of large-scale carbon sequestration facilities. Our vision of a viable strategy for carbon sequestration based on photosynthetic microalgae entails combining CO₂ from the fossil fuel combustion system and nutrients in a photobioreactor where microalgae photosynthetically convert the CO₂ into compounds for high commercial values or mineralized carbon for sequestration.

In this paper, we will present preliminary results of our research aimed at selection of species appropriate for this task based on their photosynthetic capacity, ability to produce high value products and ability to sequester carbon into carbonates.

Carbon sequestration using microalgae in large outdoor photobioreactors: from CO₂ to long term carbon sequestration

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Emissions of carbon dioxide are predicted to increase in this century leading to increased concentrations of CO₂ in the atmosphere. Most of the anthropogenic emissions of CO₂ result from the combustion of fossil fuels for energy production. As demand for energy, particularly in the developing world, is expected to increase so will CO₂ emissions.

Microalgal sequestration of CO₂ consists in growing microalgae photoautotrophically utilizing anthropogenic CO₂ as the source of carbon for biomass production. However, most of the carbon incorporated into algal biomass can be readily returned to the CO₂ pool through algal metabolism (respiration), consumption of the algal biomass by another organism or decomposition. Thus, algal biomass per se is not an optimal form for carbon storage. Some microalgae (such as coccolithophorids) can produce inorganic carbon structures (coccoliths) that are much more refractory, thus a better form for long term carbon storage. While large scale culturing of other species of microalgae (i.e., at commercial scale) has been attained in many different places, we are not aware of successful attempts to produce large amounts of coccolithophorids.

Microalgal photosynthesis affects the equilibrium of the different inorganic carbon species in the growth medium: CO₂ uptake raises the pH of the medium resulting in decreases in bicarbonate (HCO₃⁻) and CO₂ concentrations but an increase in the carbonate (CO₃²⁻) concentration. We are conducting experiments to test the hypothesis that changes in water chemistry caused by microalgal photosynthesis are conducive to CaCO₃ precipitation. The CaCO₃ thus produced can be used for long term sequestration of carbon.

Our experimental design consists in allowing microalgal cultures to raise the pH of the growth medium via photosynthetic uptake of CO₂. The raise in pH causes the dissolved inorganic carbon equilibrium to shift to CO₃²⁻. In the presence of high Ca²⁺ concentrations, CaCO₃ precipitates. In this paper we will report results of experiments conducted on a number of species. Different species produced morphologically different precipitates. We will also discuss the choice of Ca²⁺ source for the cultures from the global carbon cycle perspective. Finally we will make the argument that large scale culture of microalgal species rich in high value products (such as nutra- and pharma-ceuticals) can concurrently produce inorganic carbon compounds appropriate for long-term sequestration of atmospheric CO₂.

Production of high value products and mineral carbon from smoke stack gases using photobioreactor grown microalgae

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The objective of this work is to determine under which conditions carbon sequestration by microalgal photosynthesis is economically attractive. Microalgal sequestration of CO₂ consists in growing microalgae photoautotrophically utilizing anthropogenic CO₂ as the source of carbon for biomass and biomineral production. We qualify a carbon sequestration scheme economically attractive if the process used matches or betters the US Department of Energy cost goal of ≤\$10 per ton of CO₂ avoided.

Our approach to carbon sequestration consists in growing microalgae in large-scale outdoor photobioreactors using flue gases as the carbon source. The species grown are chosen from among the hundreds of organisms already identified as producers of high value products (e.g., carotenoids). The cultures are grown under conditions that enhance the chemical precipitation of CO₃²⁻ with Ca²⁺ to produce CaCO₃.

The first step in our project consisted in establishing a microalgal collection (78 strains representing 68 species) and determining the temperature and pH tolerances of promising microalgal strains. The second step (on going) consists in determining the tolerance of selected strains to different flue gases. Our standard culture conditions are 25°C, 60 μE m⁻² s⁻¹ under a 14:10 L:D cycle and 7.5 pH. The cultures are grown in 3.3 liter chemostats. High value product concentration (carotenoid pigments) is determined via HPLC. Based on those values and the productivity of the culture at steady state we calculate the productivity of the high value component of the biomass. The productivity of our light limited chemostat cultures is then extrapolated to that of cultures grown in outdoor photobioreactors, also light limited.

To determine the carbon mineralization potential of microalgal cultures we conducted experiments designed to test the effects of changing the CO₂ input rate on the precipitation of CaCO₃. We have found that, in the presence of high Ca²⁺ concentrations, the change in dissolved carbon species driven by photosynthesis does result in CaCO₃ precipitation.

From an industrial perspective, this process presents the possibility of decreasing carbon emissions in an economically attractive manner. We are currently scaling up our experiments to 25,000 liter outdoor photobioreactors to establish the working parameters and the economics of such a process.

**Production of high value products and mineral carbon from smoke stack gases using
photobioreactor grown microalgae**

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The objective of this work is to determine under which conditions carbon sequestration by microalgal photosynthesis is economically attractive. Microalgal sequestration of CO₂ consists in growing microalgae photoautotrophically utilizing anthropogenic CO₂ as the source of carbon for biomass and biomineral production. We qualify a carbon sequestration scheme economically attractive if the process used matches or betters the US Department of Energy cost goal of ≤\$10 per ton of CO₂ avoided.

Our approach to carbon sequestration consists in growing microalgae in large-scale outdoor photobioreactors using flue gases as the carbon source. The species grown are chosen from among the hundreds of organisms already identified as producers of high value products (e.g., carotenoids). The cultures are grown under conditions that support carbon mineralization by species known to do so (e.g., coccolithophorids) or under conditions that enhance the chemical precipitation of CO₃²⁻ with Ca²⁺ to produce CaCO₃.

The first step in our project consisted in establishing a microalgal collection (78 strains representing 68 species) and determining the temperature and pH tolerances of promising microalgal strains. The second step (on going) consists in determining the tolerance of selected strains to different flue gases. Our standard culture conditions are 25°C, 60 μE m⁻² s⁻¹ under a 14:10 L:D cycle and 7.5 pH. The cultures are grown in 3.3 liter chemostats. High value product concentration (carotenoid pigments) is determined via HPLC. Based on those values and the productivity of the culture at steady state we calculate the productivity of the high value component of the biomass. The productivity of our light limited chemostat cultures is then extrapolated to that of cultures grown in outdoor photobioreactors, also light limited.

To determine the carbon mineralization potential of microalgal cultures we conducted experiments designed to test the effects of changing the CO₂ input rate on the precipitation of CaCO₃. We have found that, in the presence of high Ca²⁺ concentrations, the change in dissolved carbon species driven by photosynthesis does result in CaCO₃ precipitation.

From an industrial perspective, this process presents the possibility of decreasing carbon emissions in an economically attractive manner. We are currently scaling up our experiments to 25,000 liter outdoor photobioreactors to establish the working parameters and the economics of such a process.

Use of microalgae for carbon sequestration from smoke stack gasses

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CO₂ released by human activities threatens the world environment since CO₂ emissions are fast outpacing CO₂ sequestration. Microalgae utilize CO₂ as one of their main building blocks and we propose that algal photosynthesis may be a viable option for anthropogenic CO₂ capture and sequestration (e.g., from smokestack emissions).

We are conducting a screening program to select microalgal species that not only show high potential for CO₂ capture and sequestration at different pHs but can also withstand the other gases present in smokestack emissions (e.g., SO_x and NO_x). Our objective is to determine under which conditions carbon capture and sequestration by microalgal photosynthesis is economically attractive when compared with other means of carbon capture and sequestration. Since microalgal culturing is expensive, we are also selecting for microalgal species that produce high value products, the revenue from which would pay for the cost of capture and sequestration.

Our experiments on >20 microalgal strains indicate that the pH of the culture medium has two main effects on the efficiency of such a system. On the one hand, photosynthetic CO₂ uptake from the medium is higher at low (6.5) than medium (7.5) and high (8.5) pH. On the other hand, the proportion of CO₂ available in the medium that is photosynthetically taken up is higher at high pH.

These findings will be discussed as they will affect the design of an industrial scale facility designed to capture and sequester CO₂ from smokestack emissions.

Appendix B

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Carbon removal through algal mediated precipitation of calcium carbonate

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Abstract

Carbon dioxide emissions have been increasing over the last century, resulting in an increase of atmospheric CO₂ concentrations ultimately affecting ocean circulation and global climate. Anthropogenic activities have resulted in a 30% increase in the amount of carbon dioxide in the atmosphere relative to the pre-industrial concentration of 280 p.p.m.v. (Stocker and Schmittner 1997). Industrial emissions provide a large portion of anthropogenic CO₂ released into the atmosphere, and therefore are a target for current carbon removal efforts. Although photosynthesis removes atmospheric CO₂, this removal is not permanent as the CO₂ can be re-released to the atmosphere through biological processes. The formation of CaCO₃ permanently removes CO₂ as a solid that cannot be biologically re-released, thereby making this process a potential tool for the elimination of some anthropogenically produced CO₂. This paper discusses experiments that were conducted to test the ability of one species of freshwater algae to alter water chemistry in order to induce the precipitation of solid CaCO₃. The estimated rate of carbon removal, via CaCO₃ precipitate, was 6.6 $\mu\text{M C hr}^{-1}$.

Keywords: calcium carbonate, carbon removal, global warming, cyanobacteria, carbon dioxide

Introduction

Increases in the concentration of carbon dioxide (CO_2) in the atmosphere are believed to be strongly associated with climatic changes and the possible alteration of the environment in the future (Physical Sciences Inc., 2000). With this threat at hand, it is beneficial to investigate methods for minimizing anthropogenic emissions of CO_2 , as anthropogenic activities have resulted in a 30% increase in the CO_2 content of the atmosphere since pre-industrial times (Stocker and Schmittner, 1997). Carbon sequestration involves the capture and storage of carbon, thereby removing it from the global carbon cycle (Physical Sciences Inc., 2000). Photosynthesis is a process whose potential for carbon assimilation is well known. It has also been determined that aquatic microalgae can use this process to assimilate carbon more efficiently than land based plants (Physical Sciences Inc., 2000). Unfortunately, this type of carbon storage is not permanent as algal cells can respire CO_2 and be consumed by other organisms that also respire the captured CO_2 .

Some species of aquatic microalgae have the ability to permanently sequester carbon through the formation of solid CaCO_3 . Carbon stored in such a way cannot be consumed and respired back into the atmosphere, making CaCO_3 a valuable sink for permanent carbon sequestration. This process occurs naturally and is known to produce such occurrences as the Bahamas whittings (Leal, 1992), and has furthermore been identified as a possible mechanism for atmospheric carbon removal in the Earth's early history (EPRI, 1993). If this natural

process can be produced commercially on a large scale, it may be possible to reduce the amount of anthropogenic CO_2 released into the atmosphere.

The hypothesis states that through photosynthesis, microalgae alter the pH of their environment, allowing for the precipitation of solid CaCO_3 . Specifically, as the algae photosynthesize, CO_2 is removed from the environment and the pH of the media is increased. This increase in pH causes a shift in ion speciation, with the percentage of CO_3^{2-} ions in the media increasing as a function of pH (Fig. 1).

BIOMASS ↑ algae $\text{CO}_2 + \text{H}_2\text{O} \leftrightarrow \text{H}_2\text{CO}_3$ $\text{H}_2\text{CO}_3 \leftrightarrow \text{H}^+ + \text{HCO}_3^-$ $\text{HCO}_3^- \leftrightarrow \text{H}^+ + \text{CO}_3^{2-}$ $+ \text{Ca}^{2+} \downarrow$ CaCO_3	pH ~ 7.0	$[\text{H}^+] > [\text{OH}^-]$	Major Carbon Species
	pH ~ 8.0	$\text{H}^+ + \text{HCO}_3^- \leftrightarrow \text{H}_2\text{CO}_3$ $\text{H}^+ + \text{CO}_3^{2-} \leftrightarrow \text{HCO}_3^-$	HCO_3^-
	pH ~ 10.2	$[\text{H}^+] < [\text{OH}^-]$ $\text{OH}^- + \text{HCO}_3^- \leftrightarrow \text{CO}_3^{2-}$	CO_3^{2-}

Figure 1. Chemical speciation pathway of CaCO_3 formation as mediated by pH. pH values for major carbon species were obtained from Stumm and Morgan (1981).

The CO_3^{2-} ions bind with Ca^{2+} ions added to the media to form solid CaCO_3 precipitate. With the addition of more CO_2 to the system, the concentration of the carbonate ions increases, making more CO_3^{2-} available for precipitation with Ca^{2+} ions. This process allows for the storage of carbon in a solid permanent form.

Materials and Methods

Experiments were conducted using a locally isolated, unidentified freshwater species of filamentous cyanobacteria (AQ0012). The cultures were grown using a freshwater photoautotrophic nutrient media developed by Aquasearch Inc., called formula 413 (FW 413). The experiments were conducted using a 14:10 light:dark

Figure 2. 3.3L Chemostat inoculated with AQ0012

cycle with a light intensity of $60 \mu\text{E m}^{-2} \text{s}^{-1}$. The species was maintained in the growth phase using a 3.3L chemostat system (Fig.2). The system provided a continuous supply of nutrients and maintained conditions necessary for biomass growth. The pH (7.8 ± 0.7) of the chemostat culture was maintained via automatic injection of CO_2 in response to changes in pH. A constant outflow of media and culture was collected in a carboy called a receiver. Cultures used for all experiments were removed from both the chemostat and receiver.

Standard methods of titration were used to determine total alkalinity. A series of algorithms were used in order to determine total alkalinity, CO_3^{2-} ion, HCO_3^- ion, free CO_2 , and total inorganic carbon concentrations. These algorithms were based on chemical speciation as dictated by thermodynamic equilibrium. A series of equations were developed to calculate total alkalinity and inorganic carbon species using well-established values for the equilibrium constants and known values of pH and ionic strength (Clesceri et al., 1995). The calcium species used in all experiments was $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ (gypsum) and will be

referred to as Ca throughout this paper. Calcium was added to the media as a saturated solution of gypsum. The gypsum was fully dissolved in the growth media.

When ready to test, the media was centrifuged, filtered, and dried in an oven. Concentrated HCl was added to the dried filtrate and the occurrence of a chemical reaction between HCl and CaCO_3 was determined through visual inspection. Bubbling of the filtrate indicated that $\text{CaCO}_3 (\text{s})$ was present. This test was used throughout all of the experiments to determine the presence of CaCO_3 . The addition of concentrated HCl to solid $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ did not produce any visible signs of a chemical reaction or bubbling. In addition to visual inspection for CaCO_3 the chemical speciation model Mineql⁺ (Schecher and McAvoy, 2001) was used to predict the formation of dissolved and solid species within the media. The model calculations predicted that the only solid species formed in the media was CaCO_3 . According to thermodynamic equilibrium, gypsum did not reprecipitate in this growth media.

The first experiment was conducted to determine the removal rate of inorganic carbon from the media as a function of increasing pH. The experiment utilized a control flask and an experimental flask. The reported results are an average of 2 trials. The control flasks were prepared with FW 413 media containing existing culture. The experimental flasks were prepared with the same inoculated media, and then saturated with Ca. Initial pH and alkalinity measurements were taken from all flasks. All flasks were grown under the previously specified conditions. Alkalinity and pH measurements were taken periodically.

After the pH of each flask reached 9.0 or higher, the experiment was terminated. The solids were harvested from the experimental flask according to the methods described above. CaCO_3 (s) was determined as previously stated.

The second experiment was designed to test the feasibility of a continuously replenished system to precipitate CaCO_3 as a function of increasing pH. A chemostat system was established with a culture of cyanobacteria AQ0012 grown in FW 413 media that had been saturated with Ca. Initial measurements of alkalinity and pH were taken from 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 remained relatively constant (7.8 ± 0.7). Once a constant supply of new calcium saturated media was initiated to the chemostat, the media and culture overflow from the chemostat were directed to a receiver. The pH of the receiver was not controlled. Excess media and culture were removed from the receiver periodically to prevent overflow. Alkalinity and pH measurements were taken daily from both the chemostat and receiver cultures.

Results

All of the experiments containing calcium in the media resulted in white particles observed in suspension among the biomass (Fig.3). Results from the first experiment with AQ0012 demonstrated a significant decrease in the concentration of total inorganic carbon from 1.26 mM C to 0.77 mM C ($19 \% \text{ day}^{-1}$) relative to the control ($5 \% \text{ day}^{-1}$) (F g. 4 & 5). Since no precipitate

Figure 3. AQ0012 + Ca under scope, CaCO_3 on left, algal filaments on right

was formed in the control, the removal of inorganic carbon was attributed to biomass incorporation. The white amorphous particles found within the experimental media were determined to be CaCO_3 (s) after being tested for reaction with concentrated HCl. The estimated rate of carbon removal was

Figure 4. Concentrations of dissolved inorganic carbon species and pH in freshwater 413 media using unknown cyanobacteria AQ0012. There was no calcium added to the media. The pH was allowed to increase. (control flask)

Figure 5. Concentrations of dissolved inorganic carbon species and pH in freshwater 413 media using unknown cyanobacteria AQ0012. Calcium was added to the media. The pH was allowed to increase. (experimental flask)

calculated to be $6.6 \mu\text{M C hr}^{-1}$ (Fig. 6). The removal rate of inorganic carbon was determined by the difference in the concentration of total inorganic carbon between the control media and the experimental media as a function of time. The calculations were based on the assumption that the control biomass equaled the experimental biomass since both were incubated under the same conditions and were inoculated with equal volumes of seeded media containing equal cell densities.

Figure 6. Carbon Removal per hour, calculated from the difference in total inorganic carbon between unknown cyanobacteria AQ0012 with no Ca^{2+} and AQ0012 with Ca^{2+} .

The results from experiment 2 indicated that the total inorganic carbon concentration in the chemostat decreased from 1.3 to 1.0 mM C ($1.0 \text{ \% day}^{-1}$), while the pH remained relatively constant (Fig. 7). The concentration of total organic carbon in the receiver, which received no pH control via CO_2 addition, decreased from 1.74 to 0.54 mM C ($3.2 \text{ \% day}^{-1}$) (Fig.8). Speciation calculations indicated that the concentration of CO_3^{2-} ions increased from 0.2% of the total inorganic carbon

Figure 7. Dissolved inorganic carbon species and pH using unknown cyanobacteria AQ0012 with freshwater 413 media and calcium in the chemostat. The pH was maintained between 7.5-9.0 by additions of carbon dioxide gas.

at the beginning of the experiment to 15-33% of the total inorganic carbon at the end of the experiment (Fig. 8). The particulate matter was determined to be $\text{CaCO}_3(s)$ upon testing with concentrated HCl.

Figure 8. Dissolved inorganic carbon species and pH using unknown cyanobacteria AQ0012 with freshwater 413 media and calcium in the receiver. The pH was allowed to increase.

Discussion

Experimentation with cyanobacteria species AQ0012 yielded promising results. The results indicated that carbon was removed from the system as solid CaCO_3 . Prior examination of calcium carbonate formation by cyanobacteria shows that under certain conditions, filaments of the organism can become encrusted with CaCO_3 (Merz-Preiss; 2000). Examination of the AQ0012 culture, indicated that particulate CaCO_3 was not encrusted on the cells of the organism, however white particles were abundant in close proximity to clumps of the algal filaments (Fig. 3).

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. Although we were unable to quantify the difference, it was visually determined that more precipitate

was formed in the receiver than in the chemostat. There was an increase in CO_3^{2-} ions available for the production of CaCO_3 in both the chemostat and receiver, but higher pH mediated carbonate ion concentrations in the receiver explain why more precipitate was formed in this vessel. This data demonstrates that it is possible to have a continuously replenished system to precipitate CaCO_3 ions as the pH of the media is increased biologically.

From an industrial perspective, this process has the possibility of decreasing carbon emissions that lead to global warming. This species of cyanobacteria has demonstrated its ability to form an environment conducive to CaCO_3 formation, and is a potential candidate for use in large-scale sequestration experiments of an industrial capacity. As the sequestration method requires a calcium supply, a relatively inexpensive source is $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$, 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 media. The use of this mineral has limited potential due to its relatively low solubility. $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ 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 more soluble source of Ca could be used to provide more Ca^{2+} ions to a medium. Future research should target other species that may increase pH at a faster rate, allowing a greater rate of solid precipitate formation in the media. This research demonstrates the feasibility of

this method, although more information is necessary to successfully establish an industrial scale carbon sequestration system. This process may prove to be affordable to industry and environmentally beneficial. Further studies are needed to determine a method for the physical separation of the biomass from the precipitate in order to collect the solid, as well as a method for quantifying the amount of carbon incorporated into biomass and the inorganic precipitate.

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Appendix C

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Commercial development of microalgal biotechnology: from the test tube to the marketplace

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Abstract

While humans have taken limited advantage of natural populations of microalgae for centuries (Nostoc in Asia and Spirulina in Africa and North America for sustenance), it is only recently that we have come to realize the potential of microalgal biotechnology. Microalgal biotechnology has the potential to produce a vast array of products including foodstuffs, industrial chemicals, compounds with therapeutic applications and bioremediation solutions from a virtually untapped source. From an industrial (i.e., commercial) perspective, the goal of microalgal biotechnology is to make money by developing marketable products. For such a business to succeed the following steps must be taken: Identify a desirable metabolite and a microalga that produces and accumulates the desired metabolite, establish a large-scale production process for the desired metabolite, and market the desired metabolite. So far, the commercial achievements of microalgal biotechnology have been modest. Microalgae that produce dozens of desirable metabolites have been identified. Aided by high throughput screening technology even more leads will become available. However, the successes in large-scale production and product marketing have been few. We will discuss those achievements and difficulties from the industrial point of view by considering examples from industry, specially our own experience at Mera Pharmaceuticals.

1. Introduction

Microalgae are an extremely heterogeneous group of organisms. To be called a microalga, the organism needs to be small (usually microscopic), unicellular (but can be colonial with little or no cell differentiation), colorful (due to photosynthetic and accessory pigments), occur mostly in water (but not necessarily) and most likely be photoautotrophic (but not necessarily all the time). Phylogenetically, microalgae can be prokaryotic or eukaryotic and, in evolutionary terms, recent or very ancient. This very diversity makes microalgae, as a group, a potentially rich source of a vast array of chemical products with applications in the feed, food, nutritional, cosmetic, pharmaceutical and even fuel industries.

The history of microalgal utilization from natural populations is centuries old (Nostoc in Asia and Spirulina in Africa and Mexico). However, the purposeful cultivation of microalgae is only a few decades old. During the 20th century, researchers and commercial producers developed several cultivation technologies that are in use today to produce microalgal biomass: open ponds [1], enclosed photobioreactors-PBRs [2] and fermentation reactors [3].

The status of microalgal applications in aquaculture, food, specialty chemicals and environmental applications has been reviewed recently [3,4,5,6]. In this paper we will concentrate on aspects of algal biotechnology that are related to producing high value compounds such as nutraceuticals and pharmaceuticals.

Microalgae are not a well-studied group from a biotechnological point of view. Of the tens of thousands of microalgal species believed to exist, only a few thousand strains are kept in collections around the world, only a few hundred have been investigated for chemical content and only a handful have been cultivated in industrial quantities (tons per year quantities). Because they are largely unexplored, the microalgae represent a rich opportunity for discovery; the expected rate of rediscovery (finding metabolites already described) is expected to be far lower than for other groups of better-studied organisms [7] (Figure 1).

Figure 1. A big advantage of using little studied organisms such as microalgae is an expected much lower rediscovery rate of compounds. This translates into higher probability of developing new drugs.

* Based on 178 bioactive compounds from our contracted collection at University of Hawaii (G. Patterson, personal communication).

2. Discovery

Natural products are a consistent source of new drugs [8]. As opposed to other techniques used to generate compounds (e.g., combinatorial chemistry), natural products offer much diversity and chemistries that are under-represented in synthetic compounds. One can also expect that natural compounds (i.e., those made by living organisms) inherently possess advantageous properties such as water solubility, cell membrane permeability and bioavailability that need to be engineered into synthetic chemicals by trial and error. Whether through classic extract and fraction screens or newer pure compound libraries, it is expected that natural products will continue to be an excellent source of new compounds. This, combined with combinatorial biosynthesis techniques, offers a rich future in new

compound discovery [9]. Furthermore, new techniques are expected to produce compound leads from presently unculturable microorganisms, including microalgae [10,11].

Several groups are actively screening microalgal isolates for high value compounds such as secondary carotenoids [12-16], fatty acids [17,18], polysaccharides [19,20] and other active compounds [21-24]. Except for microalgal strains that may be found in sufficient quantities and purity in nature (e.g., cyanobacterial mats), a minimum of laboratory scale up is necessary for this phase of the discovery efforts [25].

At Mera Pharmaceuticals, we are conducting two discovery programs to develop new compound leads. First, through a licensing agreement, we are working on developing new pharmaceuticals from a collection of over 2000 Cyanobacterial strains kept at the University of Hawaii. The UH collection has already produced over 100 bioactive molecules. We intend to revisit these compounds and, utilizing enzymatic biocatalysis techniques, multiply the number of compound leads. By creating new compound leads we expect to not only increase the number of compounds but also produce new compounds that may offer desirable characteristics (more potency, less toxicity) and that are unknown (i.e., patentable). We believe that the generated compound libraries will be sought after by the pharmaceutical industry (Figure 2).

Our second discovery program deals not only with the specific identification of new compounds but also with the scale up of culture volumes needed to produce enough material for structural elucidation and further testing, including clinical trials. This program is supported in

Figure 2. Techniques such as enzymatic biocatalysis can be used to multiply the number of bioactive leads in drug discovery. Here, each bioactive compound (gray circles) is transformed into a compound library, multiplying the probability of producing chemicals with desired characteristics.

part by a grant awarded by the United States Department of Energy (DOE) to a consortium formed by Physical Sciences Inc., the Hawaii Natural Energy Institute and Mera Pharmaceuticals to study the suitability of utilizing microalgae for carbon sequestration. The goal of the DOE is to find technologies that will lower the cost of CO₂ capture and sequestration. The costs of removing CO₂ from a conventional coal-fired power plant with flue gas desulphurization is estimated to be in the range of \$35 to \$264 per ton of CO₂ [26]. DOE's goal is to reduce the cost of carbon sequestration to below \$10 /ton of avoided net cost.

Our vision of a viable strategy for carbon sequestration based on photosynthetic microalgae entails combining CO₂ from the fossil fuel combustion system and nutrients in a photobioreactor where microalgae photosynthetically convert the CO₂ into either compounds of high commercial value or mineralized carbon for sequestration (Figure 3). While the cost of producing microalgae is much higher than \$10 per ton of biomass, this cost can be offset entirely by using microalgae that produce high value compounds. We have embarked in a search and discovery effort consisting in screening a substantial number of microalgae to determine their suitability for this purpose. Specifically, we are searching for species that

- can withstand warm growth temperatures (up to 35°C, since one could expect that flue gases would raise the algal medium's temperature),
- show broad pH optima (since one could expect changes in rate of CO₂ supply from a combustion source as, for example, power demand changes),
- can withstand the mixture of gases that would accompany the CO₂ in typical combustion systems (e.g., natural gas-, fuel oil-, and coal-fired) such as SO_x and NO_x gases,
- accumulate high value metabolites under stressing and non-stressing growth conditions, and
- are scalable to industrial-sized PBRs.

Our efforts so far have resulted in the identification of several microalgal strains that not only can withstand warm temperatures, changes in pH and thrive in combustion gas mixtures but that also accumulate high value carotenoids at a culture scale of 3.3 liter chemostats (Table 1). By combining our high value compound discovery efforts with the carbon sequestration efforts, we are able to lower the effective cost of sequestration and generate valuable byproducts in the process.

Figure 3. Diagram showing our concept for a microalgal-based carbon capture and sequestration scheme. High value products obtained from the microalgal biomass would offset the cost of CO₂ removal.

Table 1. Highest percent carotenoid per dried biomass obtained in growth experiments and predicted pigment production rates at a biomass production rate of 13 g dry biomass m⁻² d⁻¹, a typical rate for microalgae grown in Mera Growth Modules (25,000 liter PBRs).

Strain ID	Treatment which Gave Highest % Pigment	Compound	Cont. as %DW	Predicted Production Rate
AQ0011 (Chlorophyte)	5 hours strong sunlight	Lutein	0.28	0.037 g m ⁻² d ⁻¹
AQ0011 (Chlorophyte)	5 hours strong sunlight	Zeaxanthin	0.12	0.016 g m ⁻² d ⁻¹
AQ0012 (Cyanobacterium)	Standard conditions	Zeaxanthin	0.15	0.020 g m ⁻² d ⁻¹
AQ0033 (Rhodophyte)	Standard conditions	Zeaxanthin	0.21	0.027 g m ⁻² d ⁻¹
AQ0036 (Rhodophyte)	Standard conditions	Zeaxanthin	0.13	0.017 g m ⁻² d ⁻¹
AQ0052 (Chlorophyte)	Standard conditions	Lutein	0.21	0.027 g m ⁻² d ⁻¹
AQ0052 (Chlorophyte)	8 hours strong sunlight	Zeaxanthin	0.05	0.006 g m ⁻² d ⁻¹
AQ0053 (Chlorophyte)	5 hours strong sunlight	Lutein	0.35	0.049 g m ⁻² d ⁻¹

3. Production

Scale-up

One of the major problems with the development of drugs from natural products is the fact they are in limited supply (by definition this is precisely the case with non-common or low abundance organisms such as microalgae). While such organisms offer advantages for the discovery phase, the availability of material needed for further testing may be very limited [27].

Over the last decade, the consensus among microalgal biotechnologists is that commercial photoautotrophic production of high value metabolites from microalgae requires outdoor enclosed photobioreactors [2,28-31]. Tredici [2] has reviewed the development of PBRs over the last few decades. While many experimental PBRs have been designed, constructed and deemed successful, very few have actually succeeded at commercial scale. The commercial application of PBR technology remains limited mainly to the production of two Chlorophyte algae: *Chlorella* and *Haematococcus* [31,32].

Scale up of research PBRs to commercial scale is not trivial (see Tredici [2] for examples of two commercial failures due to improper scale up). PBR scale up needs to take into consideration changes in illumination, gas transfer and temperature (all three affected by the turbulence in the reactor) and their control. Indeed, scale up is an engineering problem, not a biological one. Much work has been done to describe the light field inside PBRs and general recommendations as to possible maximum scales have been made [33].

Our own scale up procedure involves establishing culture conditions in computer-controlled experimental outdoor pilot PBRs of up to 2,000 liter capacity. The design is essentially the same as for our commercial scale PBR (the 25,000 liter capacity Mera Growth Module, MGM) except for the diameter of the reactor itself (0.18 m vs. 0.41 m). We are using these scale up reactors in our DOE program on carbon sequestration and high value compound discovery efforts. The scale up reactors allow us to produce enough biomass for thorough testing of bioactivity and to establish the general parameters for production of the desirable strains.

There are two significant differences between laboratory chemostats and the MGM that could affect culture productivity at this scale. These differences concern (1) light field and (2) the mixing, dissolution and distribution of gases. The purpose of the pilot MGM experiments

is to examine scale-related effects precisely. The design of the MGM permits us to change the flow characteristics at will (Reynold's number between 2×10^3 to 2×10^5) to study these effects. So far, we have successfully scaled up two Cyanobacteria (Lynghya sp. and another unidentified filamentous strain), two Chlorophytes (Haematococcus pluvialis and an unidentified small-5 μ -coccoid strain) and a Rhodophyte (Porphyridium sp.).

Commercial photobioreactors

From a commercial (i.e., business) point of view, a PBR must have as many of the following characteristics as possible:

- high area productivity ($\text{g m}^{-2} \text{d}^{-1}$), since many costs scale with plant size;
- high volumetric productivity ($\text{g l}^{-1} \text{d}^{-1}$), since some costs scale with the amount of water needed for culture;
- large volume (l PBR^{-1}), since some costs scale with the number of reactors needed;
- inexpensive to build and maintain ($\text{\$ PBR}^{-1}$);
- easy to control culture parameters (temperature, pH, O_2 , turbulence); and
- reliability.

Photobioreactors of different designs attempt to achieve these characteristics differently [2]. Obviously, from a commercial point of view, the optimum PBR design will be the one that reliably produces the high value compound sought at the best possible quality for the least amount of money. Examples of commercial applications of PBR technology today are those used for Chlorella and Haematococcus production [31,32].

Our own photobioreactors have capacities of up to 25,000 liters and occupy an area of just 100 m^2 . They are of the serpentine type (Figure 4) and made of clear polyethylene tubing (41 cm diameter) and PVC parts (bends and control unit). Temperature and pH are computer-controlled, which provides for very tight tolerances independent of variability in ambient conditions (Figure 5), which is necessary to produce a consistent product.

Harvest

Harvesting entails concentrating the biomass produced from a concentration of $<1 \text{ g DW l}^{-1}$ in the PBR to as much as 250 g DW l^{-1} . The harvesting technique to be used is dependent on characteristics of the microalgae, such as size

Figure 4. The Mera Growth Module, a 25,000 liter photobioreactor.

Figure 5. A. Temperature (broken line) and pH (solid line) traces for a 25,000 liter MGM culture showing the tight tolerances maintained over a 7-day period (e.g., over 97% of the pH values are between 7.3 and 7.8). B. Trace of solar irradiance measured near the surface of the MGM for the same period.

and density. Reviews of the different techniques available (including flocculation, filtration, centrifugation and air flotation) have concluded that centrifugation is possibly the most reliable technique and only slightly more expensive than other techniques [1,34].

In the case of Haematococcus biomass for the production of astaxanthin (Mera's first high value product), we take advantage of the fact that *Haematococcus* cells become large and heavy during the carotenogenesis and encystment phase. The cysts coalesce into larger flocks that settle out of the growth medium quickly; we have observed settling velocities of $>1 \text{ cm min}^{-1}$ for single cells and even faster for flocks (unpublished observation, see also [35]). This provides for an efficient concentrating step (15x) in the cultivation units. The slurry thus produced is further concentrated with centrifuges (concentration factor 22x).

Separation and recovery

Here we have assumed that the goal of microalgal biotechnology efforts is to recover a high value product from the microalgal biomass. Thus, the high value product needs to be separated from the biomass. Depending on the process, the microalgal cells may need to be physically disrupted. Both ball mills and high pressure homogenizers have been used successfully to disrupt microalgal cells [36,37] to enhance recovery of astaxanthin from Haematococcus at commercial scale, but other methods may be possible [38]. Use of solvents and enzymes might help with cellular disruption and product recovery but care must be taken regarding what aids are used if the product is intended for human consumption.

Depending on the product to be recovered, the next step in the process might entail reducing the water content of the microalgal biomass. Absence of water in the biomass enhances the recovery of lipid soluble components such as astaxanthin and β -carotene. Microalgal biomass can be dehydrated in spray driers, drum dryers, freeze dryers and sun dryers. In the case of heat sensitive compounds such as astaxanthin, commercial producers have developed technologies that limit exposure of astaxanthin to conditions known to cause degradation (specially high temperatures and oxygen [32]). Following dehydration, astaxanthin is recovered from the biomass using supercritical CO_2 extraction or oil extraction techniques. At the present time commercial producers of astaxanthin do not purify astaxanthin from the extract thus obtained.

In some cases the biomass may not need to be dehydrated, and extraction and fractionation can be carried out on the wet biomass (e.g., fatty acids [39], biliproteins [40], carotenoid pigments [41]). Further downstream processing may be needed to isolate the active compound depending on the intended final product [34,42].

End products and formulation

There are very few commercial microalgal high value products in the market today (e.g., fatty acids-FAs- and carotenoids). We assume that future drugs developed from microalgal products would be prepared and packaged as other pharmacological compounds are today.

In the case of extracted FAs and carotenoids, these products can be offered in bulk at different purities, incorporated into other products or encapsulated. For example, Martek (www.martekbio.com) is a successful producer of docosahexaenoic acid (DHA) from Cryptocodinium cohnii. They market the bulk, blended product, to infant formula manufacturers but also offer it in capsules.

To illustrate the learning curve that sometimes needs to occur when putting together a new product we will look at nutraceutical astaxanthin. Dried, astaxanthin-rich, Haematococcus algal meal can be pressed into tablets. However, the astaxanthin in these tablets is degraded easily by oxidation. Producers of astaxanthin have attempted to suspend Haematococcus biomass

in edible oils instead, expecting that the oil would create a barrier between atmospheric oxygen and the astaxanthin-rich biomass. Cyanotech (www.cyanotech.com) tried suspensions in rosemary oil but found that astaxanthin was very unstable in this formulation [43]. Our own observations are that dried particles suspended in oil can cause leaks in gelatin capsules resulting in a product unacceptable to the consumer. The solution to these issues has been the development of extraction methodologies using non-petrochemical solvents. For human applications, use of petrochemical solvents could create health and/or acceptability concerns because of possible residues in the final product. Mera Pharmaceuticals has developed a proprietary oil extraction method in which edible oils are used as the extraction solvent. Alternatively, super-critical CO₂ extraction can be used to produce an astaxanthin-rich oleoresin that is then diluted with edible oils to the appropriate concentration for encapsulation [43].

4. Marketability and profitability

In the end, the objective of microalgal biotechnology is to make money by selling a product for a higher price than it costs to produce. To sell a product there must be a market, a group of consumers that are willing to purchase the product. The preferred approach is to first find a market and, then provide the product desired. “The goal is to develop a product that fills a need; one should avoid developing a product in search of a use” [44]. Indeed, the marketers should be the ones guiding the efforts of the researchers.

There are very few commercial high-value products from microalgae available today. Perhaps the three best known are β -carotene (Dunaliella), DHA (Cryptocodinium), and astaxanthin (Haematococcus). We will use astaxanthin as a case study to illustrate some of the pitfalls that may be encountered when bringing a microalgal biotechnology product to market. Astaxanthin case study

The largest consumer of astaxanthin today is the salmon feed industry. In the 1980's and 1990's, Haematococcus was identified as an organism that could be cultivated as a rich source of astaxanthin with a readily identified market, the salmon feed industry. Over the last 15 years several companies have attempted to establish commercial operations to supply natural astaxanthin to the feed market. In practice, reliable production of Haematococcus astaxanthin at industrial scale was not accomplished until the late 1990's [36,37].

However, the largest producers of astaxanthin today do not produce astaxanthin from Haematococcus but via chemical synthesis (BASF and Hoffman-La Roche). Synthetic astaxanthin has a different ratio of stereoisomers (mainly 3R,3'S) than natural astaxanthin (3S,3'S in, e.g., Haematococcus). The 3S,3'S is the main stereoisomer found in wild Pacific and Atlantic salmon species. Since salmon are unable to modify the chemical configuration of the astaxanthin molecule, one can detect whether an individual fish was fed natural or synthetic astaxanthin [45].

Why does the salmon feed industry use synthetic astaxanthin? First, microalgal producers had assumed that Haematococcus astaxanthin would be cheaper to produce. The synthetic producers have established the world market price for astaxanthin at about US\$ 2000 kg⁻¹. We suspect that the actual production cost for synthetic astaxanthin may be <US\$ 1000 kg⁻¹. To beat this cost, and assuming a 3% astaxanthin content, Haematococcus biomass would need to be produced at significantly less than \$30 kg⁻¹. Considering the added costs of producing astaxanthin (e.g., cell breaking), we feel that this low cost cannot be achieved by commercial producers at this time. It is possible that as the production technology is optimized (e.g., find a

strain that accumulates 10% astaxanthin) and production is transferred to low cost locales (e.g., China) Haematococcus astaxanthin will become cost competitive as a feed supplement.

Second, producers of Haematococcus astaxanthin had also assumed that natural astaxanthin would have better acceptability than the synthetic counterpart would. However, the public, at large, does not appear to demand and is not willing to pay a higher price for naturally pigmented salmon. We feel this is due to a lack of awareness by the consumer. Most consumers probably do not realize that most of the salmon consumed today is farmed, that pigment is added to their diets and that the pigment added is a synthetic product. As long as the consumer is uninformed there will be very little demand for natural astaxanthin-fed salmon, and astaxanthin producers will have to compete on price alone. Once the consumer is educated, or regulations favor the use of natural products, we would expect to see a premium price for natural versus synthetic astaxanthin as has occurred in the vitamin E and β -carotene markets [46,47].

There are a few applications where natural astaxanthin is preferred over the synthetic product (koi, chicken, red seabream diets) because of enhanced deposition of pigment in the tissues or regulatory requirements. However, these markets, at the present time, appear too small to sustain an enterprise engaged in the commercial production of astaxanthin.

Astaxanthin's second chance

In the 1990's the antioxidant characteristics of the astaxanthin molecule became well established. Several in vitro and animal model studies demonstrated a number of possible roles for astaxanthin in disease treatment and prevention [48]. This has opened the possibility of a new market for Haematococcus astaxanthin: human nutraceuticals. Retail price of nutraceutical grade astaxanthin is $>\$100,000\text{ kg}^{-1}$ [32] which more than justifies the increased cost of producing natural astaxanthin from Haematococcus.

The size of the astaxanthin nutraceutical market today is probably less than a few million US\$. However, we estimate the current production capacity for nutraceutical Haematococcus astaxanthin to be at least 40-50 million US\$ (retail) in Hawaii alone (Cyanotech, Mera Pharmaceuticals and MicroGaia). Thus, the industry is poised for rapid growth. However, most consumers remain unaware of astaxanthin, much less understand why it is good for them! We expect that as the producers undertake consumer awareness campaigns (advertising and public relations), demand will rise and will outpace present production capacity.

Future of Haematococcus astaxanthin

We believe that, through consumer education and by lowering production costs, the future of Haematococcus astaxanthin is bright. As mentioned above, while astaxanthin is a valuable product with important benefits for human health [48], most consumers have never heard of it. Thus, the market is still very small, and it is up to the producers to create awareness for the product. Doing so will require significant capital.

As price is always a factor in consumer acceptance, we are pursuing two strategies to lower our production costs. First, we are continuously improving our production technology to produce Haematococcus biomass with a higher final astaxanthin content. Any improvement in astaxanthin content of the final product translates directly into lower costs, since the costs are proportional to the amount of biomass produced and processed. Second, we plan to lower our production costs by expanding our production capacity into locales with lower land, labor and energy costs such as China.

Lessons learned from Haematococcus astaxanthin

Haematococcus astaxanthin has not yet realized its potential because of mistakes made by the producers. These can be summarized as:

- overly optimistic cost projections,
- lack of market research (consumers don't care...but would they if they knew?),
- an "if we make it consumers will buy it" attitude,
- lack of marketing resources to build consumer awareness, including distribution of product safety and efficacy studies results.

5. Future of microalgal biotechnology

Microalgal biotechnology has not yet attracted the attention of large (i.e., have money to fund research) pharmaceutical companies. This may be because of the lack of success stories so far. While we, microalgal biotechnologists, are convinced of the potential of microalgal biotechnology, we have little success to justify our optimism.

Moving forward, microalgal biotechnology may fulfill the following roles:

Drug and high value chemical discovery. This is perhaps the most promising aspect of microalgal biotechnology. As stated earlier, the diversity of the microalgae leads us to believe that this is a very fertile ground for search and discovery with low rediscovery rates. Generation of compound libraries based on bioactive microalgal metabolites could attract the attention of biopharmaceutical companies with the necessary resources to fund clinical trials; bringing a new drug to market costs hundreds of millions US\$ [49], which your average microalgal biotech does not have. Furthermore, photobioreactor technology has advanced to the point where it is relatively easy to scale up cultures to produce enough material for research efforts beyond initial discovery.

Drug and high value chemical manufacture. While microalgae can be fast growers (high primary productivity) many desirable chemicals are the product of secondary metabolism triggered under conditions not conducive to fast growth. In addition, once a chemical is discovered and characterized it might be produced synthetically. Furthermore, the biochemical pathway that results in the desired chemical may be transferred to an easily cultivable organism (e.g., [50]). It would seem that the future of microalgae in manufacturing might be limited to chemicals complex enough that they cannot be chemically synthesized or the pathways of which cannot be transferred to other organisms. For those chemicals that will be produced by microalgae we will need to develop new strains (faster growth, higher chemical concentration), whether by classical selection or genetic manipulation, and improve photobioreactors to the point where 40-60 g m² d⁻¹ of microalgal biomass are produced consistently.

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