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Abstract

This quarterly report documents significant achievements in the Enhanced Practical Photosynthetic CO_2 Mitigation project during the period from 10/3/2001 through 1/02/2002. Most of the achievements are milestones in our efforts to complete the tasks and subtasks that constitute the project objectives. Our research team has made significant progress towards completion of our Phase I objectives, and our current efforts remain focused on fulfilling these research objectives in accordance with 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.

Specific results and accomplishments for the fourth quarter of 2001 include:

- New procedures and protocols have been developed to increase the chances of successful implementation in the bioreactor of organisms that perform well in the lab. The new procedures include pre-screening of organisms for adhesion characteristics and a focus on identifying the organisms with maximum growth rate potential.
- Preliminary results show an increase in adhesion to glass and a decrease in overall growth rates when using growth media prepared with tap water rather than distilled water.
- Several of the organisms collected from Yellowstone National Park using the new procedures are currently being cultured in preparation for bioreactor tests.
- One important result from a test of growth surface temperature distribution as a function of gas stream and drip-fluid temperatures showed a high dependence of membrane temperature on fluid temperature, with gas stream temperature having minimal effect. This result indicates that bioreactor growth surface temperatures can be controlled using fluid delivery temperature. The possible implications for implementation of the bioreactor concept are encouraging, since it may be possible to use the bioreactor with very high gas stream temperatures by controlling the temperature of the organisms with the fluid temperature.
- Investigation of growth surface materials continues, with Omnisil and Scotch Brite emerging as the leading candidates. More investigation of these and other material types is still needed to determine the best material for particular combinations of organisms and harvesting methods.
- Tests of harvesting methods and harvesting system designs have shown that desirable levels of "percentage algae removal" can be achieved for particular organisms and growth surface materials, for example Cyanidium on polyester felt. Additional testing continues to better characterize sensitivity of the "percentage removal" to various system design parameters, but these tests have been delayed due to the lack of suitable organisms for the tests.
- The solar collectors and the pilot-scale bioreactor light distribution panels for the deep-penetration hybrid solar lighting system have been designed. One solar lighting system (solar collector tracking unit, fiber optic light transmission cables, light distribution panels) is almost completely prepared for installation during the next quarter in the pilot scale bioreactor system.
- Pressure drop results from tests on the enhanced mass transfer CO_2 absorption technique (the translating slug flow reactor) are encouraging, with reasonable values of 2.5 psi maximum over an 11.48 meter distance between pressure taps for test conditions of 0.6 m/sec slug velocity and approximately 10 m/sec gas velocity. Preparations are under way for CO_2 scrubbing tests.

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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)

Cyanidium was investigated as a potential organism for use in the bioreactor. This organism was found to grow quickly in culture and to tolerate 50-55^{o}C. However, in testing this organism in the bioreactor it was found that it did not sufficiently adhere to any of the test screening materials. Therefore we have concluded that *Cyanidium* is not suitable for the bioreactor and other organisms will be investigated. The researchers at Montana State University have isolated many (100+) strains that may potentially be used. These strains will not impose the same problem (non-adherence to the material) as the *Cyanidium* because they were isolated using new procedures based on placing actual growth surface materials in the hot springs to serve as "traps". The lab at Montana State University is testing strains for growth potential and sending the strains that grow most quickly to Ohio University. A new protocol at Ohio University has been created to streamline the procedures for testing the suitability of organisms for the bioreactor. The procedure is outlined below:

1. Cooksey lab will send 3 cultures of the best growing organisms as soon as possible. The main criterion in organism selection is doubling time and the secondary criterion is relative adhesion. Growth rate and adhesion criteria are equally ranked. The final choice of organism will be a compromise. We will provide information on both, but the experiments will be carried out separately- growth first and then adhesion.
2. Vis lab will grow the 3 cultures in 5-gallon culture containers and monitor growth daily (should take ~ 2 weeks for sufficient growth).
3. When sufficient growth has occurred in one of the cultures, part of that culture (volume to be determined) will be transferred with a minimum amount of culture media to a new rectangular container specially sized for colonizing CRF screens.
4. A CRF screen of Omnisil fabric will be placed in the rectangular loading tank and will be monitored periodically (visual checks along with digital photos) throughout the first 24 hours to determine if it is being colonized.
5. After 24 hours if the screen is sufficiently colonized, a final digital photo will be taken and the screen will undergo a 1 week survival test as discussed below.

In parallel the following:

1. The colonized Omnisil screen will be placed in the CRF and will be monitored twice a day until the organism is washed off or dies, or until it has proven it can survive for one (1) week. CRF parameters will be based on the standard methods defined above (unless otherwise instructed by Bayless) and will be monitored.
2. At the same time a small amount of the organisms will be tested for CO toxicity (realistic level in FLUE gas 20-30ppm). See testing strategies for procedure.
3. If the organisms appear to be surviving in the CRF and pass the CO test, they will be used to inoculate the 30-gallon culture. If the first organism type fails either test, one of the other two candidate organisms will be tested using the same procedure as followed for the first organism, and this will continue until an organism is found that can pass both tests.

4. While the organisms are colonizing the 30-gallon culture, the following experiments will be run in parallel (but Dip tests must precede Time Series tests):
    a) Dip tests for loosely attached organisms on Omnisil screens. See testing strategies for procedure.
    b) Time Series attachment tests for the organism on Omnisil screens. See testing strategies for procedure.
    c) When there is sufficient growth of the 5-gallon culture, more Omnisil screens will be loaded using time duration and a dip-wash as determined from the dip tests and time series tests.
    d) The colonized screens will be quantitatively tested in the CRF for up to one (1) week and if all goes well, the 105-gallon culture will be inoculated to provide plenty of biomass to run more CRF tests.
5. Presently a new organism (Tr9.4wf) is being cultured for use in the new protocol.)

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

1. Dr. Keith Cooksey, sub-contractor from Montana State University visited Ohio University in November 2001 for two days of talks concerning the CO_2 remediation project. As a result of the visit, integration of the two parts of the project being carried out in Montana and Ohio is now more closely coordinated, and the following points and protocols were developed.
    a) It is possible to use de-chlorinated tap water for growth media. This should have considerable economic impact at the pilot-plant stage and later.
    b) Only cyanobacterial isolates able to grow well at 50-55 °C will be provided to Ohio University.
    c) A method to determine cyanobacterial chlorophyll a.
    d) A protocol for scaling-up culture volumes to inoculate biomass carriers in the Carbon Remediation Facility (CRF). Particular parameters are volumes of culture to be transferred, control of medium pH, light level and light period for incubation, biomass needed at each stage of the process).
    e) A method to estimate the effect of CO on growth of organisms.
2. Based on its chemical and physical properties, Omnisil appears to be a primary candidate for the construction of the biomass carriers for the CRF.
    a) A potential list of high value products to be extracted from cyanobacterial biomass was made.
    b) Specific Results: Introduction of cyanobacterial isolates into culture and their purification
    c) During the 4th quarter of 2001 we continued the introduction of cyanobacterial samples collected in Summer field period into culture. In particular, they are samples from Rabbit Creek (right shore) and Black Sand Pool. All primary isolates were divided into three parts, each of which was then inoculated into three media: BG-11, D and DH. The letters BG-11, D or DH added after the organism designation indicate the medium upon which the isolates were initially grown. It is important to realize that all isolates were taken from experimental surfaces placed in the field (e.g. Scotch Brite, etc.) and collected when they had

been colonized. Generally speaking, this approach has led us to realize that there are significant differences in the species profiles of primary cultures of some isolates. For instance, the culture CS Trap 3 BF BG-11 contains only 2 species – *Synechococcus* sp. and *Chlroflexus* sp. At the same time, the culture CS Trap 3 BF DH contains 4 species – *Synechococcus,* a filamentous cyanobacterium (likely to be a *Phormidium* sp), a round cell cyanobacterium (unidentified) and a *Chloroflexus* sp. Thus medium DH allowed a larger number of isolates to grow than did BG –11. We expect that this approach will allow us to increase the diversity of species in our cyanobacterial collection.

d) In parallel, we are decreasing the volume of the existing collection by discarding samples exhibiting very poor growth (about 20 samples). During the 4^{th} quarter, twenty-seven (27) samples were frozen for long-term storage. After the purification we now have nine (9) uni-algal cultures of cyanobacteria from Yellowstone National Park. They are: 1.2 (6 single clones), 8.2.1, 3.2.2 (single clone) and one Tr8.2BF. Their species designation requires additional work.
e) Properties of photosynthetic organisms
f) During the 4^{th} quarter we studied the properties of a number of cultures growing under elevated amounts of CO_2 (5% CO_2 in air, flow rate 12% v/v/min). We concentrated on some isolates from an unnamed pool in the Rabbit Creek area (left shore). The reason for this concentration effort is the high level of cyanobacterial biomass in this pool (Fig. 1) as well as its temperature (55 – 58^{o}C) and pH (8.5 – 9.0). Both parameters are appropriate for our project.

Fig. 1. Unnamed pool in Rabbit Creek area (left shore)

g) In the first series of experiments we studied the rate of growth of 6 isolates in 50mL BG-11 medium supplemented with 5% $NaHCO_3$ and aerated with 5% CO_2.

The rate of gas flow was 6-8 mL/min., while the level of illumination was about 75µE. m^{-2} sec^{-1}.

h) It was found that the doubling time for this group of isolates varied between 8 and 19 hours (Fig. 2).

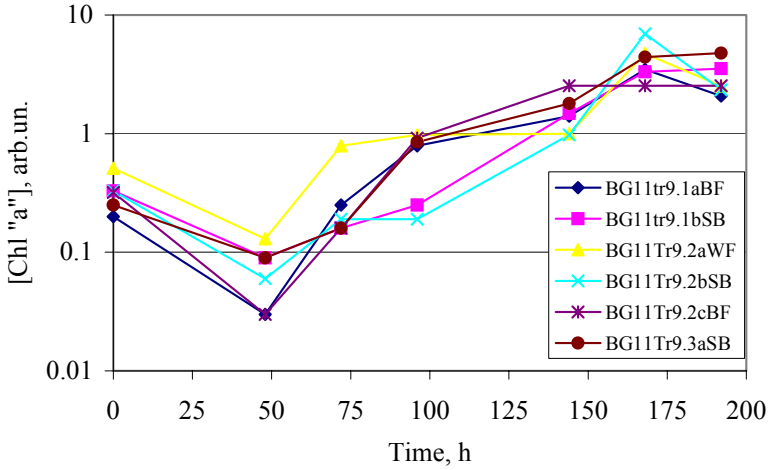


Fig. 2. Growth of the isolates from Rabbit Creek area into BG-11 medium supplemented with 5 mM NaHCO3 and aerated with 5% CO_2 in air.

i) It should be noted that the cultures began to be aerated 48 hours after inoculation, which is why the decrease of chlorophyll concentration was observed during the first 48 hours of incubation. Aeration of the cultures led to the stimulation of growth. The exponential phase occurred from approximately 50-150 hours (fig2). Macroscopic *in situ* observation of bioreactor tubes with cyanobacterial cultures suggests that the growth rate of some cultures did not appear to be logarithmic because significant adhesion to glass surfaces of the vessel was observed.

j) Figure 3 shows that when cultures of cyanobacteria are in the lag phase, the incubation media becomes acidified from slow utilization or non-utilization of the gassing phase. The beginning of the exponential growth phase coincided with the relative stabilization of the pH of the growth media. Results led to the assumption that at this time there was a balance between the rate of photosynthetic fixation of CO_2/HCO_3^- and the rate of CO_2 supply.

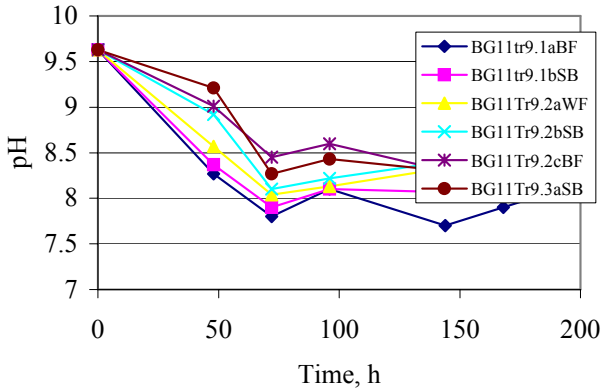


Fig. 3. pH dynamics in BG-11 medium, supplemented with 5 mM NaHCO3 and aerated with 5% CO2 in air during cultivation of isolates from Rabbit Creek area

k) In the next series of experiments growth of another group of isolates using the original media of their isolation was observed. The conditions of cultivation were changed slightly. In particular, aeration of the media began simultaneously with the inoculation of cultures.

l) Figure 4 indicates that in 5 days under these conditions, cyanobacterial biomass increased up to 400 relative chlorophyll a units from less than 1 unit. It was found that the average doubling time of this group of isolates approached 10 hours in the logarithmic phase of growth. In the late log phase, cultures began to cause the pH of the medium to rise. It is possible that at this time the rate of CO_2 input was less than its consumption rate by cyanobacteria. Therefore it is assumed that 5% CO_2 in air is not the upper concentration limit for the pilot plant cultivation of these isolates from Yellowstone National Park.

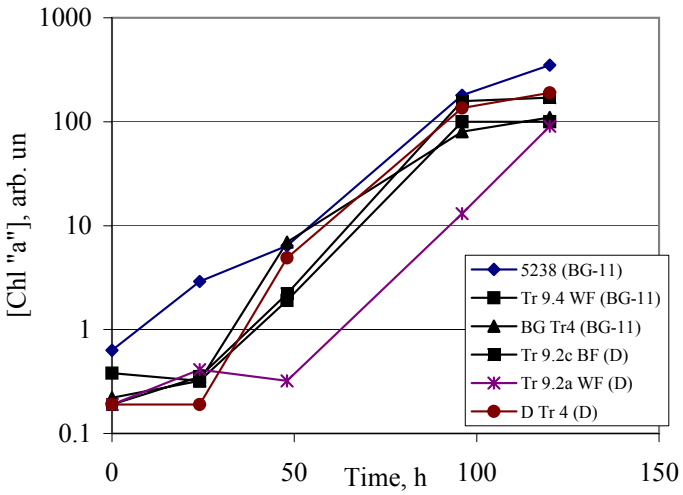


Fig. 4. Growth of the isolates from Rabbit Creek area in BG-11 medium supplemented with 5mM NaHCO3 and aerated with 5% CO_2 in air. (doubling times are similar; lag times and cell yields are different)

Since the use of distilled water may be precluded eventually, even at the pilot-plant stage, experiments were also carried out to investigate whether some isolates will grow in BG-11 medium prepared with tap water. These experiments were done without aeration with 5% CO_2 in air. Good growth of cultures D CS Tr2WF, D Tr 9.2a WF, D Tr 9.2c BF, and D Tr4 was observed under the described conditions (Fig.5).

During these experiments it was found that tap water in the town of Bozeman has a slightly alkaline pH and a higher concentration of calcium than is found in medium prepared with distilled water. Visual observation suggested that the medium prepared with tap water stimulated the adhesion of cyanobacterial cells to the glass, which is perhaps connected with the elevated concentration of calcium in the Bozeman tap water. This may be an important observation and work will continue to investigate the role of calcium ions in cellular adhesion. Also the BG-11 prepared with tap water had a significant brown color, perhaps due to the iron precipitation.

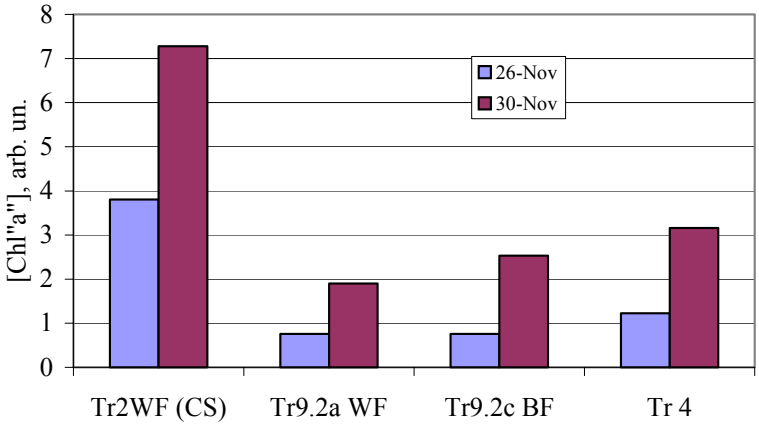


Fig. 5. Growth of 4 cultures in BG-11 medium prepared in Bozeman tap water

Since doubling times of several isolates cultivated in BG-11 medium prepared with tap water are longer than those obtained with distilled water medium (Fig. 6), the conclusion was reached that this medium may be less useful for the cultivation of cyanobacteria in the laboratory stage. However at the pilot–plant stage, this deficiency may be less important, given the financial savings provided by the use of tap water. Figures 7 - 9 demonstrate that even in tap water, the buffer system $NaHCO_3/CO_2$ keeps pH of cultures at a moderately alkaline level comparable to the pH of the pool where the cells were isolated.

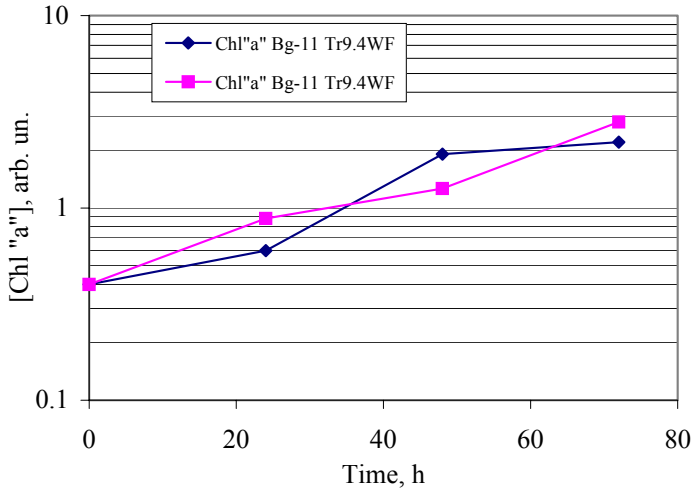


Fig. 6. Growth of Bg-11Tr9.4WF isolate (in duplicate) in BG-11 medium prepared with tap water and supplemented with 5 mM of NaHCO3 + 5 % CO_2 (doubl. time ~ 48 h)

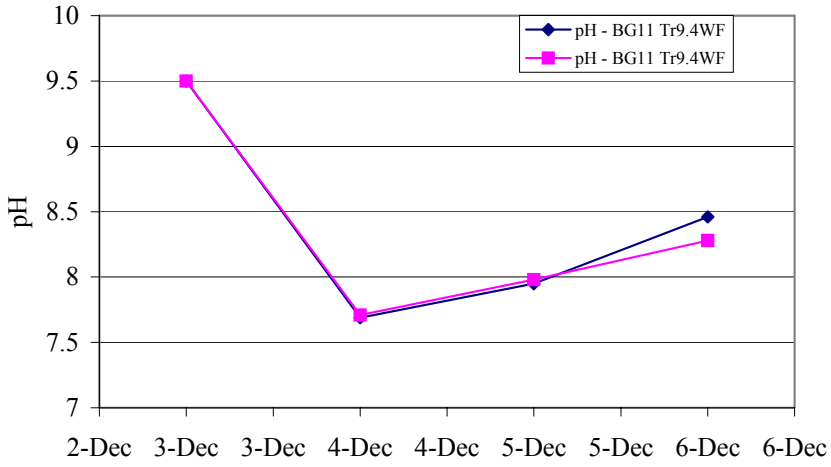


Fig. 7. pH dynamics during growth of Bg-11Tr9.4WF isolate (in duplicate) in BG-11 medium prepared with tap water, supplemented with 5 mM of NaHCO3 and aerated with 5 % CO_2 in air.

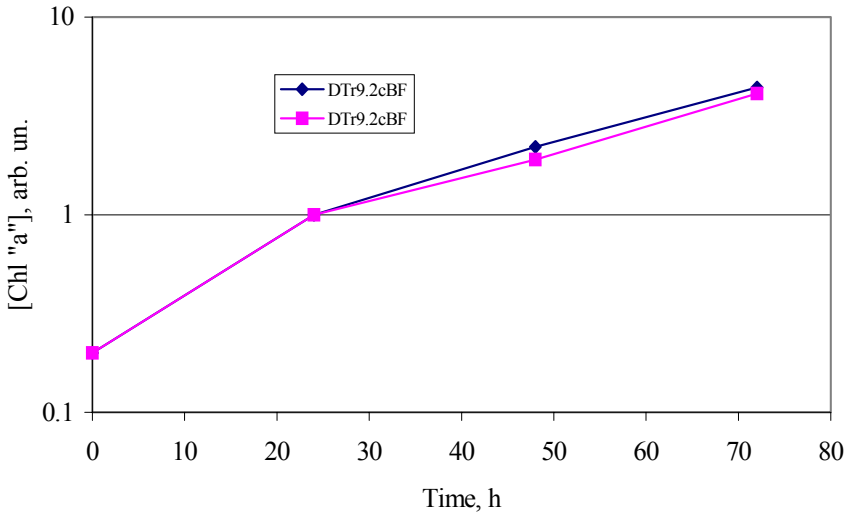


Fig. 8. The growth of D Tr9.2cBF isolate (in duplicate) in BG-11 medium prepared with tap water and supplemented with 5 mM of NaHCO3 (doubl. time =24 h)

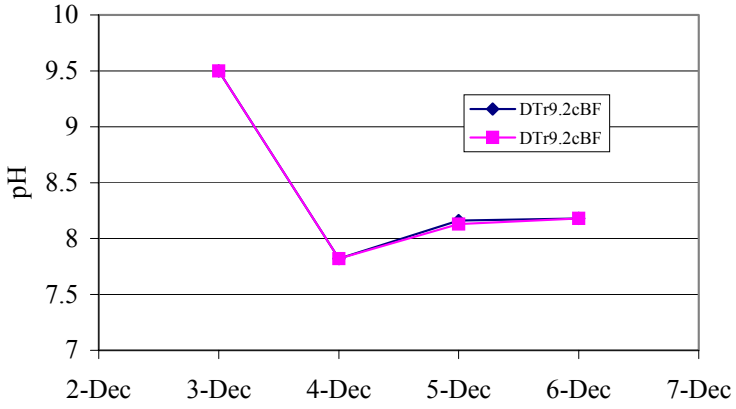


Fig. 9. pH dynamics during growth of D Tr9.2cBF isolate (in duplicate) in BG-11 medium prepared with tap water and supplemented with 5 mM of NaHCO3

In the next experiment, therefore, two factors were studied:

1. the importance of complex iron, which is normally included in BG-11 medium;
2. the possibility to grow some isolates under aeration with 5% CO_2 without supplementation of the medium with $NaHCO_3$.

To stabilize the pH of the above-described medium pH 7, 30 mM of HEPES buffer were added. Figure 10 shows that the complexed iron, which is a feature of BG-11 medium, is important for the growth of this cyanobacterial isolate, even in a medium prepared with Bozeman tap water containing 0.04 ppm of iron. It is essential that the experiment be repeated with Ohio tap water before using tap-water medium in the CRF.

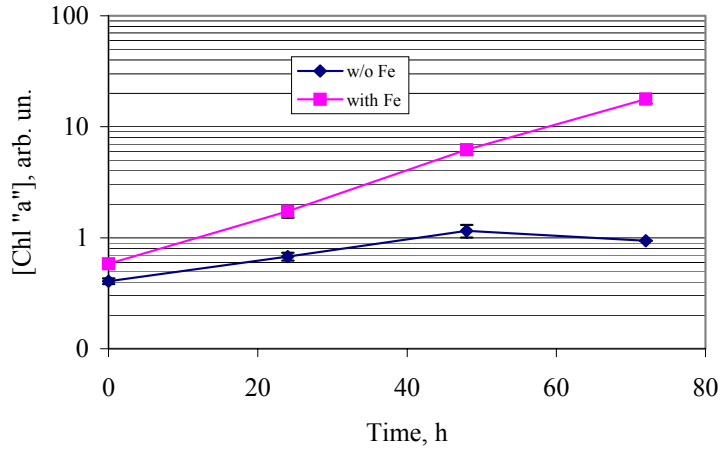


Fig. 10. The growth of the isolate D Tr 9.2c BF in BG-11 media prepared with tap water with or without iron

This experiment also allowed the observation of the slow, but permanent decrease in pH of the growth medium aerated with CO_2 in the absence of NaHCO3, even though 30 mM HEPES buffer was added to the medium (Fig.11). This is almost certainly related to the fact the pKa of HEPES is 7.2. HEPES was selected as a buffer initially because of its lack of toxicity to cyanobacteria, not because of its pKa. We are checking the possibility of the use of Bis-Tris Propane which has two pKa values, one at 6.8 and the other at 9.0., which should facilitate buffering around pH 8. However its toxicity is yet to be assessed.

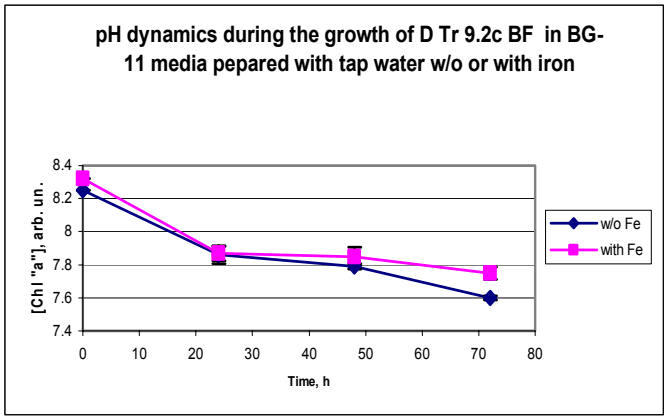


Fig. 11. pH dynamics during the growth of D Tr 9.2c BF in BG-11 media pepared with tap water w/o or with iron

Bioreactor development and testing at Ohio University

Bioreactor testing is on hold while the new organism is cultured to acceptable levels. Construction and testing of the generation 2 model scale bioreactor and the pilot scale bioreactor are continuing. One important result from a test of growth surface temperature distribution as a function of gas stream and drip-fluid temperatures showed a high dependence of membrane temperature on fluid temperature, with gas stream temperature having minimal effect. This result indicates that bioreactor growth surface temperatures can be controlled using fluid delivery temperature. The possible implications for implementation of the bioreactor concept are encouraging, since it may be possible to use the bioreactor with very high gas stream temperatures by controlling the temperature of the organisms with the fluid temperature.

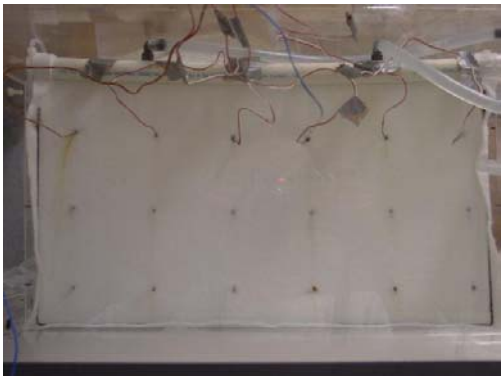


Fig. 12. Test plane in CRF system

Experimental Data:

1. Temperature Distribution of Membrane (6 points, 0C):
   a) Without External Bottom Tank Heating

	Col 1	Col 2	Col 3	Col 4	Col 5	Col 6	time
row1	35.2	33.4	34.6	37.6	35.8	39.7	2:35PM,01/04
row2	36.7	34.8	39.3	36.8	37.3	39.3	4:00PM,01/04
row3	No Water	38	38.2	38.3	38.8	41.6	2:00PM,01/05

   b) With External Bottom Tank Heating

	Col 1	Col 2	Col 3	Col 4	Col 5	Col 6	Time
row1	52.1	50.6	55.6	50.6	50.6	52.4	4:00PM,01/05
row2	46.9	45	49	46.5	49.2	51.1	5:30PM,01/05
row3	49.3	49	51.7	51.9	51.7	51.9	11:00PM,01/05

2. Circulation Gas Composition(CO_2%, O2%, CO ppm)
   a) Without External Bottom Tank Heating

	2:35PM,01/04	4:00PM,01/04	2:00PM,01/05
O2 %	7.8	9.1	9.4
CO2 %	6.1	5.4	5.1
CO ppm	53	49	45

   b) With External Bottom Tank Heating

	4:00PM,01/05	5:30PM,01/05	11:00PM,01/05
O2 %	5.3	5.5	5.9
CO2 %	7.4	7.3	7.1
CO ppm	80	52	53

3. Main Stream Simulated Flue Gas Temperature(0C)
   a) Without External Bottom Tank Heating

	2:35PM,01/04	4:00PM,01/04	2:00PM,01/05
Main Stream Temp.	53	53	53

   b) With External Bottom Tank Heating

	4:00PM,01/05	5:30PM,01/05	11:00PM,01/05
Main Stream Temp.	57	57	58

4. Bottom Tank Temperature (0C)
   a) Without External Bottom Tank Heating

	2:35PM,01/04	4:00PM,01/04	2:00PM,01/05
Bottom Tank Temp.	35	35	36

   b) With External Bottom Tank Heating

	4:00PM,01/05	5:30PM,01/05	11:00PM,01/05
Bottom Tank Temp.	50.3	50.6	54

5. Top Tank Temperature (0C)
   a) Without External Bottom Tank Heating

	2:35PM,01/04	4:00PM,01/04	2:00PM,01/05
Top Tank Temp.	31	32.5	33.8

   b) With External Bottom Tank Heating

	4:00PM,01/05	5:30PM,01/05	11:00PM,01/05
Top Tank Temp.	43.8/46.7	43.5	46.8

6. Gas Supply Composition (Air flow Rate, Methane flow rate)
   a) Without External Bottom Tank Heating

	2:35PM,01/04	4:00PM,01/04	2:00PM,01/05
Methane	1	1.1	1.1
Air	64	65	65

   b) With External Bottom Tank Heating

	4:00PM,01/05	5:30PM,01/05	11:00PM,01/05
Methane	1.1	1.05	1.1
Air	65	66	65

from Flow Meter Chart: 60 – 19139 ml/Min
70 - 22059 ml/Min

7. *Drainage Part Temperature of Chamber (0C)
   a) Without External Bottom Tank Heating

No reading

   b) With External Bottom Tank Heating

	4:00PM,01/05	5:30PM,01/05	11:00PM,01/05
Drainage Part Temp.	49	45.4	48

Summary
Comparing steady-state maximum and average membrane temperatures (from 1a and 1b) with and without water tank heating with the corresponding gas stream temperatures and fluid temperatures (3a,b and 4a,b) shows that the circulating fluid temperature has a much larger effect on membrane temperature than the gas stream temperature.

Subtask 1.2 Design deep-penetration light delivery subsystem

Oak Ridge National Laboratory (ORNL) has made significant progress on the solar collector as noted by the prototype now under test at their facility (Fig. 13). ORNL has likewise completed

three light distribution sheets (Fig.14). Light distribution results indicate relatively even illumination over the desired target area (Fig. 15). Specifications for the light distribution sheets are provided in Figure 16a,b,c. The additional sheets required for the initial pilot scale implementation will be fabricated once additional fiber is received.

Fig. 13. Solar collector

Fig. 14. Light distribution sheet

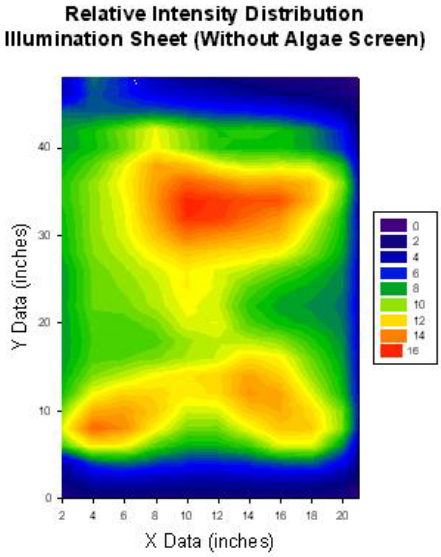
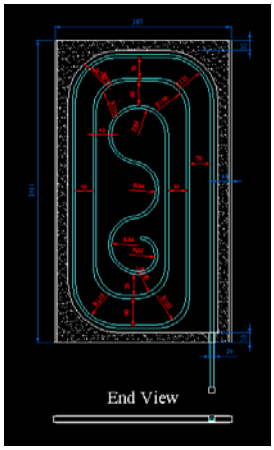


Fig. 15. Light intensity plot for light distribution sheet



a

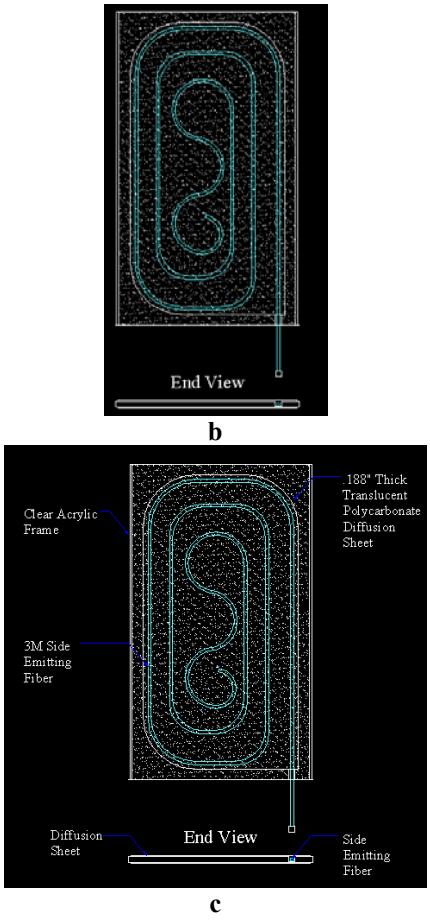


Fig. 16. AutoCAD Images of the light distribution sheet

Subtask 1.3 Investigate growth surface subsystem design

During the 4th quarter researchers at Montana State studied the growth of cyanobacterial isolates in the presence of Omnisil. In initial experiments with Omnisil, cultures died one day after inoculation. Measurement of pH showed dramatic decrease of pH in the medium to pH 3. To remedy this problem the Omnisil fabric was incubated for one day in 0.1 N KOH followed by thoroughly rinsing with water. Incubation of the washed Omnisil in BG-11 medium for one day did not cause the pH (pH 8.2) to fall significantly.

Further experimentation showed that cyanobacteria grew well in the presence of Omnisil treated with KOH (Fig.17).

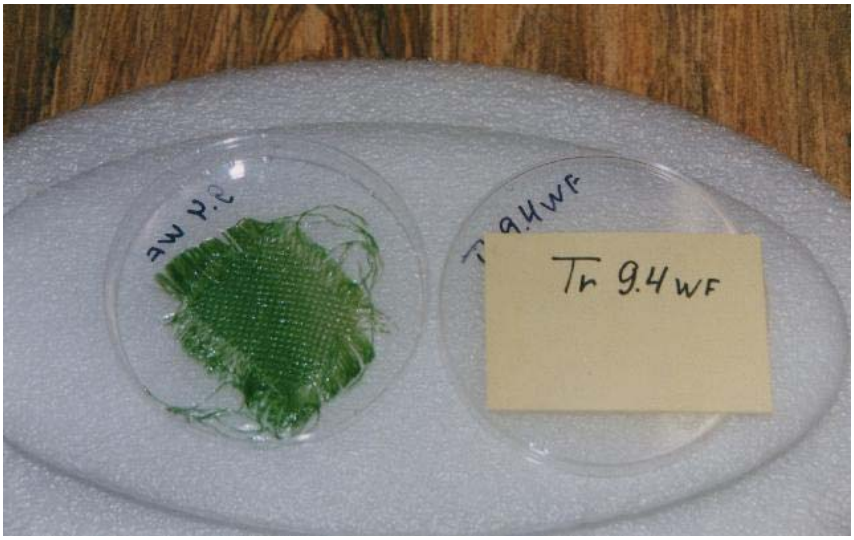


Fig. 17. Omnisil incubated 2 weeks with Tr9.4 WF isolate (BG-11 medium)

A fragment of this piece of Omnisil (Fig. 17) was examined under the Scanning Electron Microscope. It was found that the colonization of Omnisil (Fig. 18) is less than the level of that of Scotch Brite (Fig. 19).

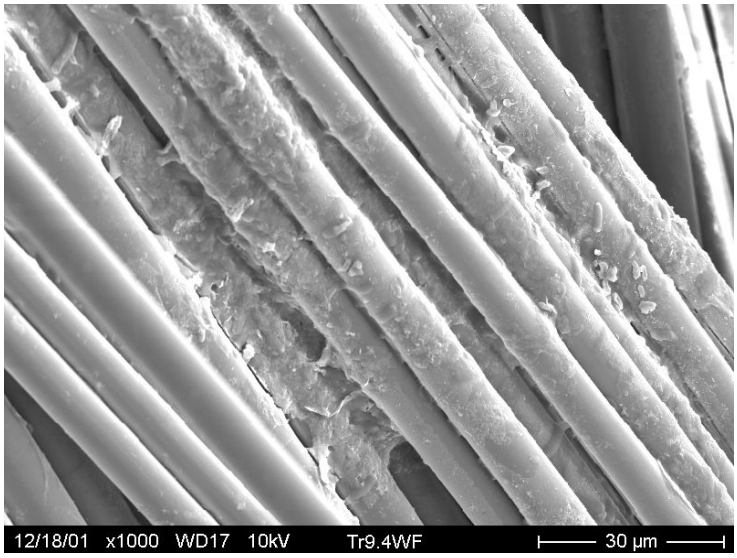


Fig. 18. The SEM picture of the colonization of Omnisil by the isolate Tr 9.4 WF

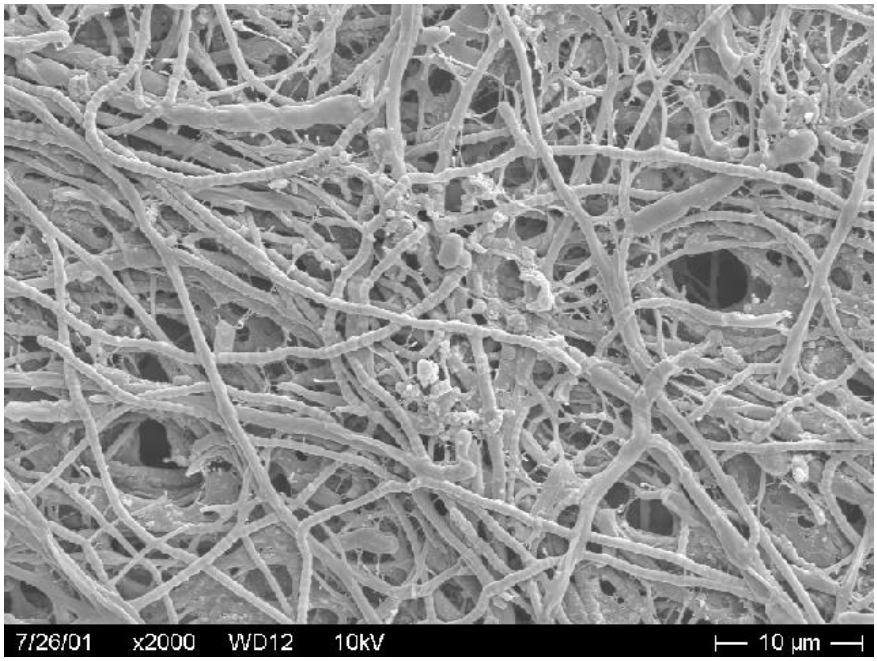
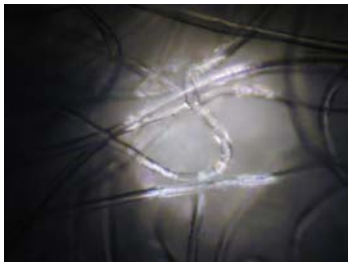


Fig. 19. SEM picture of colonization of Scotch Brite by cyanobacteria in the field

On the basis of these results it is possible to conclude that Scotch Brite is a more favorable surface for the development of a cyanobacterial biofilm than Omnisil. There may, however, have been significant losses of biofilm during the preparation of the Omnisil for SEM examination. This possibility is being examined.

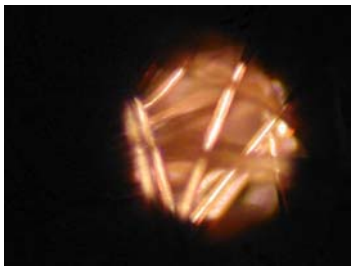
Ohio University Growth Surface studies

To better understand the properties of various candidate growth surface materials and their potential for use in the bioreactor, researchers at Ohio University have conducted microscopy work under various "wetting" conditions. This work was motivated by larger variations in the capillary action of some materials on the second wetting cycle as discussed in previous reports. For polyester felt, the material currently being used in bioreactor tests, Fig. 20 shows that the clusters have reduced significantly before and after wetting, that is, the spaces have increased. The flow properties have improved significantly to the point where the flow pattern is uniform. The time needed for the second wetting is slightly less than for the first wetting. For Omnisil material (and similarly for Teflon), flow properties improved considerably after the first wetting, with the wetting process occurring rapidly and uniformly with good capillary action displayed.



Polyester 10 x magnification

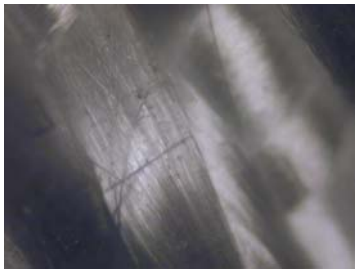
Scotch brite 10 x magnification



Teflon 10 x magnification

Omnisil 5X magnification

a: Pictures of virgin materials



Di - water 5x

Tap - water 5x

b: Pictures of the Omnisil material after wetting

Fig. 20. Pictures of the growth surface materials before and after wetting

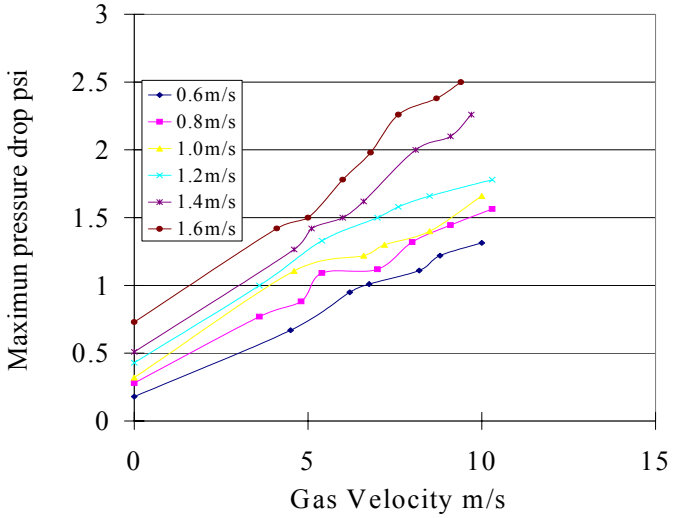
Subtask 1.4 Investigate the use of a hydraulic jump to improve the system's overall CO2 conversion efficiency

Subtask 1.4.1 Pressure drop test

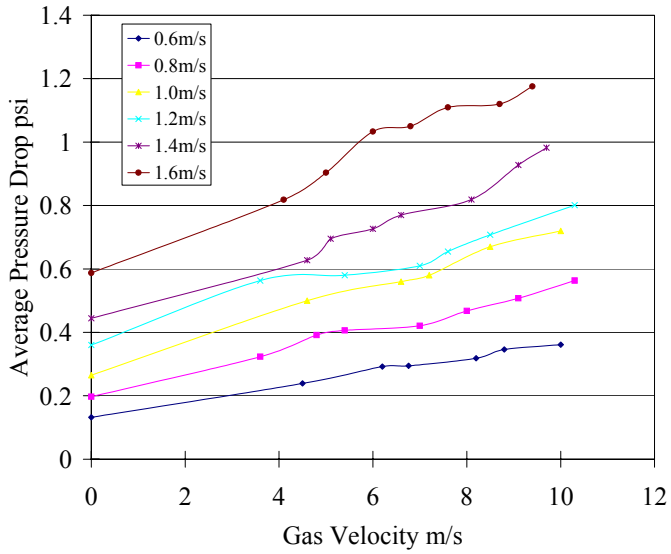
The pressure drop tests were continued in order to understand the basic pressure drop across the translating slug flow reactor. The pressure drops were measured with respect to various liquid and gas velocities in the slug flow reactor.

One thousand data of pressure drop of each condition were obtained by the data acquisition

system. Because of the fluctuation of the pressure drop, maximal pressure drop and the average pressure of each condition was recorded. The following graphs (Figure 21a,b) show the results of the pressure drop test. The distance between the two pressure taps for measuring pressure drop is 11.48m.



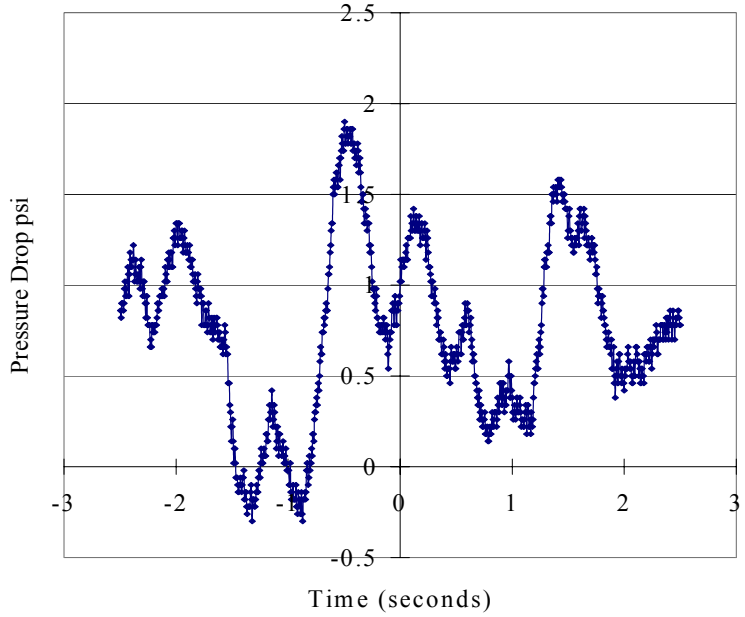
a. Maximum pressure drop



b. Average pressure drop

Fig. 21. Results of the pressure drop test

The pressure drop of slug flow fluctuates because the slugs pass through the pressure taps periodically. Figure 22 shows the change of pressure drop with respect to time.



Liquid Velocity=1.0m/s, Gas velocity=10m/s.

Fig. 22. Pressure drop fluctuations

Subtask 1.4.2 Preparation for CO_2 scrubbing test

CO_2 electrode and pH electrode were ordered from Fisher and were calibrated. The solutions for the test were made for the analysis of CO_2 and bicarbonate concentrations. The work includes:

1. Make standard $NaHCO_3$ solutions of 0.01M, 0.001M, 0.0001M and 0.00001M.
2. Make and purify NaOH solutions for sampling water from the system.
3. Make N/50 H_2SO4 solution for titration.

Subtask 1.4.3 Test the method for measuring CO_2 and bicarbonate

1. Method: when sampling CO_2 from the pipeline, part of the CO_2 will leave the solution due to the decrease of CO_2 partial pressure. In order to get the actual concentrations of gas CO_2 and bicarbonate in the solution, two samples will be taken at the same time. One sample is mixed with NaOH solution immediately, in order to capture the CO_2 gas before it leaves the solution; the other sample has no NaOH solution. For the sample with NaOH, decrease the pH to 4.5 by titration with Ion Strength Solution, and then measure the total CO_2 concentration in the solution with a CO_2 electrode. For the other sample

(without NaOH), its pH is reduced to 4.5 by titration with H_2SO4, then the bicarbonate concentration in the solution can be calculated based on the volume of sample and H_2SO4. The difference of the total CO_2 in the first method and the bicarbonate in the second sample is the gas CO_2 in the solution.

2. Test procedure:
    a. Calibrate the CO_2 electrode with NaHCO3 solutions with concentrations of 0.0001M, 0.001M and 0.01M. Make NaOH solution and dilute it to a very low concentration. Measure the bicarbonate concentration in the NaOH solution.
    b. Run the slug flow system with pure CO_2 and sample the solution.
    c. Both samples were titrated to pH 4.5 according to methods mentioned in section a.
    d. Calculate the gas bicarbonate and gas CO_2 concentrations.
3. Test results:
    a. The bicarbonate concentration in the NaOH solution is very small (about 0.003M.)
    b. The total bicarbonate and CO_2 concentration in the solution is 0.00368M before testing.
    c. For the sample without NaOH, the concentration is unchanged after testing because the liquid in the system has run with CO_2 several times and it was saturated for atmosphere pressure.
    d. For the sample with NaOH, the total CO_2 and bicarbonate concentration increased from 0.00368 to 0.0224M because the CO_2 dissolved in the liquid in the pipeline was completely captured. The difference is the gas CO_2 concentration in the liquid at 1 atm CO_2 pressure.
4. Conclusion:
    a. The proposed methods could be used to measure CO_2 and bicarbonate concentrations for the CO_2 scrubbing test by slug flow.

Comment: I can't follow this sentence.

Subtask 1.5 Design harvesting subsystem

Our main activities this quarter were again focused on design and experimental work for the integrated screen wetting/ harvesting system, and specific activities and accomplishments for the quarter include:

1. Determination of effects of temperature and humidity on high-resolution mass balance measurements to explain inconsistent mass measurements that have been confounding use of the dry-mass method. It was determined that high temperature materials could not be weighed using the current mass balance because results were not repeatable.
2. Elimination of the effect of high temperature on the mass readings for the filter and screens and to follow standard procedures, the off-line harvesting test procedure was revised to include cooling items in a desiccator before weighing them.
3. Results of four sets of high pressure/ low flow rate off-line harvesting tests to determine cleaning effectiveness for the current integral harvesting system screen design with Cyanidium on polyester felt. These tests showed repeatable results of near 70% algae removal for test conditions of 90 psi water pressure and 2 minute cleaning duration. For the calculation of test-to-test variation of "% of algae removed from screen" one data

point was thrown out and the variation calculation was focused on the other three data points as shown below.

Data points:

Screen No.	% of algae removed from screen
H2a	P1 = 70.37%
H3a	P2 = 69.35%
H3b	P3 = 66.80%

Find σ:

P_{avg} = (P1 + P2 + P3)/3 = (70.37% + 69.35% +66.80%)/3 = 68.84%

σ = {Σ(Pi – P)^2/(n-1)} ^ ½

= {[(70.37% - 68.84%)^2 + (69.35% - 68.84%)^2 + (66.80% - 68.84%)^2]/(3-1)} ^ ½ = 1.84%

Find t multiplier from student's t table assuming normally distributed data
With 95% confidence, for n = 3, t = 4.303
Therefore, tσ = 4.303 * 1.84% = 7.92%

Conclusion:
We expect that 95% of the time, if we repeat this test, we will get a '% of algae removed from the screen' in the range: P_{avg} +/- tσ = 68.84% +/- 7.92%, or between 60.92% and 76.76%.

4. Completion of uncertainty analysis to verify that the combined uncertainty of the mass gain calculation was in an acceptable range. Results showed: U_{total} = 0.00032g, for a single mass measurement and U_m = 0.000453g for a mass gain calculation based on two mass measurements. The total uncertainty of 0.000453g is small relative to the magnitude of most changes in mass computed from measurements made with the scale (usually between 0.003 grams to 0.25 grams). Only mass gains that are much larger than the measurement uncertainty (at least 10X) will be certified as actual mass gains rather than possible variations in the mass measurements.
5. Calculation of the total uncertainty for the percent of algae removed was also completed, with results for the three tests at 70.37% +/- 0.08%, 69.35% +/- 0.32%, and 66.80% +/- 0.11%. On an absolute percentage basis, the largest uncertainty is approximately 0.5%. This level is clearly acceptable, but it also points out that the effects of the measurement uncertainties are lessened when we deal with larger quantities of algae.
6. Results from qualitative harvesting tests in the off-line chamber for cyanidium on Scotch Brite showed good cleaning based on visual observation. Plans for quantitative harvesting tests for Scotch Brite are being considered along with the possibility of switching focus towards Omnisil material.

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

A literature search was conducted on the process of *combustion* and *reformulation* in reference to the algae. The results in short are tabulated as follows

1. Reformulation has mostly been done in the case of gasoline, and it is a highly developed process with respect to gasoline.

2. We have reformulation in methane and ethane as well.
3. Reformulation in methane is useful to us.

The report on the post harvesting

The most important utilization of the biomass is regarding the manufacture of biofuels. A very important and unique feature about biofuels is that they can be produced locally with local resources and technology. Municipalities or industries pay to dispose of and/or treat the organic wastes they generate, but since these can be converted to biogas, it is possible to reduce costs or generate income from the production of biofuels. Biofuels also emit less pollutants than fossil fuels therefore their popular use would reduce air pollution and carbon dioxide emissions. However the price of biofuels cannot compete with petroleum prices yet unless more efficient technology is used to produce these fuels and/or changes and creation of government policies, taxes and tariffs are accomplished to favor the production and use of biofuels.

The methods briefly described below focus on manufacturing of hydrogen by utilization of the biomass.

1. In the first case the site www.celltech.com/resources/vt/Algaeenergy.asp gives us a comprehensive plan applicable for the use of bacteria or culture and the method of 'biophotolysis', which is used to convert organic carbohydrates to hydrogen. The method is described as follows:
   a. Through a process called "biophotolysis" (breaking or splitting, utilizing an organism and light), green algae can split water into hydrogen and oxygen. There are two organisms required for this reaction:
      1) the green algae species Chlamydomonas MGA 161 and
      2) the photosynthetic bacterium, Rhodovulum sulfidophilum W-1S.

   Chlamydomonas MGA 161, when allowed to ferment, produces carbohydrate organics. Under anaerobic (without the presence of oxygen) conditions and in the presence of argon gas, the photosynthetic bacterium Rhodovulum sulfidophilum W-1S converts the organic carbohydrates to hydrogen. This experiment has been successfully performed in the laboratory at the Kansai Electric Power Company in Japan. Used on a large scale, this process is capable of producing millions of cubic feet of hydrogen gas, some of which can be directly converted to electricity using the fuel cell described above, and the rest stored in bottles for future use. Hydrogen is an absolutely clean fuel, the only waste being pure water.

   As we do not know the applicability of strains of Chlamydomonas and Rhodovulum sulfidophilum to our project and the strains that are being provided for us are certainly not those hence the applicability of this material was doubtful.

2. The second case reviews the paper presented by Dr. Anastasio Melis at UC Berkley, which is printed on the web site: http://www.uop.edu/research/publications/pdf/doe2000.pdf
3. The paper states that hydrogen was obtained from algae by simply denying sulfur in the nutrient mix and then keeping the samples in a sealed flask which caused them to opt for

an alternative pathway that triggered formation of hydrogen. This experiment was of interest to me as the main source of pollution in the case of hydrogen has been sulfur. By taking this experiment into consideration it has been deleted right from the start.

4. The document gives us the description of photobioreactor technology and commercial uses of algae, and also provides us with knowledge of the benefits of the carbon sequestration process. (This part of the report is useful to us if the applications of the post harvesting have not yet been submitted). The web site address is: http://www.netl.doe.gov/publications/proceedings/01/carbon_seq/p15.pdf

5. The details of the process on the site http://www.fao.org/docrep/W7241E/w7241e0g.html will be investigated by someone from the biology department.
6. The site informs briefly about the various kinds of bacteria (micro algae and cynobacteria) which again cause biophotolysis of water and also the enzymes, hydrogenase as nitrogenase which are both capable of hydrogen production the steps taken to treat them to utilize them.

Comment: Pollution? Sulfur? What is "it" referring to?

Comment: and?

Comment: Doesn't make sense to me (the lay person).

The following is quoted from the website cited above:

> The future of biological hydrogen production does not only depend on research advances i.e. improvement through efficiency of genetically engineering microorganisms and/or the development of bioreactors, but also on economic considerations, social acceptance, and the development of hydrogen energy systems.

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 Task 3.0 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 complete, but tests with organisms are on hold until new candidate organisms are cultured to sufficient quantities to allow population of the growth surfaces. 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 construction at the Ohio University Corrosion Center is proceeding on schedule. Representatives from Oak Ridge National Laboratories will visit Ohio University on 1/16/2002 to ensure proper placement of the solar collectors and proper fit of the light distribution panels in the pilot scale bioreactor. The construction of the bioreactor test setup at an off-site location is proceeding ahead of schedule and will allow quick installation once the site prep work is completed.

Web page

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

Specific results and accomplishments for the fourth quarter of 2001 include:

1. Discovery of significant limitations of Cyanidium, one of our candidate bioreactor organisms, with respect to the adhesion and growth rate requirements for bioreactor organisms.
2. Procedures and protocols have been developed to increase the chances of successful implementation in the bioreactor of organisms that perform well in the lab. The new procedures include pre-screening of organisms for adhesion characteristics and a focus on identifying organisms with maximum growth rate potential. The use of *in-sutu* growth surface material traps addresses adhesion concerns directly, and inoculation of each trap in three different media types has greatly enhanced the diversity of species identified since different organisms have been found to flourish in different media.
3. Several organisms collected from Yellowstone National Park using the new procedures have been delivered to Ohio University for culturing in preparation for bioreactor screening tests. The Tr9.4WF organism showed very rapid doubling times and overall good characteristics for the bioreactor in the laboratory tests. Our efforts are currently focused on successful implementation of this organism.
4. For controlling costs associated with large-scale implementation, experiments were run to test the effect of preparing growth media with tap water rather than distilled water. Preliminary results show an increase in adhesion to glass and a decrease in overall growth rates. These tests are being continued.
5. Bioreactor testing is on hold while the new organism is cultured to acceptable levels. Construction and testing of the generation 2 model scale bioreactor and the pilot scale bioreactor are continuing. One important result from a test of growth surface temperature distribution as a function of gas stream and drip-fluid temperatures showed a high dependence of membrane temperature on fluid temperature, with gas stream temperature having minimal effect. This result indicates that bioreactor growth surface temperatures can be controlled using fluid delivery temperature. The possible implications for implementation of the bioreactor concept are encouraging, since it may be possible to use the bioreactor with very high gas stream temperatures by controlling the temperature of the organisms with the fluid temperature.
6. Investigation of growth surface materials continues, with Omnisil and ScotchBrite emerging as the leading candidates. More investigation of these and other material types is still needed to determine the best material for particular combinations of organisms and harvesting methods.
7. Tests of harvesting methods and harvesting system designs have shown that desirable levels of "percentage algae removal" can be achieved for particular organisms and growth surface materials, for example Cyanidium on polyester felt. Additional testing continues to better characterize sensitivity of the "percentage removal" to various system design parameters, but these tests have been delayed due to the lack of suitable organisms for the tests.
8. The solar collectors and the pilot-scale bioreactor light distribution panels for the deep-penetration hybrid solar lighting system have been designed. One solar lighting system (solar collector tracking unit, fiber optic light transmission cables, light distribution

panels) is almost completely ready for installation in the pilot scale bioreactor system next quarter.

9. Pressure drop results from tests on the enhanced mass transfer CO_2 absorption technique (the translating slug flow reactor) are encouraging, with reasonable values of 2.5 psi maximum over an 11.48 meter distance between pressure taps for test conditions of 0.6 m/sec slug velocity and approximately 10 m/sec gas velocity. Preparations are under way for CO_2 scrubbing tests.

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