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Abstract

This report documents significant achievements in the Enhanced Practical Photosynthetic CO_2 Mitigation project during the period from 10/03/2000 through 10/02/2001. Most of the achievements are milestones in our efforts to complete the tasks and subtasks that constitute the project objectives. This is the fourth quarterly report for this project, so it also serves as a year-1 project review. We have made significant progress on our Phase I objectives, and our current efforts are focused on fulfilling these research objectives "on time" relative to the project timeline. Overall, we believe that we are on schedule to complete Phase I activities by 10/2002, which is the milestone date from the original project timeline.

Our results to date concerning the individual factors which have the most significant effect on CO_2 uptake are inconclusive, but we have gathered useful information about the effects of lighting, temperature and CO_2 concentration on one particular organism (Nostoc) and significant progress has been made in identifying other organisms that are more suitable for use in the bioreactor due to their better tolerance for the high temperatures likely to be encountered in the flue gas stream. Our current tests are focused on one such thermophilic organism (Cyanidium), and an enlarged bioreactor system (CRF-2) has been prepared for testing this organism.

Tests on the enhanced mass transfer CO_2 absorption technique are underway and useful information is currently being collected concerning pressure drop. The solar collectors for the deep-penetration hybrid solar lighting system have been designed and a single solar collector tracking unit is being prepared for installation in the pilot scale bioreactor system currently under construction. Much progress has been made in designing the fiber optic light delivery system, but final selection of the "optimum" delivery system design depends on many factors, most significantly the configuration and orientation of the growth surfaces in the bioreactor.

For the growth surface subsystem we have identified advantages and disadvantages for several candidate growth surface materials, we have built and tested various "screen" systems and fluid delivery systems, and we continue to test compatibility of the candidate materials with the organisms and with the moisture delivery and harvesting system designs. These tests will be ongoing until an "optimum" combination of growth surface material / organism type / harvesting system is identified. For the harvesting system, a nozzle-based water jet system has been shown to be effective, but it has disadvantages for the overall system design in terms of space utilization. A streamlined and integrated screen wetting / harvesting system design is currently under development and will be the focus of harvesting system tests in the foreseeable future.

This report addresses each of the key project tasks as defined in the statement of work, giving both a summary of key accomplishments over the past year and a plan for future work.

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Results and Discussion

Task 1.0. Evaluate and rank component and subsystem level alternative design concepts
Subtask 1.1 Investigate critical properties of alternative photosynthetic agents (cyanobacteria)

Significant progress has been made by researchers at Montana State in the effort to identify alternative photosynthetic agents for use in practical bioreactor systems. Their reports on the collection of organisms from various sites within Yellowstone National Park and the subsequent culture purification in the laboratory are included below.

Isolation of organisms and their introduction into culture.

Yellowstone National Park has been visited four times: July 10 and 23; August 15-16 and 30-31. Samples were taken from the following pools and springs. See Figure 1 for pictures and more information about the sampling sites.

a) A roadside pool at Corwin Hot Springs - between 6 and 7 miles on the road Gardiner – Livingston; this spring is characterized by an obvious deposit which may be an iron oxide ,
b) Black Sand Pool - about 10 miles before Old Faithful geyser; specific property of this pool – very low amount of Ca^{2+}, but high concentration of dissolved silicon;
c) Angel Terrace (Mammoth area); specific property of this pool – the continuous deposition of CO_2 into travertine, sometimes called "tufa". It consists of calcite that is precipitated from water in hot or cold springs;
d) Unnamed spring, close by the south shore of Rabbit Creek; judging by its color, this spring is characterized by a very large biomass of phototrophs.

Two approaches were used for the sampling during 3rd quarter: the traditional method of scraping of biological samples from rock surfaces, and an alternative method of selecting organisms from the environment with the help of anchored pieces of potential growth surface materials. Scotch Brite, white felt (polypropylene), and brown felt (Teflon) were the three candidate materials tested this quarter. We have referred to such substrates as "traps" for *in situ* colonization by cyanobacteria. The rationale here is that we wish to find cyanobacteria which we know will grow well on the experimental substrata, so these surfaces are used in the *initial* isolation of the organisms from the natural environment

Twenty-nine samples from thermal inventories have been collected during 3rd quarter. Sixteen of them were collected with help of traps. Almost all samples are now in the culture. Each sample has been divided on three parts and then inoculated separately in three media: BG-11; D and DH. [see Appendix I]

The majority of samples grew best in BG-11 medium, though some of them showed better growth in D or DH media. It is planned to classify organisms based on their ability to grow in these three media and to select the most promising organisms for use in the pilot facility at Ohio University. At the moment BG-11 medium might be the one we would recommend for future cultivation of thermotolerant and thermophylic cyanobacteria in the large scale bioreactors used at Ohio University.

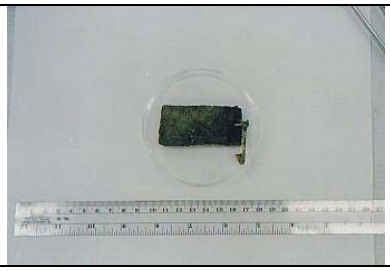
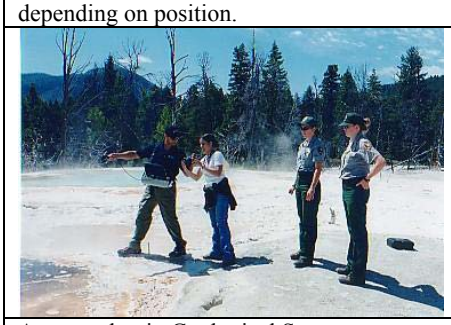
		
Fig.1a Corwin Spring, roadside. This spring is located 6 miles before Gardiner MT. pH 6-8. 57°C, It appears that the water of this spring has a very high level of iron oxide. There are two fresh traps in the picture.	Fig. 1b. Corwin Spring, riverside. pH 6-8. 57°C, The water of this spring is less saturated with iron oxide	Fig. 1c. The trap made from poly-propylene is sown after two weeks of incubation in riverside stream.
		
General picture of Black Sand Pool. Temperature varies between 41-75°C, depending on position.	Trap 1, ScothBrite, after three weeks exposure in Black Sand Pool. 72°C, pH8.5.	Trap 1 (from previous figure) seen from its bottom side. There are no obvious cells on the upper side.
		
A co-worker in Geological Survey team shoots reflected spectrum of bacterial community in a thermal pool on upper terrace of Angel Terrace (7/23/01).	The panorama of a pool on upper terrace of Angel Terrace on 7/23/01. 44°C, pH 7.9. The water is saturated with CO_2 and Ca^{2+}.	Trap 8.1 after three weeks of exposure in this area.
		
Unnamed pool in Rabbit Creek area. 57°C, pH 8.8.	Trap 9.1 (ScotchBrite) in two weeks exposure to this pool.	Trap 9.1b (Polypropylene) after two weeks of exposure in this pool.

Fig. 1. Pictures of sampling sites and the characteristics of these sites.

Of the three candidate growth surface materials the best trap for cyanobacteria in thermal springs and pools was Scotch Brite. This is probably related to the macroporous structure of this

substratum. Polypropylene possesses second place. Microscopic observations suggest that Brown Felt can be fouled by cyanobacteria only as secondary process.

The sampling on the Angel terrace in Mammoth area on July 23 was carried out in co-operation with representative of US Geological Survey. He measured the spectral characteristics of the places of sampling and promised to supply us with these results but as yet has not done so.

Culture purification in the laboratory

The purification of cultures is carried out on the basis of some specific properties of cyanobacteria. In particular, we used the motility of the trichome forms of cyanobacteria on semisolid surface [agarose] to isolate them from non-motile forms. Using this approach we have prepared almost pure cultures of *Oscillatoria princeps, Leptolingbia sp*., *Spirulina sp., Masticogladus sp.,* and others not yet identified Non-motile organisms were streaked on the surface of solid media[agar]. We have isolated a number of single colonies, which are now growing in liquid media. Nonetheless, preliminary microscopical observations suggest that representatives of species of *Synechococcus, Cyanothece,* and *Synechocystis* are the most evident non-motile forms of cyanobacteria inYNP samples we have taken. At the moment organism identities are based solely on microscopic examination.

The main problem with the isolation of pure cultures of cyanobacteria is that almost all cyanobacterial samples from sites are heavily contaminated with *Chloroflexus sp*., a non-sulfur green bacterial species. The requirement to separate cyanobacterial cultures from *Chloroflexus sp*. was unforeseen, and consequently, represents an additional task for this project**.**

K-Bogoria, provided by the Oregon University Culture Collection, has been grown in the tubular bioreactor using BG-11 medium with 5 mM or 10 mM NaHCO3 in addition to bubbling with 5% CO_2 in air. It was found (Fig. 2) that both concentrations of $NaHCO_3$, 5 and 10 mM, can be used for the cultivation of K-Bogoria, though the onset of the log-phase was more rapid in the presence of 5 mM $NaHCO_3$.

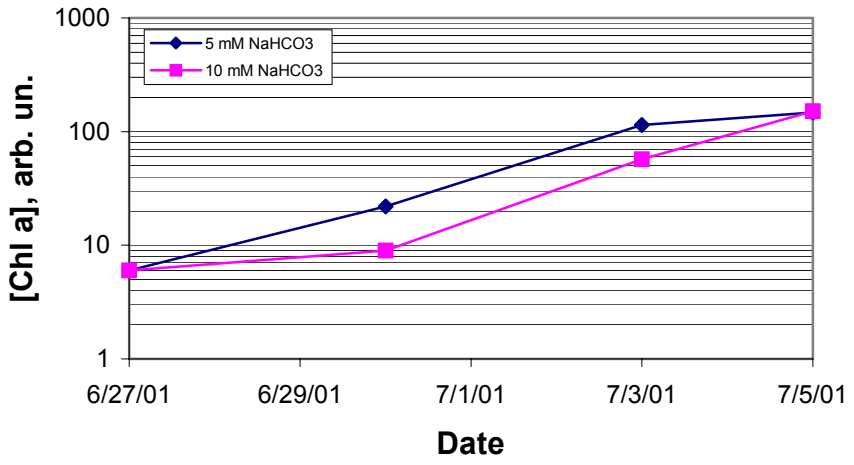


Fig. 2. K-Bogoria growth in BG-11 media, supplemented with CO_2 and $NaHCO_3$

Measurement of pH in growth medium (Fig. 3) showed that bubbling the media using a flow rate of 20-30 ml/min 5%CO_2 in air kept the pH of media at about 8.0. Without CO_2 bubbling, the pH rose to about 10.0.

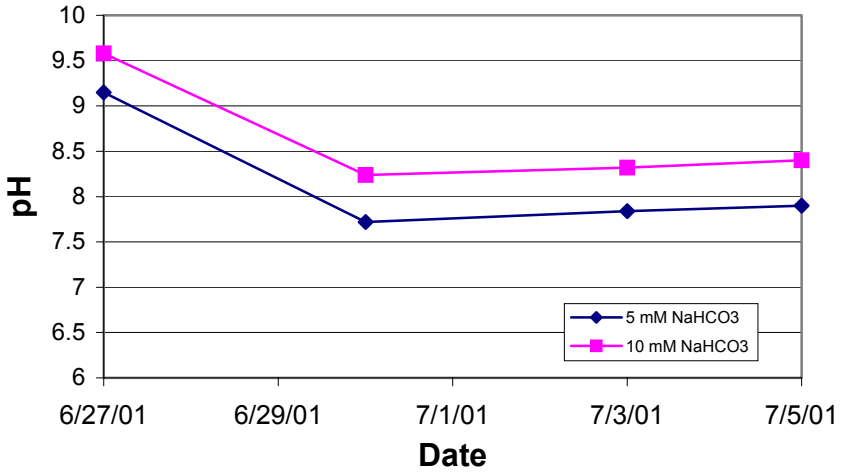


Fig. 3. pH dynamics during K-Bogoria growth in BG-11 media, supplemented with CO_2 and different amount of $NaHCO_3$

Information Gathered from VIIth Cyanobacterial Workshop

Under other auspices, Igor Brown attended the VIIth Cyanobacterial Workshop, which was held at Pacific Grove, CA, June 27-31, 2001. He gleaned the following information, which to some extent could influence the direction that this DOE project may proceed. The main topic of this workshop was the study of the mechanisms whereby cyanobacterial cells receive changes in environment and how these environmental signals influence gene expression as well as the activity of certain enzymes. In particular, some reports presented at VIIth Cyanobacterial Workshop were dedicated to the study of the mechanisms of ion homeostasis in cyanobacteria.

Dr. Hans Matthijs mentioned that only 4 % of total photosynthesis may be processed through photosystem 1 (PS 1) in higher plant chloroplasts, while in cyanobacteria this number is flexible and can increase to over 20 % when external stress requires faster turnover of adenosine three phosphate (ATP). We might assume, therefore, that PS 1 in thermotolerant cyanobacteria is already working under a high level of stress and that PS1 is working at close to its maximal activity. Consequently, the cultivation media should be low in any toxic compound that may inhibit PS 1.

Studying the expression of hydrogenases in cyanobacteria, Dr. P. Lindblad mentioned that structural studies have revealed that [NiFe] hydrogenases coordinate [FeS] clusters and harbor an active site with nickel and iron, and carbon monoxide and cyanide as ligands. That is why, we may assume that nitrogen fixing cyanobacteria might be more sensitive to CO contained in coal plant gas than non-nitrogen fixing cyanobacteria. Thus nitrogen fixing organisms may not be the best choice for large scale reactors

The data presented in the poster of S.R. Miller drew our attention to the toxic effect of sulfide. In particular, it was shown that sulfide resistance across species varied by approximately two orders

of magnitude. This should be taken into account when cyanobacterial species are chosen for exploration in the pilot plant.

I. Brown presented poster "Na^+ and Ca^{2+}-dependent membrane bound processes in freshwater cyanobacteria". The data presented in this poster suggest that both Na^+ and Ca^{2+} determine the efficiency of some membrane processes, e.g. O_2 evolution, motility and transmembrane electrical potential in cyanobacteria under alkaline conditions. Because we found that a number of cyanobacteria isolated in our project grow well only under alkaline conditions, further research of the optimal concentrations of Na^+ and Ca^{2+} for CO_2 mitigation process may be needed.

Investigation of cyanobacteria properties in model-scale bioreactors

The 30-gallon tank of the Yellowstone organism is well established and the organisms are growing well with little bacterial contamination. The culture continues to be grown in 1/2 strength Allen's media. A 100-gallon tank is almost ready for inoculation of organisms from the 30-gallon tank. This tank will allow 16 or more screens to be colonized simultaneously, as is required for the CRF-2 bioreactor system currently in development.

Ohio University researchers are continuing their efforts to develop improved procedures for quantifying organism growth rates on a large scale (for example in the bioreactor) by correlating algal chlorophyll levels with carbon levels to avoid the time consuming and inefficient "dry mass" method currently being used to determine primary productivity. The experimental procedure outlined in the third quarter report was executed but the results were inconclusive due to a large variation in the data set. Subsequently, two new test procedures were designed and executed to better understand the potential of using Chlorophyll-a as a measure of organism growth. A description of the tests and the results are included below.

Methodology for estimating the pre-test amount of Chlorophyll-a (Chl a)

In order to use Chl a as a measure of growth it is necessary to establish an initial Chl a estimate prior to the bioreactor test cycle for comparison to the post-test measurement. Since the Chl a measurement itself is destructive, we needed a method to establish an accurate pre-test estimate. We decided that adding an ~ 1 inch strip to each screen that can be removed before the experiment and used as a representative measure for an individual screen may give us the accuracy we desire. To test this idea, the 1 inch strip was added to 12 screens; the screens were soaked in the culturing vat until colonized by cyanobacteria and Chl-a measurements were made for both the strip and the actual screen to establish a correlation between the two. Figure 4 below shows the attempt to correlate the results for a reduced data set of 8 data pairs. The results appear to show no correlation, but this negative result is attributable to unacceptably large measurement variations associated with the use of a Spectrophotometer to measure Chl-a as shown by the results of our Spectrophotometer accuracy test.

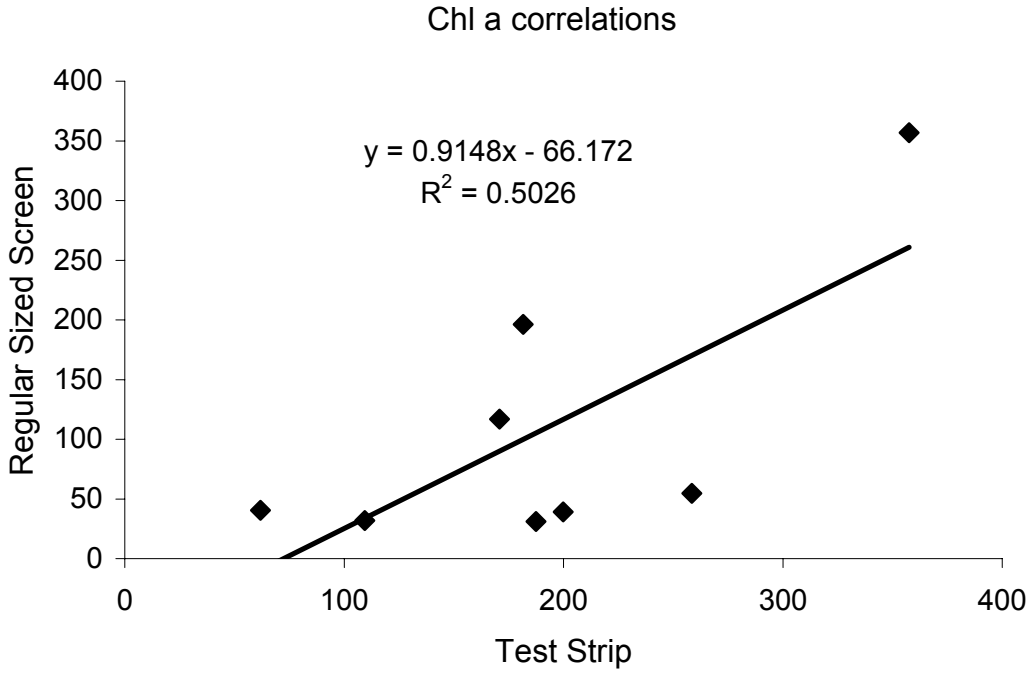


Fig. 4. Attempt to correlate disposable strip with growth surface CHL-a measurements

Spectrophotometer accuracy test

Because of the unexpected results for the first two Chl-a tests using the spectrophotometer, we executed a test to determine the accuracy of the spectrophotometer measurements of Chl a. This was accomplished by soaking 3 screens in the algae vat until they are colonized with organisms, extracting the Chl a from the screens with 90% acetone, adding all of the extracted Chl-a together, and taking 15 subsamples to be tested for Chl-a levels. Since all subsamples were expected to have the same Chl-a level, any variations in the measurements would be attributable primarily to measurement error. The actual measurement errors were found to be excessive, with over 16% uncertainty based on a student-t confidence interval and a data spread of 188. This uncertainty level makes it evident that the Spectrophotometer is not accurate enough for our intended use. In order to continue testing the Chl-a method, a more accurate measurement system must be used. We are currently investigating the use of a fluorometer for the Chl-a measurements.

Variance test	
Count	15
Mean	686
Std. Dev.	55.5
Std. Error	14.3
Min	578
Max	766
Range	188

Report on the ability of two solvents to extract putative chlorophyll from *Cyanidium calderium*.

The following report, provided by Dr. Cooksey, provides some guidance for improving future chlorophyll extraction.

In early work, we had observed that that it was difficult to extract chlorophyll pigments from Cyanidium with 90% acetone and water. The extracts, such as they were, fluoresced poorly at wavelengths known to excite higher plant and algal[diatom] chlorophylls. Since an important parameter in CO_2 conversion to organic carbon by algal or cyanobacterial biota is the efficiency with which the conversion is made, it is necessary either to measure newly - fixed carbon or an analogue of it. We have chosen to measure extractable chlorophyll fluorimetrically, since it is far easier to measure than total organic carbon and is a fairly constant fraction of the cellular biomass.

Cells of *Cyanidium calderium* were obtained from the Ohio University Carbon Sequestration group. In spite of spending a few more days than we would have liked with the Federal Express Company because of the World Trade Center attack, the organisms arrive in a good state. Microscopical observation showed them to be barely contaminated with bacteria. They were not supplied originally as an axenic culture. They were inoculated [0.5mL] into 5mL of SAG medium at pH 2.9 and incubated at 45EC under 5% CO_2 in air for 9 days. Experience has shown us that such a culture will have reached stationary phase by then. At this time after vigorous treatment of a vortex mixer, cell concentration was determined using a haemocytometer. Samples [0.25, 0.5 0.75 and 1.0mL] were filtered on to glass fiber filters[[Whatman GFC]. It was important to remove all medium because the pH of the medium will affect the stability of the chlorophyll. As an additional precaution, a suspension of $MgCO_3$ [3 drops, 10 mg/mL] was added to the vial in which the chlorophyll was to be extracted. Two series were run : one with 10 mL 90% acetone and the other with 10mL100% methanol. After 4 days at freezer temperature, the vials were shaken rapidly, the carbonate allowed to settle and the allowed to reach room temperature. The fluorescence was measured using appropriate optical filters in a Turner Model 10 fluorimeter [F_o]. Three drops of N - HCl was added and the fluorescence re-measured [F_a]. Results were calculated from F_o-F_a and any dilution factors that were employed.

The cell concentration used was 13.68 x10^6 cells /mL. Table 1 shows the results of the two series of extractions. Aqueous acetone extracts with low efficiency and unreliably. Methanol extraction on the other hand is a reliable indicator of the extractable fluorescent pigments from *Cyanidium* when the sample is about 10^7 cells or less. I make a note of caution here: The F_a measurements are usually about one half of those for F_o which suggests that pigments other than chlorophyll a were extracted. Nevertheless, extraction with methanol gave a fairly constant value for fluorescence / 10^6 cells (0.124 units /10^6 cells ± 0.011, CV=9.0%). As seen by eye, not all the green pigment was extracted from the cells, even with methanol. A second extraction did not remove all the green color, although a this extraction did remove more fluorescing material. Phycocyanin would remain in the cells in these conditions.

Table 1 Pigments extracted from *Cyanidium caldarium*

Solvent	Volume of sample ML	Relative Fluorescence	Relative fluorescence / 10^6 cells
90%Acetone	0.25	0.142	0.0415
	0.50	0.222	0.0325
	0.75	0.054	0.0053
	1.00	0.123	0.009
100% Methanol	0.25	0.410	0.120
	0.50	0.934	0.137
	0.75	1.190	0.116
	1.00	1.230	0.0899

Conclusions:

1. Methanol is a more reliable solvent to use than 90% acetone for *Cyanidium* chlorophyll determination. [We use methanol routinely for cyanobacteria].
2. A second experiment should be performed to assess how many extractions with methanol are necessary to give a reliable indicator of total carbon. It seems from this experiment that only one is required since total extraction is not necessary.
3. A sample of 10^7 cells is the maximum that can be use under these conditions. If cell number in the sample is regressed against chlorophyll fluorescence, the $R^2 = 0.9998$ for 10^7 cells or less.

Subtask 1.2 Design deep-penetration light delivery subsystem

Researchers at Oak Ridge National Laboratory provided the following report.

Overview

A deep-penetration light delivery subsystem is being developed by Oak Ridge National Laboratory to efficiently collect and deliver sunlight to the interior of a prototype bioreactor. Development of the deep-penetration light delivery subsystem is proceeding as scheduled. Three light delivery techniques (shown in Figure 5a-c) were identified and evaluated over the past several months.

The holographic diffusing technique (shown in Figure 5a) has been eliminated due to difficulties in manufacturing and limited availability of materials. Prototypes of the two remaining techniques (shown in Figures 5b,c) were developed, tested, and selected for continued investigations. Improvements to the initial prototype designs are currently underway and next-generation versions are soon to be completed. The compatibility of these luminaire designs with the bioreactor Solar Collector is being studied for potential design barriers and optimization opportunities.

Development of an Optimized Bioreactor Solar Collector

The Solar Collector, shown in Figure 6, is being developed by ORNL for use in human illumination applications and for eventual use in a pilot-scale bioreactor. Because the Solar Collector was originally developed for *human* illumination purposes, and due to the unique spectral response characteristics of the organisms used in the bioreactor, some modifications and improvements are necessary for the bioreactor application. Optimization of the primary and secondary mirror coatings to limit fiber transmission losses, increase system efficiency, and increase organic growth potentials is currently underway. Construction of a prototype system for eventual relocation to the Ohio University test facility has begun.

Testing of Next-Generation "Illumination Rod"

A next-generation "illumination rod" is being studied to determine the maximum efficiency and eventual suitability of this illumination technique for use in a pilot-scale bioreactor. Two illumination rods are used as side-illuminants to a planar plastic waveguide, resulting in a 4' x 2' sheet of even illumination. The original design of the illumination rod was flawed by fabrication difficulties. These difficulties, which were related to accurate surface scatter reproduction, have been alleviated by the current illumination rod's scattering technique, which is an experimental design conceived by 3M. The rod, shown illuminated in Figure 7, produces an even illumination of light along the length of a 1-meter cylinder. Light escapes from the illumination rod over only a limited angle, making the device well suited to effective side-illumination. Currently, the efficiency of the illumination rod is less than desirable, but potential design modifications are being tested which are aimed at increasing the design's efficiency.

Development of Next-Generation "Illumination Sheet"

The next-generation "illumination sheet" consists of 10 meters of side-emitting optical fiber sandwiched between two clear plexiglass sheets. The optical fiber is formed into a serpentine pattern before being mounted in a plexiglass frame, similar to that shown in Figure 8a,b. A mirror, fixed to the end of the optical fiber, reflects light back toward the optical source. The reflecting mirror greatly improves the efficiency and intensity distribution of the illumination sheet. The illumination sheet emits light from both sides of the sheet.

During construction of the original prototype illumination sheet, it was found that excessive losses, due to acute bends in the serpentine fiber pattern, resulted in an uneven illumination distribution (as shown in Figure 9). To avoid the uneven intensity distribution, experiments were performed to characterize the optical fiber loss as a function of bend radii. Given the ability to predict the optical loss in a fiber bend, the serpentine pattern could be modified to maximize the amount of light reaching the far side of the illumination sheet. Currently, preliminary data has been recorded for various bend angles and bend radii, and potential design changes are being considered. Additional data and testing are still required before the bend effects can be fully modeled and controlled.

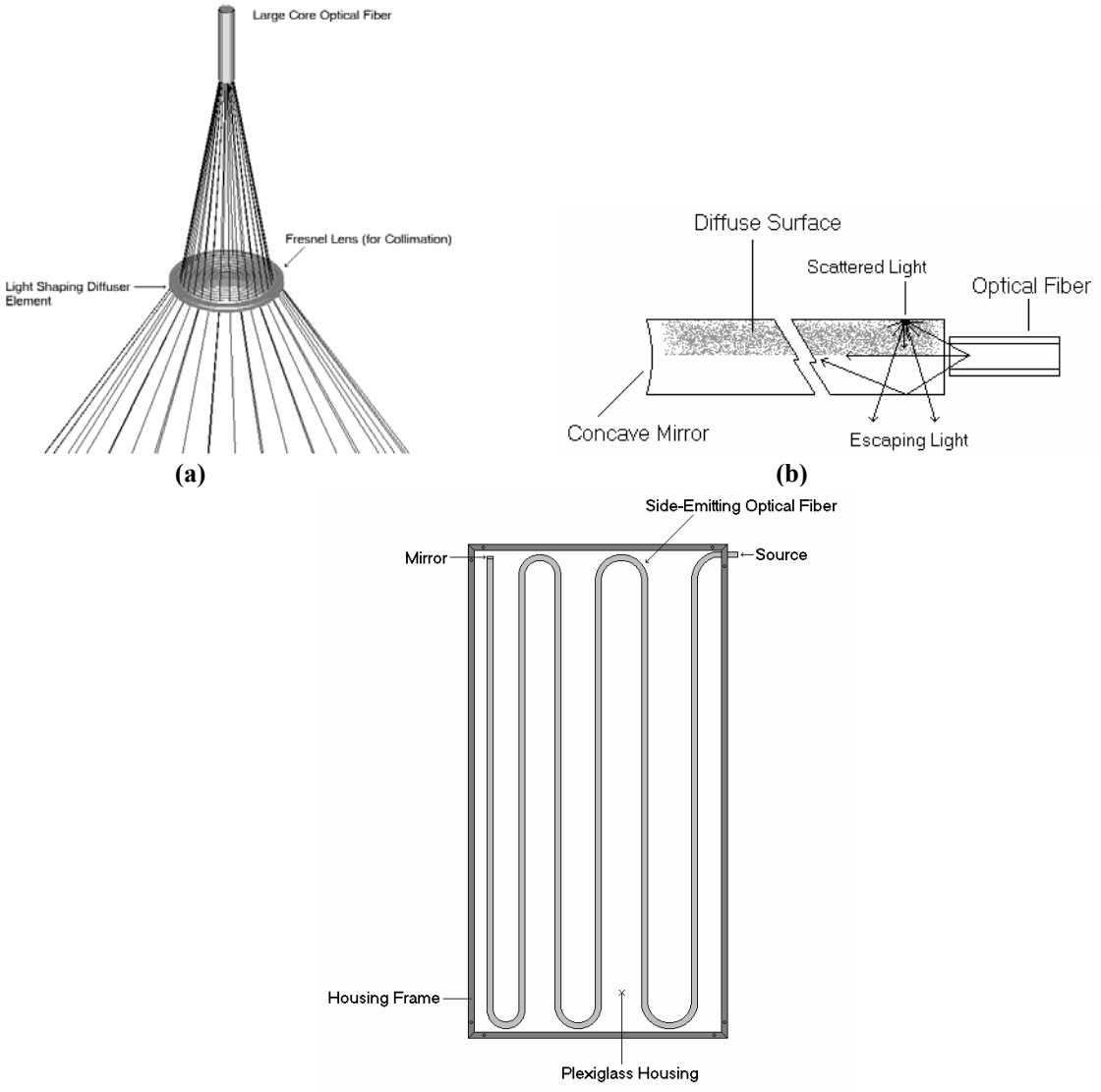


Fig. 5. a) Holographic Diffusion of a Fiber Source, b) Fiber Illuminated Cylindrical Diffusing Rod, c) Side-Emitting Fiber Illumination Sheet

Fig. 6. Bioreactor Solar Collector

Fig. 7. Illumination Rod

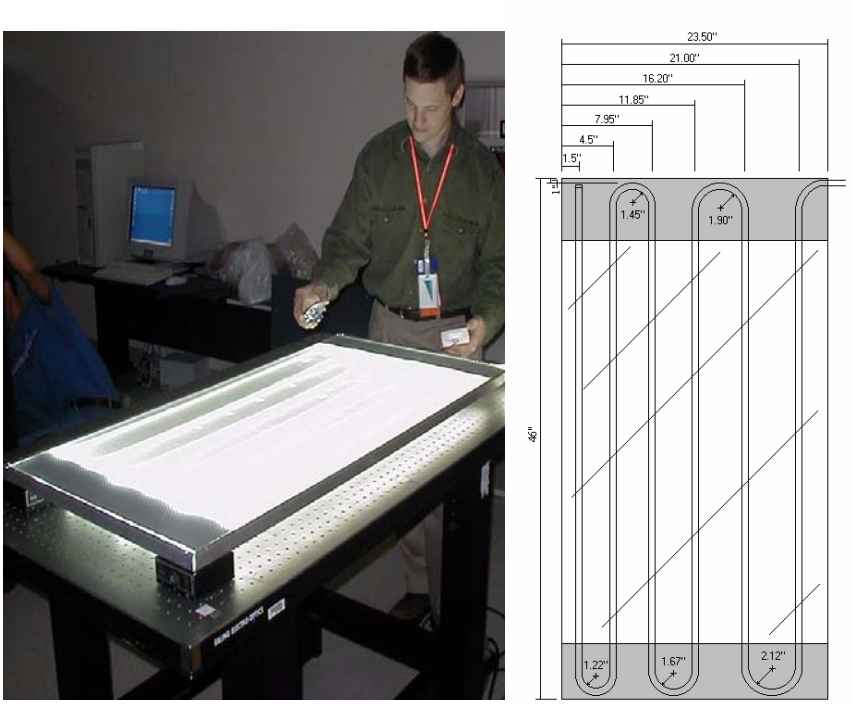


Fig. 8. a) Final construction of the illumination sheet. b) Final design of the illumination sheet

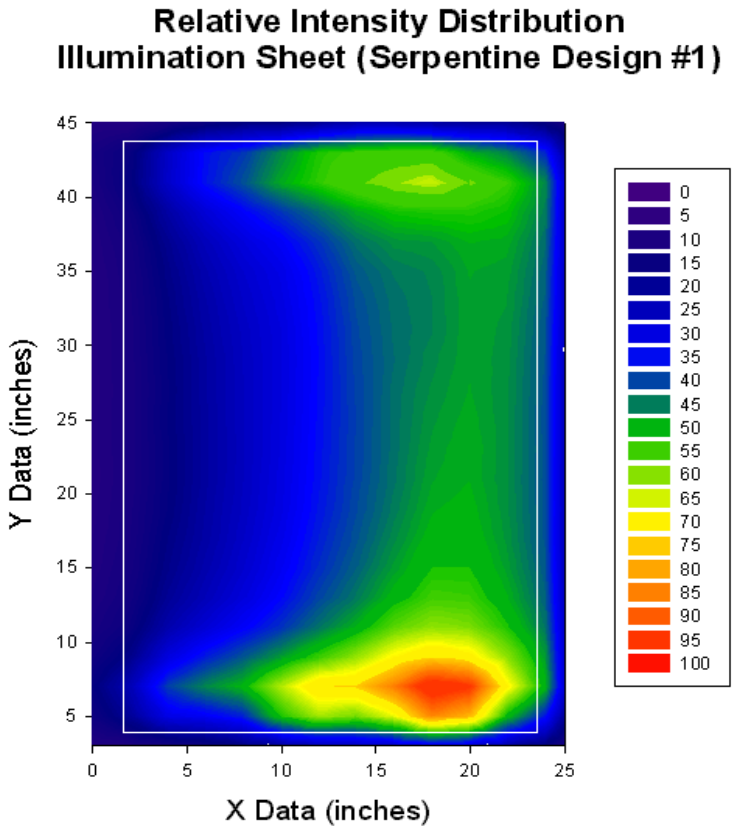


Fig. 9. Intensity Distribution of Illumination Sheet

Subtask 1.3 Investigate growth surface subsystem design

Various tests of growth surface materials are underway, looking at both appropriateness for the potentially harsh conditions in the bioreactor (due to temperature, pH level, moisture level, etc.) and compatibility with the organisms. Researchers at Ohio University have been testing numerous fabrics, including Teflon, Scotch Brite, polyester, and polypropylene felt. Teflon has advantages in its toughness and resistance to harsh conditions, and the other materials all have shown promise but have one or more issues working against them. For instance, Scotch Brite and polyester do not have the desired capillary action for uniform wetting of the surface.

Researchers at Montana State have been testing organism compatibility for three candidate growth surface materials: Scotch Brite, Teflon, and polypropylene felt. Of these three candidate growth surface materials the best trap for cyanobacteria in thermal springs and pools was Scotch Brite. This is probably related to the macroporous porous structure of this substratum. Polypropylene felt possesses second place. Microscopic observations suggest that Teflon can be fouled by cyanobacteria only as secondary process after a layer of putative red iron oxide is adsorbed.

Figures 10-12 show clean and colonized Scotch Brite. The substrata were colonized by immersing pieces of Scotch Brite in thermal pools and streams. Colonization to the level shown took less than three weeks. Small pieces of these substrata were then used to inoculate the three media mentioned [Appendix I]. Figure 10 is the clean surface of Scotch Brite showing the embedded aluminum oxide. Fig 11 is the same at a higher magnification and fig. 12 shows the complete coverage of the Scotch Brite with small rods cocci and filamentous cyanobacteria. The other two substrata did not colonize so effectively as Scotch Brite.

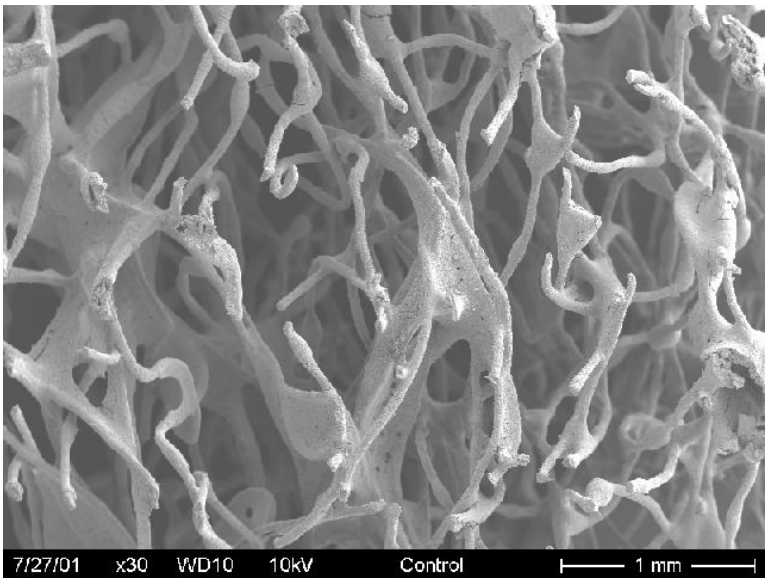


Fig. 10. Scanning electron micrograph [SEM] of a clean Scotch Brite[3M Company] surface. Note the indistinct surface caused by the embedded aluminum oxide layer.

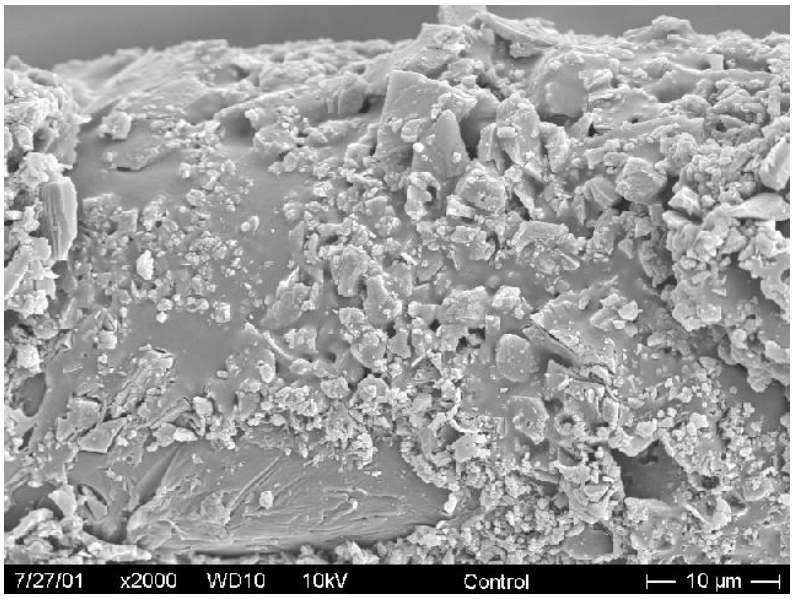


Fig. 11. As fig. 10 but at a higher magnification. The embedded aluminum oxide is obvious.

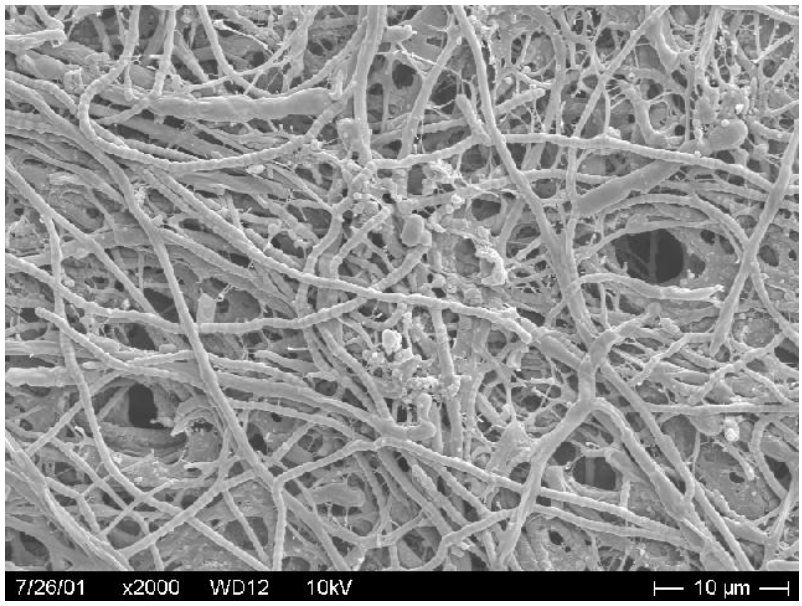


Fig. 12. Scotch Brite colonized *in situ* with cyanobacteria from Black Sand Pool, Yellowstone National Park, [see fig. 1]. Same magnification as fig.11

Subtask 1.4 Investigate the use of a hydraulic jump to improve the system's overall CO_2 conversion efficiency

The debugging work for the slug flow reactor has been finished and tests have begun. Due to its importance, the first group of tests focused on pressure drop with respect to various liquid and gas velocities. The translating slug flow reactor is made of a 4-inch diameter acrylic pipe. There are pressure taps at the inlet and at the outlet of the slug flow reactor and the distance between

the two taps is 11.48 m. A pressure transducer was used to measure the differential pressure between the two taps. The results of the tests will be used to understand the basic pressure drop of the slug flow reactor and select a gas and liquid velocity for future tests.

The liquid flow rate was measured by an orifice plate and a pressure transducer. Pure nitrogen gas was used for the tests and flow rates were measured by a gas flow meter. One thousand pressure drop data points for each condition were obtained by the data acquisition system. Because of the fluctuation of the pressure drop, we recorded the maximal pressure drop and the average pressure of each condition. The pressure drop trends with respect to the liquid and gas velocities are shown in Figure 13.

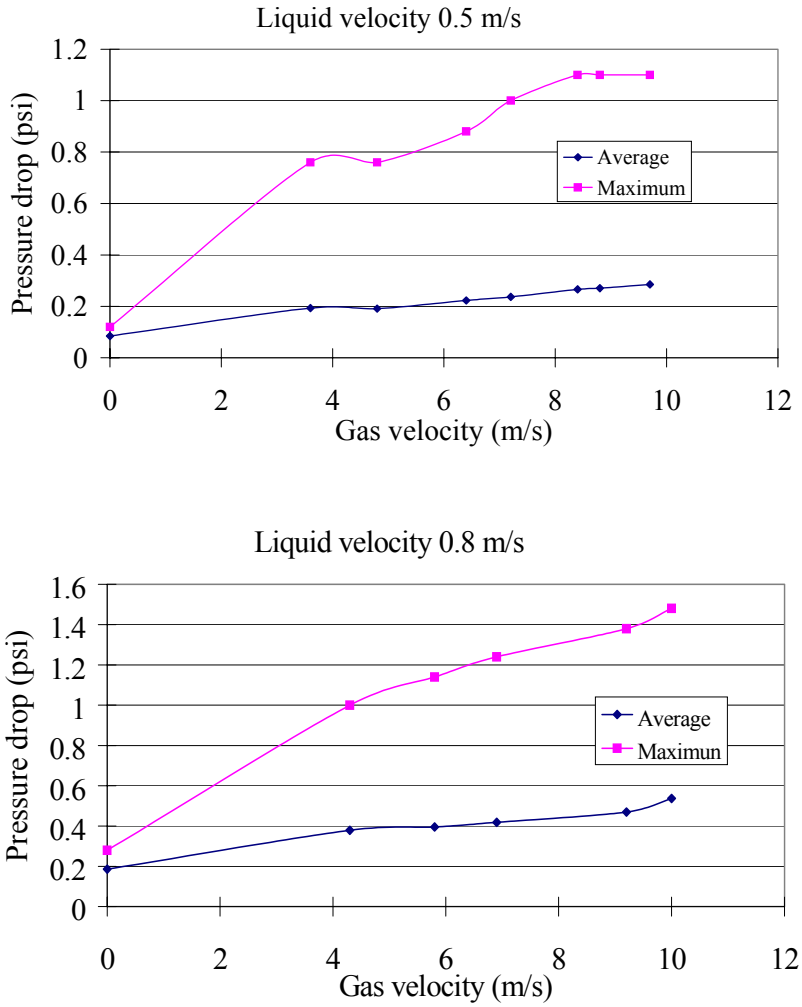


Fig. 13. Pressure drop trends with respect to the liquid and gas velocities

Subtask 1.5 Design harvesting subsystem

Our main activities this quarter were focused on design and experimental work for the integrated screen wetting/ harvesting system introduced in last quarter's status report. The system involves

a water supply system with controllable pressure/flow to allow operation as both a nutrient delivery drip system (at low delivery pressures) and an algae harvesting system (at high delivery pressures). The bioreactor screen design must be capable of delivering moisture to the screen via capillary action under normal operation, and creating a high flow "sheeting action" of fluid to displace a percentage of the algae clinging to the surface when the fluid delivery pressure is increased. Specific activities and accomplishments this quarter include:

- Redesigned header tube for improved surface wetting (see Figure)
- Designed and constructed a high pressure/low flow fluid supply system and a low pressure/high flow fluid supply system for test purposes
- Developed a test plan for quantifying the effects of various design parameters on the performance of the screen wetting/ harvesting system. Our focus will be on testing the sensitivity of the system to supply pressure and "cleaning time" to see if it is possible to strike a balance between a standard "drip operation" and on demand "harvesting mode".
- Modified the CRF-1 bioreactor to allow "long-term" testing cycles with an operational harvesting system once the off-line harvesting system tests are complete

Findings based on the tests run this past quarter include:

- Nostoc algae clings tightly to the polyester felt growth surface and qualitative tests showed that both the high pressure/low flow and low pressure/high flow systems were unable to remove the Nostoc from the polyester felt. Note that in previous tests the nozzle-based water jet systems were able to remove Nostoc from polyester felt.
- Qualitative tests have shown that Cyanidium can be removed "easily" from the polyester felt growth surface using the high pressure/low flow system design. Quantitative tests have been delayed by problems with quantifying the amount of mass removed from the screens during a harvesting cycle. The specific problem has been traced to scale repeatability issues caused by thermal effects associated with measuring materials directly after their removal from a 80C oven. We have corrected the problem by implementing a desiccator to reduce the temperature of the items to be weighed down to a level that will have no impact on the scale readings. The quantitative tests are currently being run and results will be reported in the next report.
- There are some potential problems with the use of some of the current candidate growth surface materials in the screen wetting/ harvesting system associated with their ability to "wet" via capillary action evenly and consistently. Polyester felt initially exhibits good wetting performance, but capillary action is replaced by beading and streaming after repeated uses. Scotch Brite exhibits more of a channeling action between the fibers than a slow drip, and is inappropriate for the wetting application. Work continues to better understand the properties of these and the other candidate growth surface materials.

Subtask 1.6 Quantify properties (higher heating value, elemental composition, volatile content) of dried biomass for potential end-uses.

No experimental work has been completed in this area, as current experiments are focused on identifying organisms with maximum rates of CO_2 uptake in the conditions of the bioreactor.

Task 2.0. Evaluate subsystem combinations and select an "optimum" system design

Two new system level experimental facilities are currently under construction, a new model-scale bioreactor and a pilot scale bioreactor. The pilot scale system is discussed in the Task 3.0 section of this report. The new model-scale bioreactor (CRF-2) is 4 times larger than CRF-1 in terms of growth surface area. Construction of CRF-2 and initial debugging are now complete and baseline tests are underway. As results from other tests are evaluated the "best" subsystem designs will be implemented in the CRF-2 and ultimately in the pilot scale bioreactor.

Task 3.0. Implement the optimum system in scaled model

The pilot scale bioreactor test facility is under construction at the Ohio University Corrosion Center. Ohio University technician Shyler Switzer is leading the construction effort and is working closely with representatives of Oak Ridge National Laboratories to ensure proper placement of the solar collectors. Drawings have been completed and discussions with an architect are underway to finalize plans for the enclosure and the site preparation for the solar collectors. The construction of the bioreactor test setup is being completed in an off-site location to allow quick installation once the site prep work is completed.

Webpage

The web page is running at http://132.235.19.45/DOE . All parties involved in the project have received e-mail instructions and the password to access the information.

Conclusions

This quarterly report documents significant achievements in the Enhanced Practical Photosynthetic CO_2 Mitigation project during the period from 7/03/2001 through 10/02/2001. This is the fourth quarterly report for this project, so it also serves as a year-1 project review. We have made significant progress on our Phase I objectives, and our current efforts are focused on fulfilling these research objectives "on time" relative to the project timeline. Overall, we believe that we are on schedule to complete Phase I activities by 10/2002, which is the milestone date from the original project timeline.

Our results to date concerning the individual factors which have the most significant effect on CO_2 uptake are inconclusive, but we have gathered useful information about the effects of lighting, temperature and CO_2 concentration on one particular organism (Nostoc) and significant progress has been made in identifying other organisms that are more suitable for use in the bioreactor due to their better tolerance for the high temperatures likely to be encountered in the flue gas stream. Our current tests are focused on one such thermophilic organism (Cyanidium), and an enlarged bioreactor system (CRF-2) has been prepared for testing this organism.

Tests on the enhanced mass transfer CO_2 absorption technique are underway and useful information is currently being collected concerning pressure drop. The solar collectors for the deep-penetration hybrid solar lighting system have been designed and a single solar collector tracking unit is being prepared for installation in the pilot scale bioreactor system currently under construction. Much progress has been made in designing the fiber optic light delivery system, but final selection of the "optimum" delivery system design depends on many factors, most significantly the configuration and orientation of the growth surfaces in the bioreactor.

For the growth surface subsystem we have identified advantages and disadvantages for several candidate growth surface materials, we have built and tested various "screen" systems and fluid delivery systems, and we continue to test compatibility of the candidate materials with the organisms and with the moisture delivery and harvesting system designs. These tests will be ongoing until an "optimum" combination of growth surface material / organism type / harvesting system is identified. For the harvesting system, a nozzle-based water jet system has been shown to be effective, but it has disadvantages for the overall system design in terms of space utilization. A streamlined and integrated screen wetting / harvesting system design is currently under development and will be the focus of harvesting system tests in the foreseeable future.

These activities and the others discussed in the report will be continued in the next quarter in support of the overall project objectives.

Appendix I – Compositions of three candidate media

Castenholtz D Medium

$NaNO_3$	0.7 g
Na_2HPO_4	0.11 g
KNO_3	0.10 g
$MgSO_4 \cdot 7H_2O$	0.10 g
Nitrilotriacetic acid	0.10 g
$CaSO_4 \cdot 2H_2O$	0.06 g
NaCl	8.0 mg
$FeCl_3$ solution	1.0 ml
Castenholtz micronutrient solution	0.5 ml
Distilled water to	1.0 L

pH 7.5±0.2 at room temperature

Add nitrilotriacetic acid to 500.0 ml distilled water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components and mix. Adjust pH to 7.5. Bring volume to 1.0 L with distilled water.

$FeCl_3$ solution:

$FeCl_3 \cdot 6H_2O$	2.28 g
Distilled water to	1.0 L

Castenholtz micronutrient solution:

$MnSO_4 \cdot H_2O$	2.28 g
H_3BO_3	0.5 g
$ZnSO_4 \cdot 7H_2O$	0.5 g
$CoCl_2 \cdot 6H_2O$	0.025 g
$CuSO_4 \cdot 5H_2O$	0.025 g
$Na_2MoO_4 \cdot 2H_2O$	0.025 g
H_2SO_4	0.5 ml
Distilled water to	1.0 L

Castenholtz DH Medium

This is medium D buffered at pH 8.2 with HEPES [1.2 g/L HEPES in medium D]

BG-11 Medium for Blue Green Algae

BG-11 medium (ATCC Medium 616)

$NaNO_3$	1.5 g
K_2HPO_4	0.04 g
$MgSO_4 \cdot 7H_2O$	0.075 g
$CaCl_2 \cdot 2H_2O$	0.036 g
Citric acid	0.006 g
Ferric ammonium citrate	0.006 g
EDTA (disodium salt)	0.001 g
$NaCO_3$	0.02 g
Trace metal mix A5	1.0 ml
Agar (if needed)	10.0 g
Distilled water	1.0 L

The pH should be 7.1 after sterilization

Trace metal mix A5:

H_3BO_3	2.86 g
$MnCl_2 \cdot 4H_2O$	1.81 g
$ZnSO_4 \cdot 7H_2O$	0.222 g
$NaMoO_4 \cdot 2H_2O$	0.39 g
$CuSO_4 \cdot 5H_2O$	0.079 g
$Co(NO_3)_2 \cdot 6H_2O$	49.4 mg
Distilled water	1.0 L