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Abstract

This quarterly report documents significant achievements in the Enhanced Practical Photosynthetic CO₂ Mitigation project during the period from 1/03/2001 through 4/02/2001. Many of the activities and accomplishments are continuations of work initiated and reported in last quarter's status report.

Major activities and accomplishments for this quarter include:

- Three sites in Yellowstone National Park have been identified that may contain suitable organisms for use in a bioreactor
- Full-scale culturing of one thermophilic organism from Yellowstone has progressed to the point that there is a sufficient quantity to test this organism in the model-scale bioreactor
- The effects of the additive monoethanolamine on the growth of one thermophilic organism from Yellowstone has been tested
- Testing of growth surface adhesion and properties is continuing
- Construction of a larger model-scale bioreactor to improve and expand testing capabilities is completed and the facility is undergoing proof tests
- Model-scale bioreactor tests examining the effects of CO₂ concentration levels and lighting levels on organism growth rates are continuing
- Alternative fiber optic based deep-penetration light delivery systems for use in the pilot-scale bioreactor have been designed, constructed and tested
- An existing slug flow reactor system has been modified for use in this project, and a proof-of-concept test plan has been developed for the slug flow reactor
- Research and testing of water-jet harvesting techniques is continuing, and a harvesting system has been designed for use in the model-scale bioreactor
- The investigation of comparative digital image analysis as a means for determining the "density" of algae on a growth surface is continuing

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

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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. A 30-gallon tank of the Yellowstone organism was initiated. The culture is being grown in 1/2 strength Allen's media as per the results of the earlier tests nutrient concentration with growth.
 - i. This culture is doing well and producing much biomass so that it will be ready for use in the next stage of experiments. The 5-gallon cultures of other potential organisms are being maintained.
- b. Experiments on the effects of the monoethanolamine were carried out for the thermophillic organism from Yellowstone. The experimental design was as follows: 6 replicate cultures of each of 3 treatments were utilized for a total of 18 cultures. The treatments were 10% monoethanolamine, 5% monoethanolamine and no monoethanolamine in Allen's Modified Medium that is used to grow the thermophillic organisms. We hypothesized that the monoethanolamine would have little effect on the growth of the thermophillic organisms. The cultures were sampled at Day 7 and 14. The results showed that the 10% killed the cells within a week (no cells counted) and 5% significantly inhibited growth compared with the control (Figure 1). Further testing of the monoethanolamine will be conducted in order to determine if very low levels can be tolerated.

Figure 1. Effects of monoethanolamine on the thermophillic organism from Yellowstone

- c. Yellowstone Park scientists who work with the park GPS database have been contacted. Sites have been selected which, given the temperature and pH requirements of the carbon dioxide mitigation system, are likely to hold suitable organisms. Three sites have been identified.
- d. Since the Yellowstone National Park is not open to foot traffic until May, we have obtained cyanobacterial strains from culture collections here and in other places. Dr.

- David Ward's lab [Montana State University] has helped us with some organisms, but the greatest potential source is the collection of Dr. Richard Castenholz at the University of Oregon. Dr. Castenholz is a Visiting Fellow at the Thermal Biology Institute at MSU and we expect to continue to collaborate with him. The strains we have received are : Synechococcus C-1, Synechococcus P-2, Oscillatoria sp. and K-Borgonia. Attempts to cultivate Oscillatoria sp. were unsuccessful. Synechococcus P-1 showed very slow growth and therefore we have stopped working with this culture. The doubling time for Synechococcus C-1 into media D and BG-11 is about 2-4 days. Now we are comparing the growth of K-Borgonia in media D and BG-11.
- e. Frozen stocks of Synechococcus C-1, P-2 and K-Borgonia have been prepared. Control re-growth experiments have been done. The cultures were recovered successfully.
 - f. It was shown that Chl *a* extraction with methanol is to be better than with 90% acetone in water. This is now used routinely to determine chlorophyll as a biomass indicator.
 - g. We have studied the effect of medium pH on the growth of Synechococcus C-1 in buffered BG-11 media. This experiment showed that alkalinity stimulated the growth of C-1 during lag-phase and initial log-phase. On the other hand, the alkalinity stimulated the aging (death) of cultures. Neutral pH slows down the division of Synechococcus C-1.
 - h. The addition of NaHCO₃ (1, 5 and 10 mM) shortened the lag-phase of Synechococcus C-1 in BG-11 medium, but dramatically stimulated the alkalization of media and consequently the death of cyanobacteria. Since the addition of sodium bicarbonate had negative effects, we will investigate whether this could be a general effect of alkalinity or a negative effect of increased sodium ions.

1.1.2 Quantify adhesion characteristics

- a. We have carried out a survey of potential media gelling agents for thermotolerant cyanobacteria. Agar [Difco], Gelrite [Kelco-Merck] and agarose [Seakem] were compared. Agarose appeared to have the best properties at 50°C, although with care, agar is usable at this temperature.
- b. An initial experiment to measure the adhesion to a glass surface of Synechococcus C-1 has been done. It appears that surface adhesion may involve calcium.
- c. In initial experiments, Synechococcus C-1 appeared to have better adhesion [greater biomass/area] on plastic than glass. Conditions for adhesion are being optimized.
- d. K-Borgonia adheres to both hydrophilic and hydrophobic surfaces.

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

- a. The incubation facility [light, temperature control] has been constructed. We await delivery of the tubes that will fit in the incubation system.
- b. Because it will be important to know the viability of cells harvested from the CRF before they are used for re-inoculation purposes, we have developed a live/dead test for microalgae. The test depends on staining with the fluorescent dye Sytox Green. The dye binds to DNA and can only enter a cell if the cell membrane is compromised. Dead or dying cells stain with a yellow/green fluorescence. Live cells do not take up

the stain and can be seen by their red [chlorophyll] fluorescence at another wavelength.

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.

Subtask 1.2 Design deep-penetration light delivery subsystem

Development of a deep-penetration light delivery subsystem for use in a pilot-scale bioreactor setup is proceeding as scheduled. The three unique light delivery systems illustrated in Figure 2 are in various stages of construction and testing.

Figure 2. Alternative light delivery systems

Figure 2a illustrates a technique for the high-angle dispersion of light from an optical fiber using holographic diffusers. Holographic diffusers (Light Shaping Diffusers®) are used in combination with a collimating Fresnel lens to provide a potentially efficient (>85%) and controllable method for diffusing light across a large area. Figure 2b illustrates a technique for creating a fiber-illuminated cylindrical diffusing rod. This “illumination rod” is designed to mimic the dimensions of a standard T-8 fluorescent tube and creates diffuse illumination across a 180° angle below the rod. Figure 2c illustrates a technique for creating a planar sheet of near constant illumination. The “illumination sheet” utilizes a side-emitting fiber, which can be formed into various patterns, to produce various desired intensity distributions. All three techniques appear promising and the status of efforts to test and construct each device are described below.

High-Efficiency Holographic Diffusing Element

The design and construction of a high-efficiency holographic diffusing element is on hold pending the arrival of several Light Shaping Diffusers® from Physical Optics Corporation. Expected delivery is the end of April.

Design and Construction of an “Illumination Rod”

A design for the cylindrical diffusing rod, shown in Figure 2b, was developed using ZEMAX optical modeling software. The design consisted of a 2.54 cm diameter, 1.0 meter long, optically clear cylinder with a polished lower hemisphere and a diffuse upper hemisphere. Light launched from a butt-coupled optical fiber scatters from the diffuse upper surface of the cylinder and escapes through the polished lower surface of the cylinder. To improve efficiency, upward-scattered light is redirected back toward the lower hemisphere of the diffusing rod through a silver coating on the rod’s upper hemisphere. A silver-coated concave surface at the end of the rod is used to diverge low-angle incident light that has reached the end of the rod unscattered. This addition improves the optical efficiency of the diffusing rod while also improving the overall uniformity of the scattered light. A series of parallel illumination rods, placed within close proximity to one another, would provide an approximately uniform plane of light. The number of illumination rods required would be dependent on the amount of light desired and the total area to be illuminated.

An illumination rod was constructed from a 2.54 cm diameter, 1 meter long, cast acrylic rod, with high optical clarity and optically smooth outer surface. The rod was diamond-machined on one end to create a concave surface with a radius of curvature of 4.0 cm, and polished on the other end to create a planar optical surface suitable for butt-coupling to a large-core optical fiber. The top hemisphere of the rod was sandblasted to produce a uniform scattering surface and both the top hemisphere and concave end-mirror were coated with aluminum.

Preliminary testing of the cylindrical diffusing rod revealed a discrepancy between the desired modeled surface scatter of the rod’s top hemisphere and the actual surface scatter created by the sandblasting technique. Because optical scattering is often difficult to accurately pre-model in software, the result was not entirely unexpected. The difference in surface scatter unfortunately created a diffusing rod with an uneven illumination and low efficiency, see Figure 3.

Figure 3. Illumination Rod

However, now given the correlation between the modeled scattering values and the manufactured scattering values, it may be possible to re-simulate and re-design the current illumination rod to produce a more uniform intensity distribution. Before revisions are undertaken, additional factors related to optical efficiency, construction costs, and integration issues are first being evaluated.

Design and Construction of an “Illumination Sheet”

Greater success was achieved with the development of the “illumination sheet” shown in Figure 4. The illumination sheet consisted of 10 meters of side-emitting optical fiber, which is sandwiched between two clear plexiglass sheets. The optical fiber is formed into a serpentine pattern before being mounted in the plexiglass frame. Aluminum c-channels hold the assembly together. A mirror, fixed to the end of the optical fiber, reflects light back toward an 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, an additional 1/8” thick, 2’ x 4’ prismatic diffusing sheet was added to each side of the illumination sheet to improve diffusion. In addition, a 4” strip of highly-reflective tape was added across the top and bottom portions of the illumination sheet. The reflective tape redirected light being lost at the curved portions of the serpentine fiber. The finished 7/8” thick illumination sheet is shown in Figure 4.

a) Final Design of Illumination Sheet. b) Final Construction of Illumination Sheet

Figure 4. Illumination Sheet

A measurement of the illumination sheet's intensity distribution was acquired 2.75" above the surface of the illumination sheet. No algae screen or other diffusing material was placed in the plane of measurement. The normalized intensity distribution (normalized to produce a range from 0% to 100% illuminance) is shown in Figure 5. The white box in Figure 5 identifies the approximate location and size of a typical algae screen relative to the illumination sheet.

Relative Intensity Distribution Illumination Sheet (Serpentine Design #1)

Figure 5. Intensity Distribution of Illumination

Figure 5 shows areas of increased illuminance (“hot spots”) corresponding to light leakage near fiber bends and near “scarred” portions of the fiber. Reductions in the occurrence and magnitude of these “hot spots” should be possible in future designs through more careful construction and assembly techniques. The average intensity difference across the sheet falls from 49% (far right) to 21% (far left). Ideally, a 1:1 intensity ratio across the width of the sheet is desired, however, the current 2.3:1 intensity ratio is very promising. Immediate improvements can be made to the current design, and are underway, to more closely approach a 1:1 intensity ratio.

When a diffusing material such as an algae screen is placed in the plane of measurement, the current illumination sheet’s intensity distribution is observed to become considerably more uniform across the surface of the screen. The screen’s natural surface structure combines with the reflective properties of the illumination sheet’s plexiglass housing to act as a large optical integrator, which effectively smoothes the intensity distribution shown in Figure 5. However, because the algae screen may exhibit various surface properties (sometimes wet, sometime dry, etc.), the optical properties of the algae screen must be considered but NOT depended upon for the proper performance of the illumination sheet. Therefore, design improvements and additional testing must still continue to create an illumination sheet that properly performs and exhibits a 1:1 intensity under all circumstances.

The 2' x 4' illumination sheet shown in Figure 4 is designed to be cascade-able. For example, three illumination sheets can be connected together to create one 4' x 6' illumination sheet. Each 2' x 4' section requires one optical fiber to supply the necessary amount of solar light. Consequently, a 4' x 6' illumination sheet would require three fiber connections to an outside solar collector. This scalable property greatly simplifies the requirements on the design of the solar collector.

Currently, the illumination sheet design appears to be the most promising technique for uniform illumination of close proximity algae screens. This technique will continue to be studied and improved with additional prototypes constructed and tested. Variations in the serpentine pattern of the side-emitting optical fiber, improvements to the method of mounting the optical fiber, and reductions in the optical loss associated with bend radius will be studied. Accurate efficiency measurements of all the techniques will be conducted with the arrival of new large-area camera-based optical measurement tools.

Subtask 1.3 Investigate growth surface subsystem design

One of the first choices for a potential support for the biomass in the carbon remediation facility was Scotch Brite [3-M Co.]— a nylon based polymer with aluminum oxide grafted onto it. This product proved to be toxic to at least one cyanobacterial species with which we are working.

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

All the modifications of the slug flow reactor system housed at the Institute for Multiphase Technology at Ohio University have been finished. Identified modifications include:

- (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;
- (7) Addition of a heater and its controller prior to the gas inlet of the reactor;
- (8) Additions of N₂ and CO₂ flow meters prior to the gas inlet of the reactor.

In order to get more accurate data of gas concentration changes, a decision was made to sample the gas continuously at the exit of the reactor, as well as at the inlet of the reactor. As a result, two problems appear. The first is that two gas analyzers are needed to detect two samples at the same time. The second is the liquid that goes out with gas when sampling at the exit end should be removed before entering the analyzer. To solve the first problem, another gas analyzer was ordered and will arrive soon. As for the second problem, we have identified a continuous gas sampling system that not only removes the water but decreases the test error to a minimum.

Pure water and Sodium Hydroxide solution will both be used to scrub CO₂ from flue gas in the coming experiments. 50kg Sodium Hydroxide was bought to make the solution. To measure the actual concentrations of carbonate and bicarbonate ions, titration will be applied. Sulfuric Acid, which will be used as titration solution, was ordered and arrived in the lab. The essential equipment for titration, pH meters and burets, was prepared.

Tests will start in the weeks ahead. The experimental parameters were decided (see following tables).

- (1) Liquid velocity: 1m/s (125gpm), gas velocity: 6m/s (102scfm), CO₂ concentration: 15%; liquid: water;
- (2) Liquid velocity: 1m/s (125gpm), gas velocity: 6m/s (102scfm), CO₂ concentration: 15%; liquid: NaOH + water solution;
- (3) Liquid velocity: 1m/s (125gpm), gas velocity: 2m/s (34scfm), CO₂ concentration: 15%; liquid: NaOH + water solution;
- (4) Liquid velocity: 1m/s (125gpm), gas velocity: 10m/s (170scfm), CO₂ concentration: 15%; liquid: NaOH + water solution;

For each above condition, we can achieve the following data from the meters, transducers and analyzers with the time change.

Time	min	0	1	2	3	4	5	6	7	8	9	10
Superficial gas velocities	m/s	*	*	*	*	*	*	*	*	*	*	*
Superficial liquid velocities	m/s	*	*	*	*	*	*	*	*	*	*	*
Total pressures	PSI	*	*	*	*	*	*	*	*	*	*	*
Differential pressures	PSI	*	*	*	*	*	*	*	*	*	*	*
Inlet CO₂ concentrations	%	*	*	*	*	*	*	*	*	*	*	*
Outlet CO₂ concentrations	%		*		*		*		*		*	
Inlet liquid OH⁻ concentrations	mol/mol		*		*		*		*		*	
Inlet liquid HCO₃⁻ concentrations	mol/mol		*		*		*		*		*	
Inlet liquid CO₃²⁻ concentrations	mol/mol		*		*		*		*		*	
Outlet liquid OH⁻ concentrations	mol/mol		*		*		*		*		*	
Outlet liquid HCO₃⁻ concentrations	mol/mol		*		*		*		*		*	
Outlet liquid CO₃²⁻ concentrations	mol/mol		*		*		*		*		*	
Inlet gas temperatures	°C		*		*		*		*		*	
Inlet liquid temperatures	°C		*		*		*		*		*	
Outlet gas temperatures	°C		*		*		*		*		*	
Outlet liquid temperatures	°C		*		*		*		*		*	

Note: * indicate the time to acquire data.

Subtask 1.5 Design harvesting subsystem

In an effort to develop a harvesting system for use in the model-scale to pilot-scale bioreactors, we have continued our examination of alternative harvesting methods and have also focused on improving our test facilities and procedures to provide more meaningful experimental results. Specific activities supporting the design of the harvesting system are detailed below.

Subtask 1.5.1 Examine harvesting methods for efficiency of biomass removal

As previously reported, a harvesting method that provides partial cleaning (maintaining a certain percentage of the organisms in the cleaned area) appears desirable to avoid growth lag. The commonly used water-jet cleaning method is currently the leading alternative for providing a gentle and partial cleaning of the growth surfaces. This is because of the wide variety of nozzle types available and the good controllability of the cleaning action via control of supply pressure, offset distance, and incidence angle. Current harvesting system experiments are focused on proving that the water-jet system will work well for this application and determining target parameter values that work best for specific combinations of organisms and growth surfaces.

Summary of water-jet system design parameters currently under investigation
<p>1. Nozzle types</p> <p>A series of 12 tests have been completed investigating the cleaning performance of two different types of nozzles for removing Nostoc algae from polyester fabric growth surfaces. A 90 degree low-flow full-cone whirl nozzle and a 90 degree flat-fan nozzle have both been tested and their practical coverage area and percent algae removal have been determined for common spray conditions (incidence angle approximately normal to the surface, with 10 psi gage supply line pressure and a spray time of 5 seconds). See Figures 6-8 below for nozzle spray patterns. Results from these tests showed that: a) the full-cone nozzle provides a good balance between a large coverage area and a gentle partial cleaning for these test conditions, b) the flat fan nozzle could be effective in applications where it is rotated or swept but it is not appropriate for fixed nozzle applications because of its significantly reduced coverage area, and c) the actual coverage area for the full-cone nozzle is about 85% of the theoretical coverage area based on the manufacturer's specifications.</p>
<p>2. Incidence angle of water-jet centerline (relative to plate being cleaned)</p> <p>The original harvesting test experimental setup had the plates and the nozzles set at a fixed orientation angle. This setup has recently been modified to run a new set of experiments to determine the effect of the angle of incidence between the water jet and the growth surface on the cleaning effectiveness in the area of direct impact. Algae will be placed on the screens only in the area of direct impact (rather than completely covering the screens) so that a direct determination of percent of algae removed in the direct impact area can be found from a comparison of the dry mass of algae removed from the screens and the dry mass of algae remaining on the screens. Various incidence angles will be run and results of these tests will be used to inform the design of the harvesting systems for the CRF-1 and the pilot scale bioreactor. These experiments are underway and results will be reported when available.</p>
<p>3. Impact velocity of the water-jet on the area to be cleaned</p> <p>The required level of water-jet impact velocity is currently unknown, and it is expected that it will vary considerably for different combinations of organisms and growth surfaces due to differences in adhesion properties. Once more information is gathered on adhesion properties for specific organisms we will design and conduct experiments to test the required level of impact velocity. The experiments will have to account for the fact that impact velocity is a function of many factors, including nozzle type, offset distance, and supply pressure.</p>
<p>4. Spray time, or the amount of time the spray is held on the area to be cleaned</p> <p>This is an additional parameter that will eventually be tested, especially in relation to larger screen sizes. No experiments have been run yet for this parameter.</p>

Figure 6. Plate and nozzle orientations and parameters

Figure 7. Full-cone nozzle coverage patterns

Area in gray (FGHI) is the effective cleaning area every theoretical coverage of the 90 degree flat fan nozzle. Assuming nozzles spaced transversely at every coverage width. Spray angle = 45 degree (according to the table below)

$$\text{Area of FGHI} = 140 \text{ cm}^2$$

Figure 8. Theoretical coverage pattern for Flat fan Nozzle.

Improving/Expanding the Experimental Facilities for Harvesting Tests

The current harvesting system test setup is separate from the CRF test facility, which is acceptable for studying basic parameters and “proof-of-concept.” Since an enlarged bioreactor test system (CRF-2) has been constructed, the original CRF will soon be available for harvesting system experiments. Testing harvesting systems in the CRF will lead to more realistic results since the algae will have time to grow and attach to the growth surfaces before the harvesting step is initiated, and the CRF system can be run continuously after harvesting to determine the sustained growth of the algae remaining on the screens. Various harvesting schedules and “removal percentages” will be tested. An analytic study of nozzle types, plate and nozzle spacings, and nozzle orientation angles based on existing experimental results has been completed to determine a preliminary harvesting system design. The proposed design for the CRF water-jet harvesting system uses full-cone whirl nozzles with fixed position and orientation, with the constraint that the number and the spacing of the growth plates should be kept the same as it has been in the previous tests. For the current CRF growth plates, 9 nozzles oriented at an angle between 30 and 40 degrees (relative to the vertically suspended plates) are needed for each plate to achieve direct impact coverage of the entire surface. The current CRF “processing chamber” that holds the plates will have to be modified to allow extra space for the water-jet equipment since we want to keep the screen sizes the same as in the previous CRF tests. The current plan is to build an extension on top of the current box large enough to house the water-jet harvesting system.

Comparative Digital Image Analysis to Improve the Experimental Procedure

As previously reported, comparative image analysis is being investigated because it appears to have the potential to simplify data collection over the duration of this project and to offer a means for automated monitoring of growth surface status during experiments which may allow

for an intelligent harvesting system. We are still working to establish the calibrations and to better understand the capabilities of the image analysis process (accuracy, sensitivity, etc.), but preliminary results are encouraging.

An image similarity analysis was completed for three digital images of growth surface plates (shown in Figure 9), a new screen (with no algae loaded), a screen “fully loaded” with algae, and the same screen after a cleaning cycle with the full-flow cone nozzle setup. An Edge detected Radon transformation algorithm was used to compute similarity measures for the three images. The “fully loaded” screen had a similarity measure of 0.0026 relative to the new screen (meaning they are not at all similar), and the “cleaned area” of the screen had a similarity measure of 0.8306 relative to the new screen (meaning that they are about 83% similar). These results seem very meaningful relative to the area of the images being compared. We will continue to investigate the correlation between image analysis similarity measures and the experimentally determined dry mass percentage differences using these images and images from future harvesting tests. For future tests we will focus on getting better pictures that are similar in all manners except the amount of algae on the screen, making sure the frame and background do not influence the results.

a) Corner of Original screen – Before algae applied

b) Corner of screen after algae was applied

c) Corner of screen after water-jet cleaning

Figure 9. Growth surface images before and after water-jet cleaning, used in the comparative image analysis

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. The investigation of alternative end-uses of biomass other than as a directly combustible fuel is continuing. A detailed literature and internet search has been completed and several potential end-uses have been identified, but more information is needed.

Other Experiments

In addition to the work reported above for the specific subtasks, a large amount of work went into the development of improved model-scale experimental facilities as well as preliminary testing in the existing bioreactor facility (the Carbon Recycling Facility). The results of two bioreactor test runs in the existing bioreactor facility are summarized below.

Summary of Test Run 01/17-22/01

The test specimen for *Test Run 01/17-22/01* 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 3.53%, 9.90%, and 36.32 ppm for oxygen, carbon dioxide, and carbon monoxide, respectively. The 120-hour temperature average was 120.9°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. The intensity averages for the four membranes were 117.5 $\mu\text{mol/s}\cdot\text{m}^2$ for membrane-1, 130.9 $\mu\text{mol/s}\cdot\text{m}^2$ for membrane-2, 141.5 $\mu\text{mol/s}\cdot\text{m}^2$ for membrane-3, and 150.3 $\mu\text{mol/s}\cdot\text{m}^2$ for membrane-4

Test Run 01/17-22/01 had a total dry algae mass gain of 1.37 grams, or a 31.1% increase over the estimated initial dry mass. The following table describes each membrane and final test results.

Membrane Number	1	2	3	4
Membrane Initial Dry Mass (no loading) (g)	15.86	15.90	16.51	15.88
Membrane and Algae Final Dry Mass (g)	17.17	17.07	17.62	17.28
Final Dry Mass of Algae (g)	1.32	1.16	1.11	1.40
Membrane Loading Estimated Dry Mass (g)	1.18	1.05	0.95	1.24
Mass Gain for Each Membrane (g):	0.14	0.11	0.16	0.16
Percent Mass Gain for Each Membrane (%)	12.0%	10.8%	16.5%	13.2%
Mass Gain From Filter (g)	0.44			
Mass Gain from Beaker (g)	0.36			
Total Estimated Initial Dry Mass (g)	4.42			
Total Mass Gain (g)	1.37			
Total Percent Mass Gain (%)	31.1%			

The uncertainty analysis for this test run is based on the results from the *Algae Dry Mass Determination Test* and the final results of *Test Run 01.17-22.01*. 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 membrane. 4.4581% of the total loading wet weight was used as the initial estimated 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 four weighed samples to load each membrane.

Data from *Algae Dry Mass Determination Test*, *Test Run 01.17-22.01* and Student's t-distribution:

Mean = 4.4581%
Std. Dev = 0.1933%
Degree's of freedom = 11 (based on Student's t-distribution for 12 samples)
 $t_{90} = 1.796$ (Student's t-distribution for 12 samples)

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

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

$$\text{Uncertainty}_{90} = \frac{1.796 \times 0.1933\%}{\sqrt{4}} = 0.1735\%$$

Algae wet weight applied to Membrane-1 = 26.3690 grams

Uncertainty of estimated dry weight = $26.3690 \times 0.001735 = \pm 0.04575$ grams

Estimated initial dry weight = $26.3690 \times 0.044581 = 1.1755 \pm 0.04575$ grams

Mass gain for Membrane-1 = Membrane-1 final dry weight – initial dry weight = 0.1413 grams

$$\text{Uncertainty}_{90} = \frac{0.04575 \text{ g}}{0.1413 \text{ g}} \times 100 = 32.4\% \text{ (uncertainty of mass gain at 90\% confidence)}$$

The following table depicts the uncertainty of the mass gain for each membrane.

Uncertainty of Mass Gain	Membrane-1	Membrane-2	Membrane-3	Membrane-4
At 90% Confidence	32.4%	36.0%	23.6%	29.6%
At 95% Confidence	39.7%	44.1%	28.9%	36.2%
At 99% Confidence	56.0%	62.2%	40.7%	51.1%

The second uncertainty analysis is on a total mass gain basis for the four membranes, instead of the mass gain for each membrane. The benefit of this uncertainty analysis is that the four membranes are looked at as four samples for the total mass gain, which will increase the number of samples in the uncertainty equation. All other values remain the same as in the above calculation.

$$\text{Uncertainty}_{90} = \frac{1.796 \times 0.1933\%}{\sqrt{4}} = 0.1736\%$$

Total wet weight applied to membranes = 99.0896 grams

Uncertainty of total estimated dry weight = $99.0896 \times 0.001736 = \pm 0.1720$ grams

Estimated initial dry weight = $99.0896 \times 0.044581 = 4.4175 \pm 0.1720$ grams

Total algae mass gain (from membranes) for test run = 0.5758 grams

$$\text{Uncertainty}_{90} = \frac{0.1720 \text{ g}}{0.5758 \text{ g}} \times 100 = 29.9\% \text{ (uncertainty of mass gain at 90\% confidence)}$$

The following table depicts the uncertainty values on a total mass basis for the four membranes combined.

Uncertainty of Total Mass Gain	
At 90% Confidence	29.9%
At 95% Confidence	36.6%
At 99% Confidence	51.7%

The results conclude with 90% certainty that the mass gain of *Test Run 01.17-22.01* was 1.3724 ± 0.4103 grams, i.e. the mass gain was between 0.9621 grams and 1.7827 grams.

The total uncertainty associated with the scale (used for mass measurements) is estimated as a root-sum-square of the repeatability uncertainty, the resolution uncertainty, and the calibration uncertainty. The following calculation is the uncertainty at 95% confidence.

$$\text{Uncertainty}_{95} = \sqrt{(2 \times 0.00015 \text{ g})^2 + (0.0001 \text{ g} / 2)^2 + (0.0002 \text{ g})^2} = \pm 0.00036 \text{ g}$$

This would be the uncertainty for a single measurement made with this device. Since the uncertainty associated with the scale is so small, with respect to the uncertainties calculated for the mass gains, the uncertainty in the scale is neglected.

Summary of Test Run 2/28/01-3/5/01

The test specimen for *Test Run 02/28/01-03/5/01* 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 3.30%, 9.98%, and 42.58 ppm for oxygen, carbon dioxide, and carbon monoxide, respectively. The 120-hour temperature average was 120.2°F.

The lighting was not altered from *Summary of Light Intensity Test for Test Run 2.28.01-3.5.01* and was cycled 12-hours on, and 12-hours off. The intensity averages for the four membranes were 47.3 μmols/s·m² for membrane-1, 54.0 μmols/s·m² for membrane-2, 50.9 μmols/s·m² for membrane-3, and 62.4 μmols/s·m² for membrane-4

Test Run 01/17-22/01 had a total dry algae mass gain of 1.33 grams, or a 33.6% increase over the initial estimated dry mass. The following table describes each membrane and final test results.

Membrane Number	1	2	3	4
Membrane Initial Dry Mass (no loading) (g)	17.65	17.71	17.90	17.94
Membrane and Algae Final Dry Mass (g)	18.81	18.72	18.81	19.13
Final Dry Mass of Algae (g)	1.16	1.01	0.91	1.19
Membrane Loading Estimated Dry Mass (g)	1.12	0.95	0.84	1.04
Mass Gain for Each Membrane (g):	0.04	0.06	0.07	0.15
Percent Mass Gain for Each Membrane (%)	3.9%	6.1%	8.0%	14.4%
Mass Gain From Filter (g)	0.98			
Mass Gain from Beaker (g)	0.03			
Total Estimated Initial Dry Mass (g)	3.96			
Total Mass Gain (g)	1.33			
Total Percent Mass Gain (%)	33.6%			

The uncertainty analysis was conducted following the same procedure outlined above. The following tables depict the uncertainty of the mass gain for each membrane and for the four membranes combined.

Uncertainty of Mass Gain	Membrane-1	Membrane-2	Membrane-3	Membrane-4
At 90% Confidence	99.1%	64.0%	48.4%	27.0%
At 95% Confidence	121.4%	78.4%	59.3%	33.1%
At 99% Confidence	171.2%	110.6%	83.6%	46.7%

Uncertainty of Total Mass Gain	
At 90% Confidence	48.1%
At 95% Confidence	59.0%
At 99% Confidence	83.2%

The results conclude with 90% certainty that the mass gain of *Test Run 02.28-3.5.01* was 1.3281 $\pm$ 0.6388 grams, i.e. the mass gain was between 0.6893 grams and 1.9670 grams.

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. If access is needed, please contact Dr. Bayless at Ohio University.

Conclusions

The activities and accomplishments detailed throughout this report indicate significant progress towards completion of project objectives. However, since we have just completed the second quarter of the project most of the test results are preliminary and the majority of the activities underway are focused primarily on development and improvement of test facilities rather than on definitive testing. The most significant preliminary test results and test facility developments are summarized below.

- Test results concerning organism responses to pH levels and chemical additives are being used in the selection of organisms for the bioreactor and for setting optimal conditions in the bioreactor.
- A side-emitting fiber illumination sheet has been identified as the leading alternative design for deep-penetration light delivery in the pilot-scale bioreactor.
- An improved model-scale bioreactor has been constructed and is currently undergoing proof testing.
- A slug flow reactor system has been modified and is nearly ready for proof-of-concept testing.

These activities and the others discussed in the report will be continued in the next quarter in support of the overall project objectives. Additionally, the search for appropriate thermophilic organisms in Yellowstone National Park will now be able to proceed since all regulatory impediments have been removed and the weather conditions will be favorable. Also, tests of one previously cultured Yellowstone thermophilic organism will be able to begin next quarter in the newly constructed model-scale bioreactor system since an adequate supply of the organism is now available and longer duration model-scale bioreactor tests will be initiated in the original model-scale bioreactor after it has been retrofitted with a water-jet harvesting system.