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Abstract

This quarterly report documents significant achievements in the Enhanced Practical Photosynthetic CO₂ Mitigation project during the period from 4/2/2001 through 7/01/2002. 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, our efforts are focused on improving the design of the bioreactor test system, evaluating candidate organisms and growth surfaces, and scaling-up the test facilities from bench scale to pilot scale.

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

Organisms and Growth Surfaces:

- Our collection of cyanobacteria, isolated in YNP was increased to 15 unialgal cultures.
- Illumination rate about 50 $\mu\text{E}/\text{m}^2/\text{sec}$ is not saturated for the growth of 1.2 s.c. (2) isolate. The decrease of illumination rate led to the decrease of doubling time of this isolate.
- The positive effect of Ca^{2+} on the growth of isolate 1.2 s.c. (2) without Omnisil was revealed, though Ca^{2+} addition was indifferent for the growth of this isolate at the presence of Omnisil.
- Calcium addition had a positive effect on the generation of cyanobacterial biofilm on Omnisil surface.
- The survivability problems with the Tr9.4 organism on Omnisil screens in the CRF2 model-scale bioreactor have been solved. The problems were related to the method used to populate the growth surfaces. When pre-populated screens were placed in the bioreactor the microalgae died within 72 hours, but when the microalgae were cultured while in place in the bioreactor using a continuous-population method they grew well inside of the CRF2 test system and survived for the full 7-day test duration. CRF2 tests will continue as soon as the new combined drip system / harvesting system header pipe design is refined.

Bioreactor support systems and test facilities:

- A solar collector, fiber optic light cables, and 8 light distribution panels have been installed at the pilot-scale bioreactor site. The “natural” lighting system is ready for the pilot-scale bioreactor system-level debugging tests scheduled for early to mid July.
- CO₂ scrubbing tests were carried out to understand the CO₂ scrubbing capability of translating slug flow under various conditions. Within experimental error, for liquid velocities of 0.6 m/s and 1m/s it was shown that different gas velocities (including 4.8, 6.6 and 9m/s) cause no significant change in CO₂ concentration.
- The harvesting tests were put on hold while an alternative loading method was developed and to concentrate our resources on the design of the new combined drip system / harvesting system header pipe. A new header pipe design has been completed and proof-of-concept tests have shown good performance in the drip loading mode. Tests have also shown that we can create preferable conditions for harvesting with this design, and actual harvesting tests will be run as soon as screens are available that have achieved a sufficiently “thick” growth state such that harvesting is desired.

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

Report of I. Brown's participation in 1st Congress of International Society on Applied Algology

In the end of May, 2002 Dr. I.I. Brown participated in the 1st Congress of the International Society for Applied Phycology, what was held in Spain. Author's group including D. Bayless, I. Brown, B. Cooksey and K. Cooksey presented a poster "Thermotolerant cyanobacteria from Yellowstone national Park for the practical photosynthetic CO₂ mitigation". Presented data attracted the attention of some experts in the field of CO₂ sequestration, e.g. Drs. O.Pulz, M. Borowitzka, and J. Beneman. M. Borowitzka expressed an idea, that obtained cyanobacterial biomass should be burned after extraction of valuable products to decrease the fuel consumption. Dr. Otto Pulz (Deputy Director of Institute for Cereal Processing, Germany) expressed an interest to collaborate with Dr. Bayless on the development of new type photobioreactor, used in Ohio. Dr. O. Pulz believes that our project may have good perspectives because our cultures, isolated from YNP, have doubling time shorter than 24 hours. He has carried out similar project in Germany and could not get economical effectiveness because he used tubular bioreactor of very big size and the doubling time of his culture was significantly bigger than 24 h.

Several research teams made also presentations, dedicated to CO₂ mitigation. Huesemann et al use empirical model that incorporates a Monod type relationship between growth rate (μ) and light intensity (I) as well as light attenuation by biomass in a well mixed light-limited photobioreactor, predicts that the optimum of CO₂ biofixation potential (P_{max}) is a function of the maximum specific growth rate (μ_{max}), the half-saturation constant (K_I) and the biomass light absorption coefficient (K_a). M. Borowitzka presented poster dedicated to use coccolithophorids in carbon dioxide bioremediation. He believes that the coccolithophorids are of particular interest for use to fix atmospheric carbon dioxide as they not only photosynthesize, but also produce calcium carbonate plates or coccoliths. Fernandes et al presented abstract "Flue gas containing high CO₂ level use as raw material by photosynthetic organisms to produce valuable biomass". They isolated an *Oscillatoria* spp. culture from hot spring in Portugal that tolerates high temperature and very high CO₂ concentration appears as a potential good candidate to be grown under industrial flue gas constraints. Being filamentous organism, harvesting of biomass is easily accomplished by filtration. Olaizola et al presented poster "Production of high value products and mineral carbon from smoke stack gases using photobioreactor grown microalgae". The objective of their work was to determine under which conditions carbon sequestration by microalgal photosynthesis is economically attractive. They qualify a carbon sequestration scheme economically attractive if the process used matches or betters the US DoE cost goal of \$10 per ton of CO₂ avoided. The species grown are chosen from organisms already identified as producers of high value products (e.g. carotenoids).

Organism Isolation/Behavior/Growth: Specific Results : Introduction of cyanobacterial isolates into culture and their purification

During 2nd quarter of 2002 we continued the introduction into culture of cyanobacterial samples collected in summer field period of 2001. The description of sampling sites as well as of purification methods was given in the report for 1st quarter of 2002.

By today 6 single colonies from 8.2.1.trap cord isolate have been received. Some of them are introducing in culture right now. Early, we have unialgal culture of *Oscillatoria* sp. 8.2.1 (Fig.1), but this one should be tested for upper temperature limit.



Fig. 1. Microphoto of *Oscillatoria* sp. 8.2.1 (bright light)

Besides, we have also purified unialgal culture of *Phormidium* sp. from 8.2.1 isolate. This culture can grow at 55° C, although we are not sure that such temperature is an optimum for this *Oscillatoria*. Nonetheless, this culture needs special attention, because it has very bright blue color. We assume that this culture might be potential producer of c-phycyanin.



Fig. 2. Microphoto of *Phormidium* sp. 8.2.1 (phase contrast)

During the 2nd quarter of 2002 we also received 7 single colonies from Corwin Springs Tr.9.4 (Scotch Brite) isolate, grown on BG-11 medium, solidified with 1.5 % of agarose. Now we are introducing them into culture.

We also partially purified the culture of thermophilic cyanobacterium *Fischerella* sp. (*Masticogladus laminosum*) from 2.1 isolate. We had serious problem with the segregation of this culture from *Chloroflrxus*, because *Fischerella* did not appear real growth on media, solidified with agarose. Due to this we have begun to use Marine agar (MarBio, Canada). Recently this culture has already purified from *Chloroflexus*. Now we are growing this unialgal isolate to send to Ohio about August 1. In parallel, we are decreasing the volume of the existing collection by discarding samples exhibiting very poor growth (about 15 samples). During 2nd quarter of 2002, 12 samples have been frozen for long-term storage.

Properties of photosynthetic organisms.

During the 2nd quarter of 2002 we studied the dependence of 1.2. s.c.(2) isolate growth from light illumination level. This experiment was carried out in glass fermenter. We used 12 tubes for cultivation of this isolate in BG-11 medium supplemented with 30 mM HEPES. The medium in tubes was also aerated with 5 % CO₂ in air. Gas flow was about 10 mL/min for each tube.

Tubes were separated for 4 series. First three tubes were uncoated. Second, third and fourth series of tubes were consequently covered with 1, 2 and 3 layers of window mesh of gray color (Fig. 3). Integrated levels of the illumination of tubes are shown in Table 1.

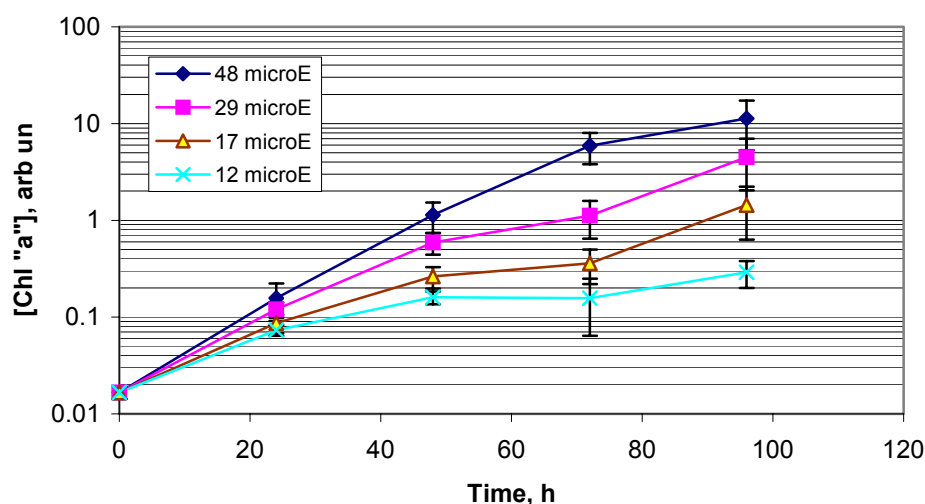


Fig. 3. General view of the photobioreactor with tubes coated with window mesh layer.

Table 1. Illumination Data

The number of mesh layers	Integrated illumination
0	48 $\mu\text{E}/\text{m}^2/\text{sec}$
1	29 $\mu\text{E}/\text{m}^2/\text{sec}$
2	17 $\mu\text{E}/\text{m}^2/\text{sec}$
3	12 $\mu\text{E}/\text{m}^2/\text{sec}$

Upper curve on Fig. 4 suggest that doubling time of 1.2 s.c. (2) isolate was ~ 8 h under integrated illumination level 48 $\mu\text{E}/\text{m}^2/\text{sec}$. We should notice that such doubling time of cyanobacterial culture is very desirable for many microalgal biotechnologists (personal communication of Dr. O. Pulz). However, we do not know still the doubling time of this culture on plant scale. The decreasing of integrated illumination level below 48 $\mu\text{E}/\text{m}^2/\text{sec}$ led to the decrease of doubling time of isolate 1.2 s.c.(2) (Fig.4).

**Fig. 4.** The dependence of the growth of 1.2 s.c. (2) isolate on the rate of illumination

To understand whether integrated level of illumination of 48 $\mu\text{E}/\text{m}^2/\text{sec}$ is a saturated level, we prepared a curve of culture division rate between 24 and 48 hours versus illumination rate (Fig.5).

Because the character of a curve on Fig. 5 does not seem to be complete “S”, we may assume that illumination rate about 50 $\mu\text{E}/\text{m}^2/\text{sec}$ is not yet saturated level of the illumination. It would be risky to recommend increasing significantly the rate of culture illumination, because we microscopically observed during this experiment that the rate of cyanobacterial adhesion to internal wall of glass tubes was higher in tubes with one layer of window mesh, although biomass yield was smaller. It would be rationally to screen effect of stronger light on both processes of cyanobacterial growth and adhesion. But such experiment needs more power light-emitting facility.

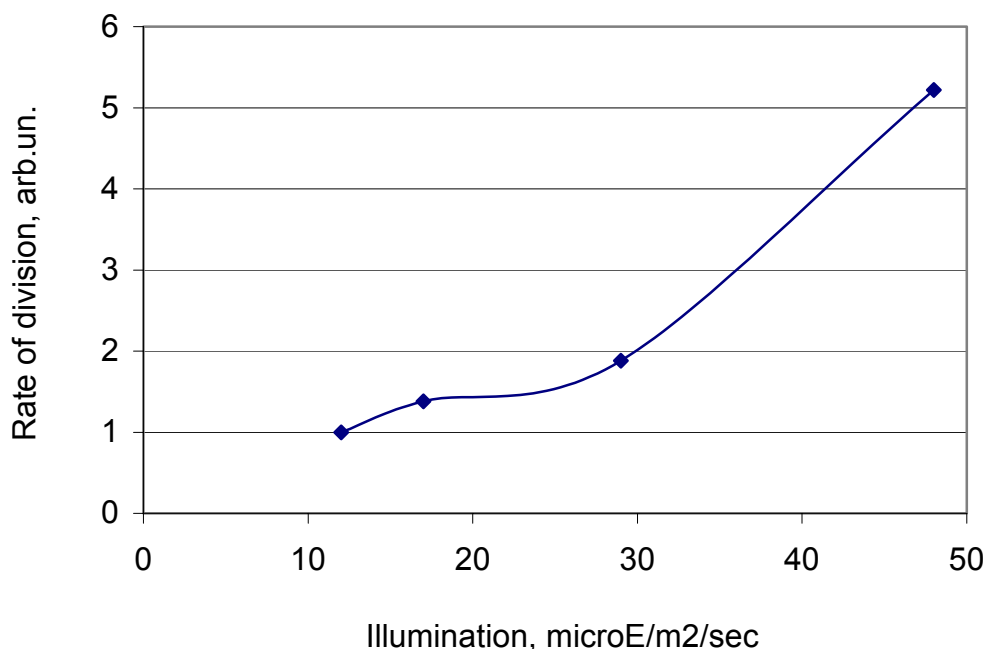


Fig. 5. The dependence of 1.2 s.c. (2) division rate upon illumination rate

Report from the researchers at Ohio University

The organisms TR9.4 and 1.2. S.C.(2) continue to be grown in 5-gallon containers for offline testing. These organisms differ in their morphology with TR9.4 being a unicell and 1.2. S.C.(2) being a chain of cells. As such the 1.2. S.C.(2) organisms tends to form aggregations in large culture but the TR9.4 remains evenly dispersed. Presently, 1.2. S.C.(2) is growing in our 100-gallon culture facility. The growth rate of all cultures is monitored via Chl. a measurements and pH, orthophosphate and nitrogen are also monitored.

The BG-11 medium for both organisms has been modified by adding 10mM CaCl₂ for better growth and adhesion as shown in the Cooksey laboratory experiments (see subtask 1.1.2). A qualitative observation is that the organisms appear to be growing much more vigorously on the walls of the culture facilities.

Experiments on HEPES (inorganic buffer) concentration needed for large-scale culturing were conducted. It was found that using 7.5mM was sufficient in the large cultures to prevent pH drop. This information is useful since HEPES is an expensive chemical for the medium and would increase the overall cost of maintaining the system with these organisms.

CRFII Bioreactor tests

The concern of CRF II test focused on the survival test and growth productivity of microalgae. The microalgae species, membrane materials and growth medium are listed in Table 2.

Table 2 Microalgae species with the relative membrane and growth medium

Microalgae Species	Membrane Material	Growth Medium
<i>Cyanidium</i>	Polyester	Allen
<i>TR 9.4</i>	Omnisil	BG-11
<i>I.2. S.C.(2)</i>	Omnisil	BG-11

Two basic methods are developed for loading microalgae into the CRF II test system: “pre-populated method” and “continuous-population method”.

In the pre-populated test, several processes are critical. (1): Before the test, the microalgae culturing solution in the 30-gallon tank are examined using Chlorophyll-a count, which is used to determine whether the culture are healthy enough to transfer to CRF II test or not. Then the membranes in racks are immersed in the culturing solution for colonizing. Next the membranes will be checked every 12 hours up to the time when the color of the membrane changes observably to green. After the membranes are totally changed to green, we believe that the microalgae have colonized on the membrane. This procedure is meant to establish relatively consistent and stable microalgal coverage on the membranes prior to installing them in the photobioreactor for CRF II test. (2): After the membranes are colorized, they will be installed in the photobioreactor for CRF II test. The photobioreactor has been cleaned up and pre-heating for 12 hours maintaining stable gas level, gas temperature, solution temperature and light intensity. The recirculating solution is growth medium only. Before installing the membranes, digital pictures should be taken for each membrane. (3): After loading, experimental data which should be written down included: gas level, gas temperature, growth medium temperature and pH of growth medium. Also pictures should be taken periodically.

The big difference between “pre-populated test” and “continuous-population test” is the colonizing method. In continuous-population test, the microalgae culturing solution in 30-gallon tank are also examined by Chlorophyll-a count first. And then 20 gallons of microalgae culturing solution are taken and transferred into CRF II test for recirculating. The photobioreactor is also preheated 12 hours maintaining stable gas level, gas temperature and light intensity. Unloaded membranes are then installed inside of photobioreactor. The microalgae will colonize and grow on the membrane during recirculating.

The survival tests are performed on microalgae *Cyanidium* colonized on polyester membrane in 24 hours using the pre-populated method. After 72 hours running in the CRF II test system, the membrane turned white and the microalgae loaded on the membrane were flushed away which can be found on the in-line filter. Fig 6 illustrates the pre-test, during-test and post-test polyester membrane.

A second test was run using microalgae species *TR 9.4* loaded on omnisil membrane, but the culturing time was increased to 48 hours. After running 72 hours, the membrane turned white again, indicating that the organisms did not survive. However, using the continuous-population method, the survival test gives an encouraging result. Microalgae *TR 9.4* are cultivated in the 30-gallons tank and then 10 gallons of culture are transferred into the CRF II test system with adding 10 more gallons of BG-11 growth medium. The blank omnisil membrane is loaded inside

of the CRF test system for colonizing. Within 1 hour some growth was evident, and within 28 hours a very healthy culture was present. The healthy culture persisted through the 7 day test, and Fig. 7 illustrates the colonizing result after 100 hours. Note that the areas of the screen that are not colonized are due to problems with the header pipe design, not the organism or growth surface, and these problems are being addressed for future tests. See section 1.5 for details on the new header pipe design for the combined drip system / harvesting system.



a. Pre-loading Polyester membrane



b. Polyester membrane loaded with Cyanidium inside of CRF II test



c. White polyester membrane after 72 hours' running in the CRF II test

Fig 6: Polyester membrane loaded with Cyanidium using pre-populated method

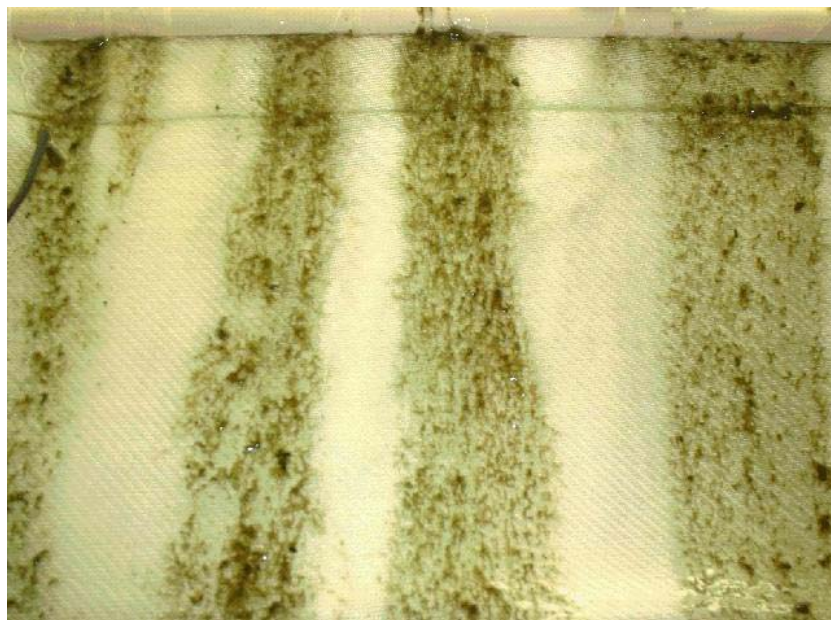


Fig. 7: TR 9.4 colonizing on omnisil membrane using continuous-population method

Conclusion of the survival test focuses on the testing method. In pre-populated method, the microalgae loaded inside of CRF II test system are always flushed away. But in continuous-population method, the microalgae grow well inside of CRF II test system. And next the growth productivity test will be set up.

Some initial testing to establish initial and final organism mass estimates for the CRF II quantitative tests has been completed. For a healthy culture of the Tr9.4 organism growing in the 5 gallon container, a 6-sample test using 50 ml samples showed that the dry mass of a 50 ml

sample was 0.0055 grams \pm 0.0013 grams with 95% confidence. In other words, the 95% confidence interval for a single sample is \pm 24% of the sample mean value, and the 95% confidence interval for the mean value is \pm 0.000531 grams or \pm 9.6% for 6 samples. We will use these results and similar ones for the 1.2. s.c.(2) organism to estimate the number of samples required to get an acceptably low amount of error for the total mass gain estimates in the CRF II test procedure.

Subtask 1.2 Design deep-penetration light delivery subsystem

ORNL has been supporting the pilot-scale bioreactor setup, and has successfully installed a solar collector, fiber optic light cables, and 8 light distribution panels. The “natural” lighting system is ready for the pilot-scale bioreactor system-level debugging tests. Figure 8 shows four of the light distribution panels illuminated by natural sunlight in the pilot-scale bioreactor.

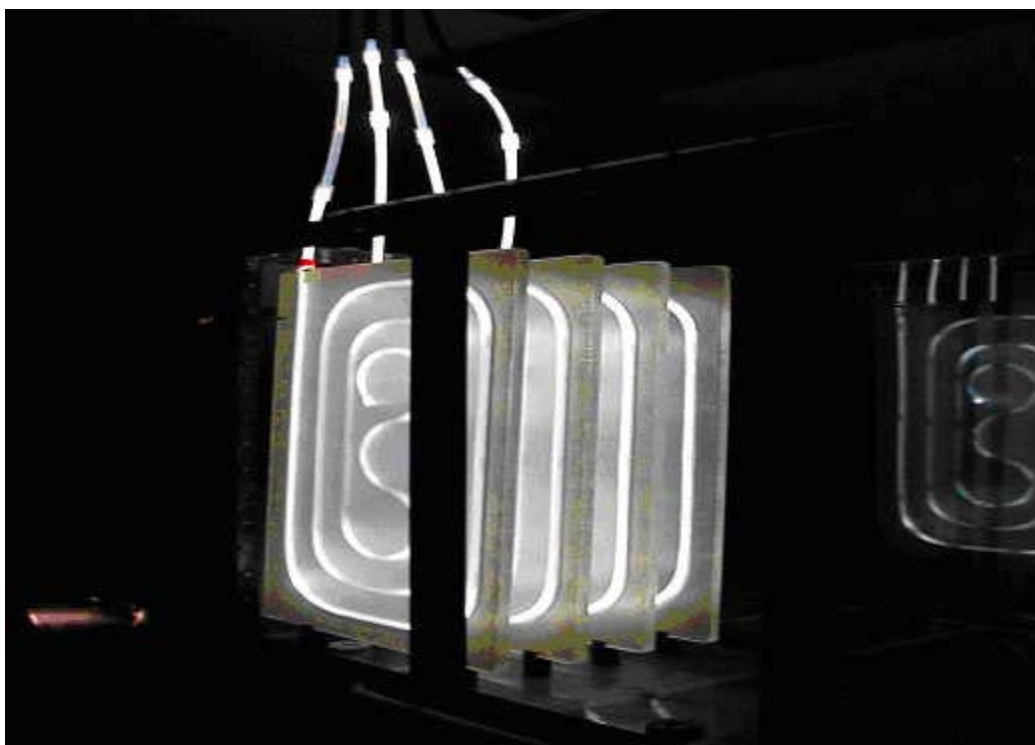


Fig. 8: Light distribution panels in the pilot-scale bioreactor

Subtask 1.3 Investigate growth surface subsystem design

During second quarter researchers at Montana State studied the effect of Ca^{2+} on the growth of 1.2 s.c. (2) isolate and its adhesion to Omnisil. The experiment was carried out with isolate 1.2 s.c. (2) (presumably *Nostoc*) in glass fermenter. All tubes were filled with BG-11 medium, supplemented with 30 mM HEPES. Initial pH in control tubes was 8.06, in tubes with CaCl_2 – 8.02. All tubes were aerated with 5 % CO_2 balanced with air.

We measured chlorophyll concentration and pH daily in all tubes. Fig.9, captured on second day of incubation, shows a dissymmetry in chlorophyll concentration between tubes w/o calcium (1,5,6 from left to right)) and tubes with calcium (2-4 from left to right).



Fig.9. Second day of incubation

Fig. 10, captured on fourth day of incubation, shows that calcium addition (10 mM) in cultivation medium stimulates cyanobacterial cell adhesion to tube walls as well as the generation of big flakes of cyanobacterial cells.



Fig.10. Fourth day of incubation

The growth curves of isolate 1.2 s.c. (2) (Fig.11) suggest that calcium addition has an stimulating effect on the doubling of 1.2 s.c. (2) culture. At this time calcium addition slowed down slightly the alkalization of growth medium (Fig.12).

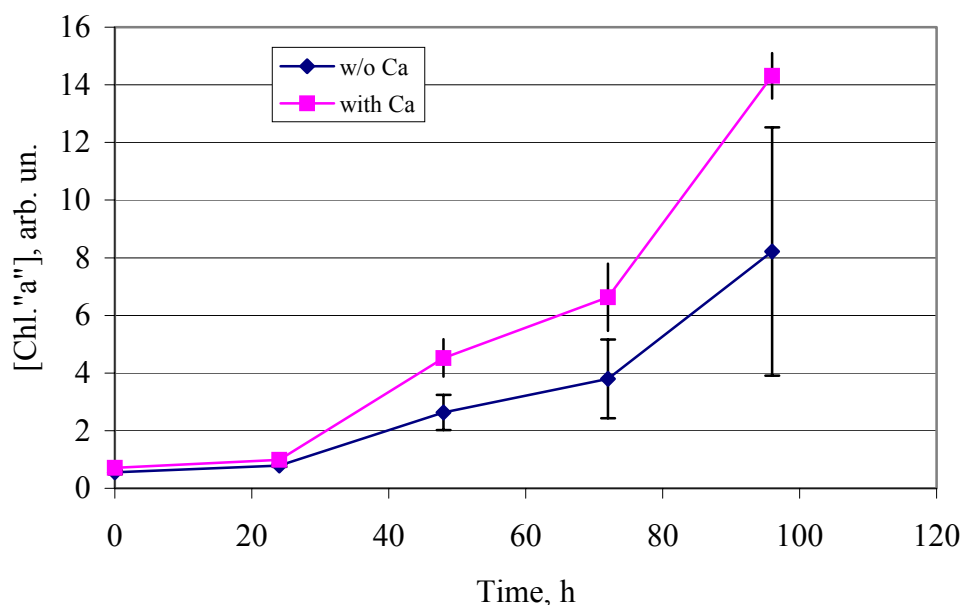


Fig.11. The effect of CaCl_2 on the growth of 1.2 s.c. (2) isolate in BG-11 medium with 30 mM HEPES and aerated with 5 % CO_2

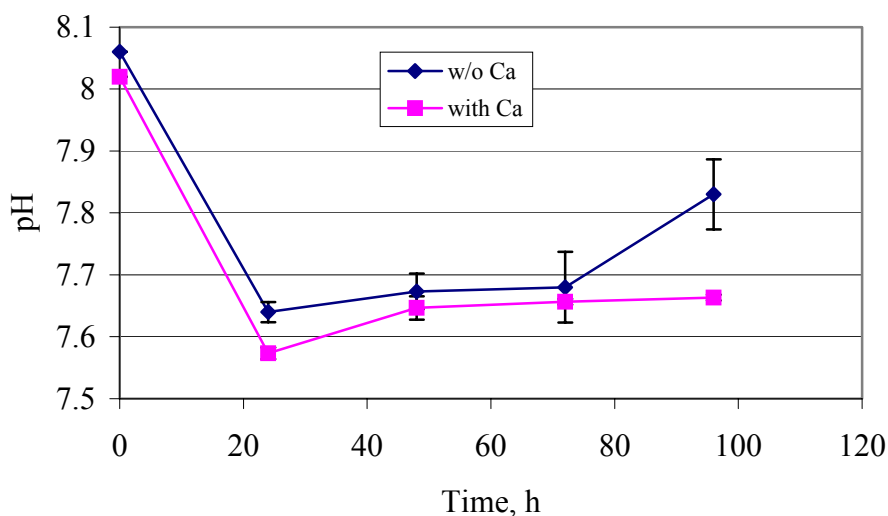


Fig.12. Effect of CaCl_2 on pH dynamics during growth of isolate 1.2 s.c. (2) in BG-11 medium, supplemented with 30 mM HEPES and 5 % CO_2

In the next series of experiments we studied the effect of calcium addition on the adhesion of 1.2 s.c. (2) isolate to Omnisil. The coupons of this substratum were washed and autoclaved in BG-11 medium supplemented with 30 mM HEPES, pH above 8. Coupons were put in tubes with the same medium, but 3 tubes were filled with BG-11 + 30 mM HEPES + 10 mM CaCl_2 .

Fig. 13 shows the shape of coupons at the day of inoculation. Despite the fact that the inoculum was very weak we could observe real colonization of edges of coupons by isolate 1.2 s.c.(2) on next day (Fig.14).



Fig.13. Coupons at the day of inoculation



Fig.14. Coupons at the day after inoculation *Black arrows point out that Omnisil coupon edges are colonized more significantly than central zones of these coupons.*

Fig.15 shows the general view of Omnisil coupons on fourth day of their incubation. Despite the fact that the coupons are colonized very well, the planktonic growth of isolate 1.2 s.c.(2) was not observed (please compare with Fig.10 when incubation was carried out without Omnisil).

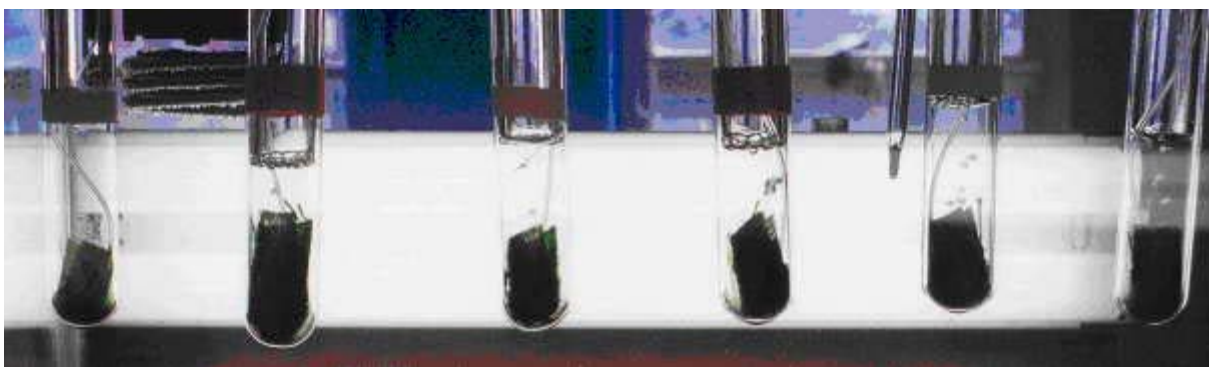


Fig. 15. Coupons at the fourth day after inoculation

Fig. 16 reflects the final yield of cyanobacterial biomass in the tubes without and with calcium. No significant difference between variants without calcium and with calcium was found.

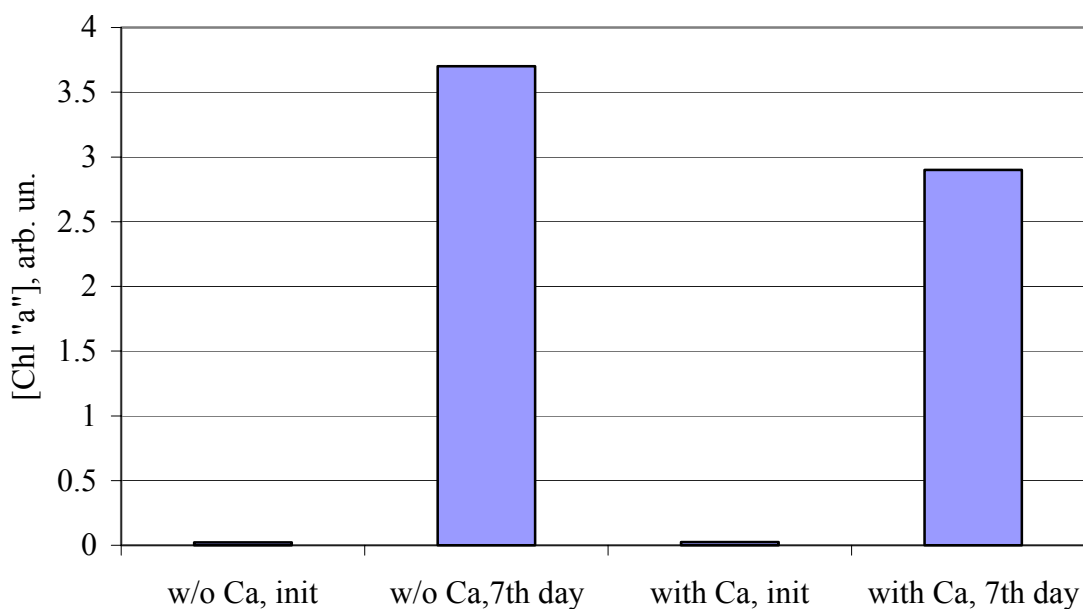


Fig. 16. Total chlorophyll yield of 1.2 s.c.(2) isolate grown in the presence of Omnisil

After removing the coupons from the tubes, the coupon # 1, incubated without calcium and coupon # 6 incubated at the presence of calcium were rinsed 3 times in fresh BG-11 medium with 30 mM HEPES. You can see on Fig. 17 that calcium stimulated the adhesion of cyanobacterial cells to Omnisil.

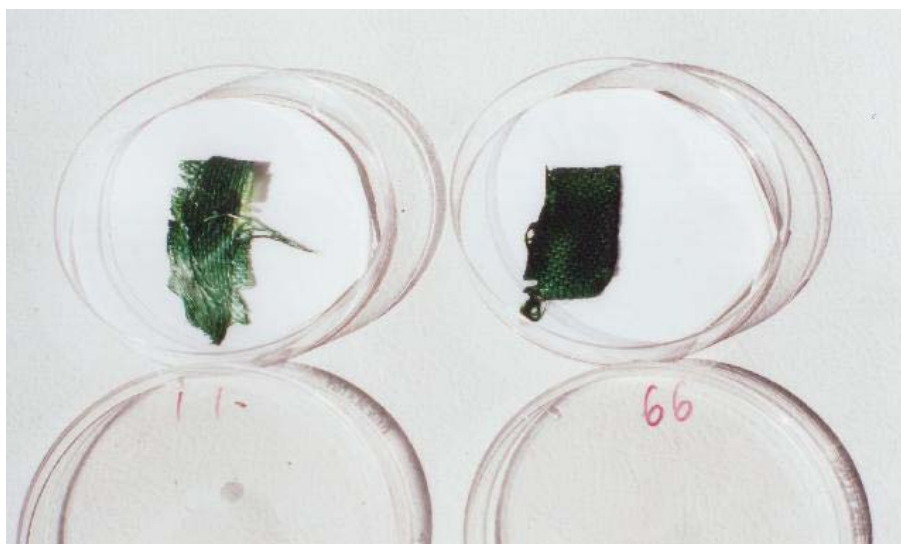


Fig. 17. Coupons showing increased adhesion via calcium addition

Surfaces of coupons # 1 and 6 were studied with help of Scanning Electron Microscopy. Small squares of colonized coupons (approx. 4x4 mm) were cut off and coated with gold. The comparison of figures 18 and 19 suggests that the biofilm generated by isolate 1.2 s.c.(2) on Omnisil surface in BG-11 medium supplemented with 10 mM of CaCl_2 is more thick and dense.

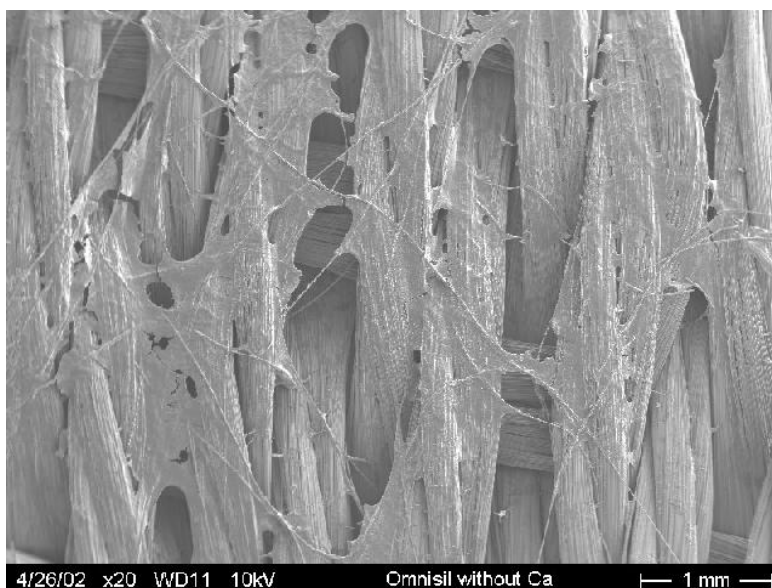


Fig. 18. SEM of attachment without calcium

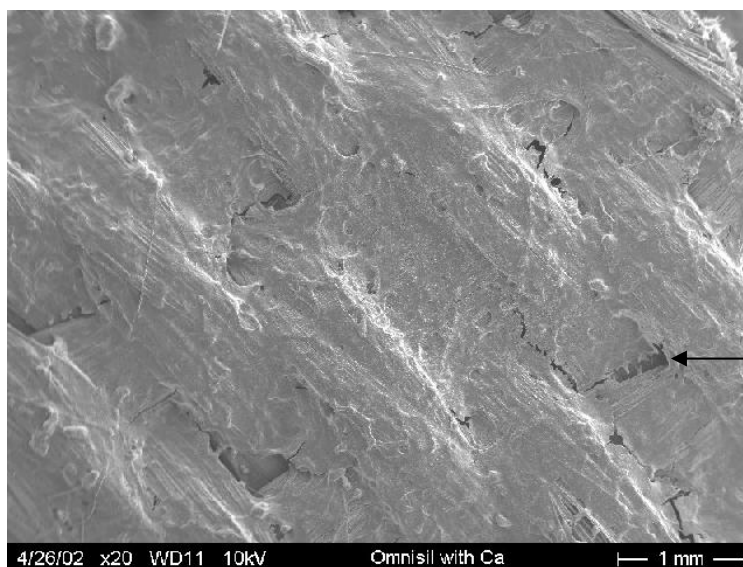


Fig.19. SEM of attachment with calcium *Arrow points out on the knob on biofilm surface.*

We are unable to see bunches of Omnisil fibers on Fig.19, although it can be done very easy on Fig. 18, when isolate was incubated without Ca^{2+} addition.

The further magnification of studying samples up to 400 X (Fig.20 and 21) confirmed the conclusion that calcium addition to the incubation medium increases the density of biofilm generated by isolate 1.2 s.c.(2). It should be noticed that calcium also stimulates the bumpiness of biofilm surface (Fig.21), although the surface of biofilm on Fig.20 seems to be smoother. One single typical bump is shown on Fig. 22.

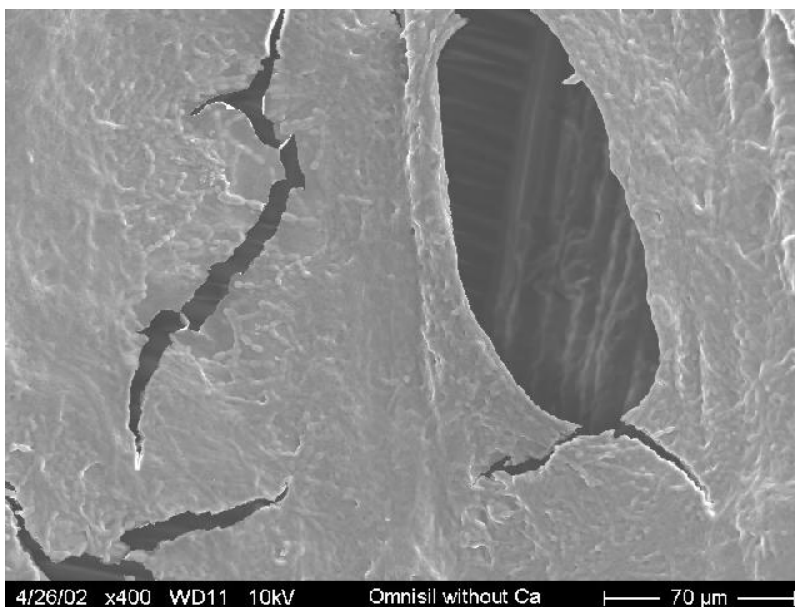


Fig.20. Increased magnification SEM image of biofilm #1

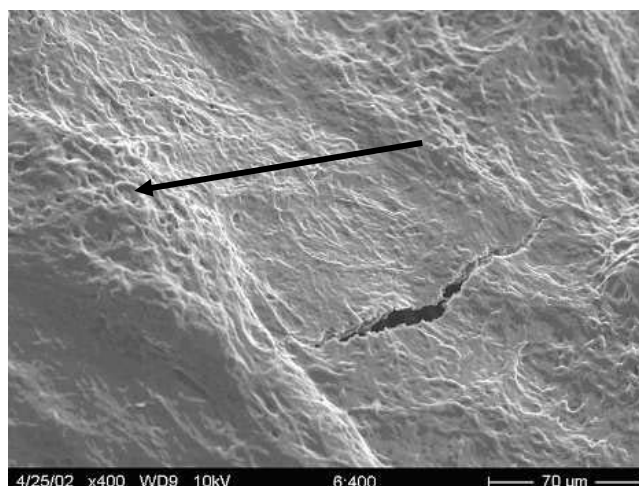


Fig.21. Increased magnification SEM image of biofilm #2 *Black arrow points out on the extracellular polymer substance*

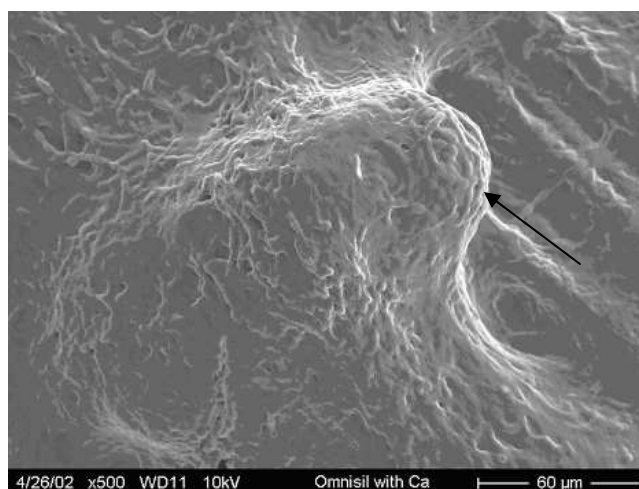


Fig. 22. Isolated biomass “bump”

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

CO₂ scrubbing tests were carried out to understand the CO₂ scrubbing capability of translating slug flow under various conditions. The liquid velocities for the test range from 0.6m/s to 1.0m/s and the gas velocities from 4.8m/s to 9.0m/s. The liquid used in the test is tap water. The gas consists of 15% of CO₂ and 85% of N₂ to simulate the flue gas in the power plant.

1.4.1 Method for CO₂ Scrubbing test

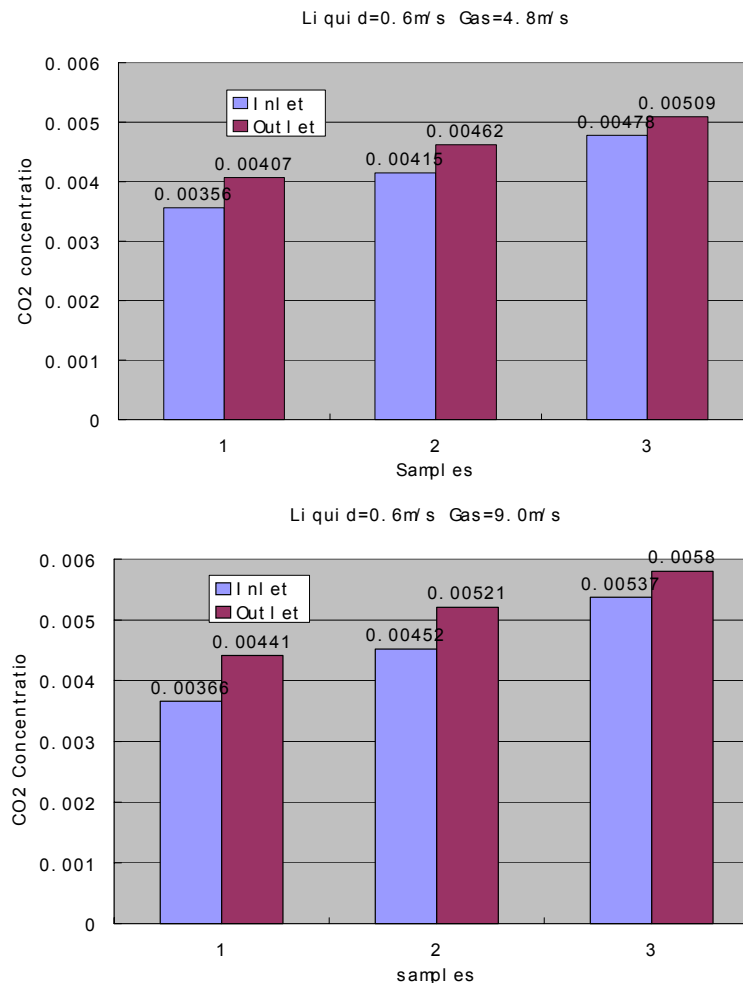
Liquid samples were taken at both the inlet and outlet of the reactor simultaneously. When sampling CO₂ from the pipeline, part of CO₂ will leave the solution due to the decrease of CO₂ partial pressure. In order to prevent sampling process from degassing, a syringe is used to

withdraw the liquid sample from the slug. The sample then is slowly injected into NaOH solution so that the gas phase CO₂ can be totally captured in the solution. Finally, titration method will be conducted to measure the CO₂ concentration in the solution.

1.4.2 Test results

The CO₂ concentrations at several representative test conditions are shown in Figure 23. Figure 24 compares the CO₂ concentration increase at different gas velocities including 4.8, 6.6 and 9m/s. The result shows that when the liquid velocity is 1m/s, the CO₂ concentration increase has no obvious change. We can explain the phenomenon in two ways: one is that the turbulence inside the slug does not change a lot with the increase of gas velocity, so the mass transfer rate does not change with the gas velocity. The second explanation is that the turbulence increase with the gas velocity, however, the residence time of slug decreased, so the overall effect does not change obviously.

Figure 25 shows the CO₂ concentration increase with the gas velocity when liquid velocity is 0.6m/s. The CO₂ concentration increase is less when the gas velocity is 4.8m/s because the turbulence in low gas velocity is not as intense as at higher velocity, causing lower mass transfer rate.



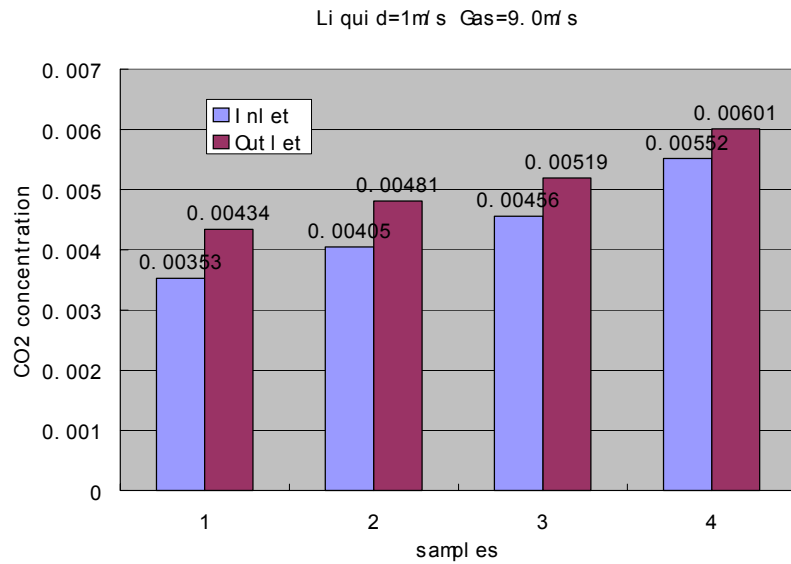
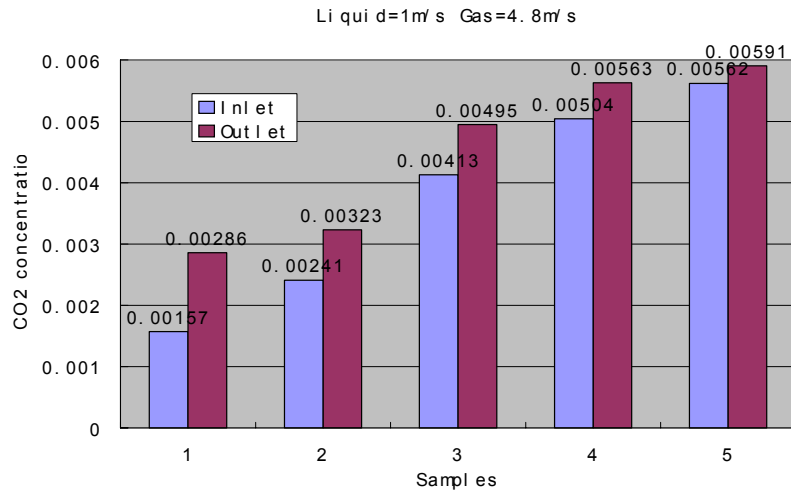


Fig. 23: CO₂ scrubbing test concentrations at several representative test conditions

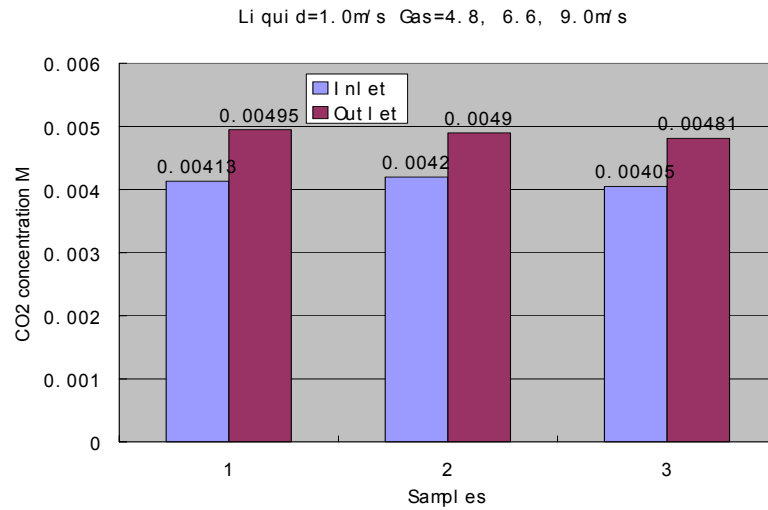


Fig. 24. Comparison of scrubbing results at 4.8 m/s

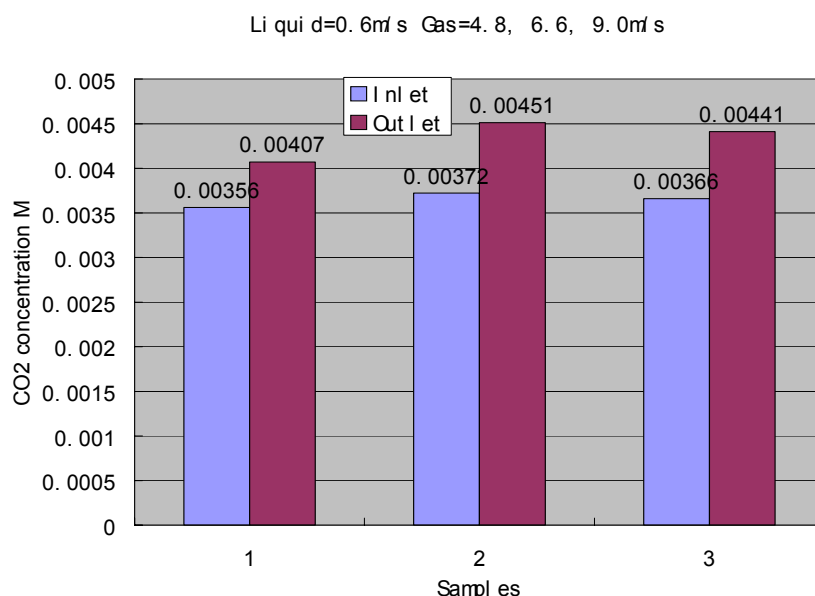


Fig. 25. Comparison of scrubbing results at 0.6 m/s

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 with two candidate screen materials (Omnisil and carbon fiber) and two candidate organisms (Tr9.4 and 1.2. s.c.(2)). The last quarterly report discussed problems we were having harvesting Tr9.4 from Omnisil screens. Further investigation showed that the tests were not completely representative since the screen loading method did not produce a sufficient loading condition to get a useful result. In other words, the loading of the organisms on the screen was so light that no harvesting was necessary, and in order for the tests to be meaningful we needed to have screens on which the organisms were “thick” enough so that harvesting would be desired.

A test with the Tr9.4 organism on a Carbon fiber screen was attempted but yielded no useful result. With the black carbon fiber screen it is difficult to tell if and when the screens are cultured. After 7 days of incubation time by immersion in a culturing vat, the screen was removed and a harvesting test was run, but visually no organisms appeared to be removed from the screen. A post test check of the screen to see if any organisms were left on the screen showed no organisms, so it appears that the Tr9.4 organism does not populate the carbon fiber screens using the immersion culturing method.

The test results with both material types have shown that the method of loading the organisms on the screens by immersion in a culturing vat does not work well for the Tr9.4 organism. Therefore, the harvesting tests were put on hold while an alternative loading method was developed. The “continuous-population method” described in Section 1.1 shows good promise, and harvesting tests are scheduled to resume as soon as the new header pipe design for the

screens is perfected. The work completed to date on the new header pipe design is detailed below.

Combined drip system/harvesting system design

- a. A new screen based on the flow control idea has been built up. Some slight changes had been made to the method of attaching membrane. Instead of needle bar, we use a thin plastic plate to screw the fabric up with the central plate. Please refer to the drawings (Figure 26) for detail.
- b. The first proof-of-concept test was run in the modified off-line chamber on Monday to discover how this flow control design was going to meet our functional requirements. Our main concerns for this design were the uniformity of water distribution and slot clogging problem. And the results from this test seemed promising. The off-line chamber was modified to be able to re-circulate water in a closed loop. One gallon solution of 1.2. S.C.(2) was re-circulating in this system during testing. The test lasted about one hour until no algae remained in the water circulating in the chamber by visual observation. One side of the membrane was found to have more water streams than the other side. Pictures were taken to track the performance of this screen every 30 minutes. We got a pretty good loading effect during this one hour as you can see from Figure 27. We observed evenly spread small water streams on one side throughout the whole test, which showed that the water (solution) was uniformly distributed as we wished. In order to check the clogging of slots, we took out the central plate and found that the slots on one side of the plate with more smooth water flow were not clogged up but some of those on the other side with fewer flow got clogged. The reason more water flowed down one side of the membrane seems to be related to the fact that the slot in the bottom of the header pipe that the screen passes through was too wide, and the side of the material that was in contact with the header pipe had good flow properties while the side separated from the header pipe slot edge by a gap did not perform as well. Additional tests with a reduced slot width have shown excellent results, so the new design should be ready for use after some additional tests to refine the design.

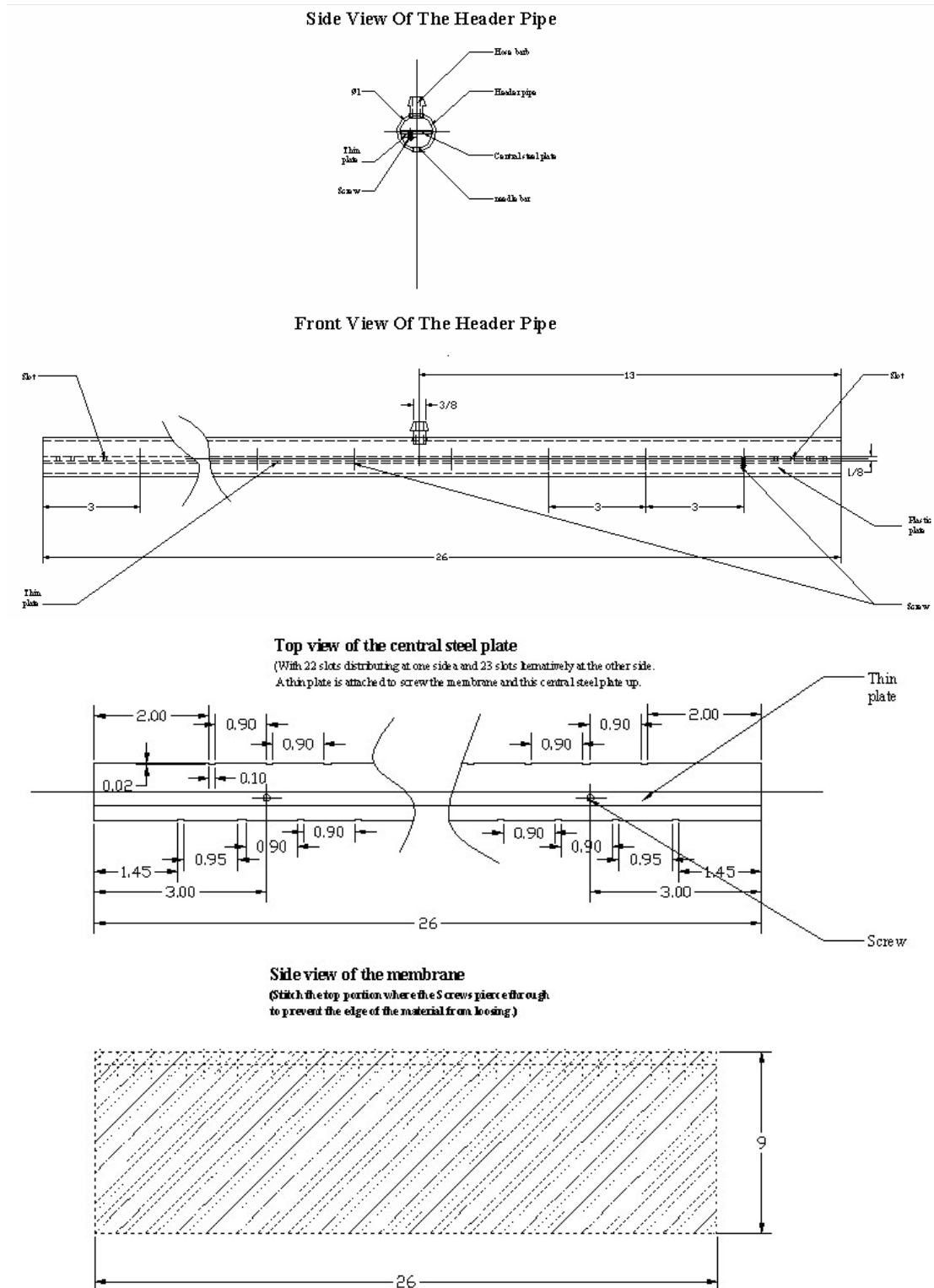


Fig. 26: Drawings of header pipe flow control design



a. Screen at start of loading 1.2. s.c.(2)



b. Observation of flow distribution after re-circulating 1.2. s.c.(2) for 30 mins



c. Screen loading effect after re-circulating 1.2. s.c.(2) for 60 mins

Fig. 27: Drip loading of 1.2. s.c.(2) on Omnisil using the new header pipe design

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

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 CRF II system has completed debugging tests and 7-day survivability tests have been run successfully. The up-flow bioreactor has been designed but is temporarily on hold awaiting the new header-pipe design for the combined drip system / harvesting system. 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 phase 1 construction of the bioreactor is complete, and the solar collector and light distribution system (including 8 light distribution panels in the bioreactor chamber) have been

installed and are functioning correctly. A week long system-level debugging test is planned in early July.

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 second quarter of 2002 include:

Organisms and Growth Surfaces:

- Our collection of cyanobacteria, isolated in YNP was increased to 15 unialgal cultures.
- Illumination rate about 50 $\mu\text{E}/\text{m}^2/\text{sec}$ is not saturated for the growth of 1.2 s.c. (2) isolate. The decrease of illumination rate led to the decrease of doubling time of this isolate.
- The positive effect of Ca^{2+} on the growth of isolate 1.2 s.c. (2) without Omnisil was revealed, though Ca^{2+} addition was indifferent for the growth of this isolate at the presence of Omnisil.
- Calcium addition had a positive effect on the generation of cyanobacterial biofilm on Omnisil surface.
- The survivability problems with the Tr9.4 organism on Omnisil screens in the CRF2 model-scale bioreactor have been solved. The problems were related to the method used to populate the growth surfaces. When pre-populated screens were placed in the bioreactor the microalgae died within 72 hours, but when the microalgae were cultured while in place in the bioreactor using a continuous-population method they grew well inside of the CRF2 test system and survived for the full 7-day test duration. CRF2 tests will continue as soon as the new combined drip system / harvesting system header pipe design is refined.

Bioreactor support systems and test facilities:

- A solar collector, fiber optic light cables, and 8 light distribution panels have been installed at the pilot-scale bioreactor site. The “natural” lighting system is ready for the pilot-scale bioreactor system-level debugging tests scheduled for early to mid July.
- CO_2 scrubbing tests were carried out to understand the CO_2 scrubbing capability of translating slug flow under various conditions. Within experimental error, for liquid velocities of 0.6 m/s and 1m/s it was shown that different gas velocities (including 4.8, 6.6 and 9m/s) cause no significant change in CO_2 concentration.
- The harvesting tests were put on hold while an alternative loading method was developed and to concentrate our resources on the design of the new combined drip system / harvesting system header pipe. A new header pipe design has been completed and proof-of-concept tests have shown good performance in the drip loading mode. Tests have also shown that we can create preferable conditions for harvesting with this design, and actual harvesting tests will be run as soon as screens are available that have achieved a sufficiently “thick” growth state such that harvesting is desired.

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