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

Quarterly Technical Progress Report for the Period Ending 31 March 2001

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ABSTRACT

Most of the anthropogenic emissions of carbon dioxide result from the combustion of fossil fuels for energy production. Photosynthesis has long been recognized as a means, at least in theory, to sequester anthropogenic carbon dioxide. Aquatic microalgae have been identified as fast growing species whose carbon fixing rates are higher than those of land-based plants by one order of magnitude. Physical Sciences Inc. (PSI), Aquasearch, and the Hawaii Natural Energy Institute at the University of Hawaii are jointly developing technologies for recovery and sequestration of 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 January to 31 March 2001 in which Aquasearch tested 24 different species of microalgae for growth at three different temperatures. Eleven species were analyzed for the presence of high-value pigments. Most of the algae analyzed were good sources of industrially valuable pigments. Analysis of the methods for introducing and dissolving CO₂ in the commercial bioreactor was begun this quarter.

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1. INTRODUCTION

Emissions of carbon dioxide are predicted to increase in the next 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 10-15 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.

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

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

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

2. EXECUTIVE SUMMARY

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. 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. The advantages of the proposed process include the following.

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

The proposed program calls for development of key technologies pertaining to: (1) treatment of effluent gases from the fossil fuel combustion systems; (2) transferring the recovered CO₂ into aquatic media; and (3) converting CO₂ efficiently by photosynthetic reactions to materials to be re-used or sequestered. A description of the work plan for accomplishing these objectives is presented in Section 2.2 of this report.

The work discussed in this report covers the reporting period from 1 January to 31 March 2001. The goals for the first year (October '00- September '01) of the "Capture and sequestration of CO₂ from stationary combustion systems by photosynthesis of microalgae" project are to survey 20 different species of microalgae, at laboratory scale, for their capacity to

- Withstand high growth temperatures
- Withstand the non-CO₂ gases generated during the combustion of fossil fuels
- Present high CO₂ uptake rates
- Either mineralize the CO₂ taken up (i.e., produce refractory inorganic carbon compounds such as carbonate minerals) or produce high value products than would offset the cost of carbon sequestration.

The result of this first year of research will be to select 3 species that mineralize CO₂ and three species that produce high value products utilizing flue gas mixtures as the cultures' carbon source. These selected species will then be used in years 2 and 3 in demonstration studies of microalgal carbon sequestration at commercial scales in outdoor photobioreactors.

Up to this point in time we have

- Tested 24 different species of microalgae for growth at three different temperatures (15, 20 and 25 °C)
- Analyzed 11 different species for high-value pigments
- Started testing 1 species at the chemostat level for carbon uptake rate
- Estimated the global carbon sequestration efficiency of a commercial microalgal facility to be used as a benchmark.

Our preliminary results indicate that

- Out of 24 species of microalgae tested so far 22 grew at 15, 20 and 25 °C (one species did not grow at 15 °C and one species did not grow at 25 °C)
- Out of the species analyzed so far for high value pigment content one appears to be a good source of the carotenoid zeaxanthin, 7 appear to be good sources of phycocyanin and 4 appear to be good sources of phycoerythrin
- Based on preliminary analysis of the first chemostat-grown culture, the estimated efficiency of CO₂ uptake by the culture is several fold higher than our benchmark, a commercial microalgal facility.

In the photobioreactor, we aim to maximize the transfer of CO₂ to the water, while minimizing the amount of CO₂-stripping by the air that is being injected. At the level of this analysis, it is obvious that if the CO₂ is to be injected separately from the air, then the size of the bubbles from the air injectors should be large to produce the largest possible bubbles. This will reduce CO₂-stripping. At the same time, the bubble size for the CO₂ inlet stream should be as small as possible. A strong dependence of mass transfer on diameter was noted.

3. EXPERIMENTAL

3.1 Subtask 2.1 - Characterization of Physiology, Metabolism and Requirements of Microalgae

3.1.1 Microalgal Culture Collection

The Aquasearch Culture Collection consists at the present time of 48 different strains of microalgae representing an estimated 39 species (Figure 2). Forty two strains have been isolated locally (i.e., in Hawaii) by the staff at Aquasearch and are maintained as unialgal cultures. It is expected that strains isolated in Hawaii are adapted to relatively high temperatures. Furthermore, six strains have been imported from established culture collections and about 40 more strains will be imported over the following two months. The strains are maintained on an agar-based nutrient medium. When needed for an experiment, cells from the agar cultures are transferred to test tubes containing liquid growth medium. After a few days of growth (may vary depending on the strain) the cultures are transferred to larger containers such as 250 ml Erlenmeyer flasks. Further scale up is performed according to the type of experiment planned.

Figure 2. The Aquasearch Culture Collection. At the present time 48 strains of microalgae are maintained. Forty more strains will be added over the next 2 months.

In February '01 scale up of the microalgal cultures was started. Up to this point the cultures had been maintained in Petri dishes on a solid nutrient medium (agar based). The cultures were started in liquid nutrient medium in small test tubes (5 ml). The cultures were grown under a 14:10 hr light:dark cycle in a temperature-controlled room ($24^{\circ}\text{C} \pm 2^{\circ}\text{C}$) until enough biomass was produced to inoculate Erlenmeyer flasks (250 ml flasks with 100-150 ml of nutrient medium).

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

Table 1. List of Strains that Have Already Been Grown on Liquid Medium as Well as the Largest Cultivation Container to Which They Have Been Scaled (Tt = test tube, E2 = 250 ml Erlenmeyer flask, CH = 3 liter chemostat). For proprietary considerations the strains are only identified by their culture collection identifier. The dates indicate when the different growth experiments were started.

AQ Strain Number	Largest Scale	15°C Growth	20°C Growth	25°C Growth	30°C Growth	35°C Growth	Chemostats	HPLC
AQ0001	Tt							No
AQ0002	Tt							No
AQ0003	Tt							No
AQ0008	Tt							No
AQ0009	Tt							No
AQ0011	E2	3/24/01	3/24/01	3/24/01				No
AQ0012	CH	3/17/01	3/17/01	3/17/01			4/17/01	Yes
AQ0013	E2	3/24/01	3/24/01	3/24/01				No
AQ0014	Tt							No
AQ0015	Tt							Yes
AQ0016	E2	4/18/01	4/18/01	4/18/01				Yes
AQ0017	E2	4/18/01	4/18/01	4/18/01				No
AQ0018	E2	3/17/01	3/17/01	3/17/01				Yes
AQ0019	E2	4/18/01	4/18/01	4/18/01				No
AQ0020	E2	4/18/01	4/18/01	4/18/01				No
AQ0021	E2	4/18/01	4/18/01	4/18/01				Yes
AQ0022	E2	3/24/01	3/24/01	3/24/01				No
AQ0023	E2	3/17/01	3/17/01	3/17/01				Yes
AQ0024	E2	3/24/01	3/24/01	3/24/01				No
AQ0025	E2	3/24/01	3/24/01	3/24/01				No
AQ0026	Tt							No
AQ0027	E2	4/18/01	4/18/01	4/18/01				No
AQ0028	E2	3/24/01	3/24/01	3/24/01				No
AQ0029	E2	3/24/01	3/24/01	3/24/01				No
AQ0030	E2	4/18/01	4/18/01	4/18/01				Yes
AQ0031	E2	4/18/01	4/18/01	4/18/01				Yes
AQ0032	E2	3/17/01	3/17/01	3/17/01				Yes
AQ0033	Tt							No
AQ0034	Tt							No
AQ0035	Tt							No
AQ0036	Tt							No
AQ0037	E2	4/18/01	4/18/01	4/18/01				Yes
AQ0038	E2	4/24/01	4/24/01	4/24/01				No

Table 2. List of Strains that Have Already Been Grown on Liquid Medium as Well as the Largest Cultivation Container to Which They Have Been Scaled Were Started (Continued)

AQ Strain Number	Largest Scale	15°C Growth	20°C Growth	25°C Growth	30°C Growth	35°C Growth	Chemostats	HPLC
AQ0039	E2	4/18/01	4/18/01	4/18/01				Yes
AQ0040	E2	3/24/01	3/24/01	3/24/01				No
AQ0041	E2	3/24/01	3/24/01	3/24/01				No
AQ0042	E2	3/24/01	3/24/01	3/24/01				No
AQ0043	E2	3/24/01	3/24/01	3/24/01				No
AQ0044	Tt							No
AQ0045	Tt							No
AQ0046	Tt							No
AQ0048	Tt							No
AQ0049	Tt							No
AQ0050	Tt							No
AQ0051	Tt							No

Culture Growth

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:

$$\mu = \text{Ln} \left(\frac{F_2/F_1}{\Delta T} \right) \quad (1)$$

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.

To determine the growth rates of microalgae at different temperatures, cultures were incubated submerged in temperature-controlled water baths at $60 \mu\text{E m}^{-2} \text{s}^{-1}$ of PAR. The cultures were grown in 250 ml Erlenmeyer flasks. The flasks were agitated three times daily.

3.1.2 High Value Pigment Analysis

Microalgae are a diverse group of over 30,000 species of microscopic plants that have a wide range of physiological and biochemical characteristics. Microalgae produce many different substances and bioactive compounds that have existing and potential applications in a variety of commercial areas, including human nutrition, pharmaceuticals, and high value commodities. Algal pigments (carotenoids and phycobiliproteins) are one such group of molecules. Examples of natural algal pigments that already have been commercialized include B-carotene (food additive grade worth about \$1,400 per kg, market size estimated >\$500 million per year),

astaxanthin (feed additive grade worth about \$2,500 per kg-market size about \$200 million- but up to >\$100,000 per kg for nutraceutical grade-market size not know at this point).

Phycobiliprotein Pigments

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

Carotenoid Pigments

Carotenoids are analyzed via High Performance Liquid Chromatography (HPLC). The carotenoid pigments are extracted from the algal biomass with a mixture of methanol, ammonium acetate and dimethyl sulfoxide. The extract is then injected into the HPLC system. Our system consists of a Beckman System Gold with a model 126 programmable solvent module, a model 168 diode-array detector, and a model 508 injector with a 100 uL loop. The column is a Supelco Discovery C8 column 150x4.6 mm, 5 um particle size. The solvent system consisted of

- A. MeOH: Acetonitrile: Acetone at 20:60:80
- B. MeOH: Ammonium Acetate 0.25M: Acetonitrile at 50:25:25

using the following time program:

Solvent B	100%	→	60%	over 22 minutes
Solvent B	60%	→	5%	over 6 minutes
Solvent B	5%			for 10 minutes
Solvent B	5%	→	100%	over 2 minutes.

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

3.1.3 CO₂ Utilization Efficiency

CO₂ Utilization Efficiency of a Commercial Microalgal Facility

We have estimated the CO₂ utilization efficiency of Aquasearch's commercial facility which produces a high value pigment (astaxanthin) from the microalga *Haematococcus pluvialis*. The efficiency was calculated as the ratio of the amount of carbon contained in the biomass of *H. pluvialis* produced by Aquasearch to the amount of CO₂ that Aquasearch purchases for biomass production. The result is given in Section 4.2.1.

CO₂ Utilization Efficiency of an Experimental Chemostat

The CO₂ utilization efficiency of our first experimental chemostat culture was calculated as the ratio of the amount of carbon taken up by the experimental culture per unit time to the amount of CO₂ that is fed into the chemostat per unit time. The uptake of CO₂ by the culture is estimated from changes in the concentration of CO₂ in the growth medium as indicated by changes in pH and from changes in the biomass. The amount of CO₂ that is fed into the chemostat is estimated from the flow rate of the CO₂ gas times the period of time during the CO₂ is flowing into the chemostat. The CO₂ utilization efficiency is given in Section 4.2.1.

The chemostat cultures are temperature and pH regulated. A pH probe is immersed in the growth medium and automatically logs the pH of the culture with a resolution of 15 seconds. A computer is programmed with the high and a low pH set points. When the pH of the culture rises, caused by CO₂ uptake by the microalgal cells, above the high set point, a stream of CO₂ is automatically injected into the culture causing a drop in pH. When the pH becomes lower than the low set point, the CO₂ stream is automatically shut off. The flow rate of the CO₂ stream multiplied by the duration of the injection results in the total amount of CO₂ injected. The amount of carbon actually taken up by the cells in the culture is estimated from the biomass of the culture and its growth rate.

4. RESULTS AND DISCUSSION

4.1 Task 1.0 - Supply of CO₂ from Power Plant Flue Gas to Photobioreactor

4.1.1 Subtask 1.3 - Carbon Dioxide Dissolution Method

In the small, laboratory scale reactors used in Task 2, any introduced gases are effectively mixed instantaneously throughout the culture medium. This is not so in larger scale reactors (particularly the 25,000 L AGM), due to the physical configuration of the photobioreactor.

In this task, we will undertake theoretical and experimental investigations of the optimum method for dissolving carbon dioxide from the flue gas mixtures into the aqueous environment of the bioreactors. In the current Aquasearch commercial reactor, there are two gas streams added to the photobioreactor. A large stream of air is added using multiple injectors arranged radially near the walls of the photobioreactor. Large (~ ½ inch) diameter nozzles are used. This air is used to add momentum to the liquid and promote liquid circulation in the long tube that comprises the photobioreactor. In the current design, a high-volume, low-pressure flow of filtered ambient atmosphere is introduced in a 2-m vertical airlift section of the reactor in which fluid rises, creating the head pressure necessary for recirculation.

Slightly upstream of the air injection location, CO₂ is added through a small pipe with a sparger on the end to produce small bubbles. The CO₂ is introduced in a 2-m “down-flowing” section, where it rises against the fluid flow. This procedure dramatically improves the dissolution of CO₂. CO₂ is not added continuously, but rather is added when the pH of the liquid rises to a certain level. CO₂ is used both to provide carbon for growth of microalgae and to keep the pH in an optimum regime for growth.

In the commercial-scale photobioreactors, air is needed for circulating the liquid. In the smaller chemostats that are used to grow microalgae in the laboratory, CO₂ is sometimes used by itself, without adding any air to promote mixing. This can result in much higher carbon conversion (or efficiency of CO₂ utilization) than in the larger scale system. As far as the growth rate of microalgae is concerned, air has both advantages and disadvantages.

The chief advantage of air is that it removes some of the dissolved oxygen in the water. Photosynthesis results in the production of O₂ by the microalgae. Under some conditions, the water can be supersaturated with oxygen. When this occurs, the rate of photosynthesis (and growth) falls rapidly. The relatively large flow of air through the photobioreactors strips out some of the dissolved oxygen and prevents high levels of supersaturation.

The chief disadvantage of air addition is that it strips CO₂ as well as O₂ from the water. The removal of CO₂ lowers the efficiency of carbon utilization by 50% to 80%. Thus, in the commercial photobioreactors, the efficiency of CO₂ utilization (based on carbon production and CO₂ usage) is only 12.5%.

What determines the efficiency of CO₂ utilization by the microalgae: the rate of incorporation of CO₂ into the liquid or the rate of uptake of CO₂ by the microalgae? In the

former situation, the microalgal growth is limited by the rate that CO₂ is dissolved into the liquid. In the latter, there is adequate CO₂ in the water and the rate is limited by the available sunlight. We would like to find a balance in which as much of the CO₂ added as possible is incorporated into biomass.

If we introduce a flue gas containing 5 to 10% CO₂ in the downflowing section where pure CO₂ is currently introduced, the 10 to 20-fold increase in flow rate could create a substantial back-pressure on the airlift-driven circulation. We may be able to solve this problem by simply increasing the flow rate of the airlift supply to overcome the flow rate of the flue gas. However, the solution may not be so simple. The flue gas supply will be pulsed (because it is used to regulate pH), whereas the airlift is continuous. Thus, we might create a strongly modulated fluid flow rate that is not favorable to the microalgae cultures. Other solutions could involve (a) decreasing bubble size of the flue gas to provide for higher dissolution rates, or (b) automatic modulation of the airlift flow rate to offset the counter-flow of flue gas.

In this quarter, we began a theoretical investigation to explore the limits of the mass transfer and the dependence of mass transfer on operating parameters, in preparation for a more detailed experimental and theoretical investigation. The mass transfer rate to bubbles is controlled by:

- The concentration driving force between the interface and the bulk liquid;
- The interfacial area for mass transport, a ;
- The mass transfer coefficient in the liquid, k_x .

The resistance on the gas-side in the bubble is usually negligible unless the gas in question is very soluble in the liquid; therefore, mass transfer in the gas will be neglected. The overall mass transfer rate, in moles per sec per volume of liquid is:¹

$$N_A/V = k_x a (x_i - x) \quad (2)$$

where x and x_i are the mole fractions of component A in the liquid at the bulk and interface, respectively. Once again, a is the interfacial area per unit volume and k_x is the mass transfer coefficient in the liquid.

For small bubbles (diameters less than 0.5 mm), the liquid mass transfer coefficient is calculated from the following relationship:¹

$$\frac{k_x D_p M_\ell}{\rho D v} \quad (3)$$

where D_p is the bubble diameter
 M_ℓ is the mean molecular weight of the liquid
 ρ is the liquid density

$\mathcal{D}_v$ is the diffusivity of the dissolved gas in the liquid
 μ is the liquid viscosity
 $\Delta\rho$ is the difference between the liquid and gas density ($\sim\rho$)
 g is the gravitational acceleration

For large bubbles (diameters greater than 2.5 mm), the following should be used:¹

$$\frac{k_x D_p M_\ell}{\rho \mathcal{D}_v} = 0.42 \left(\frac{\mu}{\rho \mathcal{D}_v} \right)^{1/2} \left(\frac{D_p^3 \rho \Delta \rho g}{\mu^2} \right)^{1/3} \quad (4)$$

In order to use Eq. (2), we need to have the mean diameter of the bubbles and the interfacial area. The interfacial area can be calculated from:¹

$$a = \frac{6\varepsilon}{D_p} \quad (5)$$

where ε is the gas hold-up (the relative volume of the dispersed phase, i.e., the gas). Equation (5) was used to calculate the interfacial area using values for ε taken from Figure 18-129 in Reference 2.

Using Eq. (2), the maximum rate of mass transfer can be estimated by setting the bulk concentration of the gas species A to zero and by using the saturated value for the interfacial concentration. Thus

$$N_A/V \approx k_x a x_i \quad (6)$$

In Figure 3, this maximum mass transfer rate is plotted as a function of diameter using the "small" bubble relationship (Eq. (3)) and the "large" bubble relationship (Eq. (4)). The calculations were carried out for pure water at 20°C. One set of curves applies to O₂ and the other, to CO₂. The differences in solubilities between the two molecules account for the differences in maximum amount of mass transfer.

The small bubble regime is probably more typical of the CO₂ injection sparger, which is designed to produce fine bubbles. The large bubble regime is more like the air injectors which are one-half inch pipes. For formation of single bubbles from a submerged orifice of diameter D_o , the bubble diameter is given by:¹

$$D_p = \left[\frac{6D_o \sigma}{g \Delta \rho} \right]^{1/3} \quad (7)$$

where σ is the interfacial tension. Using this equation, the diameter of the bubbles from the air nozzles in the Aquasearch photobioreactors is estimated to be 8 mm.

Figure 3. Maximum mass transfer rate from bubbles in water at 20°C as a function of diameter.

In the photobioreactor, we would like to maximize the transfer of CO₂ to the water, while minimizing the amount of CO₂-stripping by the air that is being injected. At the level of this analysis, it is obvious that if the CO₂ is to be injected separately from the air, then the size of the bubbles from the air injectors should be large to produce the largest possible bubbles. This will reduce CO₂-stripping. At the same time, the bubble size for the CO₂ inlet stream should be as small as possible. This is what one would expect, of course, but Figure 3 shows how strong the dependence is on diameter.

4.2 Task 2.0 - Selection of Microalgae

4.2.1 Subtask 2.1 - Characterization of Physiology, Metabolism and Requirements of Microalgae

Culture Growth

Figure 4 summarizes the results of culture growth for 24 strains at three different temperatures. The data indicate there is a large degree of uncertainty about the mean growth rate of each culture (standard deviation > mean in all cases, not shown). This is the case for two reasons. First, these experiments were designed to quickly provide information on temperature tolerances for the different strains. As such the cultures were grown in batch mode. Cultures in batch mode show different growth rates at different stages of the cultures' growth curve. Second, a number of these cultures are of a filamentous and clumping nature. Thus the cells are not uniformly distributed throughout the growth medium. This translates into inherently noisy data. However, from the point of view of the objective of the experiment (to determine the temperature tolerances of the different microalgal strains) the results clearly show that, except for one, the strains tested to date grow well at up to 25 °C. Further experiments will test growth at 30 and 35°C.

Figure 4. Results of growth rate measurements on 24 species of microalgae at three different temperatures.

High Value Pigment Analysis

So far we have analyzed the pigment content of 11 different microalgal strains. This first batch of strains tested is made up entirely of microalgae from the Cyanobacteria. This group was tested first since the Cyanobacteria are good potential candidates as sources of high value pigments. Two different cultures were analyzed from each strain; a relatively young culture and a relatively older culture

The results of our analysis of carotenoid pigments are summarized in Figure 5. The most abundant carotenoids of these strains are zeaxanthin and B-carotene and are reported as the mass ratios to chlorophyll-a, an indicator of algal biomass. B-carotene is used widely as a food coloring in margarine, butter, drinks, cakes and candies. It is also sold as a nutritional supplement or nutraceutical. Zeaxanthin is a carotenoid believed to be important in human nutrition and, specially, eye health. Of the 11 strains tested, AQ0012 showed potential as a source of zeaxanthin, a high value carotenoid pigment believed to be important in human nutrition. Five other strains may be considered potential sources of B-carotene, also a high value pigment. All strains had phycobiliproteins as part of their pigment complement (characteristic of the Cyanobacteria). Of those, seven strains contained phycocyanin and four contained phycoerythrin. The phycobiliproteins are high value molecules used to produce fluorescent probes useful in diagnostic biochemistry.

Figure 5. Summary of carotenoid pigment analysis of 11 strains of Cyanobacteria. Zea/Chl: mass ratio of zeaxanthin to chlorophyll-a, B-car/Chl: mass ratio of B-carotene to chlorophyll-a, Zea/Bcar: mass ratio of zeaxanthin to B-carotene. The values reported are the average of measured concentrations in two different cultures of each strain.

CO₂ Utilization Efficiency

CO₂ Utilization Efficiency of a Commercial Microalgal Facility

The calculated CO₂ utilization efficiency for Aquasearch's commercial facility for the production of astaxanthin from *H. pluvialis* is about 12.5 %. This means that 12.5% of the CO₂ purchased by Aquasearch to control the pH of, and provide carbon nutrition to, its cultures is captured in the biomass harvested.

CO₂ Utilization Efficiency of an Experimental Chemostat

Figure 6 is made up of two photographs of the same chemostat containing a culture of microalgal strain AQ0012 but at different times (April 20 and April 27, 2001, respectively) and it shows the potential for carbon sequestration of microalgal cultures. Figure 7 shows the changes of pH in this chemostat over a two day and one night period (39 hr). The upward changes in pH are produced by photosynthetic uptake of CO₂ from the culture medium, the drops are produced by automatic injection of CO₂ into the culture medium. The data is collected by our automatic monitoring and control system and is used to estimate the amount of CO₂ injected into the culture.

Figure 6. Photographs of the same chemostat culture (AQ0012) seven days apart showing the large capacity for carbon sequestration of microalgal cultures. The panel on the left shows little biomass, mostly concentrated in 3-4 mm clumps. The panel on the right is the same culture after seven days of photosynthetic growth.

Figure 7. Computer generated trace of culture pH measured in the chemostat photographed in Figure 6. Rises in pH are caused by photosynthetic uptake of CO_2 by the algal cells. When the pH reaches the high set point (7.6 pH in this case), CO_2 is automatically injected into the culture medium and the pH drops. The broken line represents the periods during which the culture received light. The slope of the rise in pH, driven by photosynthesis, indicates carbon uptake by the culture.

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

5. CONCLUSION

Up to this point in time we have

- Tested 24 different species of microalgae for growth at three different temperatures (15, 20 and 25 °C)
- Analyzed 11 different species for high value pigments
- Started testing 1 species at the chemostat level for carbon uptake rate
- Estimated the global carbon sequestration efficiency of a commercial microalgal facility to be used as a benchmark.

Our preliminary results indicate that

- Out of 24 species of microalgae tested so far 22 grew at 15, 20 and 25 °C (one species did not grow at 15 °C and one species did not grow at 25 °C)
- Out of the species analyzed so far for high value pigment content one appears to be a good source of the carotenoid zeaxanthin, 7 appear to be good sources of phycocyanin and 4 appear to be good sources of phycoerythrin
- Based on preliminary analysis of the first chemostat-grown culture, the estimated efficiency of CO₂ uptake by the culture is several fold higher than our benchmark, a commercial microalgal facility.

In the photobioreactor, we would like to maximize the transfer of CO₂ to the water, while minimizing the amount of CO₂-stripping by the air that is being injected. At the level of this analysis, it is obvious that if the CO₂ is to be injected separately from the air, then the size of the bubbles from the air injectors should be large to produce the largest possible bubbles. This will reduce CO₂-stripping. At the same time, the bubble size for the CO₂ inlet stream should be as small as possible. A strong dependence of mass transfer on diameter was noted.

Within the next quarter we expect to

- Obtain 40 more microalgal strains known to produce high value products or mineralize carbon
- Continue to carry out temperature experiments (up to 30 and 35 °C)
- Carry out carbon sequestration experiments in chemostat cultures, and
- Set up the hardware necessary for simulated flue gas experiments in chemostats.
- Obtain 40 more microalgal strains known to produce high value products or mineralize carbon.

One of our sources for the microalgal strains will be the University of Hawaii. In January 2001, Aquasearch executed an agreement to develop pharmaceuticals from a unique collection of approximately 2,000 strains of microalgae held by the University of Hawaii. The agreement gives Aquasearch exclusive rights to more than 100 unique, bioactive compounds from microalgae contained in the collection. Two of the compounds are now in Phase II clinical trials for treatment of cancer. Pharmaceuticals derived from microalgae are expected to be worth 1 to 10 million per kg, which would more than offset the cost of CO₂ sequestration utilizing microalgae.

In the photobioreactor, we would like to maximize the transfer of CO₂ to the water, while minimizing the amount of CO₂-stripping by the air that is being injected. At the level of this analysis, it is obvious that if the CO₂ is to be injected separately from the air, then the size of the bubbles from the air injectors should be large to produce the largest possible bubbles. This will reduce CO₂-stripping. At the same time, the bubble size for the CO₂ inlet stream should be as small as possible. A strong dependence of mass transfer on diameter was noted.

6. REFERENCES

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