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Abstract

This quarterly report documents significant achievements in the Enhanced Practical Photosynthetic CO_2 Mitigation project during the period from 1/3/2001 through 4/02/2002. Most of the achievements are milestones in our efforts to complete the tasks and subtasks that constitute the project objectives, and we are currently on schedule to complete Phase I activities by 10/2002, the milestone date from the original project timeline. As indicated in the list of accomplishments below, we are continuing to evaluate candidate organisms and growth surfaces, and we are expanding the test facilities in preparation for scaled up system-level testing.

Specific results and accomplishments for the first quarter of 2002 include:

Organisms and Growth Surfaces:

- Isolate 1.2 s.c. (2) has been selected for further investigations because of its favorable growth properties.
- Research on optimal conditions for the growth of cyanobacterial isolates from YNP should be carried out using distilled water which has more stable chemical parameters, although tap water use may be permissible during full scale operations (at the cost of longer organism doubling times).
- Tr. 9.4 WF is able to generate a biofilm on an Omnisil surface. Over the long term Omnisil does not inhibit the growth of TR 9.4 isolate, though it does elongate the lag phase of growth of this isolate.
- Initial survivability tests for the TR 9.4 organism on Omnisil screens in the CRF2 model-scale bioreactor are underway. We have experienced problems keeping the organisms alive for more than three days, but we are currently investigating several possible causes for this unexpected result.
- Accelerated materials testing have shown that Omnisil fabric has acceptable strength properties for use in a practical bioreactor system.

Bioreactor support systems and test facilities:

- Several CO_2 scrubbing experiments have been completed in the translating slug flow test system, however the error introduced by the original process for measuring CO_2 concentration in the solution was so big that the resulting data was unreasonable. A new sampling method to prevent degassing of the liquid sample is being implemented, and a new set of tests has been scheduled for the week of 4/15/2002.
- Qualitative harvesting tests of TR 9.4 on Omnisil have been completed but the results are inconclusive. Very little harvesting effect was observed with the current harvesting system design, but the results were greatly impacted by the minimal amount of organism growth on the screens at the time of the harvesting tests. Measures are being taken to extend the colonization time to achieve a screen loading condition that represents a more realistic harvesting condition, and additional tests will be run in the near future with these screens.
- Significant work has been completed in the design of a new up-flow bioreactor test facility, using a vertical flow as expected in practical applications as opposed to the horizontal flow used for convenience in our current CRF test systems.
- A reasonably priced location has been selected for the pilot scale bioreactor system, and construction can now proceed in order to prepare for the installation of the solar collectors and the bioreactor.

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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)

Report from the researchers at Montana State

During 1st quarter I. Brown gave two talks in the Thermal Biology Institute seminar series concerning results of our studies as well as the general principal of modes of intracellular pH homeostasis in cyanobacteria. An abstract "Thermotolerant cyanobacteria from Yellowstone National Park for the practical photosynthetic CO_2 mitigation" has been accepted by the 9th International Conference on Applied Algology. I. Brown will attend this conference at the end of May'02. Conference materials will be published in the special issue of "Applied Algology" Journal. The authors are I.Brown, B.Wigglesworth-Cooksey, K.Cooksey and D. Bayless.

A. Organism behavior and growth.

Specific Results : Introduction of cyanobacterial isolates into culture and their purification

During 1st quarter of 2002 we continued the introduction into culture of cyanobacterial samples collected in Summer field period. In particular, they are samples from Rabbit Creek (right and left shores), Black Sand Pool, Angel Terrace (Mammoth area) and Corwin Spring. We note that thermal springs on Angel Terrace have highest concentration of CO_2 in the thermal springs and pools of YNP. We assume therefore that the isolates from Angel Terrace may have maximal affinity for high CO_2 concentrations. The isolates from Corwin Spring may also be suitable for the biotechnological CO_2 mitigation because they require elevated amounts of iron in the media and could grow well on steel mesh if it used as biofilm supports. We believe it would be rational to repeat the isolation of cyanobacteria in this area using metallic meshes instead of the plastic polymers employed last summer. Primary isolates from Angel Terrace and Corwin Spring were divided and have consequently been inoculated into six media: original BG-11, D and DH, as well as in same media with addition of calcium carbonate (5 mM) or $FeCl_3$, correspondingly. This approach has allowed us to verify a species in Angel Terrace, which has not been described previously.

After the purification we now have 13 unialgal cultures of cyanobacteria from Yellowstone National Park. They are: 1.2 (6 single clones), 8.2.1, 3.2.2 (single clone), TR8.2BF, Corwin Spring Tr2WF, Corwin Spring Tr4SB, 2.1(III), 8.2.1 film from travertine surface II, 8.1(III). Their species designation requires additional work.

In parallel, we are decreasing the volume of the existing collection by discarding samples exhibiting very poor growth (about 15 samples). During 1st quarter of 2002, 27 samples were frozen for long-term storage.

During 1st quarter of 2002 we studied also 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). In particular, we have concentrated on the isolate Tr 9.4WF and unialgal isolate 1.2 s.c.(2).

Properties of photosynthetic organisms.

We have studied the rate of growth of the isolate TR 9.4WF in 50mL BG-11 medium, prepared with tap water and supplemented with 5 % of $NaHCO_3$ and aerated with 5 % of CO_2. The rate of gas flow was 6-8 mL/min. The level of illumination was about 75 μE. m^{-2} sec^{-1}.

The doubling time the isolate 9.4WF was 48 h in BG-11 media prepared with tap water, though its doubling time of this isolate in BG-11 medium prepared with distilled water was about 10 h. (Fig. 1). Thus tap water is usable in the media, but at some cost in terms of growth rate. Note that the two cultures run at the same time duplicate each other.

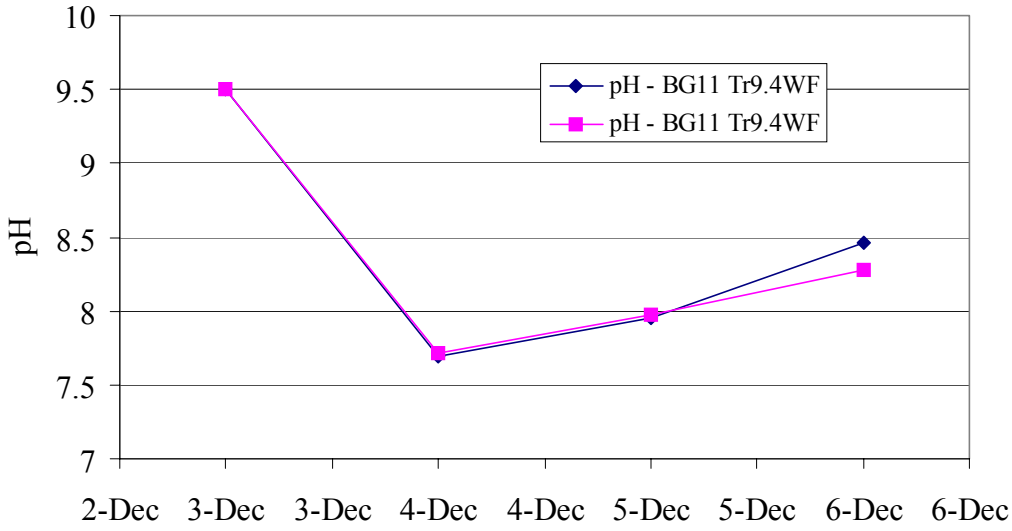


Fig. 1. pH dynamics during growth of Bg-11Tr9.4WF isolate (in duplicate) in BG-11 medium prepared with tap water and supplemented with 5 mM of $NaHCO_3$

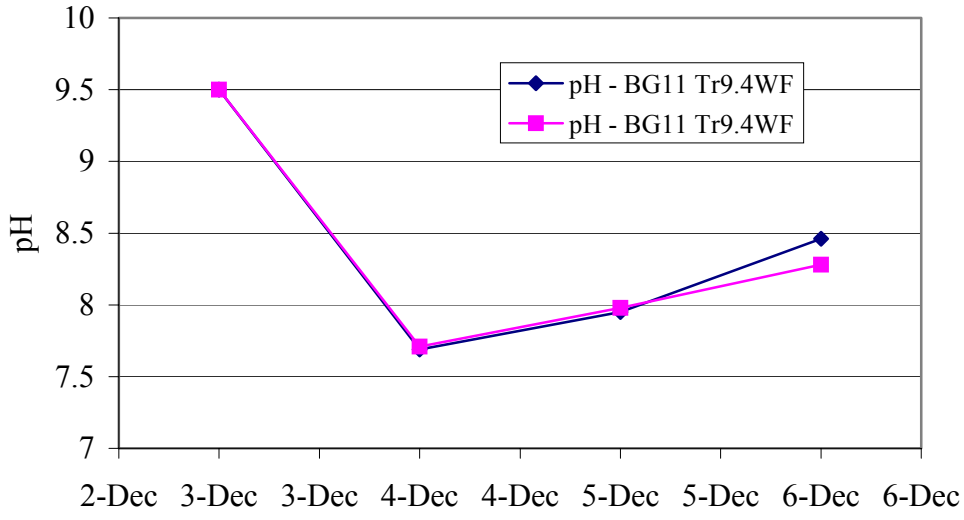


Fig. 2. pH dynamics during growth of Bg-11TR9.4WF isolate (in duplicate) in BG-11 medium prepared with tap water and supplemented with 5 mM of $NaHCO_3$

Fig.2 suggests that tap water does not decrease the buffering properties of BG-11 medium supplemented with 5 mM of sodium bicarbonate.

In the second series of experiments we studied the growth changes of isolate Tr 9.4WF growth in samples of tap water collected on different days. The rationale for the experiment was that the tap water could vary considerably from day to day as the local water treatment plant adjusts the pH and iron content of the product water.

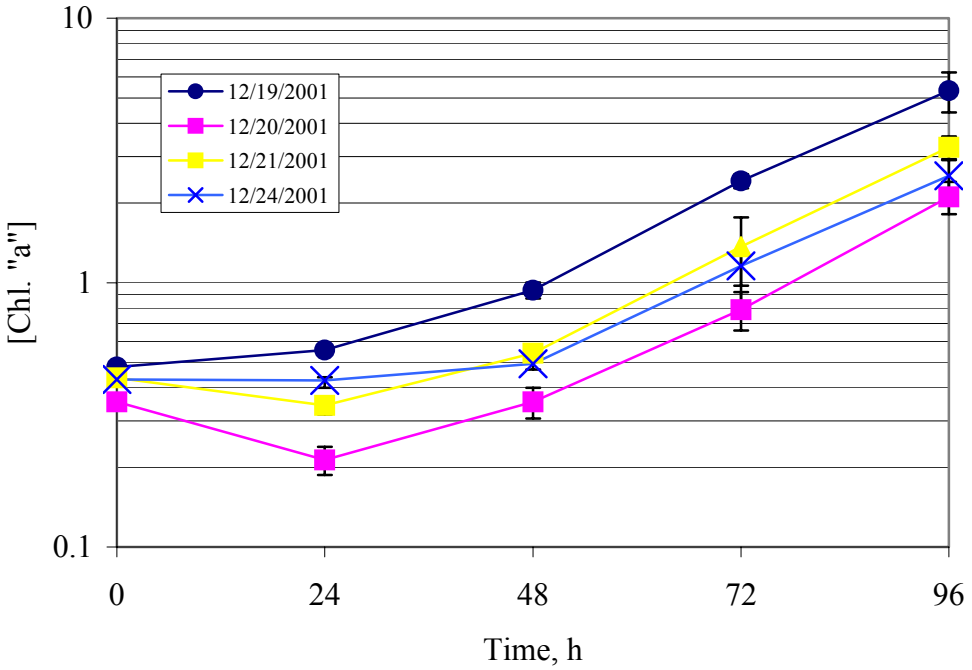


Fig. 3. Growth curves of Tr9.4WF isolate in BG-11 medium, prepared with tap water collected on different days

Fig. 3 shows that the growth of Tr. 9.4WF isolate did not have uniform character in BG-11 media, prepared with tap water collected on different days. On the basis of this experiment we conclude that research on optimal conditions for the growth of cyanobacterial isolates from YNP should be carried out using distilled water which has more stable chemical parameters, although tap water use may be permissible during a full scale operations.

We have begun work with unialgal isolates 1.2 s.c. (1) and 1.2 s.c. (2), isolated on right shore of Rabbit Creek (Fig. 4). On morphological criteria this unialgal culture appears to belong to genus *Anabaena*.

The cultivation of these unialgal isolates in BG-11 medium supplemented with 30 mM HEPES and aerated with 5 % CO_2 showed that these cultures have doubling time equal to 12.5 and 10.1 h respectively (Figs. 5-6).

We have chosen isolate 1.2 s.c. (2) for further investigations, because its growth in replicate bioreactors tubes was more reproducible than isolate 1.2 s.c. (1).

Fig.4. Area of the sampling of the isolate 1.2 on right shore of Rabbit Creek (July, 2001).

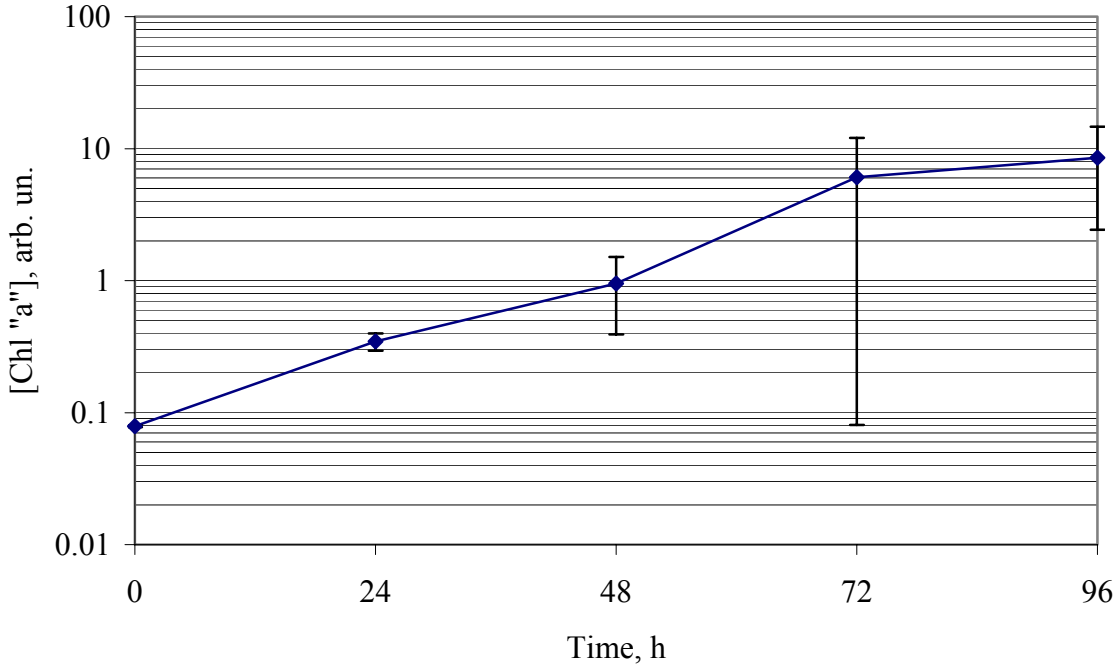


Fig.5. Growth curve of unialgal isolate 1.2 s.c. (1) in BG-11 medium + 30 mM HEPES, aerated with 5 % CO_2 (Aver. D = 12.5 h) No $NaHCO_3$ added

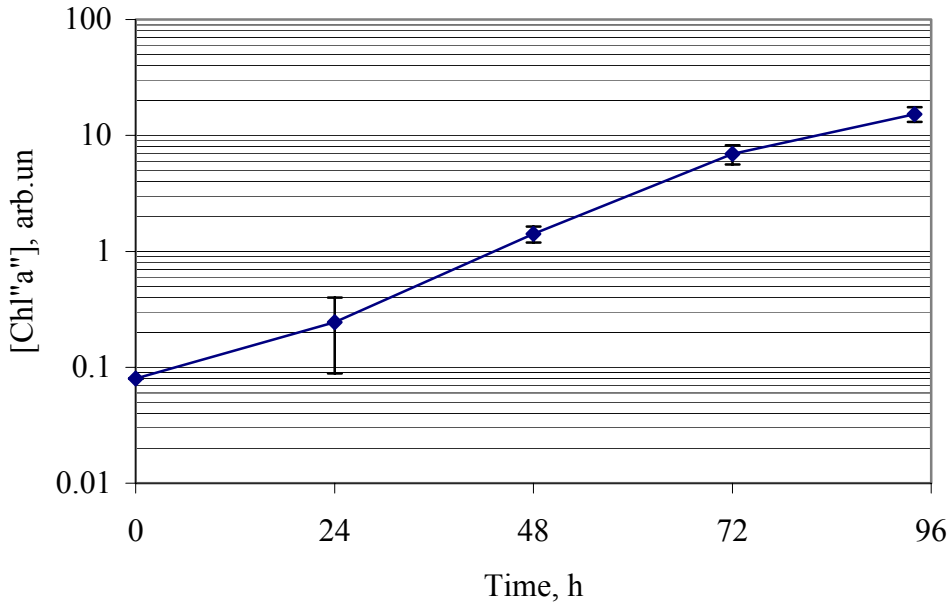


Fig.6. Growth curve of unialgal isolate 1.2 s.c. (2) in BG-11 medium + 30 mM HEPES, aerated with 5 % CO_2 (Aver. D = 10.1 h) No $NaHCO_3$ added

Bioreactor development and testing at Ohio University

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

A CO tolerance test was run for the TR9.4 organism. The results of the growth during the 30 ppm CO test are shown in Figure 7. This level of CO was not a problem for the organism as evidenced by the continued growth during CO exposure.

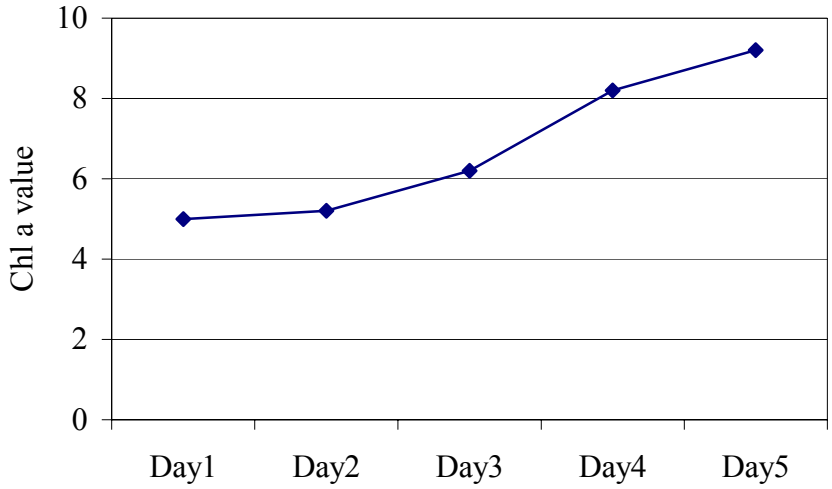


Figure 7: CO Tolerance test results for TR9.4

Construction of the scaled up version of the CRF bioreactor test facility (CRF2) is completed (see Figure 8) but debugging work is still occurring during the initial CRF2 tests. Two survivability tests for the Tr9.4 organism on Omnisil screens have been attempted, but both tests were aborted after 3 days since the organism appeared to have died. More information of required washing and preparation of the Omnisil material, as well as growth lags and colonization times are being used to prepare for a new survivability test to be run during the week of 4/15/02. Also, significant work has been accomplished in the design of a new up-flow bioreactor test facility, using a vertical flow as expected in practical applications as opposed to the horizontal flow used for convenience in our current CRF test systems.

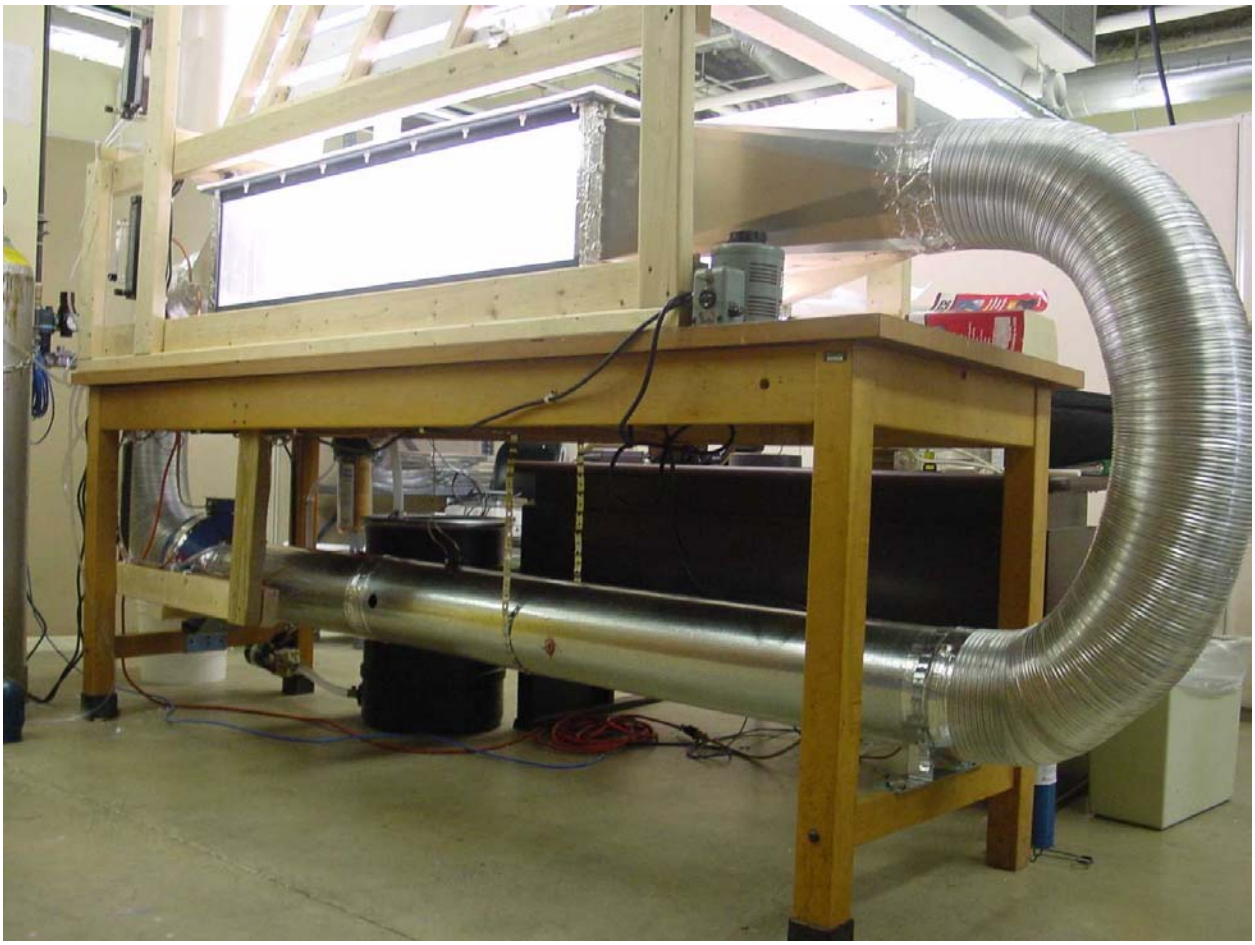


Figure 8: Scaled up version of the CRF bioreactor test facility (CRF2)

Designing the Upflow reactor

The major design decision for the reactor was whether make the growth surfaces continuous or modular. Modular construction would be easier, but there was a question about the ability to load the organisms on the individual screens. An experiment was run in which a membrane (omnisil) was loaded with algae solution by using gravity wetting and then letting it germinate in an incubator. With the success of the experiment it was determined that the reactor could be modular with the basic reactor framework consisting of a honey-comb arrangement of hexagonal

cage-like structures with the membrane wrapped around each unit, and with the lighting source in the center of each hexagonal unit (See Figure 9). A single hexagonal structure will be prototyped, but the single structure can be placed with many such units to make up a big reactor. Another cause of concern was the lighting system of the reactor, which was finally decided as going with four tubes of 40 Watts each.

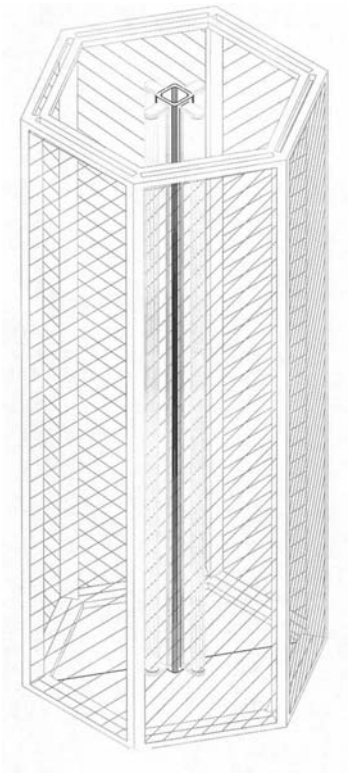
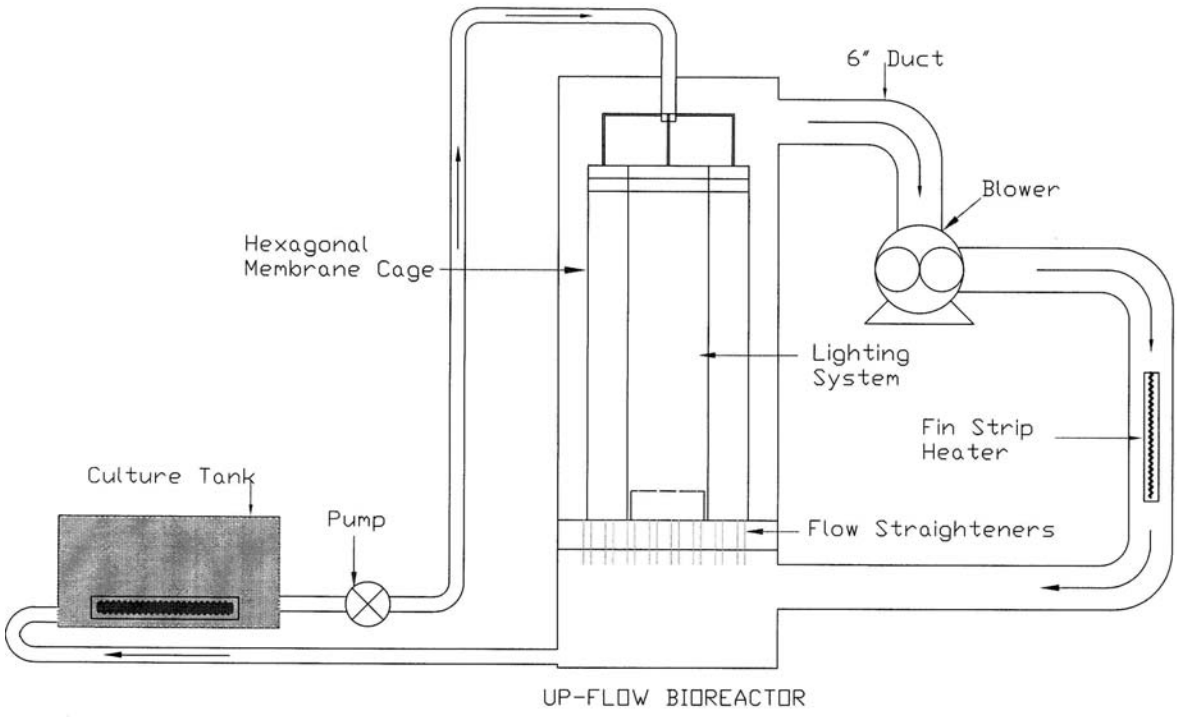


Figure 9: Single Upflow bioreactor module, showing hexagonal cage and lighting system

The reactor unit was then incorporated in a system consisting of (See Fig. 10).

a) Tank
b) Immersion heater
c) Ductwork
d) Blower
e) Fin heater
f) Flow straighteners
g) Pump

The cage will be made up of rods of very small diameters having a ductwork at the top to let the algae and the fluid flow down the membrane through the slits provided.



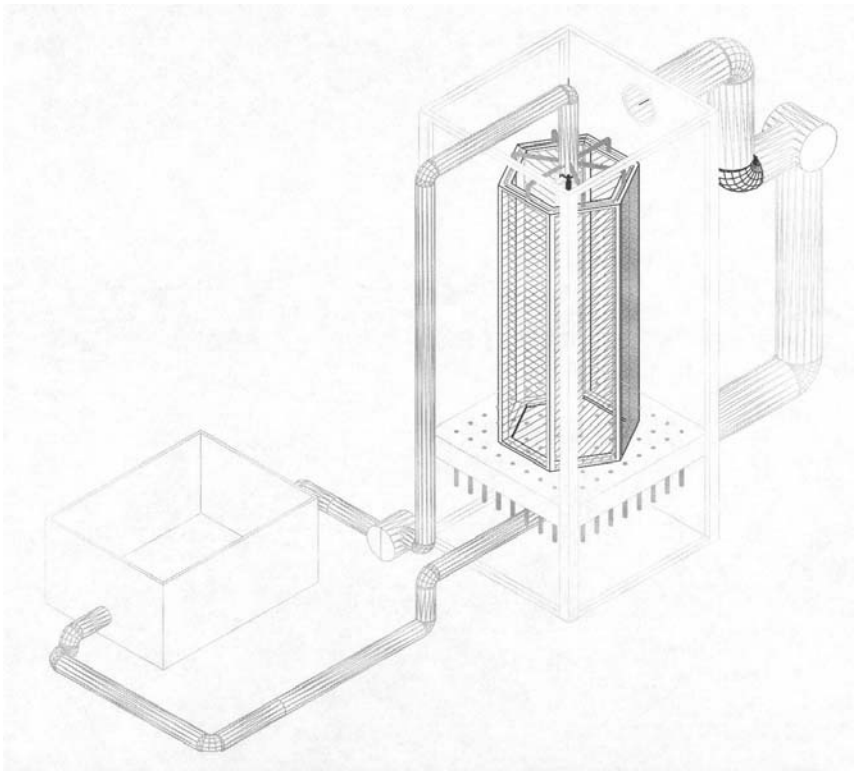


Fig. 10: Two views of the Up-Flow Bioreactor

Subtask 1.2 Design deep-penetration light delivery subsystem

No report was received from ORNL this quarter.

Subtask 1.3 Investigate growth surface subsystem design

During 1st quarter 2002 researchers at Montana State studied the growth of cyanobacterial isolates TR. 9.4WF in the presence of Omnisil and the colonization of this substratum by isolate TR. 9.4WF.

The experiment was carried out in glass bioreactor. All tubes were filled with BG-11 medium, supplemented with 30 mM HEPES. Initial pH was 8.25. Omnisil coupons(5x3 cm) were placed in 3 tubes and 3 were used as controls (Fig.11). All tubes were aerated with air + 5 % CO_2.

Fig.11. Tubular bioreactor

We measured chlorophyll concentration daily only in tubes without Omnisil because the Omnisil coupons carried a large proportion of the biomass. At this time pH, as a relative index of cyanobacterial growth, was also measured in both sets of bioreactor tubes at appropriate times. The growth curve of the culture without Omnisil (Fig.12) suggests that doubling time of TR 9.4 WF culture was about 20 h under described conditions. At this time the doubling time of this culture, incubated in BG-11 and supplemented with 5 mM $NaHCO_3$ was about 8 h. Perhaps bicarbonate anion is more effective for the growth of this culture than CO_2, although at this pH either culture should have had sufficient bicarbonate present.

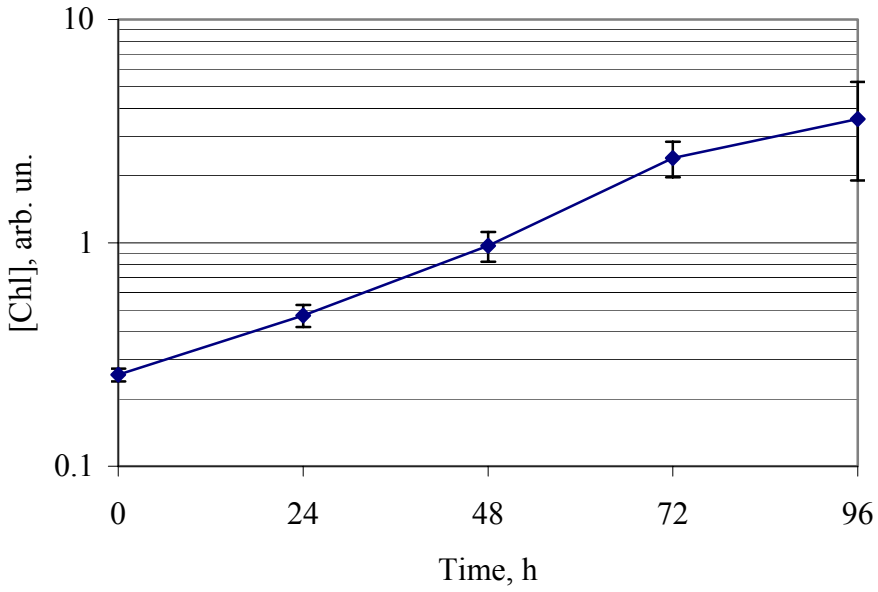


Fig.12. Growth curve of TR 9.4 WF isolate in BG-11 medium + 30 mM HEPES, aerated with 5 % O_2 (Aver. D = 20 h) No $NaHCO_3$ added

The graph (Fig.13) of pH dynamics in TR 9.4WF culture, grown without Omnisil, suggests that the exponential growth of cyanobacteria (24 – 96 h) is accompanied with alkalinization of growth medium.

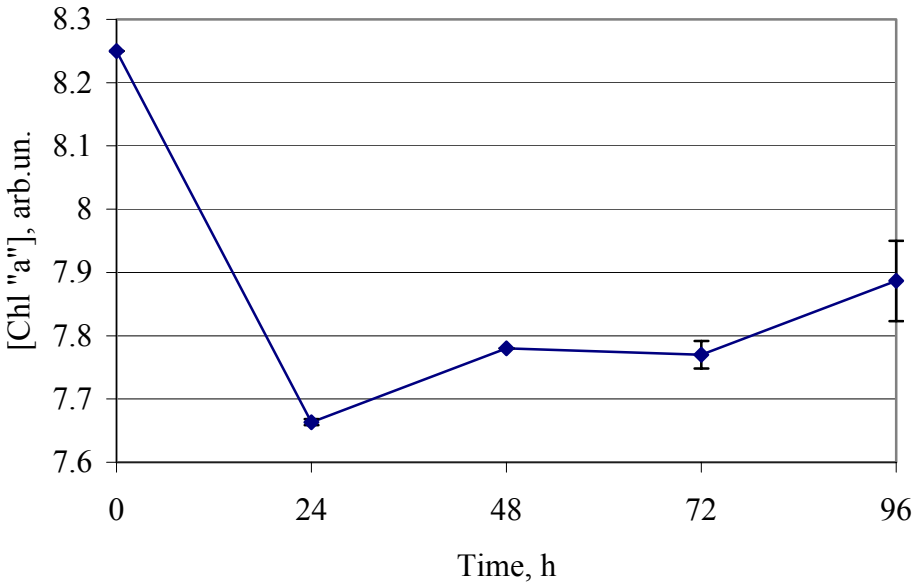


Fig.13. pH dynamics of BG-11 medium supplemented with 30 mM HEPES and aerated with 5 % CO_2 during cultivation of TR 9.4WF isolate

At 72 h, planktonic growth of the isolate TR.9.4 WF in the tubes with Omnisil (Fig. 14) was not observed, although planktonic growth was observed in tubes without Omnisil.

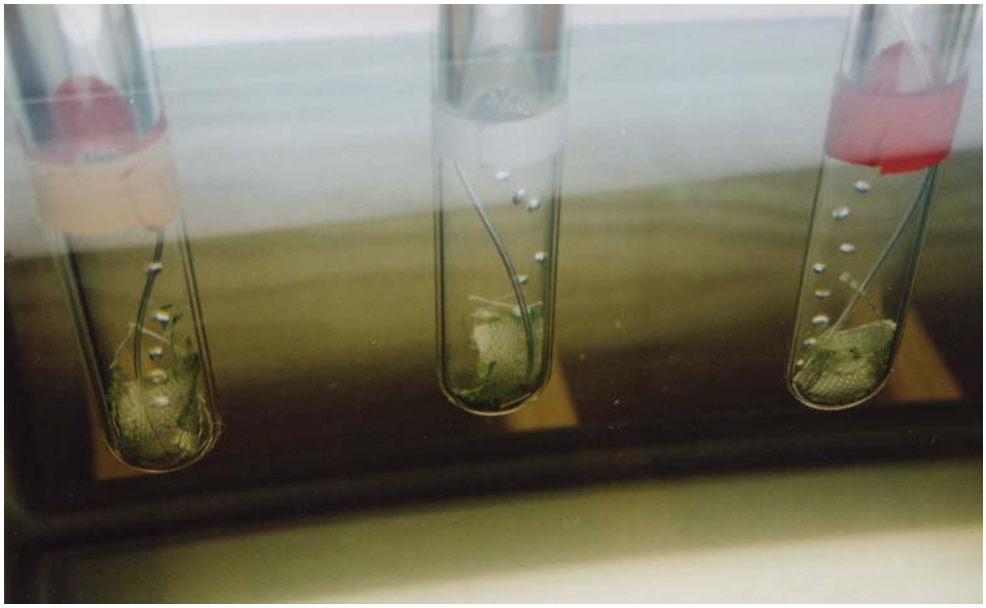


Fig. 14. TR.9.4 WF in the tubes with Omnisil

Further incubation of samples with Omnisil (more than 72 h) led to the appearance of a planktonic fraction of Tr. 9.4 WF isolate. (Fig.15.) Note that the edges of the Omnisil coupons are colonized more significantly than central zones.

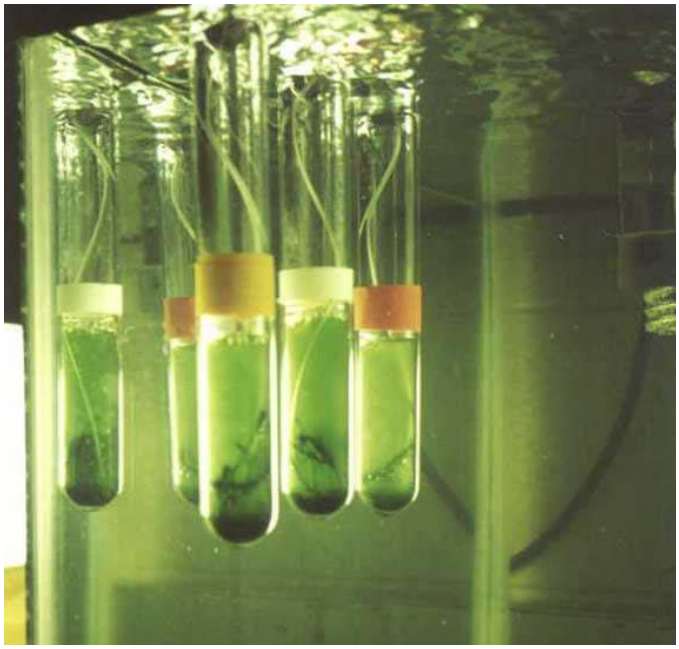
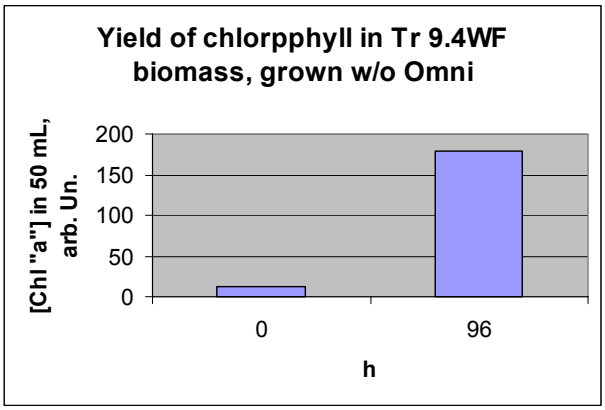


Fig. 15. Further incubation of samples with Omnisil (more than 72 h)

The final yield of biomass was not significantly different between tubes with and without Omnisil (Fig. 16 and 17).



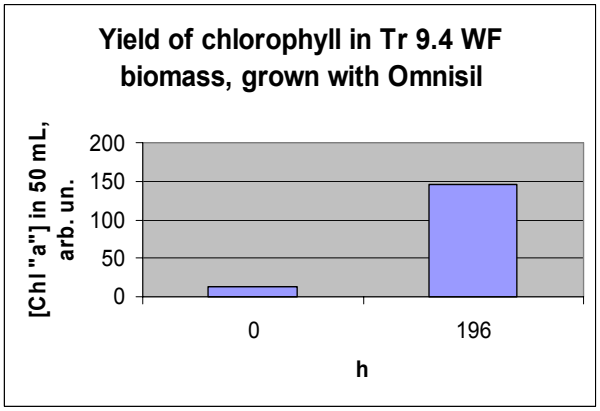


Fig. 16. Growth Chart 1

Fig.17. Growth Chart 2

Fig. 18 shows intensive colonization of Omnisil coupons by studied isolate after 7 days incubation. This picture also confirms our conclusion that cells have a larger affinity for the edges of coupons than to their central areas. We assume that single fibers of Omnisil threads are colonized more easily than the compact threads.

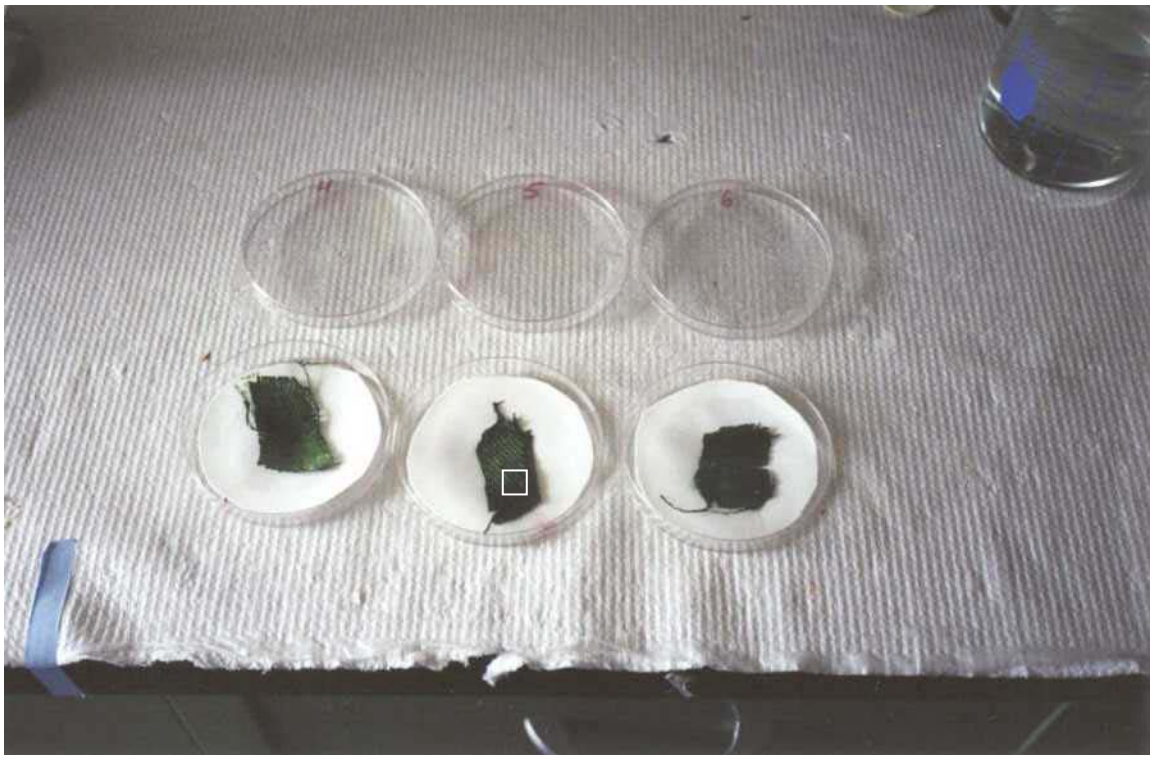


Fig. 18. Intensive colonization of Omnisil coupons
White box on center sample points out on the area isolated for further SEM.

SEM pictures were made after cryostage preparation of Omnisil coupons and then coating with gold. Figures 19 (a-d) suggest that TR.9.4 isolate generates a biofilm on Omnisil surface.

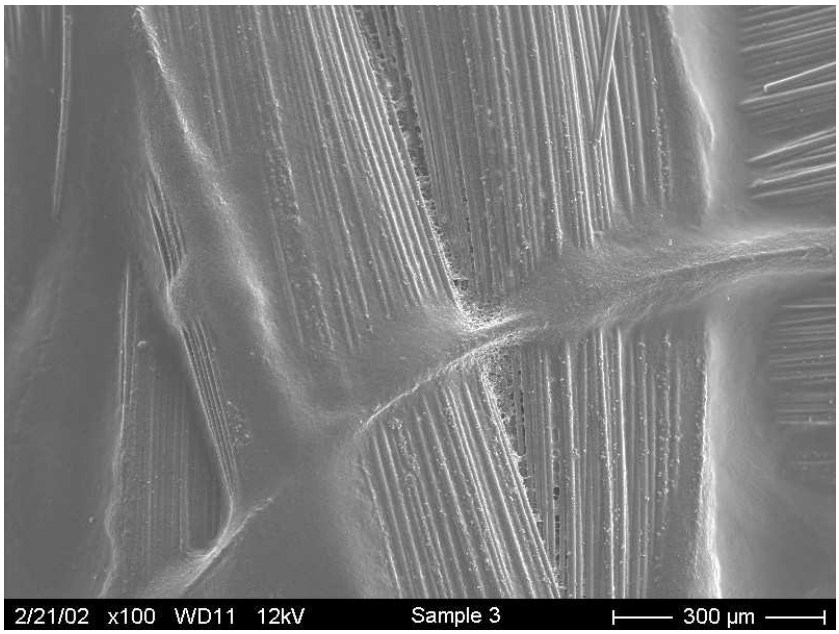


Fig. 19a – SEM photograph of TR9.4

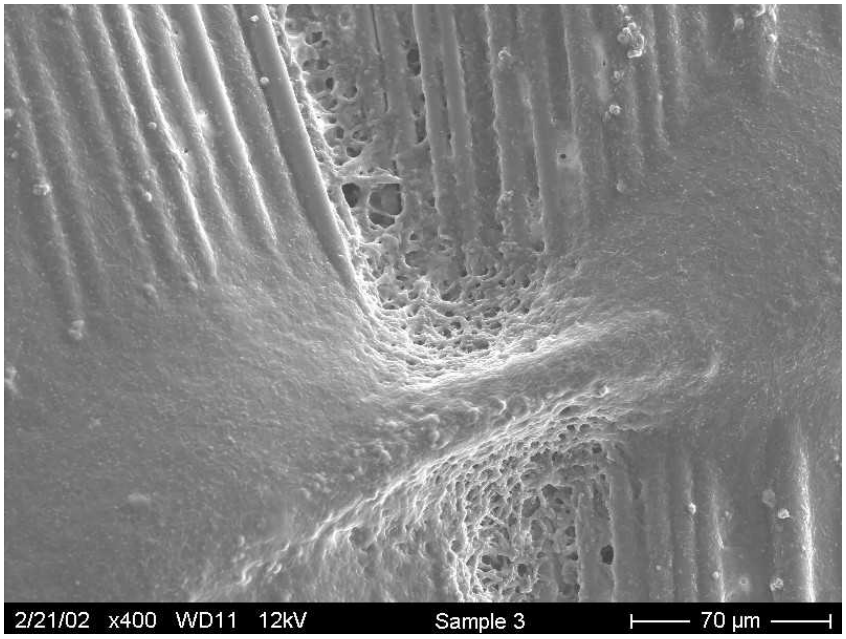


Fig. 19b – SEM photograph of TR9.4

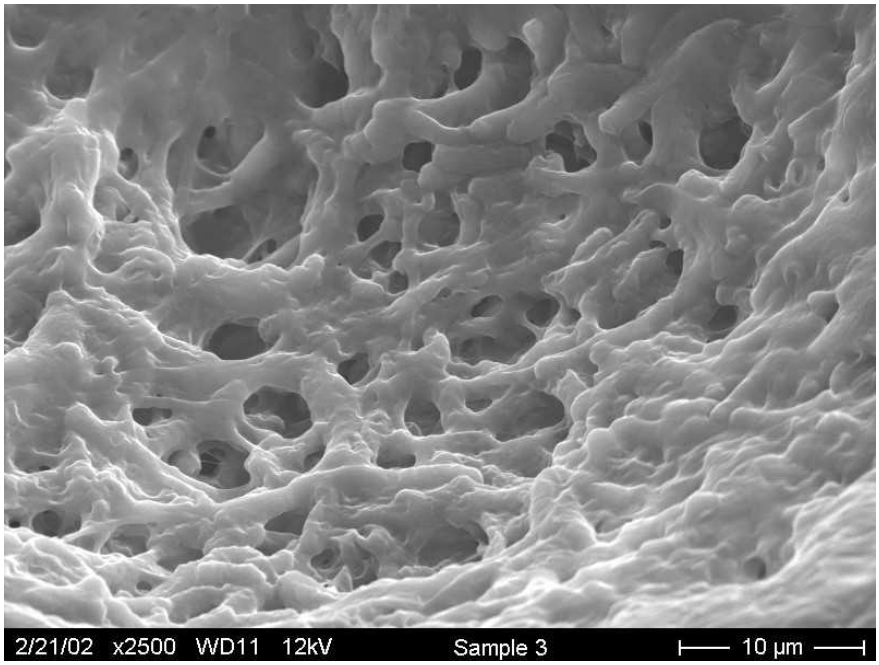


Fig. 19c– SEM photograph of TR9.4

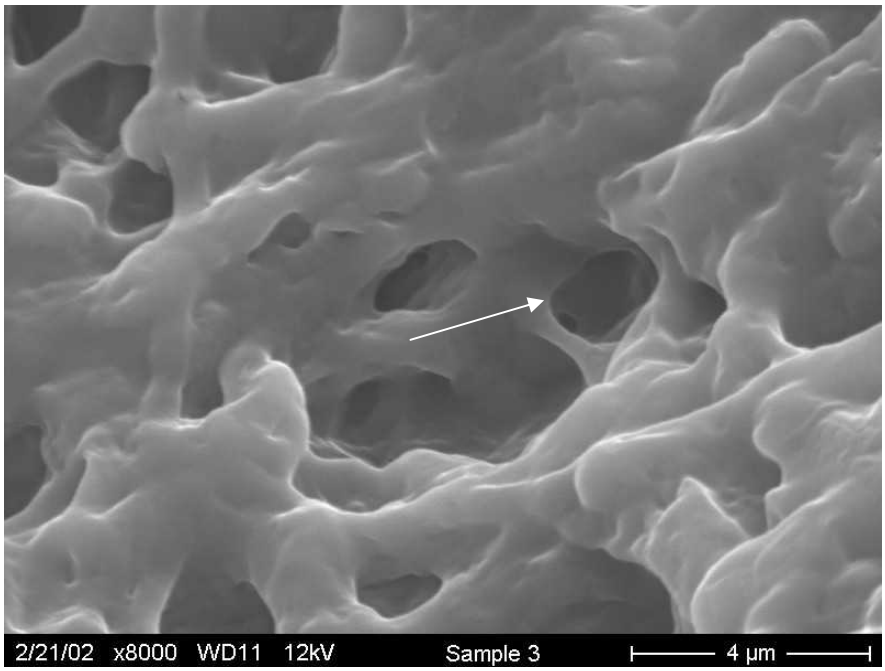


Fig. 19d: – SEM photograph of TR9.4
Arrow points out the extracellular polymer substance.

In summary, the preliminary conclusions are:

1. Omnisil does not inhibit the growth of TR. 9.4 isolate, though it elongates the lag phase of growth of this isolate.
2. TR. 9.4 WF is able to generate a biofilm on an Omnisil surface.

Ca^{2+} effect on the adhesion of the isolate 1.2 s.c. (2).

To judge the effect of Ca ions on the adhesive properties of isolate 1.2s.c.(2) we adjusted the Ca content of a series of BG –11 media and then allowed the cyanobacterial cells to attach to glass coupons for 100 min. The coupons were rinsed three times by dipping in BG-11 medium with the usual amount of Ca, i.e. 0.25mM. This technique was developed previously to judge the effects of surface chemistry on diatom attachment. The results show a positive effect of Ca on the number of cells attaching to the substrata (Fig. 20). We are following this line of investigation with a view to modifying the growth media and its use in the Carbon Remediation Facility at the Ohio University.

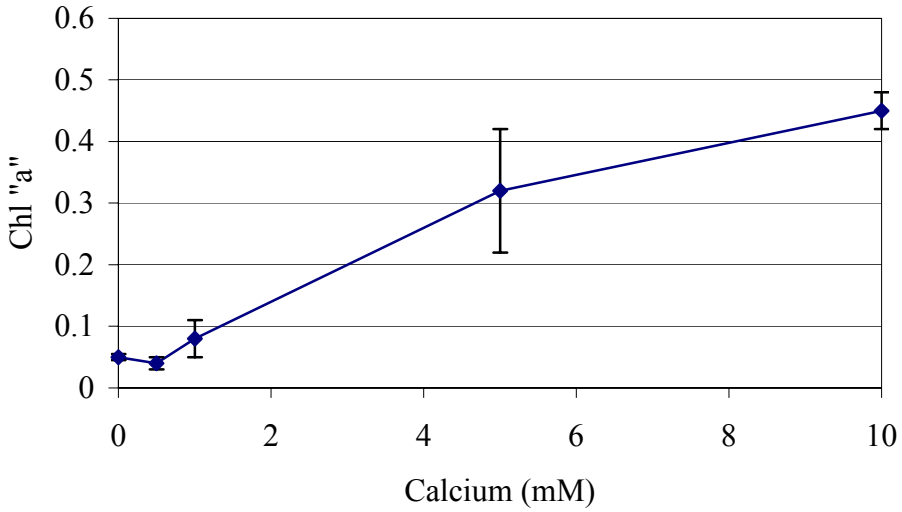


Fig. 20. Effect of Ca on Cell Attachment

Ohio University Growth Surface studies

To better understand the strength properties of various candidate growth surface materials and their potential for use in the bioreactor, researchers at Ohio University have conducted accelerated materials testing, producing the results summarized in Table 2.

Table 2: Burst strengths of various fabrics in various solutions

S.No	Chemical	Burst Strength (psi)
Omnisil 1000	Sulphuric Acid	370*
Omnisil 1000	Ammonia Solution	550
Ryton	Sulphuric Acid	500
Ryton	Ammonia Solution	550
Ryton	Power Span	190
Polypropylene	Sulphuric Acid	150
Polypropylene	Ammonia Solution	120
Fiberglass	Ammonia Solution	580
Teflon 1	Power Span	290

- - New Samples put in the Accelerated Test Rig on 03/19/02

Subtask 1.4 Investigate the use of a hydraulic jump to improve the system's overall CO_2 conversion efficiency

1.4.1 Method for CO_2 Scrubbing test

When sampling CO_2 from the pipeline, part of 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 gas CO_2 before it leaves the solution; the other sample has no NaOH solution. Then 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 CO_2 electrode. For the other sample, its pH is reduce to 4.5 by titration with H_2SO_4, then the bicarbonate concentration in the solution can be calculated based on the volume of sample and H2SO4. 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.

1.4.2 Test results

Several groups of CO_2 scrubbing experiments have been done. However, we found the error in the process is so big that the resulting data is unreasonable. There are two possible causes for the error. The first one is the degassing of CO_2 when sampling and storing the solution; and the second one is the error introduced by measurement process. We considered the first cause might be the main reason for the error.

1.4.3 Proposed Solutions

In order to prevent sampling process from degassing, a syringe will be used to withdraw the liquid sample from the slug. The sample then is slowly injected into NaOH solution so that the gas phase CO_2 can be totally captured in the solution. Finally, titration method will be conducted to measure the CO_2 concentration in the solution. Till now, two syringes and their needles were ordered. A new group of tests will be done once the nitrogen is delivered to the lab.

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. Because of many factors which showed that the organism which was previously being used in the harvesting tests (Cyanidium) would likely not be selected for further testing, we switched our harvesting tests to focus on the fastest growing of the new organism cultures provided by Montana State, Tr9.4WF. Additionally, due to screen material wetting properties, the two materials that have been found to be most suitable for the integrated screen wetting/ harvesting system screens are Omnisil and carbon fiber. Based on this information and field data that showed the Tr9.4 organisms grew well on Omnisil test samples, we began a series of qualitative and quantitative tests for harvesting the Tr9.4 organism from Omnisil screens.

Specific harvesting test activities and accomplishments for the quarter include:

- In February Omnisil screens were constructed and basic flow properties were tested in the off-line harvesting test facility. Initial debug tests were run without organisms

because there was not a sufficient amount of Tr9.4 available to populate the screens for the harvesting tests.

- In March after the Tr9.4 organism was available for screen colonization, qualitative studies of the harvesting characteristics for this particular organism and growth surface (Omnisil) were initiated. Problems were encountered with the time required to colonize the Omnisil screens. Previous organism/material combinations required 24-48 hours for colonization, but after 48 hours there was little to no attachment of the organism to the Omnisil screen. After 5 days some colonization was evident (Figure 21) so the qualitative visual test was run. Test parameters were the same as the standard high pressure / low flow harvesting tests that were successful in removing previous organisms. The qualitative results are that only a small amount of cynabacteria appeared to be washed off based on the visual observation. The reason for this could be the unsatisfactory loading since the amount of Tr9.4 attached to the screen even after 5 days of colonization was small. See Figure 21 for the post-harvesting picture showing the change due to harvesting.
- Based on data from Montana State that showed a growth lag but significant growth after 7 days for the Tr9.4 organism on Omnisil in test-tube bioreactors, the next qualitative test was done on an Omnisil screen which had been soaked in the sliver tank (filled with a medium containing Tr 9.4) in the incubator for 7 days. The loading condition was better than the previous one, but the growth was still not to the point where harvesting would be desired in the actual system. The harvesting test was run under the same high pressure / low flow conditions but again there was no obvious removal of algae from the screens. The washing water showed no green color as would be expected if organisms were washed off. Figures 22 and 23 show the qualitative results from this test.
- The next qualitative test will involve periodic monitoring of the colonization of the screens in the incubator, allowing as much time as necessary so that the screens get to the point where harvesting would be desired in the actual system before the harvesting test is run. The need for the excessively long colonization time is being investigated, and if it cannot be reduced testing of other combinations of organisms and growth surfaces will be accelerated.

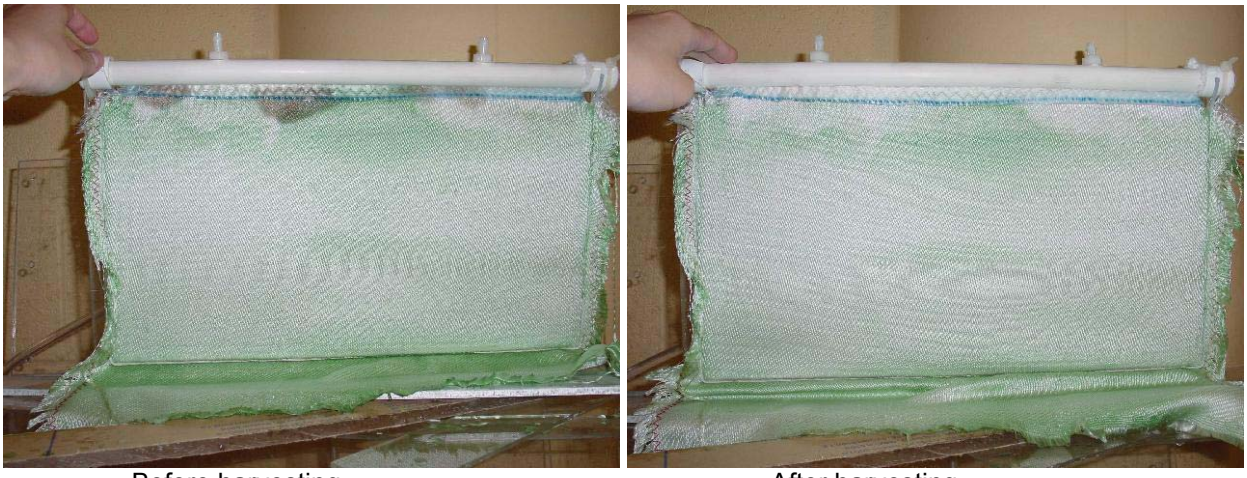
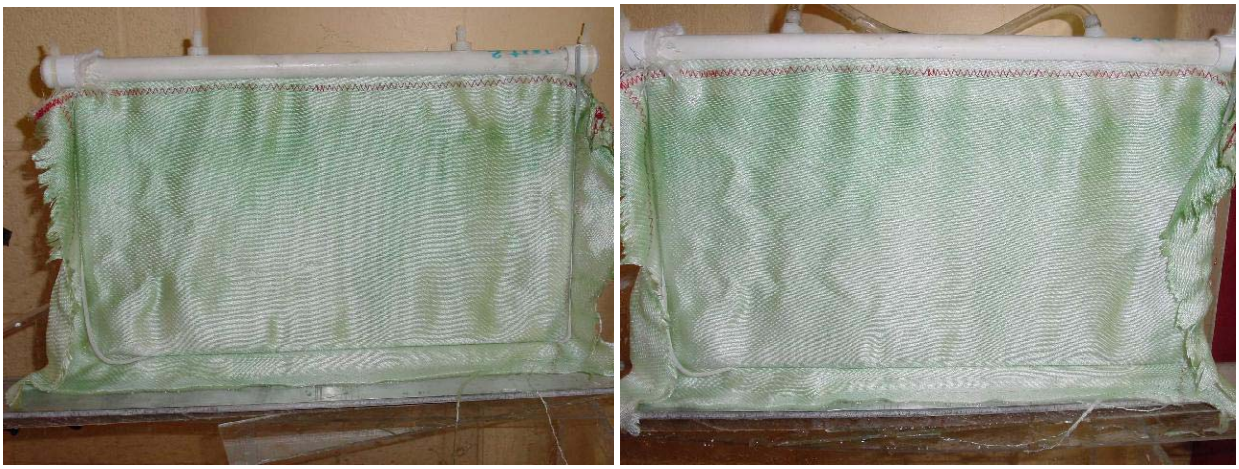


Figure 21. Omnisil with TR 9.4 —5 days loading before harvesting test



Before harvesting After harvesting

Figure 22. Omnisil with TR 9.4 —7days loading before harvesting test

Figure 23. Clear water during harvesting – showing lack of organism removal

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

The literature search discussed in last quarter's report has been reviewed and will be continually updated as more information is found. Experiments in this area are on hold until we get closer to final decisions between candidate organisms.

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

In order to enable system-level testing of various subsystem combinations, three new system-level experimental facilities are currently under construction: a larger scale CRF (CRF2), a model-scale up-flow bioreactor, and a pilot scale bioreactor. The larger scale CRF is ready for use but debugging continues during the first tests. The up-flow bioreactor was discussed under Subtask 1 and the pilot scale system is discussed in the Task 3.0 section of this report.

Task 3.0. Implement the optimum system in scaled model

The placement of the pilot scale bioreactor test facility has been changed from the Ohio University Corrosion Center to an off-campus lease property at 1005 E. State Street based on costs. The lease agreement has been approved and the modification costs for the space have been estimated at $6700. The construction of the bioreactor is proceeding ahead of schedule (at a separate location) and will be available for quick installation once the modification work for the site is completed.

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

Specific results and accomplishments for the first quarter of 2002 include:

Organisms and Growth Surfaces:

- Isolate 1.2 s.c. (2) has been selected for further investigations because of its favorable growth properties.
- Research on optimal conditions for the growth of cyanobacterial isolates from YNP should be carried out using distilled water which has more stable chemical parameters, although tap water use may be permissible during full scale operations (at the cost of longer organism doubling times).
- Tr. 9.4 WF is able to generate a biofilm on an Omnisil surface. Over the long term Omnisil does not inhibit the growth of Tr. 9.4 isolate, though it does elongate the lag phase of growth of this isolate.
- Initial survivability tests for the Tr9.4 organism on Omnisil screens in the CRF2 model-scale bioreactor are underway. We have experienced problems keeping the organisms alive for more than three days, but we are currently investigating several possible causes for this unexpected result.
- Accelerated materials testing has shown that Omnisil fabric has acceptable strength properties for use in a practical bioreactor system.

Bioreactor support systems and test facilities:

- Several CO_2 scrubbing experiments have been completed in the translating slug flow test system, however the error introduced by the original process for measuring CO_2 concentration in the solution was so big that the resulting data was unreasonable. A new sampling method to prevent degassing of the liquid sample is being implemented, and a new set of tests has been scheduled for the week of 4/15/2002.
- Qualitative harvesting tests of TR9.4 on Omnisil have been completed but the results are inconclusive. Very little harvesting effect was observed with the current harvesting system design, but the results were greatly impacted by the minimal amount of organism growth on the screens at the time of the harvesting tests. Measures are being taken to extend the colonization time to achieve a screen loading condition that represents a more realistic harvesting condition, and additional tests will be run in the near future with these screens.
- Significant work has been completed in the design of a new up-flow bioreactor test facility, using a vertical flow as expected in practical applications as opposed to the horizontal flow used for convenience in our current CRF test systems.
- A reasonably priced location has been selected for the pilot scale bioreactor system, and construction can now proceed in order to prepare for the installation of the solar collectors and the bioreactor.

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