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Principal Authors: Dr. David J. Bayless
Dr. Morgan Vis
Dr. Gregory Kremer
Dr. Michael Prudich
Dr. Keith Cooksey, Montana State University
Dr. Jeff Muhs, Oak Ridge National Laboratories

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Organization:	Ohio Coal Research Center 248 Stocker Center Athens, OH 45701-2979 bayless@ohio.edu (740) 593 0264 <i>voice</i> (740) 593 0476 <i>fax</i>	Department of Microbiology LW-113B Montana State University Bozeman, Montana 59717 umbkc@gemini.oscs.montana.edu	Division of Photonics Oak Ridge National Laboratory P.O. Box 2009, MS-8058 Oak Ridge, TN 32831 um4@ornl.gov
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Abstract

This is the first quarterly report of the project Enhanced Practical Photosynthetic CO₂ Mitigation. The official project start date, 10/02/2000, was delayed until 10/31/2000 due to an intellectual property dispute that was resolved. However, the delay forced a subsequent delay in subcontracting with Montana State University, which then delayed obtaining a sampling permit from Yellowstone National Park. However, even with these delays, the project moved forward with some success.

Accomplishments for this quarter include:

- Culturing of thermophilic organisms from Yellowstone
- Testing of mesophilic organisms in extreme CO₂ conditions
- Construction of a second test bed for additional testing
- Purchase of a total carbon analyzer dedicated to the project
- Construction of a lighting container for Oak Ridge National Laboratory optical fiber testing
- Modified lighting of existing test box to provide more uniform distribution
- Testing of growth surface adhesion and properties
- Experimentation on water-jet harvesting techniques
- Literature review underway regarding uses of biomass after harvesting

Plans for next quarter's work and an update on the project's web page are included in the conclusions.

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Experimental Methodology

The experimental and test facilities are designed to simulate the flue gas emission from fossil-fired power plants. Flue gases from fossil unit scrubbers for SO_x control contain 10-15% CO₂ and have a temperature around 150°F. It is likely that thermophylic cyanobacteria, which tolerate high CO₂ and elevated temperature, would be most suitable for reducing these CO₂ emissions. The effect of temperature, gas flow rate, CO₂ concentration in the simulated flue gas and light intensity over the growth characteristics of microalgae species were examined at the test facility shown schematically in Figure 1.

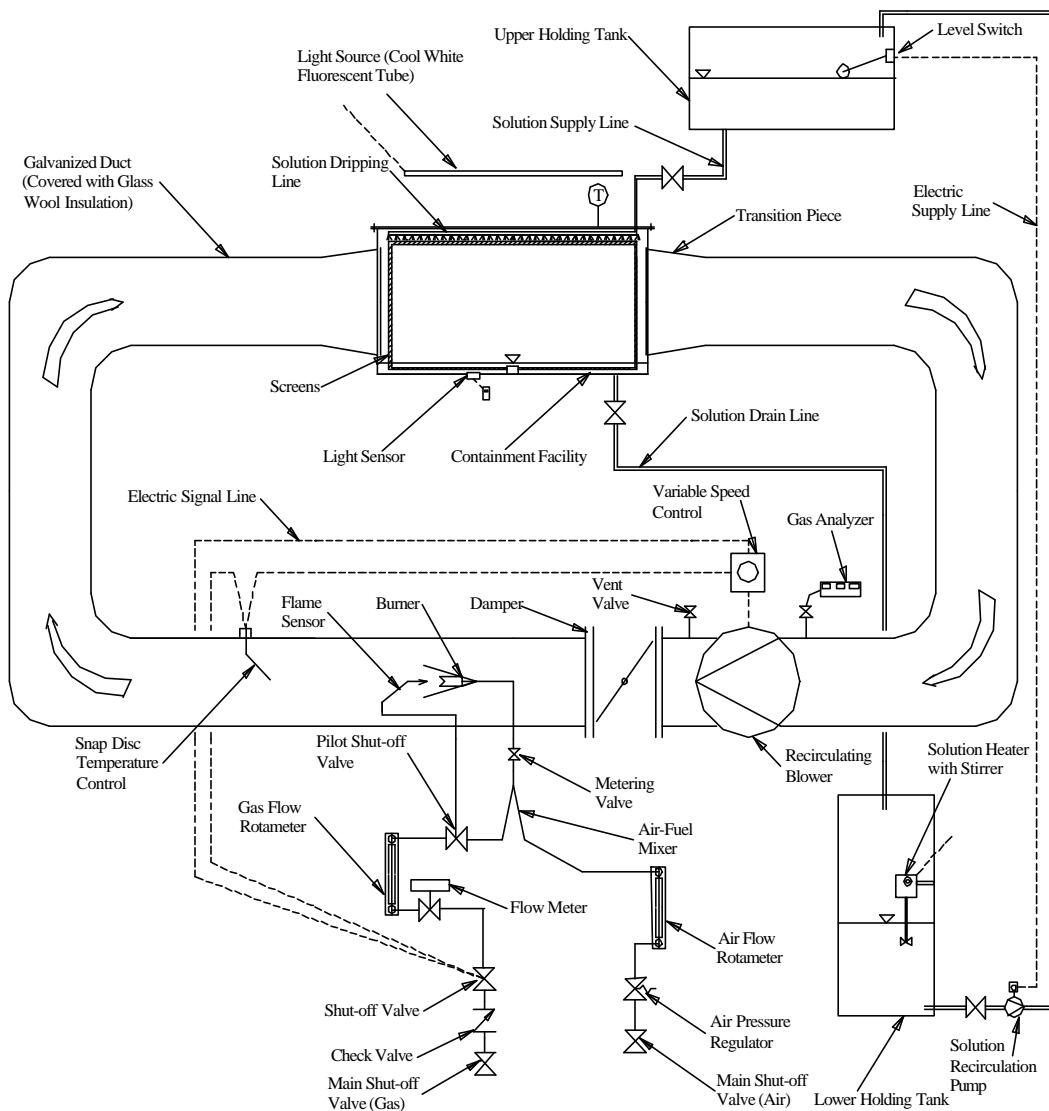


Figure 1. Schematic of the Carbon Recycling Facility

The facility aims to develop a high-density, large-volume photosynthesizing microalgal culture system to maximize the photosynthetic capacity. As with any diverse group of organisms, algae

vary in requirements, with different media and handling techniques from culture to culture. The experiment setup passes simulated flue gas over the vertically stacked screens inside the containment on which microalgae are grown. This assembly assists in reducing the pressure drop of flue gas as well as increasing the effective area for efficient trapping and bio-conversion of CO₂ in the flue gas. The experimental setup can be visualized as having the following sub-systems; a flue gas recirculation system, a gas burner system, an algae grow solution recirculation system, and an analysis system.

The flue gas recirculation system is designed to circulate hot flue gas through the algae culture in the containment facility. The recirculating fan circulates through the ductwork and containment. The simulated flue gas is found in a typical power plant that is scrubbed to remove SOx.

The containment facility is an open cubical box made of $\frac{1}{4}$ " thick Plexiglas with the top cover made of $\frac{3}{8}$ " thick Plexiglas. Plexiglas was used because it can sustain high temperatures and also provides transparency that allows visual monitoring of any changes occurring in the algae growth inside the containment. The containment facility is provided with a 1" PVC flange at the top edges, having twelve $\frac{3}{8}$ " holes. The top cover with matching holes is bolted to the flanged top. A rubber gasket is applied between the top cover and flange.

The two faces of the box (12" wide x 11 $\frac{3}{4}$ high) are provided with two rectangular openings (9" wide x 8" high). These openings serve as the entry and exit of flue gas through the containment. The other openings provided in the box include one at the bottom (sized at $\frac{1}{2}$) for solution drain and two (sized at $\frac{1}{2}$) on the top cover for the solution supply manifold and for insertion of a thermometer or thermocouple wire or light sensor. The openings are then sealed with thermal resistant glue and thermal sealing tape.

An inline centrifugal duct fan having variable speed control maintains the circulation of flue gas through the setup. Galvanized ductwork, 8" in diameter and with a total length of approximately 20', provides passageway for the circulation of flue gas through the experimental facility. The ductwork is made of galvanized sheet metal rolled into cylindrical rolls 8" in diameter x 2' in length. Each duct piece is connected to another with rivets. The ductwork is connected to the circulation fan with steel hose clamps and terminated at the containment facility with two transition pieces at either end.

Transition pieces, rectangular (9" wide x 8" high) to circular (8" diameter and 12" long), made of galvanized sheet metal are connected to the containment facility with fourteen $\frac{1}{4}$ " nuts and bolts. Transition pieces are coupled to the ductwork with rivets. All the joints on the ductwork are thermally sealed with insulation tape. Two vent valves, sized $\frac{1}{4}$ ", are fitted before and after the recirculation fan. One of the vent valves serves as a gas sample collector point for the analyzer to assess the circulation gas for concentrations of CO₂, O₂, and CO.

The gas burner system is primarily designed to maintain a suitable operating temperature range for the recirculating gas while providing sufficient levels of CO₂. The gas burner system is designed as a part of the gas recirculation system to preheat the circulating gas, which then simulates the actual power plant flue gas. The idea of burning premixed natural gas with air is to create a steady flame. Figure 2 is a schematic of the features of the burner assembly.

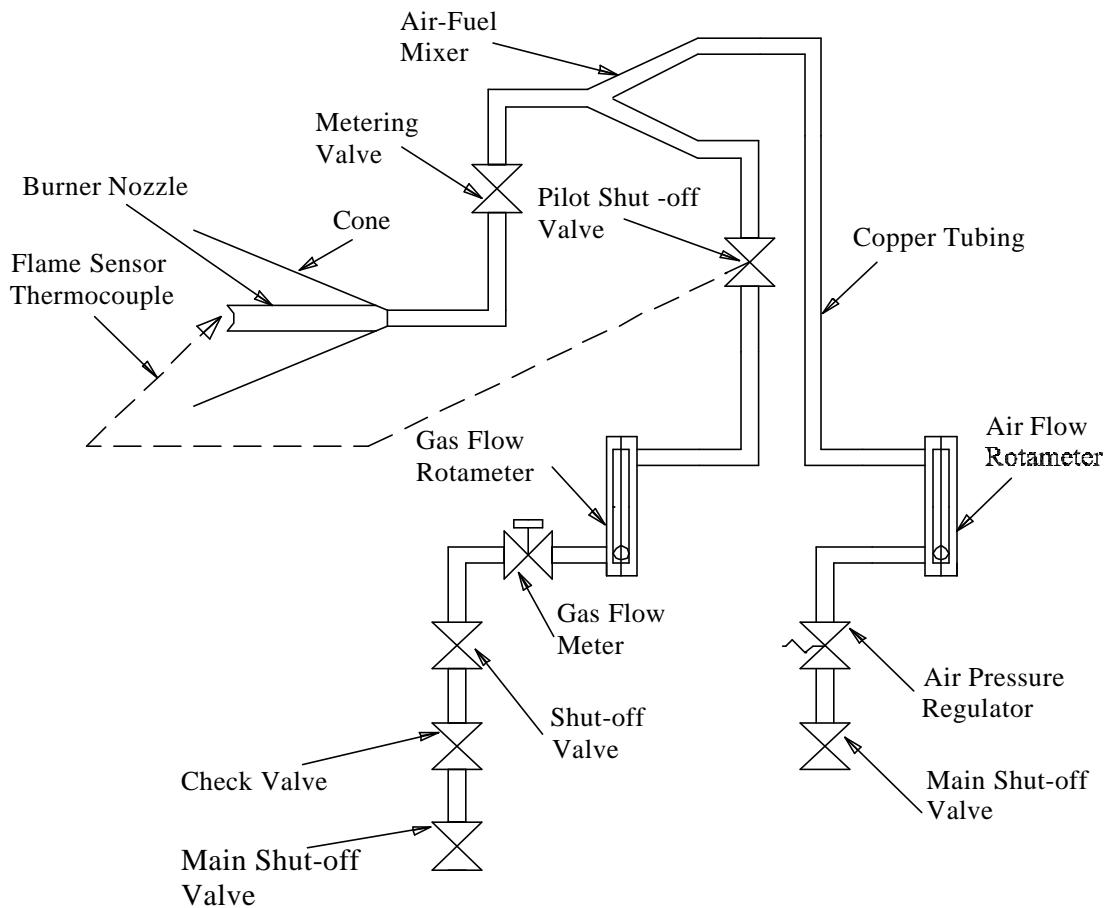


Figure 2. Schematic flow diagram showing burner assembly.

The burner assembly consists of a 2" long burner nozzle covered with a galvanized cone so as to protect the root of the flame from the flow of circulating gas. The sub-assembly of burner nozzle, cone, and flame sensor is mounted inside the ductwork and serves as a combustion chamber. This particular section of the burner assembly is accessible by opening the ductwork at the joint. The flame sensor is a safety device that senses the flame/temperature at the burner nozzle and lets the pilot valve open, maintaining the flow of gas. When there's no flame, the valve snaps shut and kills the supply of gas, preventing leakage of natural gas into the combustion chamber. Prior to entering the burner nozzle, natural gas is premixed with air in a Y-shaped air-fuel mixer. To sustain the flame in the flow field of gas, pressurized air is supplied to the burner. The premixed gas and air are metered through a metering valve after the mixer and then burned in the nozzle. The metering valve helps to provide fine control of the flame at the nozzle.

Another additional safety feature provided in the burner system is the snap disc temperature control. This temperature control is preset to 200°F so that if the temperature exceeds the preset value, the controller kicks off the recirculation fan and shuts off the gas supply valve so that no fuel is supplied. This, of course, prevents overheating of the system. Simultaneously, the pilot shut-off valve closes when no flame is sensed, adding additional safety to the setup. An air

pressure regulator with filter is provided in the air supply line to regulate the pressure of air supplied for burning. In addition, check-valves in the gas supply line and main shut-off valves are provided to manually shut off the system when not running.

A recirculation system is designed to circulate the culturing media through the algae culture, dispersed over screens, while they are subjected to the high temperature flue gas in the containment. The basic idea is to keep the algae cultures moist inside the containment and provide nourishment for them to grow even at high temperatures. Figure 3 illustrates in a schematic representation the features of the recirculation system.

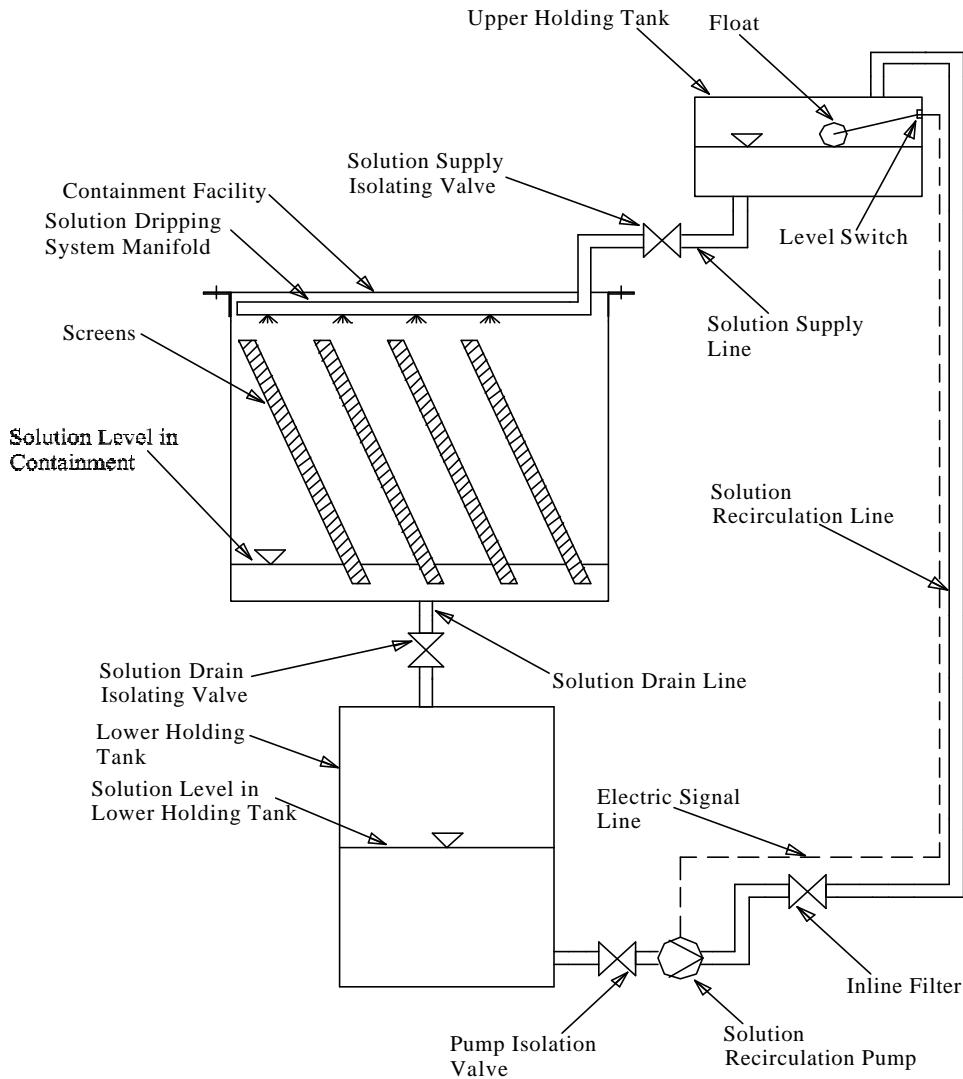


Figure 3. Schematic flow diagram showing growth solution recirculation system.

Four screens made of polyester fabric cloth fastened within the frame and loaded with algae culture are placed inside the containment at an angle of 65°. Screens are 21" long x 10½ wide with ½ frame width. Figure 4 shows the dimensional features of the screens.

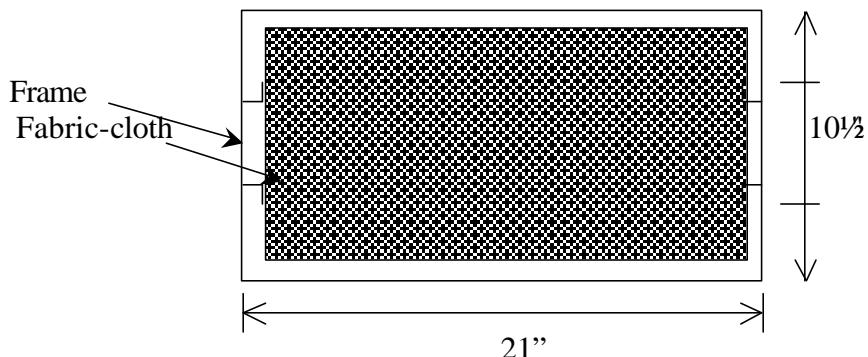


Figure 4. Dimensional features of screens.

Screens are supported inside the containment by a slotted fixture covering the width of the containment. The slotted fixture has four slots cut at an angle of 65°. The growth solution (medium I) is dripped from a plastic upper 18 gallon holding tank through a solution dripping manifold over the screens. The manifold is a $\frac{1}{2}$ PVC pipe system with the main supply line divided into four branches, 22" long for each screen. Each branch is provided with twenty $\frac{1}{8}$ " diameter holes to drip solution over the screens. Solution from the upper holding tank flows through the manifold under gravity, and flow can be controlled with the isolating valve provided in the supply line.

Solution collected in the containment is drained back to the steel lower 24 gallon holding tank. Both tanks are black to prevent any photosynthetic reaction in the solution due to external light. Algae solution from the lower holding tank is pumped back to the upper holding tank by a recirculation pump after passing through an inline 5 μm rated filter. The filter traps any algae passing through and circulates a clear solution free of algae. The upper holding tank is provided with a switch set to maintain the level of the solution in the tank so as to provide a continuous flow of solution over the screens. The float-type switch activates the recirculation pump on low level, and when the desired upper level is reached, shuts off the pump.

Analysis of the recirculating gas, growth solution, and light intensity inside the containment is performed to quantify the CO₂ absorption capacity of microalgae. The temperature of the flue gas is measured by inserting the thermocouple wire into the flue gas stream in the containment. Flue gas is then analyzed for CO, CO₂ and O₂ content using a Nova Analytical Systems Inc., model 375WP analyzer. The analyzer utilizes a sensitive infrared detector for CO₂ and disposable electrochemical sensors for O₂ and CO. A built-in sample pump draws in the sample gas from the probe for analysis. The electrochemical O₂ and CO sensors produce a small voltage, which is directly proportional to the respective gas concentration. This output is amplified and displayed on the front panel meter. A solid-state infrared detector detects CO₂, which is specific to CO₂. Flue gas after detection is vented into the atmosphere. All three gases are simultaneously detected and displayed on LCD readout meters, one for each gas. The pH of recirculating solution is measured using a Hanna Instruments made pH meter, model pHep. The range for the pH meter is from 0.0 to 14.0 pH with resolution of 0.1 pH and accuracy (@20°C) of ± 0.1 pH.

Photosynthetically Active Radiation (PAR) is measured using a Licor LI-190SA quantum sensor. The quantum sensor measures PAR received on a plane surface. A silicone photodiode with an enhanced response in the visible wavelengths is used as the sensor. Licor radiation sensors produce a current proportional to the radiation intensity. The current output of the sensor is measured over a milli-volt recorder by connecting an amplifier between sensor and recorder. The special purpose amplifier converts the micro-amp level current output of Licor light sensor to a corresponding signal voltage. LI-190SA sensor has a calibration constant of 6.67 or calibration multiplier of -149.93. The calibration multiplier is the negative reciprocal of the calibration constant and is always a negative number because the shield of the coaxial cable is positive instead of negative. This is expressed in radiation units per microamp.

The setup is provided with a cool white light bank with an effective capacity of 612W. The light bank capacity is adjustable by adding or removing the 32W tube lights from the fixtures. Nine total fixtures are fixed and a pair of tube-lights can be mounted on each. The light bank resembles an enclosed trough and can be slid over the containment.

The LI-190SA is mounted at the base of the containment. The main idea is to measure the radiation from the artificial source of light (cool fluorescent light) passing through the screens and culture media. The output of the sensor from the millivolt adapter is boosted with an amplifier and measured over a millivolt recorder (multiplier).

An experimental investigation was carried out on *Nostoc 86-3* microalgae species to determine temperature response, CO₂ absorption, and growth characteristics of the species under simulated flue gas conditions. For each experiment, the steps involved were:

- 1) preparing algae culture
- 2) sampling algae culture to determine mass of algae culture used for experimentation
- 3) preheating of containment facility and culturing solution
- 4) setup for gas analysis
- 5) measuring temperature and light sensor
- 6) trial of the experiment for a specific temperature and light intensity.

Microalgae species *Nostoc 86-3* was isolated in pure culture from soil enrichment carried out under conditions of fixed nitrogen and selection of hormogonia induced by red light. The species were cultured in 20 gallons of culture medium I (described in Appendix A). The algae culture was illuminated by a 42W cool-white fluorescent lamp at an intensity of 64 $\mu\text{mol}\cdot\text{s}^{-1}\text{m}^{-2}$ and bubbled with air and CO₂ mixed together in the ratio of 19:1. The fluidization created by the bubbled CO₂ helps in uniform defragmentation of algae samples and CO₂ transport via bulk flow diffusion.

The algae colonies so cultured are later transferred into 6-gallon plastic sampling containments, from where the algae samples are drawn out for experimentation. Each containment is provided with sample draw out isolation valve and a closed circuit heater to maintain the algae samples at 110°F and a pH level of 7.4. The reason for preheating the algae samples at 110°F arises from the effort to prevent algae samples from thermal shock when they are transferred from the culturing containment at ambient temperature to an experimental facility at test temperature. The algae samples in sampling containments are illuminated by a bank of 60W cool-white

fluorescent lamps at an intensity of 64 $\mu\text{mol}\cdot\text{s}^{-1}\text{m}^{-2}$.

Algae Dry Mass Determination Test

This summary explains the procedure that was used to estimate the percent mass of dry algae from a wet weight basis. This is also the procedure that will be used to load the membranes in all the tests. A 100-ml algae sample (including growth medium) was poured across a very fine wire mesh to filter the algae from the growth medium. The algae sample remained on the wire mesh for 9 minutes to further reduce water content. Two oven-dried and weighed crucibles were each filled with approximately 6-grams of filtered algae. The samples were placed in an oven and dried for 116 hours at 107°C. The masses of the two samples were periodically measured during the drying process to determine when all the moisture had been removed from the samples. After 75 hours of drying, there was no measurable mass difference in subsequent mass measurements and the final mass measurement (116 hours) of each sample.

The percent dry mass of algae was determined by dividing the algae's dry mass wet mass (mass loaded into the crucible). The first crucible's percent dry mass was 4.67% and the second's was 4.24%. These first two samples were taken as an initial test for the above-described method. Ten more samples were taken following the same procedure. The average percent dry mass of the ten samples was 4.46% with a standard deviation of 0.19%.

A specific amount of cyanobacteria culture is loaded over each screen, either by directly pouring the algae solution over the screens or by using a peristaltic pump to distribute it evenly over the screens. The pouring or distributing rate is adjusted so that the organisms get enough time to attach to the screen fabric.

For each trial experiment, the containment facility and algae growth solution (to be circulated through screens inside the containment) are preheated for 12 hours to the temperature at which the trial experiment is intended to be run. The containment facility's upper and lower holding tanks are cleaned off, and a new filter element is fitted to the inline filter. The filter cartridge is preheated at 180°F for 24 hours to remove any moisture content and is weighed before fitting.

To start preheating, a set of screens is fitted over the fixture at an angle of 65°. The top cover is fitted and bolted over the experimental facility and solution is dripped over the screens from the upper holding tank. The dripping rate is adjusted to maintain a level of $1\frac{1}{2}\text{-}\frac{3}{4}$ " inside the facility. The solution is allowed to circulate between the upper and lower holding tank through screens, recirculation pump, and inline filter. The main shut off valves for air and gas are opened and air pressure is adjusted to 20 psi. The temperature rating of the snap disc temperature control is adjusted to the desired operating temperature. With flow control knobs for both the air and gas rotameters closed and metering valve half throttled, the flame sensing thermocouple for the burner is heated with an external propane torch through an opening in the duct work to open the pilot valve. Air and gas flows are then adjusted to get a sharp blue flame at the burner tip. Slowly the metering valve is fully opened and again the rotameter knobs are adjusted to get a sharp blue flame at the burner tip. At this time the flue gas recirculating blower is switched on. Flow control knobs of air and gas flow rotameters are again adjusted to obtain desired temperature for preheating.

Once the experimental facility is preheated to the desired experimental temperature and samples are being cultured at 110°F, the top cover is opened and the required quantity of cyanobacteria is introduced in equal amount over each screen. While loading the algae samples over the screens, the circulation of hot gas is kept running. Once the samples are loaded over each screen, the screens are fitted back inside the containment. After the screens are inserted in the experimental containment, the top cover is fitted back and bolted down over the containment. The growth solution dripping rate is adjusted so that a level of $\frac{1}{2}$ - $\frac{3}{4}$ " is maintained in the bottom of the containment facility. The level of algae solution in the upper holding tank is maintained constant by the level switch and recirculation pump.

During each 120 hour trial experiment for specific amounts of algae samples and fixed temperature, readings for temperature, air and gas flow rotameter values, light intensity, O₂, CO₂ and CO concentration are recorded. After the experiment is complete, the algae samples from each screen are compared to the original algae sample for visible changes in color and/or molecular characteristics (like cellular density and cellular structure). This analysis provides information about algae samples regarding their health and growth characteristics at high temperatures, similar to those in power plant flue gas. Also after the experiment is over, the screens and inline filter element are dried and their weights are noted. The dry weight of screens and the filter is noted. Calculation of weight differences when compared to the original mass of algae samples loaded into the system gives information on the growth of algae when exposed to a particular temperature.

Results and Discussion

Subtask 1.1 Investigate critical properties of alternative photosynthetic agents (cyanobacteria)

1.1.1 Quantify agent growth rate characteristics in controlled experiments as a function of temperature, bicarbonate concentration, moisture content and nutrient level

- a. The published data about physiology and ecology of thermotolerant cyanobacteria have been investigated.
- b. It was found that there is additional way to enhance photosynthetic CO₂ mitigation, i.e., to use thermotolerant strains of cyanobacteria with calcium deposition potential.
- c. Two thermotolerant strains of cyanobacteria isolated in Yellowstone National Park have been introduced in culture. Permission to supply to the Ohio group cultures of Cyanidium caldarium isolated from Nymph Cr. in Yellowstone National Park has been negotiated with park authorities. Efforts to allow the archiving of these cultures are being undertaken by this group and others at MSU working in YNP.
- d. 5-gallon cultures of 3 thermophilic organisms were initiated in a newly acquired 50 C incubator. These cultures are growing well and one is ready to be transferred to a larger container for mass culturing. Experiments on the effects of nutrient level were carried out for one thermophilic organism. The experimental design was as follows:

5 replicate cultures of each of 6 treatments were utilized for a total of 30 cultures. The treatments were 2X (double normal concentration), 1X (normal conc.), 0.5X (1/2 normal conc.), 0.1X (1/10th normal conc.) and 0.001X (1/100th normal conc.) of Allen's Modified Medium that is used to grow the thermophilic organisms. We hypothesized that a higher nutrient concentration would promote growth. The cultures were sampled every three days and the number of cells per culture counted as a measure of growth. The results of our first test are presented in Figure 5.

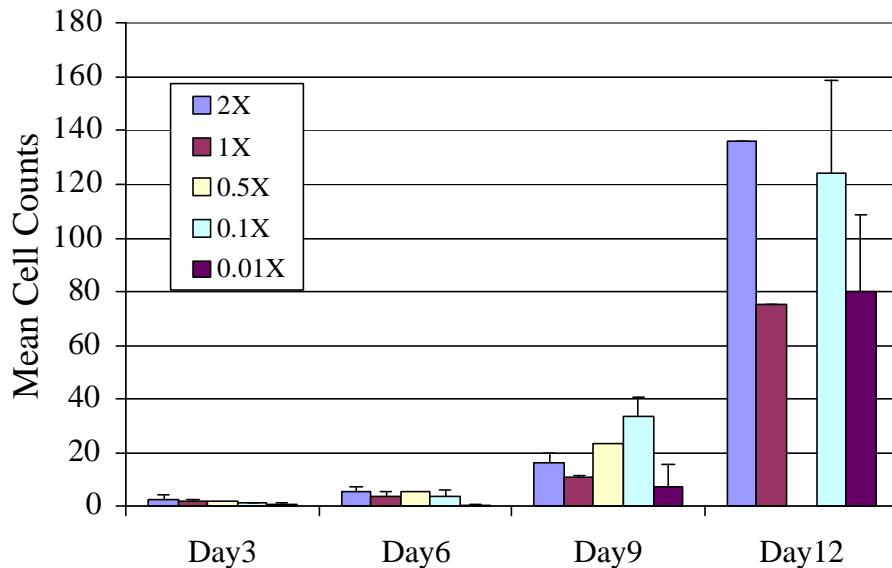


Figure 5. Results of 1st Nutrient Assay Experiment. Error bars represent standard deviation.

We had difficulties with the culture set-ups and evaporation at high temperatures. We modified the set-ups and re-ran the experiment. The results of the second experiment are shown in Figure 6. The conclusion from the second experiment is that the lower concentration of nutrients appears to favor growth of these organisms. This result is contrary to our hypothesis. One explanation may be that these organisms are adapted to low nutrient environments. We have used this information to alter our culture conditions for the organisms.

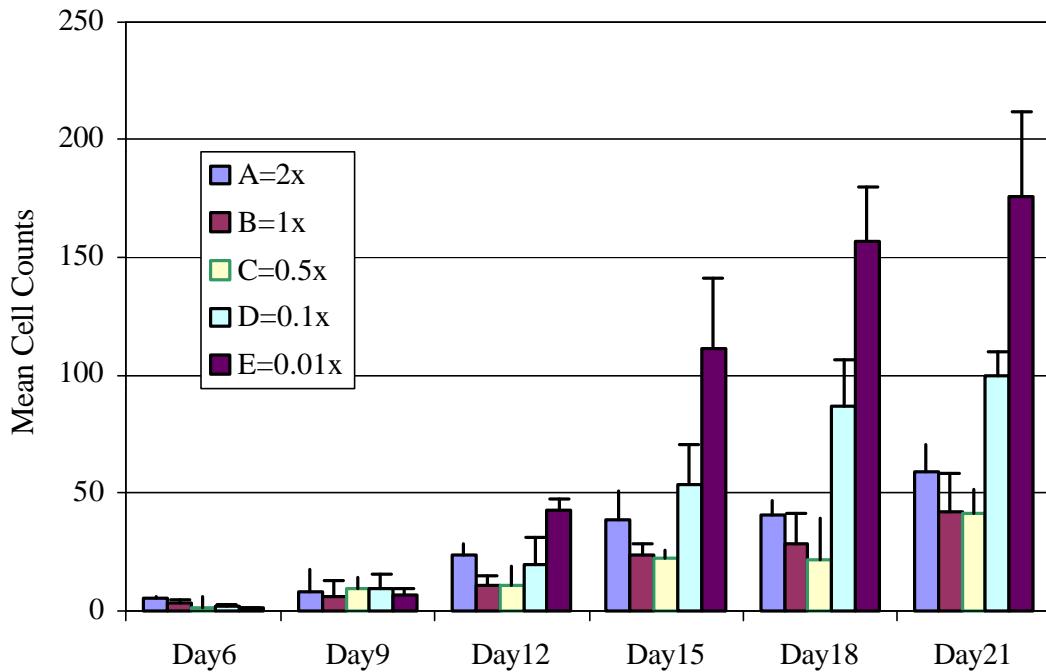


Figure 6. Results from 2nd Nutrient Assay Experiment. Error bars represent standard deviation.

1.1.2 Quantify adhesion characteristics

No progress made this quarter.

1.1.3 Quantify growth characteristics (size when mature and average time to mature) for harvesting considerations

- A lab-scale glass fermenter for the examination of different physiological properties of cyanobacteria has been designed.
- Technical documentation for this glass fermenter has been sent to 3 potential vendors for a price quotation.

1.1.4 Quantify growth characteristics at low temperatures for analysis of environmental impacts should there be loss of containment.

No progress made this quarter.

In addition to the work reported under Task 1, three test runs in the bioreactor were performed.

Summary of Test Run 10/18-24/00

The test specimen for *Test Run 10/18-24/00* was the cyanobacteria, *Nostoc 86-3*. The target values for the gas concentrations were 3%, 10%, and less than 50 parts per million for oxygen, carbon dioxide, and carbon monoxide, respectively, with a temperature range between 120°F-125°F. The gas concentration averages for the 120 hours were 2.90%, 10.05%, and 32.54 ppm for oxygen, carbon dioxide, and carbon monoxide, respectively. The 120-hour temperature average was 122.6°F. The lighting was not altered from *Summary of Light Intensity Test (thru 10-10-00)* and was cycled 12-hours on and 12-hours off.

Test Run 10/18-24/00 had a total dry algae mass gain of 1.25 grams, or a 29.8% increase over the estimated initial dry mass. The following table describes each membrane and final test results. More details are provided in *Data Sheet Test Run 10.18-24.00*.

Membrane Number	1	2	3	4
Membrane Initial Dry Mass (no loading) (g)	16.82	16.55	17.26	17.08
Membrane and Algae Final Dry Mass (g)	18.14	17.81	18.59	18.52
Final Dry Mass of Algae (g)	1.33	1.27	1.32	1.43
Membrane Loading Estimated Dry Mass (g)	1.04	1.03	1.02	1.10
Mass Gain for Each Membrane (g):	0.28	0.24	0.31	0.33
Percent Mass Gain for Each Membrane (%)	27.16	23.56	30.38	30.09
Mass Gain From Filter (g)	0.01			
Mass Gain from Beaker (g)	0.07			
Total Initial Estimated Dry Mass (g)	4.19			
Total Mass Gain (g)	1.25			
Total Percent Mass Gain (%)	29.80			

Uncertainty Analysis

The uncertainty analysis is based on the results from the *Algae Dry Mass Determination Test* and the final results of *Test Run 10.18-24.00*. From the *Algae Dry Mass Determination Test*, the average percent dry mass of the initial twelve samples was 4.46% with a standard deviation of 0.19%. The same technique used to gather the twelve initial samples was used to load the membrane. An algae sample taken from the bulk tank was poured across a wire mesh to remove most of the water content, but still retaining the algae mass. The sample remained on the wire mesh for 9 minutes to further reduce water content. The remaining sample was scooped into a beaker and weighed. The algae sample in the beaker was then applied to the membrane and the beaker was weighed again to determine the algae loading weight. This was repeated for each remaining membrane. 4.4581% of the total loading wet weight was used as the estimated initial dry mass for each membrane.

The following sample calculation is the uncertainty at 90% confidence in the estimated initial dry mass for membrane-1. It is based on the 12 samples taken from *Algae Dry Mass Determination Test*, using Student's t-distribution and using only one sample to load the membrane.

Data from *Algae Dry Mass Determination Test, Test Run 10.18-24.00* and Student's t-distribution: Mean = 4.46%, Std. Dev = 0.19%, and Degree's of freedom = 11 (based on -distribution for 12 samples), $t_{90} = 1.796$ (Student's t-distribution for 12 samples)

$$\text{Uncertainty} = \frac{t \times \sigma}{\sqrt{n}}$$

t = Student's t-distribution for 12 samples at 90% confidence

σ = Standard deviation of the 12 samples

n = Number of samples applied to membrane-1

$$\text{Uncertainty}_{90} = \frac{1.796 \times 0.19\%}{\sqrt{12}} = 0.34\%$$

Algae wet weight applied to Membrane-1 = 23.44 grams

$$\text{Uncertainty of estimated dry weight} = 23.44 \times 0.0034 = \pm 0.081 \text{ grams}$$

$$\text{Estimated initial dry weight} = 23.4396 \times 0.044581 = 1.0450 \pm 0.08136 \text{ grams}$$

$$\text{Mass gain for Membrane-1} = \text{Membrane-1 dry weight} - \text{initial weight} = 0.2838 \text{ grams}$$

$$\text{Uncertainty}_{90} = \frac{0.08136}{0.2838} \times 100 = 28.7\% \text{ (uncertainty of mass gain at 90% confidence)}$$

Summary of Test Run 11/2-7/00

The test specimen for *Test Run 11/2-7/00* was the cyanobacteria, Nostoc 86-3. The target values for the gas concentrations were 3%, 20%, and less than 50 parts per million for oxygen, carbon dioxide, and carbon monoxide, respectively, with a temperature range between 120°F-125°F. The gas concentration averages for the 120 hours were 2.96%, 19.22%, and 16.90 ppm for oxygen, carbon dioxide, and carbon monoxide, respectively. The 120-hour temperature average was 122.3°F. The lighting was not altered from *Summary of Light Intensity Test (thru 10-10-00)* and was cycled 12-hours on and 12-hours off throughout the test.

Test Run 11/2-7/00 had a total dry algae mass gain of 0.90 grams, or a 28.0% increase over the estimated initial dry mass. The following table describes each membrane and final test results. More details are provided in *Data Sheet Test Run 11.2-7.00*.

Membrane Number	1	2	3	4
Membrane Initial Dry Mass (no loading) (g)	17.29	14.85	16.99	15.57
Membrane and Algae Final Dry Mass (g)	18.31	15.86	17.89	16.57
Final Dry Mass of Algae (g)	1.03	1.02	0.91	1.01
Membrane Loading Estimated Dry Mass (g)	0.93	0.80	0.73	0.74
Mass Gain for Each Membrane (g):	0.10	0.22	0.17	0.26
Percent Mass Gain for Each Membrane (%)	10.74	27.28	23.73	35.40
Mass Gain From Filter (g)	0.04			
Mass Gain from Beaker (g)	0.10			
Total Estimated Initial Dry Mass (g)	3.20			
Total Mass Gain (g)	0.90			
Total Percent Mass Gain (%)	28.00			

Uncertainty Analysis

The following sample calculation is the uncertainty at 90% confidence in the estimated initial dry mass for membrane-2. It is based on the 12 samples taken from *Algae Dry Mass Determination Test*, using Student's t-distribution and using two weighed samples to load the membrane.

Data from *Algae Dry Mass Determination Test, Test Run 11.2-7.00* and Student's t-distribution:

$$\text{Uncertainty}_{90} = \frac{1.796 \times 0.1933\%}{\sqrt{2}} = 0.2454\%$$

Algae wet weight applied to Membrane-2 = 17.9094 grams

Uncertainty of estimated dry weight = $17.9094 \times 0.002454 = \pm 0.04395$ grams

Estimated initial dry weight = $17.9094 \times 0.044581 = 0.7984 \pm 0.04395$ grams

Mass gain for Membrane-2 = Membrane-2 dry weight – initial weight = 0.2178 grams

$$\text{Uncertainty}_{90} = \frac{0.04395g}{0.2178g} \times 100 = 20.2\% \text{ (uncertainty of mass gain at 90% confidence)}$$

Summary of Test Run 11/14-19/00

The test specimen for *Test Run 11/14-19/00* was the cyanobacteria, Nostoc 86-3. The test run was performed under ambient conditions at an average temperature of 83.2°F. The lighting was not altered from *Summary of Light Intensity Test (thru 10-10-00)* and was cycled 12-hours on, and 12-hours off throughout the test.

Test Run 11/14-19/00 had a total dry algae mass gain of 2.0 grams, or a 59.2% increase over the estimated initial dry mass. The following table describes each membrane and final test results. More details are provided in *Data Sheet Test Run 11.14-19.00*.

Membrane Number	1	2	3	4
Membrane Initial Dry Mass (no loading) (g)	16.64	16.26	15.73	15.97
Membrane and Algae Final Dry Mass (g)	17.87	17.43	17.09	17.54
Final Dry Mass of Algae (g)	1.23	1.16	1.36	1.57
Membrane Loading Estimated Dry Mass (g)	0.76	0.79	0.88	0.97
Mass Gain for Each Membrane (g):	0.47	0.37	0.49	0.60
Percent Mass Gain for Each Membrane (%)	60.83	47.29	55.33	61.34
Mass Gain From Filter (g)	0.01			
Mass Gain from Beaker (g)	0.08			
Total Estimated Initial Dry Mass (g)	3.41			
Total Mass Gain (g)	2.01			
Total Percent Mass Gain (%)	59.20%			

Uncertainty Analysis

The following sample calculation is the uncertainty at 90% confidence in the estimated initial dry mass for membrane-1. It is based on the 12-samples taken from *Algae Dry Mass Determination Test*, using Student's t-distribution and using four weighed samples to load each membrane.

Data from *Algae Dry Mass Determination Test, Test Run 11.14-19.00* and Student's t-distribution:

$$\text{Uncertainty}_{90} = \frac{1.796 \times 0.1933\%}{\sqrt{4}} = 0.174\%$$

Algae wet weight applied to Membrane-1 = 17.15 grams

Uncertainty of estimated dry weight = $17.15 \times 0.00174 = \pm 0.030$ grams

Estimated initial dry weight = $17.15 \times 0.0446 = 0.765 \pm 0.030$ grams

Mass gain for Membrane-1 = Membrane-1 dry weight – initial weight = 0.465 grams

$$\text{Uncertainty}_{90} = \frac{0.02976g}{0.4652g} \times 100 = 6.4\% \text{ (uncertainty of mass gain at 90% confidence)}$$

Subtask 1.2 Design deep-penetration light delivery subsystem

- 1.2.1 Define spatial photon delivery (lighting) requirements and model design configurations incorporating large-core optical fibers using COTS lighting design tools.
- 1.2.2 Determine preliminary solar-based photon delivery (lighting) systems spatial effect on cyanobacteria growth rates.
- 1.2.3 Test lighting cycle durations on growth rates.

During this quarter, further testing was performed to determine if artificial lighting (without fiber optics) could be used to provide a uniform lighting source. Figures 7-10 show the optimum achievable distribution, which has been determined impossible to achieve in a scaled-up reactor (with a depth over three feet) due to the high rate of attenuation.

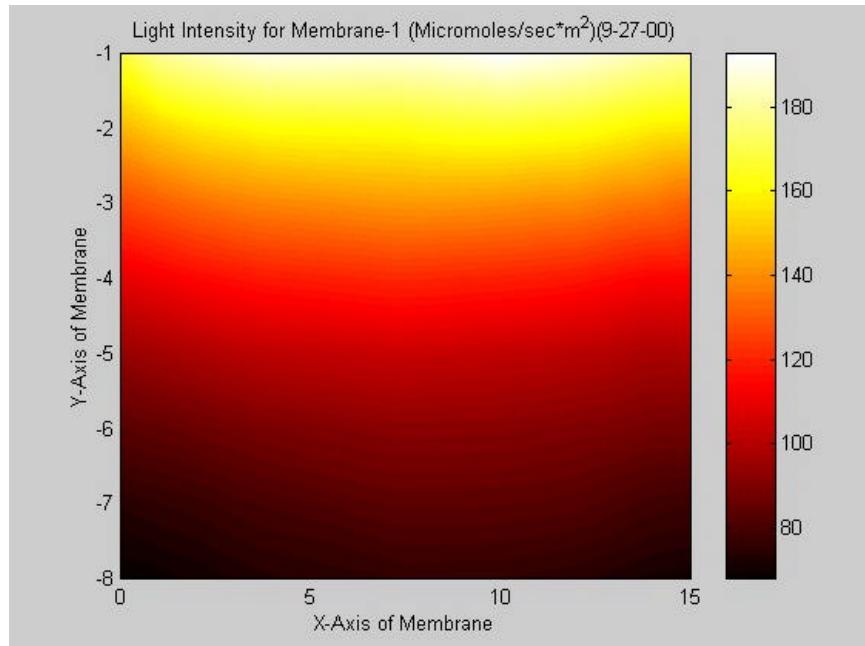


Figure 7. Optimal Photosynthetic Photon Flux on Membrane 1

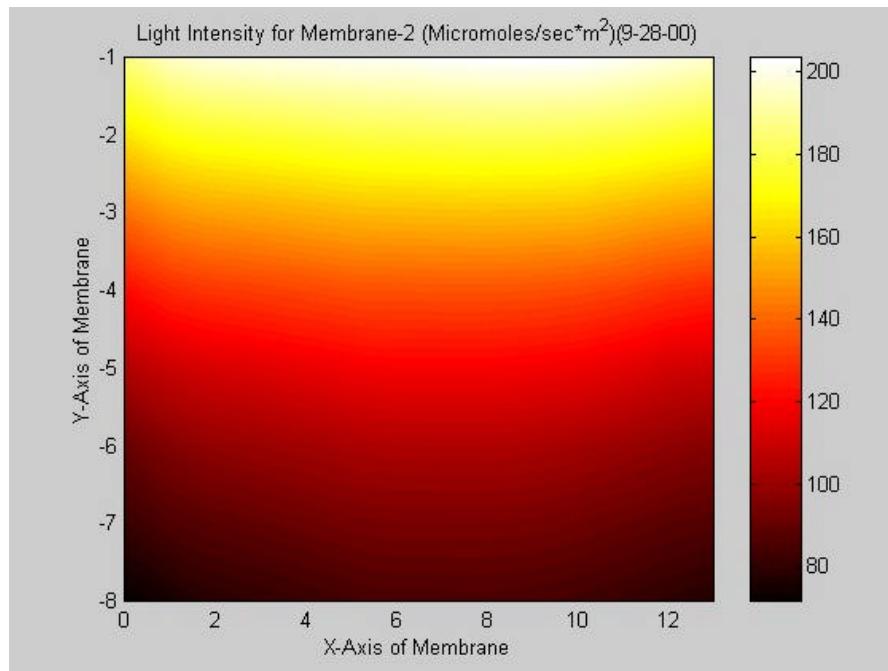


Figure 8. Optimal Photosynthetic Photon Flux on Membrane 2

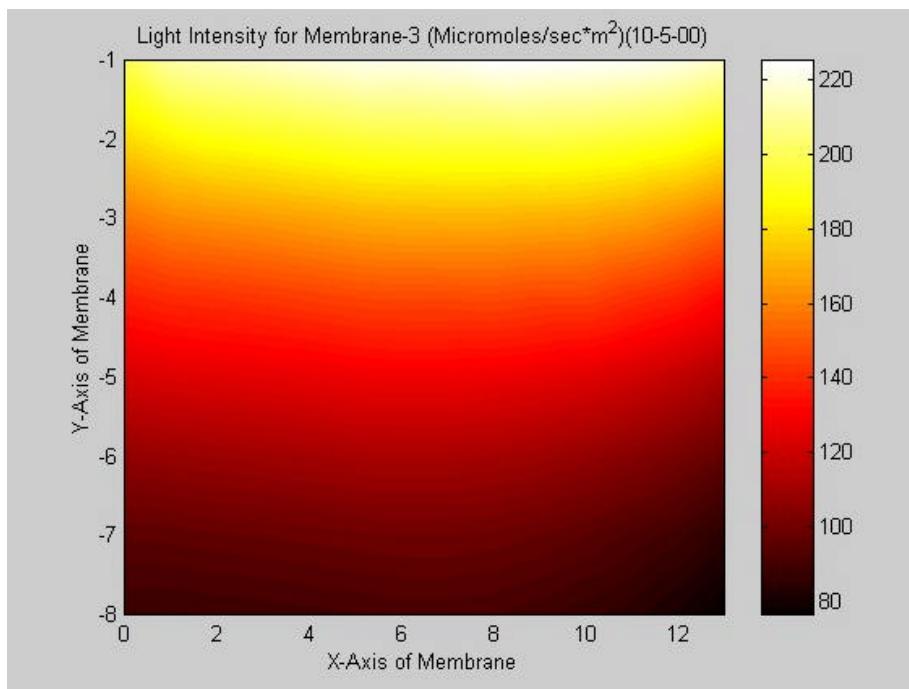


Figure 9. Optimal Photosynthetic Photon Flux on Membrane 3

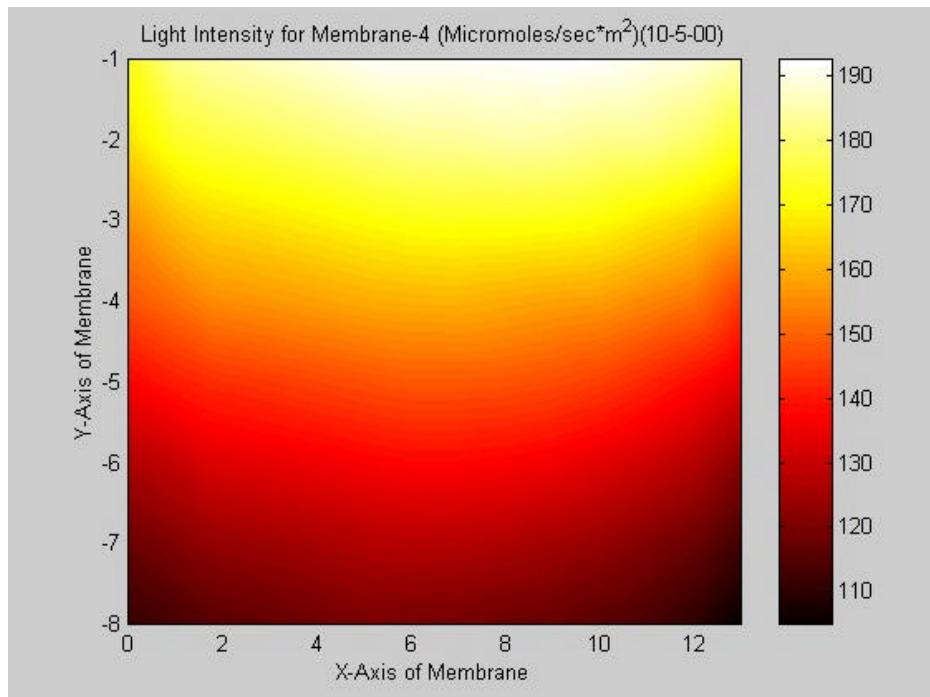


Figure 10. Optimal Photosynthetic Photon Flux on Membrane 4

Because of the poor distribution achievable with external artificial lighting, the researchers at Oak Ridge National Labs have designed a testing box for measurement of light distribution via

fiber optic cables with growth membranes in-place. The box is described in Figure 11. The unit is currently under construction at Ohio University and it is hoped that the fibers can provide a more uniform distribution of photons within the bioreactor to achieve an optimal balance between growth rate and surface area.

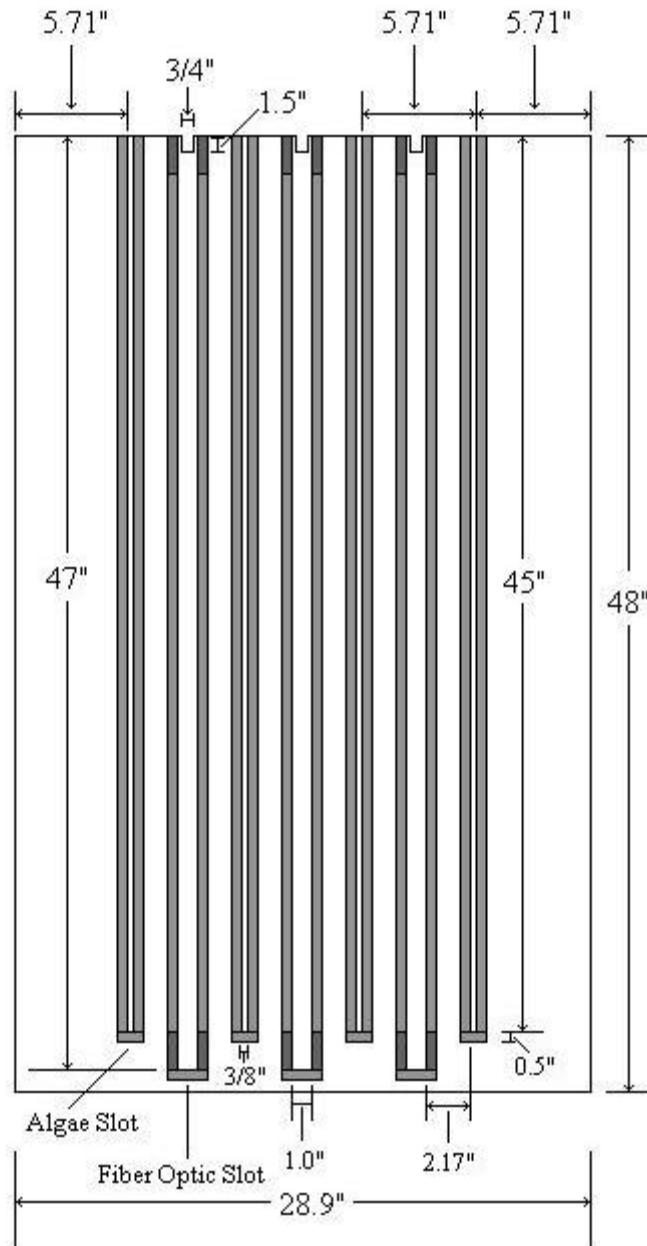


Figure 11. Schematic of the fiber optic test facility

Subtask 1.3 Investigate growth surface subsystem design

- 1.3.1 Examine surface configuration for effects on growth and harvesting
- 1.3.2 Examine surface composition for effects on growth and harvesting

The polymer "Scotch Bright" has been tested for the sterilization properties. It was found that this polymer can be sterilized at 121°C for 30 min and there was no detectable damage.

Subtask 1.4 Investigate the use of a hydraulic jump to improve the system's overall CO₂ conversion efficiency

- 1.4.1 Examine effect of hydraulic jump on HCO₃ (bicarbonate) concentration
- 1.4.2 Examine effect of hydraulic jump on exhaust gas temperature
- 1.4.3 Examine effect of hydraulic jump on need for direct flue gas exposure to promote photosynthesis in the bioreactor
- 1.4.4 Quantify costs / negative effects of hydraulic jump on the system

An early decision was made to utilize a previously designed and constructed slug flow reactor system currently housed at the Institute for Multiphase Technology at Ohio University. The current system was designed specifically for use in a situation, which was moderately different than the desired configuration. Subsequent efforts focused on the identification of system requirements for the slug flow reactor to be used for scrubbing of CO₂ from a synthetic flue gas and the modifications required in the present system. A schematic of the desired configuration is presented in Figure 12.

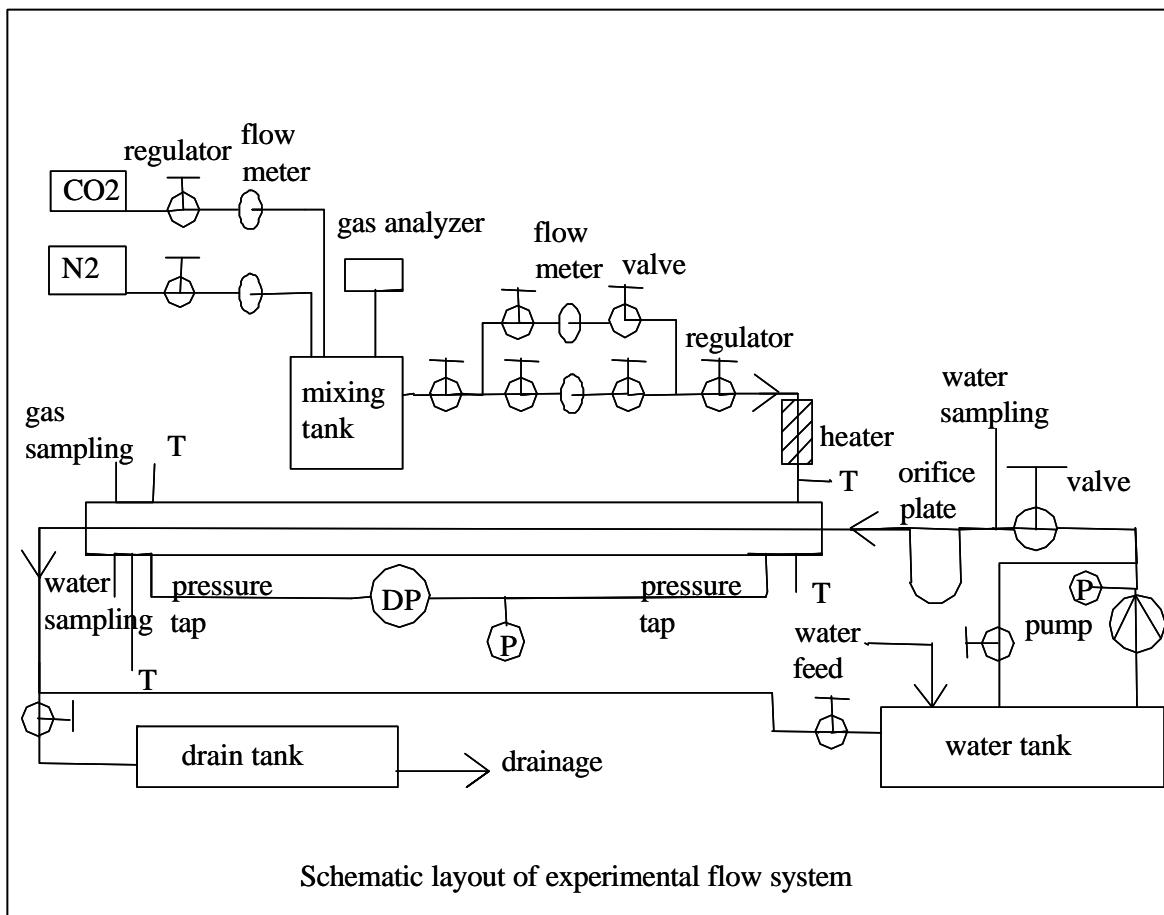


Figure 12. Proposed Translating Slug Flow Reactor Modifications

Identified modifications included:

- (1) conversion of reactor from vertical configuration to horizontal flow
- (2) addition of a second gas inlet stream for the ability to deliver 15% CO₂ in 85% N₂
- (3) addition of gas sampling ports prior to the inlet and at the exit of the reactor
- (4) addition of liquid sampling ports prior to the inlet and at the exit of the reactor
- (5) addition of thermocouples prior to the inlet and at the exit of the reactor
- (6) addition of a differential pressure transducer to monitor pressure drop in reactor

Piping for inlet gas lines has been purchased and the manifold system for gas delivery is under construction. High accuracy gas flow meters have been evaluated and appropriate units identified. These are currently in the process of being ordered. Acrylic piping for the horizontal flow configuration has been located and it is anticipated that this will be fitted with flanges and sized for installation prior to the end of January. A differential pressure transducer of the required sensitivity has been specified and an appropriate unit is being located. Location of all sampling and/or monitoring ports within the reactor system has been completed. Drilling of the reactor pipe and subsequent installation of these sampling/monitoring ports is awaiting the receipt of all units for concurrent installation.

Subtask 1.5 Design harvesting subsystem

Phase I research aims to determine which individual factors have the most significant effect on CO₂ uptake in an enhanced photosynthesis system. It also will evaluate and rank component and subsystem level alternative design concepts with respect to their ability to control or provide those factors which maximize CO₂ uptake. We have made progress in phase I level research for the harvesting subsystem as described in the following paragraphs.

We have researched the growth patterns and preferences of the various types of thermophilic organisms that are candidates for the bioreactor and have determined that a harvesting method that provides a frequent, gentle, and partial cleaning is desirable. Thorough cleaning methods (which would remove nearly 100% of the cyanobacteria in the cleaned area) are likely to result in a significant growth lag as the cleaned area is repopulated. Partial cleaning (which attempts to maintain a certain percentage of the organisms in the cleaned area) appears to be the better choice for an overall optimum level of CO₂ uptake. Research into cleaning methods has identified the commonly used water-jet cleaning method as the most likely to provide the controllability, flexibility, cost, and performance necessary for a successful harvesting system design. Therefore, experiments to date have focused on determining parameters that maximize the performance of a water-jet cleaning system for the carbon recycling facility experimental setup.

The water-jet system design parameters currently under investigation include

1. Nozzle types
2. Orientation angle of water-jet (relative to plate being cleaned)
3. Impact velocity of the water-jet on the area to be cleaned
4. Spray time, or the amount of time the spray is held on the area to be cleaned

Results to date show desirable cleaning performance from a water-jet system consisting of a 90° low-flow full-cone whirl nozzle with its centerline oriented at an angle of 46° relative to the growth surface, using 10-psi line pressure and a spray time of 5 seconds. The organisms remaining in the partially cleaned area after the spray cleaning were examined and were found to be healthy and capable of continued growth.

One of the difficulties encountered in this and other experiments is that the total mass of the cyanobacteria involved is small, and the change in mass (for instance before and after a cleaning operation) is even smaller, so special dry-weight methods for comparison are often required to make meaningful comparisons. Therefore, another focus of our efforts has been on developing improved test procedures to simplify data collection over the duration of this project. One alternative technique, being investigated for approximating changes in the mass of cyanobacteria on a growth surface, is comparative image analysis. Digital images of the growth surfaces taken at different times can be processed using image analysis techniques and characteristics of the images can be compared. If changes in certain characteristics (either color based or density based) can be reliably calibrated with measured changes in mass, then image analysis may be used to simplify and speed up the test process and ultimately automate the cleaning process (i.e. to provide a real-time determination of when the surface needs to be cleaned). We are currently working to establish the calibrations and to better understand the capabilities of the image analysis process (accuracy, sensitivity, etc.).

Only limited amounts of work have been completed on subtasks 1.5.2, 1.5.3, 1.5.4, and 1.5.5. Specifically, for subtask 1.5.2 we are waiting on more detailed results about the growth characteristics of specific thermophilic organisms before determining and testing harvesting schedules. Methods for differentiating between live and dead organisms (1.5.3) have been identified but have not been tested thoroughly, and separation methods (1.5.3) and repopulation methods (1.5.5) will be studied later in the project with reference to continuous harvesting systems. Examination of methods for processing harvested algae for end use and/or reuse in the CRF (1.5.4) have been started but there are no major findings to report.

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

A Ph.D. student involved in the project is currently researching this task. He has started by examining the literature on potential end-uses of biomass other than as a directly combustible fuel. Several potential end-uses have been identified, but more details are needed.

Webpage

The web page is running at <http://www.ent.ohiou.edu/~bayless2/CO2>. All parties involved in the project will receive e-mail instructions and the password to access the information.

Conclusions

While the data collected this quarter is insufficient to draw strong conclusions, it has helped to direct the work for the upcoming quarter. Specifically, some of the tasks that will be undertaken in the next quarter include

- When culturing has sufficiently progressed, begin growth rate measurements under extreme (140°F^+) conditions in various flue gas compositions
- Continue fundamental testing of growth surfaces, organism adhesion, and intrinsic growth rates
- Continue modification of translating slug flow reactor and begin basic testing of soluble carbon species concentrations versus flow conditions
- Deliver lighting system containment to Oak Ridge to begin fiber testing schemes
- Continue optical- and mass-based harvesting experiments with water spraying

Finally, because the rate of progress has increased greatly over the last two months, the web page with weekly progress reports to provide a greater level of project feedback.