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Abstract

This quarterly report documents significant achievements in the Enhanced Practical Photosynthetic CO₂ Mitigation project during the period from 7/2/2003 through 10/01/2003. As indicated in the list of accomplishments below we are preparing for the final tests necessary to meet our project goals.

Specific results and accomplishments for the third quarter of 2003 include:

Bioreactor support systems and test facilities:

- The solar collector used in the light delivery system showed signs of degradation and hence had to be replaced by ORNL. A set of light readings were taken after the new solar collector was installed. The readings showed an acceptable light profile.
- The CRF-2 test system has undergone major improvements to produce the high flow rates needed for harvesting (as determined by previous experiments). The main changes to the system are new stainless steel header/frame units with increased flow capacity and a modified pipe end sealing method to improve flow uniformity, and installation and plumbing for a new high flow harvesting pump. The improvements have been completed and the system is ready for testing.
- The pilot scale bioreactor is ready for testing pending some information from the CRF-2 tests.

Organisms and Growth Surfaces:

- The shape of the *Chlorogloeopsis* sp. cells (cyanobacteria) was found to be affected by environmental pH, which may be useful in culture quality control. Besides, the further investigation of this phenomenon suggested that the rate of cell adhesion to glass surface decreases upon medium alkalization. Thus, harvesting effectiveness may be improved by increasing medium pH up to 9 before harvesting of cyanobacteria from a substratum.

Table of Contents

Executive Summary	1
Results and Discussion	2
Experimental Apparatus	2
Data Collection and Reduction	2
Task 1.0 Evaluate and rank component and subsystem level alternative design concepts	2
Subtask 1.1 – Investigate critical properties of alternative photosynthetic agents	2
Subtask 1.2 – Design deep-penetration light delivery subsystem	2
Subtask 1.3 – Investigate growth surface subsystem design	10
Subtask 1.4 – Investigate the use of a hydraulic jumps to improve the system’s overall CO ₂ conversion efficiency	10
Subtask 1.5 – Design harvesting subsystem	10
Subtask 1.6 – Quantify properties of dried biomass for potential end-uses	11
Task 2.0. Evaluate subsystem combinations and select an “optimum” system design	11
Task 3.0. Implement the optimum system in scaled model	11
Webpage	12
References	12
Conclusion	12

Figures

Figure 1 – Lighting panel notes and designations	3
Figure 2 – Lighting sheets in bioreactor	4
Figure 3 – Lighting Panel Distributions.....	6-9

Executive Summary

This quarterly report documents significant achievements in the Enhanced Practical Photosynthetic CO₂ Mitigation project during the period from 7/2/2003 through 10/01/2003. As indicated in the list of accomplishments below we are preparing for the final tests necessary to meet our project goals.

The solar collector used in the light delivery system showed signs of degradation and hence had to be replaced by ORNL. A set of light readings were taken after the new solar collector was installed. The readings showed an acceptable light profile.

The CRF-2 test system has undergone major improvements to produce the high flow rates needed for harvesting (as determined by previous experiments). The main changes to the system are new stainless steel header/frame units with increased flow capacity and a modified pipe end sealing method to improve flow uniformity, and installation and plumbing for a new high flow harvesting pump. The improvements have been completed and the system is ready for testing.

The shape of the *Chlorogloeopsis* sp. cells (cyanobacteria) was found to be affected by environmental pH, which may be useful in culture quality control. Besides, the further investigation of this phenomenon suggested that the rate of cell adhesion to glass surface decreases upon medium alkalinization. Thus, harvesting effectiveness may be improved by increasing medium pH up to 9 before harvesting of cyanobacteria from a substratum.

As we finish the third year of this three year project we can report that we have made significant progress towards achieving our overall project goals and are working hard to complete all of our deliverables. All of our test facilities are developed and our test plans and procedures are in place and the CRF-2 tests that will provide the results needed to proceed with the pilot scale bioreactor testing are in process. We will proceed with the tests needed to meet our project goals and will report the results as soon as possible.

Results and Discussion

Experimental Apparatus

The experimental apparatus used include photosynthetic incubators, bench-scale bioreactors, and a pilot-scale bioreactor that have been previously characterized. Other equipment used include pipettes, electric balances (mass scales), natural gas burners, CO/O₂/CO₂ gas analyzers, and DNA sequencing equipment. Details of the specific applications of the experimental equipment are included in the discussion of the actual data collection and reduction.

Data Collection and Reduction

Task 1.0. Evaluate and rank component and subsystem level alternative design concepts

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

Report from the researchers at Montana State:

More than three months ago we started the microproject to obtain some cyanobacterial clones with elevated resistance to low pH. This attempt has been induced by the fact that the cultivation of a cyanobacterium upon elevated concentration of CO₂ required medium buffering with HEPES buffer. We used two approaches: a) to select mutant clones with elevated resistance to acid pH during the migration of isolate 3.2.2 against acid pH gradient; and b) long term incubation of 3.2.2 isolate in acid batch culture. First method did not give a positive result, while second approach gave us several clones of 3.2.2 isolate that can grow up upon pH 6. I'll try to multiply and to preserve them if I have the access to current equipment.

As previously reported, our isolate 5.2 s.c.1 did not fall in any published genotype of cyanobacteria. During current quarter we have carried out strong phylogenetic analysis of this isolate and have found that this one is a new cyanobacterial genus. We have deposited this isolate at American Type Culture Collection to open the way for us to publish a paper about this isolate.

We also found that the shape *Chlorogloeopsis* sp. cells (cyanobacterial culture is currently using by your team) is affected by environmental pH. This observation might help your colleagues to avoid confusion during culture quality control. Besides, the further investigation of this phenomenon suggested that the rate of cell adhesion to glass surface decreases upon medium alkalization. Thus, we may recommend you to increase medium pH up to 9 before harvesting of cyanobacteria from a substratum. However, we have no more time to double check this idea in model experiments with Omnisil. The pH effect on *Chlorogloeopsis* metamorphosis is also a subject for one more paper.

Subtask 1.2 Design deep-penetration light delivery subsystem)

ORNL continues to support the pilot-scale bioreactor development. For example, the solar collector used in the light delivery system showed signs of degradation and hence had to be

replaced by ORNL. A set of light readings were taken after the new solar collector was installed. The readings showed an acceptable light profile.

While the solar collector appears to be functioning well, we are still experiencing minor problems with the light distribution. Not that the organisms will die in this environment, but in the long-term, if an even light distribution can be produced, higher productivity can be achieved. Nevertheless, the distribution is clearly good enough to grow organisms (as will be seen), but the uneven quantity of photon distribution should be noted.

To quantify the photon flux in the bioreactor, each of the light sheets was divided into a grid of 3x5 to give 15 readings for each. The sheets are marked A, B, C, D, E, F, G, and H (left to right) when seen from the growth tank side. Each sheet provided 2 sets of 15 readings for both surfaces. For simplicity we have used conventions A(L) and A(R) for each sheet which represent the left and right side of the sheet (again when looked at from the growth tank side).

Let's say we call the membranes 1, 2, 3 ...etc.; then #1 will receive light from A(R) and B(L). The locations of these panels in the bioreactor are shown in Figures 1 and 2.

Figure 1. Lighting panel notes and designations

Figure 2. Lighting sheets in bioreactor

The readings and the respective graph are shown below in Table 1.

Table 1. Photon flux measurements (in $\mu\text{mol s}^{-1} \text{m}^{-2}$)

A(R)		
43.8	44.6	32.7
50.5	36.4	43.8
25.3	29.7	43.8
29.7	29.0	39.4
46.8	36.4	60.2
B(R)		
52.0	44.6	57.2
44.6	57.2	58.0
38.6	44.6	52.0
43.1	40.9	52.0
72.1	46.1	78.8
C(R)		
55.7	51.3	66.9
46.1	66.1	61.7
37.2	51.3	57.2
43.8	51.3	60.9
97.3	48.3	85.4

B(L)		
69.8	57.2	84.0
67.6	79.5	50.5
63.2	59.4	44.6
67.6	66.1	52.0
117.4	52.8	92.1
C(L)		
58.7	60.9	74.3
62.4	46.8	73.6
49.8	46.1	71.3
61.7	48.3	60.2
150.8	56.5	112.9
D(L)		
61.7	55.7	63.2
53.5	69.1	46.8
53.5	55.7	43.1
58.7	55.7	46.8
91.4	48.3	81.7

Table 1. Photon flux measurements (in $\mu\text{mol s}^{-1} \text{m}^{-2}$) con't

D(R)			E(L)		
45.3	47.6	55.0	66.1	57.2	64.6
54.2	63.2	44.6	48.3	60.9	70.3
39.4	49.0	51.3	58.7	52.8	47.6
44.6	49.8	50.5	57.2	63.9	45.3
51.3	44.6	65.4	134.5	51.3	102.5
E(R)			F(L)		
50.5	47.6	60.2	67.6	54.2	71.3
40.9	55.0	54.2	67.6	81.0	49.0
42.4	44.6	58.7	63.2	66.9	48.3
40.9	46.1	51.3	63.2	66.1	50.5
64.2	43.1	84.0	125.6	58.0	101.8
F(R)			G(L)		
57.2	53.5	69.1	58.0	51.3	76.5
44.6	63.9	56.5	61.7	66.1	51.3
43.8	49.8	59.4	56.5	67.6	51.3
44.6	52.8	60.2	54.2	47.6	46.1
63.2	49.0	98.1	138.9	71.3	105.5
G(R)			H(L)		
58.0	49.8	60.2	55.7	43.8	63.2
46.1	63.9	61.7	53.5	49.8	38.6
47.6	54.2	63.2	49.8	44.6	34.9
46.1	54.2	60.2	52.8	39.4	32.7
33.6	50.5	91.4	173.9	37.2	130.8

Table 2. Maximum, minimum, and average amount of flux delivered by light sheets.

	A(R)	B(L)	B(R)	C(L)	C(R)	D(L)	D(R)
MAX.	60.2	117.4	78.8	150.8	97.3	91.4	65.4
	50.5	92.1	72.1	112.9	85.4	81.7	63.2
	46.8	84.0	58.0	74.3	66.9	69.1	55.0
	44.6	79.5	57.2	73.6	66.1	63.2	54.2
	43.8	69.8	57.2	71.3	61.7	61.7	51.3
	43.8	67.6	52.0	62.4	60.9	58.7	51.3
	43.8	67.6	52.0	61.7	57.2	55.7	50.5
	39.4	66.1	52.0	60.9	55.7	55.7	49.8
	36.4	63.2	46.1	60.2	51.3	55.7	49.0
	36.4	59.4	44.6	58.7	51.3	53.5	47.6
	32.7	57.2	44.6	56.5	51.3	53.5	45.3
	29.7	52.8	44.6	49.8	48.3	48.3	44.6
	29.7	52.0	43.1	48.3	46.1	46.8	44.6
	29.0	50.5	40.9	46.8	43.8	46.8	44.6
MIN.	25.3	44.6	38.6	46.1	37.2	43.1	39.4
Sum	592.2	1023.9	781.6	1034.3	880.5	884.9	755.6
Avg.	39.5	68.3	52.1	69.0	58.7	59.0	50.4

Table 2. Maximum, minimum, and average amount of flux delivered by light sheets (con't)

	E(L)	E(R)	F(L)	F(R)	G(L)	G(R)	H(L)
MAX.	134.5	84.0	125.6	98.1	138.9	91.4	173.9
	102.5	64.2	101.8	69.1	105.5	73.6	130.8
	70.3	60.2	81.0	63.9	76.5	63.9	63.2
	66.1	58.7	71.3	63.2	71.3	63.2	55.7
	64.6	55.0	67.6	60.2	67.6	61.7	53.5
	63.9	54.2	67.6	59.4	66.1	60.2	52.8
	60.9	51.3	66.9	57.2	61.7	60.2	49.8
	58.7	50.5	66.1	56.5	58.0	58.0	49.8
	57.2	47.6	63.2	53.5	56.5	54.2	44.6
	57.2	46.1	63.2	52.8	54.2	54.2	43.8
	52.8	44.6	58.0	49.8	51.3	50.5	39.4
	51.3	43.1	54.2	49.0	51.3	49.8	38.6
	48.3	42.4	50.5	44.6	51.3	47.6	37.2
	47.6	40.9	49.0	44.6	47.6	46.1	34.9
MIN.	45.3	40.9	48.3	43.8	46.1	46.1	32.7
Sum	981.3	783.5	1034.3	865.6	1003.8	880.5	900.5
Avg.	65.4	52.2	69.0	57.7	66.9	58.7	60.0

Shown next (Figure 3a-h) below are the graphs corresponding to the data in Table 1. Please refer to Figure 1 to understand the position of light panels A-H.

B(R)

C(L)

C(R)

D(L)

D(R)

E(L)

E(R)

F(L)

Figure 3. Lighting Panel Distributions

Summary of Observations and Results of Lighting Tests

- 1: It can be seen from table 1 that light sheet A delivers the minimum amount of light (average $39.5 \mu\text{mol s}^{-1}\text{m}^{-2}$). It can be contributed to two factors. As shown in figure 1 there is a bend in the fiber coming from the collector. So before entering the light sheets it loses quite a good amount of light. Also the length of the fiber (which loses more light) going into light sheet A is long enough to loss light before entering into the light sheet.
- 2: Light sheets C and F deliver maximum amount of light (Average $68.9 \mu\text{mol s}^{-1}\text{m}^{-2}$). This is because length of fibers (which loses more light) going into light sheets C and F are having the minimum length.
- 3: It has been noticed that light intensity delivered from right side of each sheet is less than that of the left side. As shown in Figure 1 the right side of the sheets have the fixture for holding the fibers. This may contribute to less amount of light from the bends where there is maximum light output.

Subtask 1.3 Investigate growth surface subsystem design

No new material tests have been run. We are focusing our efforts on Omnisil fabric since it has excellent wetting and rewetting properties, organisms adhere well to it, and it has properties that are appropriate for the bioreactor environment.

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

Based on the results collected and reported in previous reports, the experiments in this area have been discontinued. The Phase I experimental evaluation of the hydraulic jump is complete.

Subtask 1.5 Design harvesting subsystem

Our main activities this quarter were focused on design, manufacturing, and experimental work for the integrated screen wetting / harvesting system, working exclusively with Omnisil fabric and 1.2. s.c.(2) organisms. The design changes to the overall test system required to produce the high flow rates needed for harvesting (as determined by previous experiments) have been completed and the system is ready for testing. The changes to the system included manufacturing new header/frame units out of stainless steel material with increased flow capacity and a modified pipe end sealing method to improve flow uniformity, identifying and purchasing a new high flow harvesting pump and installing it and plumbing it into the system, and improving the test system data acquisition unit.

The test plan includes initial qualitative tests to see how two candidate shim materials (plastic and stainless steel) perform with the new high flow rate pump needed for harvesting. Based on uniformity of flow during drip mode and harvesting mode and durability of the shims, one of the

shim types will be selected for the quantitative tests in both the CRF-2 and the pilot scale bioreactor. It is expected that these long-term quantitative tests will begin during October 2003. The long term tests will include initial growth, harvesting, and regrowth stages to simulate the long-term operating modes for the bioreactor. Data will be gathered on growth rates and harvesting effectiveness.

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

We continue to search the literature and search for potential end uses for the biomass, but experiments in this area are on hold until we get closer to making a final decision for the organism.

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

The CRF-2 is the primary test facility for evaluating the subsystem combinations. Long-term quantitative tests began in the CRF-2 bioreactor last quarter. System improvements to allow harvesting and regrowth phases of the long term testing have recently been completed. Tests with the improved system are scheduled to begin in October and the results will be combined with other test results to enable us to select the “optimum” system to be tested in the pilot scale bioreactor.

Task 3.0. Implement the optimum system in scaled model

The phase 1 construction of the bioreactor has been completed, including the solar collector and light distribution system. We are now in the phase of system improvement as we wait for CRF-2 results in order to be able to finalize the design and construction of the pilot scale system. A recent list of accomplishments and actions items for the pilot scale bioreactor is copied below.

Pilot Scale Bioreactor Group Accomplishments:

- The pilot-scale unit was tested for leaks and other defects. After fixing the same, we successfully performed a test run of the all the sub-systems operating simultaneously, to check for sustainability.
- The solar collector used in the light delivery system showed signs of degradation and hence had to be replaced by ORNL. We took a set of light readings after the new solar collector was installed and submitted it to the group. The light profile was acceptable.
- A test-run of the pilot-scale bioreactor with algae introduced for the first time in the system was started. Although the test ran for about 2 weeks, we had to discontinue the same because the burner went out consistently. This may also have interfered with the growth of the algae on the screen.
- Another test-run of the pilot-scale unit with algae was conducted after fixing the burner problem. This test was continued for 5 days before we had to discontinue it due to pump failure.

- The results of this test, as presented to the group, showed that the header pipe design with the stainless steel shim was causing a problem wherein the algae only flowed out from the ends of the header pipe and very less in the center. This can be attributed to the deformation of the shim at the ends when the end caps were tightened.
- This non-uniform distribution of the algae over the Omnisil membrane has caused us to go back and look at modifications for the design of the header pipe. As of now, we are going to try out the same design but with a plastic shim instead of stainless steel. Also a gasket will be used between the end caps and header pipe to avoid deformation of the shim material.
- Our preliminary estimations and mass calculations associated with the pilot-scale unit showed that a balance with a range of 2500g with a readability of 0.01g will have sufficient capacity and total accuracy for all practical purposes. We have initiated the ordering process for this balance.

Pilot Scale Bioreactor Action Items:

- Our goal is to conduct a long term test-run of the entire system with algae growing on the membranes to ensure survivability of the algae.
- At present we are conducting experiments on alternatives to the current header pipe design to achieve uniform distribution of algae over the entire surface of the membrane.
- We will install the parallel pump arrangement to counter the problem of pump failure and complete the necessary modifications to the plumbing.
- We will complete the plumbing required to incorporate the harvesting pump into the system and perform a test run to check proper functioning of the same.

Webpage

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

Conclusions

As we finish the third year of this three year project we can report that we have made significant progress towards achieving our overall project goals and are working hard to complete all of our deliverables. All of our test facilities are developed and our test plans and procedures are in place and the CRF-2 tests that will provide the results needed to proceed with the pilot scale bioreactor testing are in process. We will proceed with the tests needed to meet our project goals and will report the results as soon as possible.

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