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Abstract

This quarterly report documents significant achievements in the Enhanced Practical Photosynthetic CO₂ Mitigation project during the period from 1/2/2003 through 4/01/2003. As indicated in the list of accomplishments below we are progressing with long-term model scale bioreactor tests and are completing final preparations for pilot scale bioreactor testing.

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

Bioreactor support systems and test facilities:

- Long-term survivability tests for Sc1.2(2) on Omnisil have been successfully completed and results demonstrate a growth rate that appears to be acceptable. Tests were completed using two different screen header pipe designs (drilled-hole and pressure shim) for comparison. Both performed adequately but the pressure shim design will likely be selected for the future testing due to its ability to allow increased flow for harvesting.
- The results of harvesting tests show that at low flows (<2 GPM) sheeting action is not sufficient to remove Sc1.2(2) from Omnisil, but harvesting is possible using only sheeting action (no impingement jet is needed) at high flow rates (5-10 gallons per minute for the current screen size). The flow to the header for the current test system is limited by the fluid inlet tube, so we plan to modify the test system to increase maximum flow capacity and run some full screen harvesting tests in the near future.
- Tests have shown that organisms can be repopulated on a harvested section of a bioreactor screen, demonstrating that continuous bioreactor operation is feasible, with continuous cycles of harvesting and repopulating screens. Future tests will be used to quantify long-term growth productivity.
- The phase 1 construction of the pilot scale 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.

Organisms and Growth Surfaces:

- The most positive aspect of recent experiments is that we could reach a doubling time of the cyanobacterial culture about 7.5 h when the illuminating source [fluorescent tubes] were moved closer to the growth tubes. In this position, illumination was about 270 $\mu\text{E} \cdot \text{m}^{-2} \cdot \text{sec}^{-1}$. All lower levels of illumination led to a decrease in cyanobacterial growth rate.
- Our macroscopical and microscopical observations suggest that the culture of this isolate undergoes significant morphological changes after 60-70 h of incubation in the batch culture mode. First of all, the culture begins to clump. This clumping perhaps leads to the decrease of effective illumination of culture. Besides we believe that this clumping of cells may reflect a decrease of combined nitrogen in medium or a medium alkalization.
- Because of this we studied the effect of medium pH on the morphology of isolates 1.2 s.c. 2 and 6. Our observations showed that both non-starved isolate 1.2 s.c. 2 (Fig.3) and 6 have chain shaped trichomes under neutral conditions. (Neutral conditions were obtained by the addition of 30 mM HEPES to BG-11 medium). At this time, cells of 1.2 s.c. 2 isolate incubated in alkaline medium had non-typical shapes, i.e., they were tetrads (Fig.4).
- During first quarter of 2003 we have also started the purification of clonal isolates of cyanobacterium *Synechococcus* sp. which is found in the 9.4 WF mixed species culture. We

have obtained a number of filters with single colonies of *Synechococcus* from the 9.4 WF sample. However, these colonies are still too small for the further manipulation and we are growing them to obtain colonies able to be seen with the naked eye.

- During first quarter of 2003 we have also been working on the genotyping of some unialgal isolates of cyanobacteria from Yellowstone National Park.

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Results and Discussion

Experimental Apparatus

The experimental apparatus used include photosynthetic incubators, bench-scale bioreactors, and a pilot-scale bioreactor that have been characterized in previous quarterly reports. 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

We have compared the growth of 1.2. s.c. 6 isolate in BG-11 medium buffered with 30 mM HEPES (initial pH was 7.34) aerated with regular compressed air, and a mixture of air and 15 % CO₂. This experiment showed clearly that 15% CO₂ does not inhibit the growth of 1.2 s.c. 6 isolate (Fig.1). Moreover, the doubling time of this isolate between 24 and 48 h was about 7.5 h, which is highest rate of doubling time for the thermophilic cyanobacterium that we have tested.

Fig.1. Growth dynamics of 1.2 s.c. 6 isolate in buffered BG-11 medium aerated with different gas mixtures. The average D between 24 and 96 h was ~ 7.5 h.

We have repeated the experiment to compare the growth of 1.2. s.c. 6 isolate in BG-11 medium buffered with 30 mM HEPES (initial pH was 7.34) aerated with regular compressed air, and a

mix of air and 5 % CO₂ at the light level used for previous experiment with 15 % CO₂. Fig. 2 reflects the result of this experiment. The growth curve of the isolate 1.2 s.c.6 shows that doubling time of this isolate in neutral BG-11 medium aerated with 5 % CO₂ was about 7.5 h for the period between 24 and 48 h. This is the same doubling time that was found for this isolate for period between 24 and 48 when aerated BG-11 medium with 15 % CO₂ (Fig. 1).

Fig. 2. Growth dynamics of 1.2 s.c. 6 isolate in buffered BG-11 medium aerated with air and air + 5% CO₂.

On the basis of these experiments we conclude the following:

1. We are not sure that the concentration of CO₂ in liquid medium was different when we aerated media with different amounts of CO₂ in air. We are not able to measure the dissolved CO₂ concentration in a medium. It is possible that the three fold increase in CO₂ concentration in the gas phase did not affect significantly the level of dissolved CO₂ in the medium because of the high temperature (55°C). As a result we observed neither repression nor stimulation of cyanobacterial growth when medium was aerated with 15 % CO₂. Although there is no doubt that the presence of CO₂ in the gas mixture dramatically stimulated growth of the cyanobacterium in the comparison with regular compressed air (Fig.1 and 2).
2. The most positive aspect of recent experiments is that we could reach a doubling time of the cyanobacterial culture about 7.5 h when the illuminating source [fluorescent tubes] were moved closer to the growth tubes. In this position, illumination was about 270 μE .m⁻². sec⁻¹. All lower levels of illumination led to a decrease in cyanobacterial growth rate.
3. Our macroscopical and microscopical observations suggest that the culture of this isolate undergoes significant morphological changes after 60-70 h of incubation in the batch culture mode. First of all, the culture begins to clump. This clumping perhaps lead to the decrease of effective illumination of culture. Besides we believe that this

clumping of cells may reflect a decrease of combined nitrogen in medium or a medium alkalization.

4. Because of this we studied the effect of medium pH on the morphology of isolates 1.2 s.c. 2 and 6. Our observations showed that both non-starved isolate 1.2 s.c. 2 (Fig.3) and 6 have chain shaped trichomes under neutral conditions. (Neutral conditions were obtained by the addition of 30 mM HEPES to BG-11 medium). At this time, cells of 1.2 s.c. 2 isolate incubated in alkaline medium had non-typical shapes, i.e., they were tetrads (Fig.4).

Fig. 3. Non-starved isolate 1.2 s.c. 2 (Fig.3) and s.c. 6 with chain shaped trichomes

Fig. 4. 1.2 s.c. 2 isolate incubated in alkaline medium with tetrad shaped cells

Because both cultures were of the same age, we believe that the morphological changes are connected with the stress induced by alkalinity, rather than N-insufficiency. Microscopical observation therefore may help to evaluate the stage and quality of both 1.2 s.c. 2 and 6 cultures.

A good criterion for NO_3 starvation could be the appearance of heterocysts in both 1.2 s.c. 2 and 6 cultures. We are developing a simple microscopical test for heterocysts verification, since in these organisms heterocysts are less obvious than in other cyanobacteria. Provisionally, we expect to carry out the following : A suspension of cells in a microfuge tube will be centrifuged to prepare a cell pellet. 0.5mL of concanavalin A-FITC (20 microgram/mL in BG-11 medium) will be added and the cells re-suspended. The mixture will be incubated in the dark for 1hour and the cells re-centrifuged. The pellet will be washed x3 and the cells re-suspended in BG-11 for epifluorescence microscopy.

During first quarter of 2003 we have been also started the purification of clonal isolates of cyanobacterium *Synechococcus* sp. which is found in the 9.4 WF mixed species culture. To this end we used an installation shown on Fig. 5. We have obtained a number of filters with single colonies of *Synechococcus* from the 9.4 WF sample. However, these colonies are still too small for the further manipulation and we are growing them to obtain colonies able to be seen with the naked eye.

Fig. 5. Testing Facility

Also, during the first quarter of 2003, Montana State was working on the genotyping of some unialgal isolates of cyanobacteria from Yellowstone National Park. The first group of isolates was composed : 1.2 s.c. (2), 1.2. s.c. (3), 3.2.2 s.c. (1) and 8.2.1 s.c. (10). All cultures were

grown in standard BG-11 to middle of the logarithmic phase. We used Taq DNA Polymerase in Storage Buffer B, Promega Catalog # M1161, and dNTPs, Promega Catalog # C1141.

Primers: **L23cyR** (5'-TGC CTA GGT ATC CAC C); **cy781 mix** [cy781F(a) (5'-AATGGGATTAGATACCCAGTAGTC) + **cy781F(b)** (5'-AAAGGGATTAGATACCCCTGTAGTC)]

	PCR reaction:	Master Mix for Y reactions:
5	ul Taq Buffer	$(13+0.25) \times 5 = 66.25$
5	ul $MgCl_2$ (25 mM)	$(13+0.25) \times 5 = 66.25$
1	ul dNTPs (2.5 mM)	$(13+0.25) \times 1 = 13.25$
0.25	ul Taq polymerase	$(13+0.25) \times 0.25 =$
1	ul L23cyR primer (50 uM)	$(13+0.25) \times 1 = 13.25$
1	ul cy781f primer(mix) (50uM)	$(13+0.25) \times 1 = 13.25$
35.75	ul Sigma water	$(13+0.25) \times 37.25 = 493.56$
49	ul master mix volume	

All the ingredients were well mixed in one 1.5 – 2.0 ml tube, and then aliquots added to the individual 0.2 ul PCR tubes. Our DNA samples were then added separately to each small tube.

1 ul DNA sample
50 ul TOTAL sample volume

Samples:

1. 1.2 s.c.2 (:undiluted)
2. 1.2 s.c. 2 (:10)
3. 1.2 s.c. 2 (:100)
4. 1.2 s.c. 3 (:undiluted)
5. 1.2 s.c. 3 (:10)
6. 8.2.1 s.c.10 (:undiluted)
7. 8.2.1 s.c.10 (:10)
8. 8.2.1 s.c.10 (:100)
9. 3.2.2 s.c. 1 (:undiluted)
10. 3.2.2 s.c. 1 (:10)
11. P2 (:10) reference strain
12. Blank
13. Marker

PCR program used: MBUR

1. 94 C, 2 min.
2. 94 C, 1 min.
3. 55 C, 1 min.
4. 72 C, 1 min.
5. 29 times to step 2
6. 72 C, 10 min.

7. 4 C, for ever...

PCR products obtained were compared with PCR product of DNA isolated from *Synechococcus* P2- a positive control organism whose sequence is well known. This comparison suggested that DNA samples extracted from unialgal cultures had a MW very close to that of the PCR product synthesized on the basis of DNA extract from P2.

After this the PCR products were purified with the help of QIAquick PCR purification kit. Purified PCR products were run in gel parallel with different amounts of a 100bpDNA Ladder. This procedure helped us to determine amounts of PCR products required for preparation of the next generation PCR product to be used for DNA sequencing.

We used primer 1070F (5'-ATGGCTGTCGTCAGCT) for this amplification. Amplification was made on the base of ABI-Big Dye. We used Ethanol/EDTA Precipitation for the sample preparation for DNA sequencing. Sequences of DNA were obtained using a ABI Genetic Analyzer. The DNA sequences in samples isolated from 8.2.1 s.c. (10) were:

```
TTGCNACCCGCGTCTTAGTTGCACATTCAGTTGGGACTCTGGGGAGACTGCCGGT
GACAAGCCGGAGGAAGGTGCGGATGACGTCAAGTCAGCATGCCCTTACGCCCT
GGGCGTCACACGTGCTACAATGGCCAGCACAAAGGGTAGCCAGCCAGCGATGGT
GAGCCAATCCCGCAAAGTTGGTCTCAGTTCAGATCGGAGTCTGCAACTCGACTCC
GTGAAGGCGGAATCGCTAGTAATCGCAGGTCAGCCATACTGCGGTGAATACGTT
CCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGCTGGCCACGCCCGAAG
TCGTTACTCCAACCGCAAGGAGGGGAGCGCCGAAGCAGGGGCTGGTGACTGGGGT
GAAGTCGTAACAAGGTACCGTACTGGAAGTGCGGCTGGATCACTCCTTTTANGG
AGACTACCCACTCATCAACCGGTATGNTTTGGGTGGGGAGCCGGGTCATCTAAN
GGTCAATCANGTTGANTCTTTGCTGAACTTCAA
```

Downloading this sequence to the BLAST program showed 100% alignment with a previously uncultured cyanobacterium isolated from the bacterial mat on travertine depositional face of Angel Terrace, Mammoth Hot Springs, Yellowstone National Park within temperature gradient from 73 degrees to 25 degree C. The published sequence of this unculturable cyanobacterium is:

```
1 GAGTTTGATC CTGGCTCAGG ATGAACGCTG GCGGCGTGCT TAACACATGC
AAGTCGAACG
61 GGGGTGCAAA CCCTAGTGGC GGACGGGTGA GTAACGCGTG AGAACCTGTC
TCGAGGTGGG
121 GGACAACAGT TGGAACGAC TGCTAATACC CCATATGCCG AGAGGTGAAA
GATTTATCGC
181 CTTGAGGGGG GCTCGCGTCC GATTAGCTCG TTGGTGGGGT AACGGCCTAC
CAAGGTGACG
241 ATCGGTAGCT GGT TTGAGAG GACGACCAGC CACACTGGGA CTGAGACACG
GCCCAGACTC
301 CTACGGGAGG CAGCAGTGGG GAATTTTCCG CAATGGGCGC AAGCCTGACG
GAGCAACGCC
```

```

361 GCGTGTGGGA TGAGGCCTGT GGGTTGTAAA CCACTTTTCT CAGGGACGAA
      GCTCTGACGG
421 TACCTGAGGA ATCAGCCTCG GCTAACTCTG TGCCAGCAGC CGCGGTAAGA
      CAGAGGAGGC
481 AAGCGTTATC CGGAATTATT GGGCGTAAAG CGTCCGTAGG TGGTTTGACA
      AGTCTGGGGC
541 TAAAGCCCGG AGCCCAACTC CGGATCGGCC CTGGAAACTG TCAGACTTGA
      GTAAGGTAGG

```

Both sequences showed very high level of the similarity to *Synechococcus* C9 isolated by several research groups who work in YNP. Further morphological comparison of our isolate 8.2.1 s.c. (10) with *Synechococcus* C9 which is kept in the laboratory of D. Ward at MSU suggests their morphological identity. Because of these molecular and morphological coincidences we believe that isolate 8.2.1 s.c. (10) is also *Synechococcus* sp.

We obtained practically identical sequences for DNA samples extracted from isolates 1.2 s.c. (2) and (3).

```

CGAGAGGTGGGCACTCTAATGGGACTGCCGGTGACAAACCGGAGGAAGGT
GGGGATGACGTCAAGTCCTCATGGCCCTTATGGGTAGGGCTACACACGTC
ATACAATGGCGCGTACAGAGGGTTGCGAAGGGGTGACCTGGAGCCAATCC
CAGAAAGCGCGTCGTAGTCCGGATTGCAGTCTGCAACTCGACTGCATGAA
GTCGGAATCGCTAGTAATCGCGGATCAGCATGTCGCGGTGAATACGTTCC
CGGGTCTTGTACACACCGCCCGTCACACATGGGAGTGGACTGTACCAGAA
GTGGCTGGCCTAACCCGCGAGGGAGGGCGGCCCCCACGGTATGGTTCATG
ACTGGGGTGAAAGTCGTAACAAGGTAGCCGTATCGGAAGGTGCGGCTGGAT
      CACCTCCTTTCTGGACAA

```

This was not surprising, because both colonies 2 and 3 were isolated from a partially purified sample 1.2. Further search of same sequence using the BLAST program showed very good alignment of the sequence obtained with a different uncultured bacterium clone G94, from an environmental sample isolated from the Great Artesian Basin at a temperature 57 deg.C. The authors of this sample only mentioned this one as a "green filament".

```

1  tcgattagag ttgatactg gctcaggatg aacgctggcg gcgtgcctaa cacatgcaag
61  tcgaacgtga aggggcgttt tcggatgccg ctgaaagtgg cgaacgggtg agtaacacgt
121 agataacctg cctggagtg ggggataacc actggaaacg gtggctaata ccgcataggg
181 cettgaagtc gggaggcttc gaggggaaag ctccggcgct tcaggagggg tctgcgtccg
241 attagctagt tggcggggta acggcccacc aaggcgacga tcggtagctg gtctgagagg
301 acgatcagcc acacgggaac tgagacacgg tcccactcc tacggggggc agcagtgagg
361 aatattgggc aatgggcgaa agcctgatec agccatgccg cgtgcgggaa gaaggccttc
421 ggggtgtaaa ccgcttccgg cggggaccag gatcgcccgc atgaacagtg tgggtggatg
481 acggtactgg cagaagaagc accggctaatacgtgcagc agccgcggta atacgtaggg
541 tgcgagcgtt aatcggaatt actgggcgta aagcgtgcgc aggcggccct ttaagtcagg

```

However, Professor R.W. Castenholz has suggested that isolates 1.2 (2) and (3) are really the cyanobacterium *Chlorogloeopsis* sp. However, our further search for the sequence of *Chlorogloeopsis* 16SrRNA sequence on the INTERNET revealed a different sequence for 16SrRNA for *Chlorogloeopsis*. Because of this we decided to repeat the sequencing of isolates 1.2 s.c. 1, 3, 6. The DNA sequence of the sample with DNA extracted from 3.2.2 s.c. (1) revealed that this sample was contaminated with foreign DNA and should be repeated.

We then ordered new primers from Sigma Genesis. They are:

1. **1070F** (5'-ATGGCTGTCGTCAGCT)
2. **L23cyR** (TGC CTA GGT ATC CAC C)
3. **23R** (5'-ATGGCTGTCGTCAGCT)
4. **cy781F(a)** (5'-AATGGGATTAGATACCCAGTAGTC)
5. **cy781F(b)** (5'-AAAGGGATTAGATACCCCTGTAGTC)
6. **1505F** (5'-GTGAAGTCGTAACAAGG)

On February 18, 2003 we extracted DNA from isolates

14. 1.2 s.c.2
15. 1.2 s.c. 3
16. 1.2 s.c. 6
17. 3.2.2 s.c. 1
18. 5.2 Synechocystis s.c.1
19. 2.1 (III) Masigocladus s.c. 1

We used new primers **L23cyR** and equimolar mix of primers **cy781F** (a) and (b). We have obtained PCR products for isolates 3.2.2 s.c. 1, 5.2 Synechocystis s.c.1 and 2.1 (III) Masigocladus s.c. 1, but not for three unicolonial cultures of 1.2 *Chlorogloeopsis*. PCR products of samples 4-6 were used a matrix for the preparation of substances for the DNA sequence. Unfortunately, we did not get any sequence information, probably because of too low an amount of PCR products added to master mix for the sequencing. Because of this problem we decided to change the primers according to Nubel U, Garcia-Pichel F, Muyzer G. PCR primers to amplify 16S rRNA genes from cyanobacteria. Appl Environ Microbiol. 1997 Aug; 63(8):3327-32.

We purchased new primers recommended by Nubel et al. (1997). These primers are:

1. **CYA106F** CGG ACG GGT GAG TAA CGC GTG A
2. **CYA359F** GGG GAA TYT TCC GCA ATG GG
3. **CYA781R(a)** GAC TAC TGG GGT ATC TAA TCC CAT T
4. **CYA781 (b)** GAC TAC AGG GGT ATC TAA TCC CTT T

For the amplification of 16S rRNA from isolates mentioned above, we used the primer **CYA 106F** and an equimolar mix of primers **CYA781R** (a) and (b). Using this mixture as a source of primers, we obtained PCR products from all isolates. These PCR products were purified with the help Quiagen Purification Kit. Purified PCR products were amplified in parallel using either **CYA 106F** or the mix of **CYA781R** (a) and (b).

Products obtained have been sequenced. We have analyzed the sequences of two samples: 1.2 s.c. 2 and 3.2.2 s.c. 2. The sequence of 16S rRNA for the isolate 1.2 s.c. 2 is:

```
CGGTGGCTAAGACCGGATGTGCCGGGAGGTGAAAGGGAGACCGCCACAAG
AGGAGCTCGCGTCCGATTAGCTAGATGGTGGGGTAAAGGCCTACCATGGC
GACGATCGGTAGCTGGTCTGAGAGGACGAACAGCCCACACTGGGACTGAG
ACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATTTTCCGCAATG
GGCGAAAGCCTGACGGAGCAATACCGCGTGAGGGAGGAAGGCCCTTGGGT
TGTAACCTCTTTTCTCAGGGAAGAAGAAATGACGGTACCTGAGGAAAAA
GCATCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGATGCAAGC
GTTATCCGGAATGATTGGGCGTAAAGGGTCCGTAGGTGGTGTATGTATGTC
TATTGTCAAAGGTTTCTGGCTTAACCAGAGACAGGCAGTAGAACTGCAA
AACTAGAGTGCAGTCGGGGCAGGGGGAATTCCTGGTGTAGCGGTGAAATG
CGTAGAGTCAGGAAGAACACCGGGGCGAAAGCGCCCTGTAGGCT
```

The BLAST search showed a close alignment to the sequence of *Chlorogloeopsis* sp. PCC 7518 and *Chlorogloeopsis* sp. PCC 6718 with E value 0.0. (These data can be found at [<http://www.ncbi.nlm.nih.gov/blast/Blast.cgi#5814223>]).

Summarizing our morphological and molecular data we can conclude that isolate 1.2 s.c. 2 is a clone of *Chlorogloeopsis* sp. The sequence for the isolate 3.2.2 s.c. 1 is:

```
CGGCTGCTAATACCCCATATGCCGCAAGGTGAAATCTTTTTTGGCCTGAG
GATGAGCTCGCGGTGGATTAGCTAGTTGGTGGGGTAATGGCCTACCAAGG
CAACGATCCATAGCTGGTCTGAGAGGATGATCAGCCACACTGGGACTGAA
GACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATTTTCCGCAAT
GGGCGAAAGCCTGACGGAGCAAGACCGCGTGAGGGATGAAGGCCTTTGGG
TTGTAAACCTCTTTTCTCAGGGAAGAACACAATGACGGTACCTGAGGAAT
AAGCCTCGGCTAACTCCGTGCCAGCAGCCGCGGTAAGACGGAGGAGGCAA
GCGTTATCCGGAATTATTGGGCGTAAAGCGTCCGCAGGTGGCTTTCCAAG
TCTGCTGTCAAACCCGAGGCTTAACCTCGGATCGGCGGTGGAACTGGA
TCGCTAGAGTACGTACAGGGGTAGGGGGAATTCCCGGTGTAGCGGTGAAAT
GCGTAGATATCGGGAAGAACACCAGCGACGAAAGGCCCTACTGGGACG
```

These sequences were examined using the BLAST program and an excellent alignment to the thermophilic *Synechococcus* group was found. *Thermosynechococcus elongatus* and *Synechococcus elongatus* also belong to this group [www.ncbi.nlm.nih.gov/blast/Blast.cgi]. The 16S rRNA sequences we obtained vary a little but this is not so important for current stage of our project.

The sequence analysis of the other isolates which may be of use in the CRF is continuing.

Report from the researchers at Ohio University

The organism 1.2 s.c. (2) continues to be grown in large containers (from 5-gallon to 109 gallon) for use in CRF tests, offline harvesting tests, and in the planned pilot-scale bioreactor tests. Additional organisms (currently identified as organism 3 and organism 4 for simplicity) are also being cultured and organism 3 has been scaled up to a 5-gallon container.

CRFII long-term qualitative tests

Test 1: Using Drilled hole designed headers. A long run test was conducted for 5 weeks. Approximately 0.6 gpm flow rate was maintained during dripping mode to grow the organism on the screens. Organisms grew very well on the Omnisil membrane. They showed very good qualities of adhering to the membranes. All the test parameters were well maintained throughout the test, showing that the bioreactor test system is stable and ready for quantitative testing. (Temperature of the tank: approximately 50⁰C, Temperature of the CRF II chamber: approximately 50⁰C)

Test 2: using pressure shim headers. Another long run test was started and is still continuing. The dripping mode was a success and the organisms were well adhered to the membrane. The flow rates were approximately 0.5 gpm. All the parameters were well maintained. (Temperature of the tank: approximately 50⁰C, Temperature of the CRF II chamber: approximately 50⁰C)

See Subtask 1.5 for additional information related to harvesting and post-harvesting growth for these tests.

Subtask 1.2 Design deep-penetration light delivery subsystem

ORNL continues to support the pilot-scale bioreactor development. Some problems developed with the solar collector and fibers over the winter, but ORNL is scheduled to visit the site in Athens in April to fix the problems.

Subtask 1.3 Investigate growth surface subsystem design

No new tests have been run on different materials. 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 again focused on design and experimental work for the integrated screen wetting / harvesting system, working exclusively with Omnisil screens and 1.2. s.c.(2) organisms.

CRFII long-term qualitative tests with harvesting and post-harvesting growth

Test 1: Using Drilled hole designed headers. Approximately 0.6 gpm flow rate was maintained during dripping mode to grow the organism. Organisms grew very well on the Omnisil membrane. They showed very good qualities of adhering to the membranes. At the end of the 5-week CRFII test we tried to harvest the organisms from the membrane by increasing the flow rate above the drip mode level. The harvesting was not a success, partly due to mechanical problems with the header pipes. The headers had been manufactured out of clear plastic to allow visualization of the flow control system within the header pipe, but the holes drilled and tapped into the brittle plastic for the water lines developed edge cracks so the pipes would not hold pressure for the harvesting mode. The headers for the next test were made from a less brittle PVC material to avoid this problem.

Test 2: using pressure shim headers. Another long run test was started and is still continuing. The dripping mode was a success and the organisms were well adhered to the membrane. The flow rates were approximately 0.5 gpm. After sufficient growth was established on the screens (after several weeks) we performed a harvesting test by increasing the flow on one of the membranes but we were unable dislodge substantial amount of organisms from the membrane. The flow that we were able to achieve was only 2 gpm with the test system, and there was not a full "sheeting action" as desired for harvesting, so once again the test results show that a higher flow may be necessary for harvesting and the test system will have to be modified to achieve higher flow for future harvesting tests. Figures 6 and 7 show the screen on both sides before and after harvesting.

To quickly test if harvesting could be achieved using this type of header design modified to allow higher harvesting flow rates, we simulated the "sheeting action flow" (no impingement jet) by removing the inlet water pipe from the header and using the full flow rate on a small area of the screen. In this test we were able to dislodge an appreciable amount of the organism from the "harvested" section of the membrane. The flow rate was 1.55 gpm, and it was directed to the left 1/3 of the screen width on one side of the screen only (side B). The results of this test shown in Figures 8 and 9 indicate that harvesting is possible using only high-flow sheeting action (no impingement jet is needed), but we estimate that 5-10 gallons per minute may be needed to achieve the desired level of harvesting for the entire screen. The flow to the header for the current test system is limited by the fluid inlet tube, so we plan to modify the test system to have a maximum flow capacity greater than 6 GPM while still maintaining the low flow capacity for growth mode and run some full screen harvesting tests in the near future.

We were also successful in growing organisms on the "cleaned" section of the membrane after the harvesting, as shown in Figure 10. This indicates that continuous bioreactor operation is feasible, with continuous cycles of harvesting and repopulating screens. Future tests will be used to quantify long-term growth productivity.

a. Before Harvesting Test

b. After Harvesting Test

Figure 6: Screen side A before and after harvesting with 2gpm flow through the header

a. Before harvesting

b. After harvesting

Figure 7: Screen side B before and after harvesting with 2gpm flow through the header

a. Side B before harvesting

b. Side B after harvesting the left area only

Figure 8: Screen side B before and after local harvesting with 1.55 gpm on local area

Figure 9: Side B after local harvesting (close up)

Figure 10: New Growth in Harvested Area

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. As described above the qualitative tests in the CRF-2 have been successful. Quantitative tests in the CRF-2 that will enable us to select the “optimum” system to be tested in the pilot scale bioreactor will begin in April 2003.

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.

Group Accomplishments:

1. We installed the new plumbing system for the bioreactor which involved mounting the bombs, filter rig, harvesting tank, etc.
2. Manufactured the agitation system and tested it with algae, which prevents it from settling inside the culture tank. The results were encouraging which were shown to the group in the form of a video clip.
3. We notified the group and Oak Ridge National Laboratories immediately regarding the problem of the light fibers coming loose from the solar collector, and also about the partial transparency of the primary mirror on the collector. The process for repairing the same is (by folks at ORNL) underway and will take up to 3-4 weeks to complete.
4. We manufactured an Omnisil membrane using a drilled-hole header pipe design and are performing a shake down test of the entire system to check for sustainability. Uninterrupted operation of the entire system as a whole, with all subsystems (except light delivery) operational, is seen so far.
5. Set up a computer at the 1005 East State St. facility for the Data Acquisition system. For the shake down test (being performed now) we are using a DA system that Duncan Earl, from ORNL, brought with him.

Action Items:

1. Manufacture Omnisil membranes once the group decides upon the header pipe design and the frame.

2. Assist Duncan and Dave Beshears from ORNL when they come down here to dismantle the solar collector and tracker for repairs and when they return for re-installation of the same.
3. We will present the results of the current shake down test to the group and make any modifications as deemed necessary.
4. We have to come up with an idea to set up a culture facility for the pilot-scale unit. Until then we will transfer culture from the culture laboratory in 045, Stocker Center.
5. Once we have the solar collector and light sheets operational, we will start a 60-day long test to check for sustainability and also performance of the bioreactor unit.

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 proceed in the third year in this three year project we can report that we have made significant progress towards achieving our overall project goals and are working hard to get on schedule. All of our test facilities are developed and our test plans and procedures are in place and we are beginning the CRF-2 tests that will provide the results needed to proceed with the pilot scale bioreactor testing.

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