TESTING AN ALGAE-BASED AIR-REGENERATION SYSTEM (NAGW--4897) TECHNICAL SUMMARY

Introduction

The primary objective of this project was to evaluate the potential of an air-regeneration system based on the growth of unicellular algae, especially subaerial algae, on the external surface of hollow ceramic tubes. It was thought that subaerial algae, because they are exposed to a variety of environmental stresses in their natural environment, would provide a stable base for a bioregenerative system and reduce some of the stringency in the requirements of the electrical and mechanical support systems. The ceramic tubes would both mimic the algae's normal habitat (the surfaces of wood and porous rocks) and provide a means of containing the growth medium in microgravity. Because this is a novel system, most of our efforts were directed toward determining the major parameters governing the growth of the algae on the ceramic tubes using a simple test-bed developed during the first year of the project.

Summary of materials and methods.

Organisms. Six strains of unicellular algae were chosen for evaluation in the system: *Chlorella vulgaris* Beijerinck (UTEX 259), *Chlorella fusca* var. *vacuolata* Shihara et Krauss (UTEX 251), *Chlorococcum scabellum* Deason et Bold (UTEX 1233), *Neospongiococcum punctatum* (Arce & Bold) Deason (UTEX 786), *Stichococcus* sp. (VSU 105), and a cyanobacterium tentatively identified as a member of the genus *Gloeocapsa* (VSU 104); the last two were isolated from subaerial environments near Valdosta--these strains were chosen for their availability and ease of use in the system. Stock cultures were maintained in unialgal, not axenic, condition on agar slants.

Growth conditions. The algae were first suspended in liquid medium and then painted onto the upper surface of ceramic tubes with a nominal pore size of 0.3 to 0.5 μm. A minimal salts medium (BBM) was circulated through the tubes for about 30 minutes a day. Unless noted otherwise, the tubes were incubated in groups of 4 in polypropylene boxes (550 ml) under continuous light with a photosynthetic photon flux of 35 or 50 $\mu mol\ m^{-2}\ s^{-1}$.

Growth rate and CO_2 uptake. Growth of the algae was monitored visually and through periodic measurements of the rate of photosynthetic CO_2 uptake. For the latter measurements, the test unit was attached to a polycarbonate air reservoir with a volume of 6800 ml and an infrared gas analyzer (LI-6252, LI-COR, Inc.) operating in absolute mode. Ambient air, with a CO_2 concentration between 400 and 550 μmoles/mole, was circulated through the reservoir, test unit, and analyzer by means of a separate flow control unit (LI-670, LI-COR, Inc.) for 5 minutes to purge the system. After the system had equilibrated, the loop was closed. The CO_2 content of the air was recorded at one minute intervals for fifteen minutes. The rate of change of CO_2 in the system was then determined from these readings using a least-squares fit of the data. At the end of a trial, the algae were rinsed from the tubes using distilled water and a brush. Aliquots of the harvest were counted with a hemocytometer or filtered for dry weight, protein, and chlorophyll determinations. Protein was extracted with hot sodium hydroxide, chlorophyll with DMSO.

Light relations. Four trials, involving *Chlorella vulgaris*, *Neospongiococcum punctatum*, *Stichococcus* strain 105, and *Gloeocapsa* strain 104, were conducted to determine the relationship between light and CO_2 uptake in this system. The test units containing the algae were periodically attached to the CO_2 analyzer. The irradiance was varied by changing the

distance between the light source and the test unit. The resultant irradiance was measured using a quantum sensor (LI-190SZ, LI-COR, Inc.) located in a similar box at the same distance from the light. The box was purged with ambient air, then sealed and CO_2 uptake monitored for 15 minutes as before. The system was purged with air between measurements to bring the CO_2 concentration back to ambient levels.

CO_2 relations. Two trials, involving *Chlorella vulgaris* and *Gloeocapsa* strain 104, were conducted to get indication of how increased CO_2 concentrations would effect the efficiency of the system. For these trials, eight ceramic tubes were incubated in large (6800 ml) polycarbonate boxes. These were periodically connected to the CO_2 analyzer. The box-analyzer system was then purged with air with a CO_2 concentration of about 3000 ppm . After the CO_2 concentration reached an equilibrium the loop was closed and the change in CO_2 concentration monitored overnight.

Summary of results

Basic patterns of growth. Visible growth first appears on the tubes within 7 days of inoculation. The region covered by algae gradually expands until the entire upper surface, and, in some instances, part of the lower surface, of the tube is covered with a dense growth. The time required to reach complete coverage--between 30 and 60 days--appears to depend on the growth form of the alga--*Chlorella vulgaris*, a species that lacks sheaths and does not form packets, covered the surface in a shorter time than *Gloeocapsa* strain 104, possibly as the result of a more even coverage at the time of inoculation. Using a denser inoculum should reduce the time to complete coverage. Changes in the rate of CO_2 uptake during this period generally reflect the visible changes taking place on the tube. It is also possible to detect nutrient limitation from plateaux in uptake-time curves. The actual rate of CO_2 uptake 60 days after inoculation was about 90 $\mu moles^{-2} min^{-1}$ for *Chlorella vulgaris* and *Stichococcus* strain 105 at an irradiance of 66 $\mu mol\ m^{-2}\ s^{-1}$ (PPF), slightly less for *Neospongiococcum punctatum* and *Gloeocapsa* strain 105.

In three instances, the test units were maintained for extended periods. In these cases, although visible changes were minimal, the rate of CO_2 uptake increased for another 100 days, reaching a peak between 150 and 180 days after inoculation. The peak rate was about 180 $\mu moles\ m^{-2}\ min^{-1}$ at an irradiance of 66 $\mu moles\ m^{-2}\ s^{-1}$ (PPF). Apparently, during this stage of development, the mass of algae is increasing in thickness until an equilibrium is reached in which the cells closest to the tubes are dying or becoming dormant because of CO_2 and/or light limitation while new cells are added to the outer layers. Tubes can be maintained in this state indefinitely, as long as the medium is refreshed periodically and contamination from the air is minimized; in our trials fungal contamination caused a decline in net CO_2 uptake after 180 days.

The amount of biomass recovered from the tubes at the end of trial varied greatly and not necessarily in keeping with the length of the trial. For example, in four trials involving *Chlorella vulgaris*, the following dry weights were obtained: 58 gm m^{-2} after 73 days, 134 gm m^{-2} after 97 days, 40 gm m^{-2} after 185 days, and 116 gm m^{-2} after 373 days. The cause of this variation is completely understood. It is interesting to note, however, that four of the five highest values of recoverable dry weight were some of the later trials, after we had begun to circulate air through the boxes actively in response to indications of CO_2 limitation. The amount of protein recovered was generally low, roughly 5 to 10% of dry weight. This can be attributed to either an incomplete extraction or to the poor state of health of the organisms at the time of harvest or to a combination of the two.

CO_2 uptake as a function of incident light. In general, the light/CO_2-uptake curves appear to follow Michaelis-Menten kinetics, with the photosynthetic photon flux used to incubate the organisms somewhat below the light saturation point. There were some differences in the shape of the curves for the different strains, however. We were unable to achieve saturating photon fluxes for the two UTEX strains, while the two subaerial strains developed in my lab were saturated at fluxes below 150 μmoles m^{-2} s^{-1} (PPF); the half-saturation constant in these two case was below 50 μmoles m^{-2} s^{-1}. This low saturation point could have a significant impact on the design of a light-delivery system for use in a spacecraft. The highest rate of CO_2 uptake recorded in this series was 200 μmoles m^{-2} min^{-1}, at an irradiance of 225 μmoles m^{-2} s^{-1} (PPF), for *Chlorella vulgaris*, about 325 days after inoculation.

CO_2 uptake as a function of CO_2 concentration. Both strains tested appeared to follow the same Michaelis-Menten kinetics: CO_2 saturation occurs between 1000 and 1500 ppm, and the half saturation constant is between 200 and 250 ppm. These results are in keeping with the fact that these algae use a typical C_3 mechanism to fix carbon dioxide. They also indicate that while some improvement in the efficiency of the system can be expected with increased concentrations of CO_2, the amount of improvement is limited.

Conclusions

At this point we have concluded our basic tests of the system. As expected, the system is fairly robust and can be kept operational over long periods with minimal care. However, two major drawbacks of the system can be identified. First, the time taken to reach peak rates of uptake is too long. The length of time is somewhat compensated for by the fact that the system can be maintained in an operational mode for over 300 days, but is still unacceptable. This problem can be addressed in future work in three ways. First, the initial inoculum can be increased. We used a small amount of a liquid suspension in order to standardize our procedures and allow for comparisons to be made between separate trials. In an operational system, a larger inoculum directly from a solid medium, possibly another tube, would be more likely. Second, the photon flux could be increased to more closely coincide with the light saturation point through the use of a better lighting system. Third, the CO_2 concentration in the chamber could be increased to 1500 ppm, higher than the saturation point, but lower than is commonly found in spacecraft. The second drawback is that the rate of CO_2 uptake is too low for a compact system. At the maximal rates recorded, between 75 and 100 square meters of tubes would be required to meet the needs of each member of the crew. Given a good light distribution system, it should be possible to pack this area of tubes into three to five cubic meters of volume, but this is still large when compared with existing physico-chemical systems. Again, increased lighting, increased CO_2 concentrations and, possibly, better choice of organisms may reduce the volume. It should also be remembered that the major advantage of bioregenerative systems is that they can perform many functions at once: carbon dioxide removal, oxygen production, waste water treatment, nutrient recycling, and possibly even food production.

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PRELIMINARY DEVELOPMENT AND EVALUATION OF AN ALGAE-BASED AIR REGENERATION SYSTEM

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ABSTRACT

The potential of air-regeneration system based on the growth of microalgae on the surface of porous ceramic tubes is evaluated. The algae have been maintained in the system for extended periods, up to 360 days. Preliminary measurements of the photosynthetic capacity have been made for *Chlorella vulgaris* (UTEX 259), *Neospongiococcum punctatum* (UTEX 786), *Stichococcus* sp., and *Gloeocapsa* sp. have also been made. Under standard test conditions (photosynthetic photon flux ~ 66 μmol m^{-2} s^{-1}, initial CO_2 concentration ~450 μmol mol^{-1}), mature tubes remove up to 0.2 μmoles of CO_2 per tube per minute. The rate of removal increases with photon flux up to at least 225 μmol m^{-2} s^{-1} (PPF); peak rates of 0.35 μmoles of CO_2 per tube per minute have been achieved with *Chlorella vulgaris*. These rates correspond to between 120 and 210 μmoles of CO_2 removed per square meter of projected area per minute.

KEY WORDS: air-regeneration, microporous tubes, microalgae, *Chlorella*

CONTENT SENTENCE: Preliminary results from an air-regeneration system based on the growth of unicellular algae on ceramic tubes are presented.

INTRODUCTION

Current life-support systems on American space shuttles and on the Russian space station *Mir* are based on the consumption of expendable supplies. While such systems are adequate for missions in the vicinity of Earth, plans for long-term manned missions outside of Earth's orbit require the development of regenerative life-support systems that would allow significant recycling of the spacecraft's air and water supplies (4). A number of chemical, physical, and biological systems are currently being developed to meet this requirement. The system under consideration here is an air regeneration system based on the growth of subaerial algae on the surface of microporous ceramic tubes.

The subaerial algae are a phylogenetically diverse group of microorganisms defined by their presence on exposed surfaces above the soil line (5). This group seems to be particular well-suited to for space-based bioregenerative life-support systems. First, they do not need to be submerged in a liquid medium, but can be grown on porous surfaces. This should reduce the amount of liquid medium required, eliminated problems associated with maintaining a well-mixed culture, and allows for a simple mechanical harvest at the end of the useful life of a culture. In addition, because they are subjected to a variety of environmental conditions in their natural habitat, they are tolerant of inconsistencies in their culture conditions. All true subaerial forms can withstand prolonged periods without water, periods of reduced light, and wide fluctuations in temperature. These features should result in a stable system, able to survive short-term failures in mechanical or electrical support systems with little ill-effect.

The ceramic tube system is an adaptation of a system designed to support the growth of vascular plants in a weightless environment (1). However, while the ceramic tubes provide a direct analog of the algae's natural habitat and the algae grow readily on them, little was known previously concerning the parameters of growth. Therefore, the primary objective of the work presented here was to establish some of these parameters, with particular emphasis on CO_2 uptake as a function of the age of the culture. We also examined CO_2 uptake as a function of incident light with a view toward developing a more compact air-regeneration system.

MATERIALS AND METHODS

Design of the test system. For the purposes of this study a simple test unit was developed. The unit consisted of four ceramic tubes, each 14.5 cm long with an outside diameter of 1.6 cm and a nominal porosity of 0.4 μm, inserted in a polypropylene box with a volume of about 550 ml. The completed test unit was sterilized by autoclaving. The tubes were then connected to a reservoir containing 0.5 liters of liquid medium (Bold's Basal Medium, a minimal salts medium common in phycological studies). In a typical trial, a suspension of algae, containing 5 to 10 million cells ml^{-1}, was painted onto the upper surface of the tubes using sterile cotton swabs. Approximately 0.2 to 0.3 ml of suspension were added to each tube, giving newly inoculated surfaces a faint greenish tinge. It should be noted that the tubes had be dry prior to inoculation; the algae did not adhere to wet tubes. After inoculation the liquid medium was pumped through the tubes for 30 minutes. The tubes are incubated under fluorescent lights with a photosynthetic photon flux of 35 to 50 μmoles m^{-2} s^{-1}. The medium was circulated through the tubes for 30 minutes each day. Reservoirs were replaced at roughly 30 day intervals. Individual trials took

from 60 to 365 days.

Organisms. Four organisms were chosen for preliminary evaluation: the chlorophytes *Chlorella vulgaris* Beijerinck (UTEX 259), *Neospongiococcum punctatum* (Arce & Bold) Deason (UTEX 786), and *Stichococcus* sp. (VSU 105), and a cyanobacterium tentatively identified as a member of the genus *Gloeocapsa* (VSU 104). *Chlorella vulgaris* is a common unicellular green alga with a long history in research. And while this particular strain was isolated from freshwater (6), conspecifics are often encountered in terrestrial and subaerial habitats (2). *N. punctatum,* also known as *Deasonia punctata* (Arce & Bold) Ettl & Komarek (2), is a soil alga selected for its ease of use in the system. The strains of *Stichococcus* and *Gloeocapsa* used are true subaerial forms isolated from a brick wall in southeastern Georgia. Stock cultures are maintained on agar slants in a unialgal, but not axenic, condition.

CO_2 uptake. To measure photosynthetic CO_2 uptake, the test unit was attached to a polycarbonate air reservoir with a volume of 6800 ml and an infrared gas analyzer (LI-6252, LI-COR, Inc.) operating in absolute mode. Ambient air, with a CO_2 concentration between 400 and 550 μmoles/mole, was circulated through the reservoir, culture box, and analyzer by means of a separate flow control unit (LI-670, LI-COR, Inc.) for 5 minutes to purge the system. After the system had equilibrated, the loop was closed. The CO_2 content of the air was recorded at one minute intervals for fifteen minutes. The rate of change of CO_2 in the system was then determined from these readings using a least-squares fit of the data.

Irradiance was varied by changing the distance between the light source and the unit. The resultant irradiance was measured using a quantum sensor (LI-190SZ, LI-COR, Inc.) located in a similar box at the same distance. The system was purged with air between measurements to

bring the CO_2 concentration back to ambient levels.

RESULTS

Patterns of growth. Visible growth first appeared on the tubes within 7 days of inoculation. The region covered by algae gradually expanded until the entire upper surface, and, in some instances, part of the lower surface, of the tube was covered with a dense growth. The time required to reach complete coverage appears to depend on the growth form of the alga. *Chlorella vulgaris*, a species that lacks sheaths and does not form packets, covered the surface in a shorter time than *Gloeocapsa* strain 104, possibly as the result of a more even coverage at the time of inoculation.

Development of the algae on the tubes is reflected the time course of CO_2 uptake (Figure 1). All four strains tested showed a rapid increase in CO_2 uptake over the first 60 days as coverage expands. Note, however, that plateaux in the development can be discerned in *Neospongiococcum punctatum*, *Chlorella vulgaris* (trial 3), and *Stichococcus* strain 105. These were apparently related to nutrient depletion and/or contamination of the medium in the reservoir; replacement of the reservoir with fresh medium led to an additional increase in CO_2 uptake until the next plateau. Equilibrium had not been achieved in any test unit at the end of 60 days. The actual rate of CO_2 uptake 60 days after inoculation was about 0.6 μmoles per minute per unit for *Chlorella vulgaris* and *Stichococcus* strain 105 at an irradiance of 66 μmol m^{-2} s^{-1} (PPF) , slightly less for *Neospongiococcum punctatum* and *Gloeocapsa* strain 104.

In three instances the test units were maintained for extended periods: *Chlorella vulgaris*, trials 1 and 2, and *Gloeocapsa* strain 104, trial 1. CO_2 uptake peaked between 150 and 180 days

after inoculation; the peak rate was about 1.2 μmoles of CO_2 removed per unit per minute at an irradiance of 66 μmoles m^{-2} s^{-1} (PPF). At this point, the tubes were completely covered with a dense growth, so that shading of the cells closest to the tube became a limiting factor. Shortly thereafter, visible fungal contamination was noted in some of the test units; the decline in the rate CO_2 uptake seen after 180 days can be attributed at least in part the fungal contamination. Two of the units, *Chlorella vulgaris* (trial 1), and *Gloeocapsa*, were terminated. The third unit was continued. This unit apparently reached an equilibrium between the two organisms that resulted in a rate of CO_2 uptake about 50% of the peak rate. This equilibrium continued for over 100 days.

CO_2 uptake as a function of incident light. Curves of CO_2 uptake as a function of incident light for selected periods can be found in Figure 2. In all cases the algae are growing below their light saturation point, but the onset of light saturation in the two isolates from Georgia appears to take place at a lower irradiance than is the case for the two UTEX cultures. The highest rate of CO_2 uptake, 1.4 μmoles of CO_2 per minute per unit, was recorded for *Chlorella vulgaris* trial 2 at an irradiance of 225 μmoles m^{-2} s^{-1} (PPF); this value was recorded about 325 days after inoculation, well after the decline noted in the previous section.

DISCUSSION

The system as described above has at least two major drawbacks that must be addressed before it can be considered as a reasonable choice for an air regeneration system. First, the time taken to reach peak rates of uptake is too long. This is partially the result of the small inoculum used in the early trials. We have since changed our methods so that a denser inoculum of algae is

transferred directly from the agar slants to the ceramic tubes. Second, the rate of uptake is too low for a compact system. If we use the projected area of the ceramic tubes as a basis, noting that the inoculated region was only 10.5 cm in length, the 0.6 μmoles of CO_2 removed by the algae per minute is equivalent to a rate of uptake of about 90 μmol m^{-2} min^{-1}. Because humans produce about 16 mmoles of CO_2 per minute, 180 m^2 of tubes would be required for each member of the crew. The situation is somewhat improved if we use the peak rate of uptake--in which case the area of tubes required is reduced to 90 m^2 --but still unacceptable. We are currently working on ways to reduce this number. First, we have begun experimenting with higher concentrations of CO_2 in the air space. The 400-550 μmol mol^{-1} used here, while convenient for preliminary experiments, is below the CO_2 saturation point of most algae (3) and far below the concentrations found in spacecraft (4). Second, we have begun experimenting with LEDs and fiber optic light sources. These should allow us to achieve saturating photon fluxes, thereby increasing the rate of CO_2 uptake significantly. The small volume of these light sources should also allow us to design a compact integrated system of lights and algal tubes. Once these improvements are completely implemented a more reasonable estimate of the size of the final air-regeneration unit can be made.

ACKNOWLEDGMENTS

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FIGURE LEGENDS

Figure 1. CO_2 uptake per unit as a function of days after inoculation. The light regime was fixed between 60 and 66 $\mu mol\ m^{-2}\ s^{-1}$ (PPF) for all measurements except *Neospongiococcum punctatum*; these measurements were taken with a flux of 50 $\mu mol\ m^{-2}\ s^{-1}$ (PPF). The *Chlorella vulgaris* plot is a composite of three separate trials, labeled series 1, series 2, and series 3.

Figure 2. CO_2 uptake per unit as a function of incident light. Each graph is a composite of measurements taken over a period of three days late in the individual trial. *Chlorella vulgaris* measurements were taken between days 325 and 337, *Gloeocapsa* sp. between days 197 and 204, *Neospongiococcum punctatum* between days 43 and 50, and *Stichococcus* sp. between days 47 and 53. Individual series of measurements for each species are indicated.

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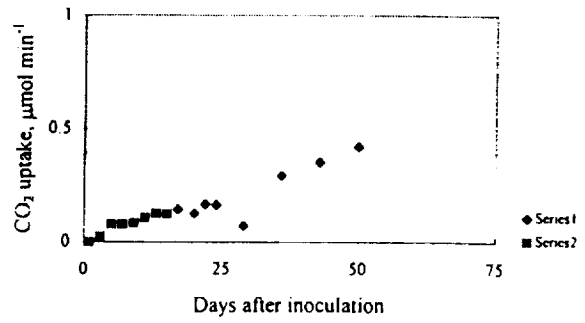
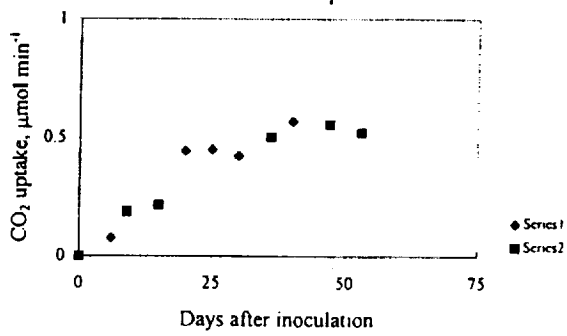
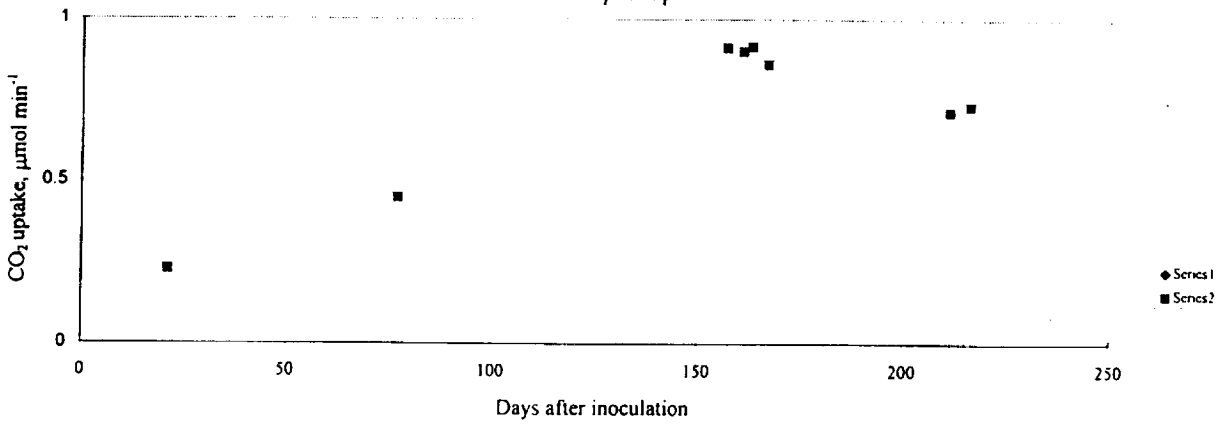


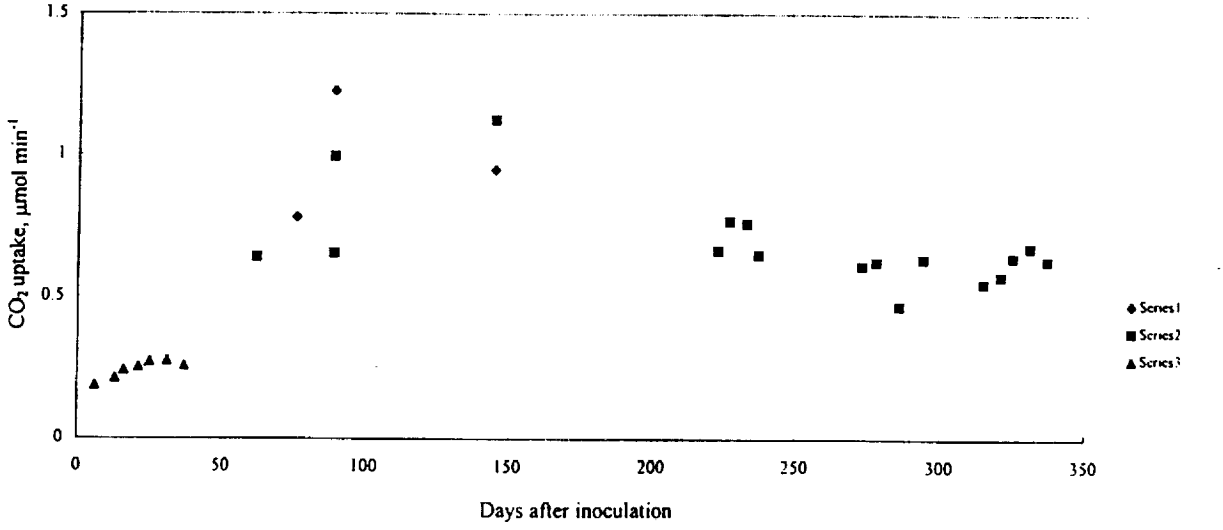
Stichococcus sp.



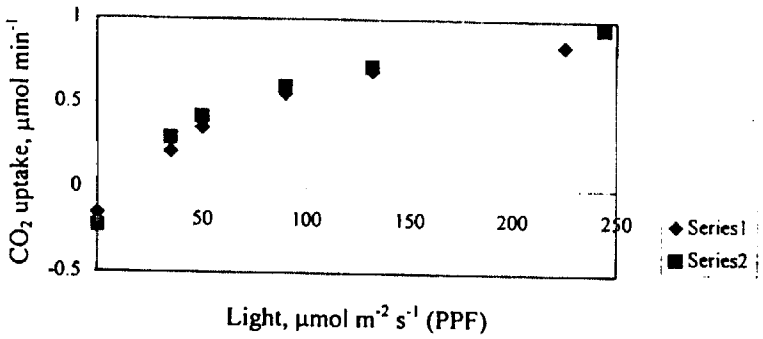
Gloeocapsa sp.



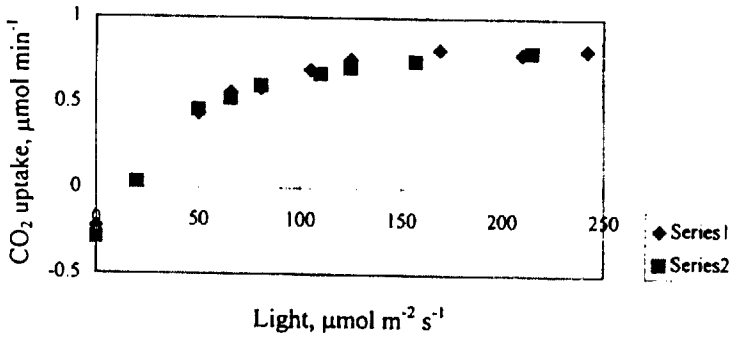
Chlorella vulgaris



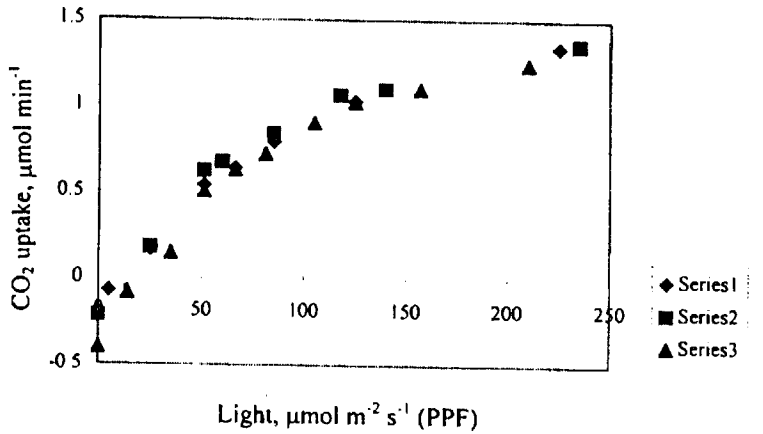
Neospongiococcum punctatum



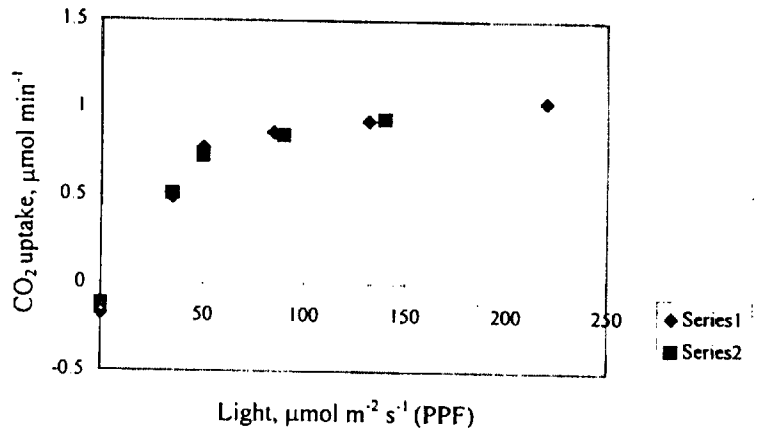
Stichococcus sp.



Chlorella vulgaris



Gloeocapsa sp.



DEVELOPMENT OF AN ALGAE-BASED AIR REGENERATION SYSTEM: CARBON DIOXIDE RESPONSE CURVES

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ABSTRACT

I am developing a air-regeneration system for possible use in spacecraft based on the growth of unicellular subaerial algae on the external surface of hollow ceramic tubes. In its current state of development, a small inoculum of algae is painted onto the surface of ceramic tubes with a nominal pore size of 0.4 μm. The tubes are then incubated under continuous light (photosynthetic photon flux 50 to 60 μmol m^{-2} s^{-1}). Nutrients are provided by circulating a minimal salts medium (BBM) through the tubes for about 30 minutes a day. Under these conditions, visible growth appears about a week after inoculation; complete coverage of the upper surface of the tubes occurs two to three weeks later.

Because this is a novel system, many of the basic parameters governing the growth and photosynthetic capacity of the algae are unknown. Of particular interest at this stage is the relationship between CO_2 concentration and the rate of CO_2 uptake. Two strains were used in this study: *Chlorella vulgaris* (UTEX 259), a eukaryotic chlorophyte, and cf. *Gloeocapsa* (VSU 104), a prokaryotic cyanobacterium. Inoculated tubes were incubated in groups of eight (total inoculated area ~130 cm^2) in polycarbonate boxes (volume ~6900 ml). At roughly weekly intervals the incubation chambers were flooded with air with a CO_2 concentration of 2960 ppm. The boxes were then sealed. Changes in CO_2 concentration were monitored overnight using an infrared gas analyzer.

The measured response of photosynthetic CO_2 uptake to changes in CO_2 concentration can be approximated by a Michaelis-Menton curve of the form

$$P = P_{max}/(1 + K/C).$$

P_{max}, the maximum rate of CO_2 uptake, increased from 0.0 to 1.5 μmol m^{-2} s^{-1} for *Chlorella* and from 0.0 to 1.1 for cf. *Gloeocapsa* over the first three to four weeks. The value of P_{max} for *Chlorella* then began to fluctuate, possibly in response to changes in the nutrient content of the medium. K, the half-saturation constant for CO_2, was relatively stable for each strain, approximately 200 parts per million for *Chlorella*, 250 for cf. *Gloeocapsa*.

Assuming a CO_2 concentration of 4000 ppm and an average human production of 300 micromoles of CO_2 per second, at least 200 square meters of inoculated surface would be needed to sustain each member of the crew. We are currently trying to reduce that number.

INTRODUCTION

NASA is currently developing plans for long-duration manned space-flights to Mars and elsewhere in the Solar System, with manned Mars flights tentatively scheduled for sometime early in the next century. Basic to these plans is the development of reliable low-cost, low-energy air and water regeneration systems that not only meet the normal life-support functions but are also capable of recycling most of the spacecraft's resources (Eckart, 1996; NASA Aerospace Medicine Advisory Committee, 1992). Bioregenerative systems seem to offer the best hope of meeting this requirement. Systems based on the activity of photosynthetic organisms, such as the system under development in my laboratory, have the added advantage of contributing to a partial closure of the carbon loop.

Basic design of the system

The system is based on the growth of subaerial and terrestrial algae on the surface of porous ceramic tubes. The subaerial algae seem especially well-suited for inclusion in advanced life-support systems. They do not need to be submerged in a liquid medium, but can be grown on moist surfaces, thereby reducing the amount of liquid medium required and potential problems with gas exchange and harvest. In addition, they are remarkably tolerant of inconsistencies in their culture conditions and are able to withstand extended periods without water, short periods of reduced light, and wide fluctuations in temperature; this feature could be important in space, where the life-support system could depend on the ability of the photosynthetic component to survive temporary failures in one or more of the mechanical or electrical support systems. The ceramic tubes mimic the algae's natural substratum. In addition, because the nutrient solutions are circulated through the centers of the tubes and only reach the surface by capillary action, the tubes provide a means of controlling the liquid nutrient solutions in microgravity (Dreschel & Sager, 1989).

Objectives of the present study

Previous work demonstrated the basic feasibility of the design. With periodic replacement of the nutrient solution we were able to maintain an active layer of algae for up to one year (Nienow, in press). However, the overall rate of CO_2 uptake was low. We are now trying to optimize the system. Of particular interest was the potential to increase the rate of uptake by

increasing the photosynthetic photon flux and the concentration of CO_2 in the air space. Preliminary results concerning the light response have been published elsewhere (Nienow, in press); here we concentrate on the CO_2 response curve.

In our previous experiments, we used ambient concentrations of CO_2, roughly 400 to 500 parts per million. These concentrations are lower than would be encountered in a spacecraft (NASA Advanced Life Support Program, 1996). It was also assumed that they were below the CO_2 saturation point of the algae, but I am unaware of any studies in which the CO_2 response of algae was measured in a subaerial setting.

MATERIALS AND METHODS

Organisms

Two strains of unicellular algae were used for this set of tests:

UTEX 259--*Chlorella vulgaris* (Figure 1a), a unicellular chlorophyte; although this strain was originally isolated from freshwater, conspecifics are common in subaerial environments.

VSU 104--cf. *Gloeocapsa* sp. (Figure 1b), a unicellular cyanobacterium; this strain was isolated from a brick wall in S. E. Georgia.

These strains were chosen for their availability, ease of use, and ability to grow well on Bold's Basal Medium (BBM). Stock cultures are maintained on BBM solidified with 1.5% agar in unialgal condition.

Inoculation of the ceramic tubes and incubation conditions

A small sample of algae was first suspended in liquid BBM and then painted onto the upper surface of the ceramic tubes. The tubes used in these trials have a diameter of 1.6 cm, a length of 13 cm, 10.5 cm of which is available for inoculation, and a nominal pore size between 0.3 and 0.5 μm. The tubes were incubated in groups of eight in sealed polycarbonate boxes under continuous fluorescent lighting (50 to 60 μmol m^{-2} s^{-1} (PPF)) (Figure 2). Liquid BBM was pumped through each set tubes for 30 minutes each day to allow for nutrient and moisture exchange. Ambient air was circulated through the boxes continuously. Individual trials lasted 2 (*Gloeocapsa*) or 3 (*Chlorella*) months.

Determination of the rate of CO_2 uptake

At one to two week intervals, the box containing the inoculated tubes was flooded with air enriched in carbon dioxide (CO_2 concentration 2960 ppm). After equilibration, the box was sealed and the internal air circulated through an infrared gas analyzer (LI-COR, model LI-6252) operating in absolute mode. The concentration of CO_2 in the air was recorded at one minute intervals, usually overnight. An initial series of measurements was made within 24 hours of inoculation to determine the time constant for leakage from the box (McDermitt *et al.*, 1989). In later series, the average rate of decrease in the concentration of CO_2 was determined for 10-minute intervals centered on selected CO_2 concentrations below 2000 μmol mol^{-1}.

These rates, corrected for leakage, were approximated by a Michaelis-Menton equation of the form

$$P = P_{max} / (1 + K/C)$$

where P_{max} is the maximum rate of CO_2 uptake as a function of CO_2 concentration, K is the half-saturation constant for CO_2, and C is the concentration of CO_2 in the system.

RESULTS

Figure 3. Changes in the concentration of CO_2 in the chamber as a function of time. Examples are given of uncorrected output from individual trials involving *Chlorella vulgaris* and cf. *Gloeocapsa*. In each instance the decline in the CO_2 concentration within the chamber is linear until the absolute concentration falls below 1000 μmol mol^{-1}. This indicates that the photosynthetic uptake of CO_2 is saturated by CO_2 at an early stage. The CO_2 compensation point for both algae is between 25 and 50 μmol mol^{-1}. The trials illustrated were run at 72 days after inoculation for *Chlorella* and 65 days for cf. *Gloeocapsa*; the output from other trials is similar.

Figure 4. CO_2 response curves. Examples are given of CO_2 response curves for *Chlorella* and cf. *Gloeocapsa*. These curves indicate the degree of fit between the corrected rates of CO_2 uptake and the Michaelis-Menton approximation. Most of the deviation from the predicted curves (solid lines) can be attributed to mixing problems in the chamber. Michaelis-Menton parameters for each example are:

Chlorella at 29 days, K = 225 μmol mol^{-1}, P_{max} = 90 μmol (CO_2) m^{-2} min^{-1};

Chlorella at 59 days, K = 225 μmol mol^{-1}, P_{max} = 72 μmol (CO_2) m^{-2} min^{-1};

cf. *Gloeocapsa* at 32 days, K = 375 μmol mol^{-1}, P_{max} = 95 μmol (CO_2) m^{-2} min^{-1};

cf. *Gloeocapsa* at 65 days, K = 225 μmol mol^{-1}, P_{max} = 66 μmol (CO_2) m^{-2} min^{-1}.

The low value of K for cf. *Gloeocapsa* at 65 days is apparently atypical for this strain.

Figure 5. Trends in P_{max} (and K) over time. The figures illustrate the overall trend in P_{max} as the culture ages. In both instances P_{max} increases as the organisms grow to cover the surface of the tube, reaching a maximum of about 100 μmol m^{-2} min^{-1} (1.5 μmol m^{-2} s^{-1}). Under ideal conditions, we would expect the rate of CO_2 uptake to stabilize once the tubes are completely covered, as continued growth at the surface leads to shading of layers next to the tube. Instead, a marked decline can be noted in both systems. This was brought about in each case by a failure of the nutrient delivery system; at one point cf. *Gloeocapsa* was completely desiccated. While unintentional, this demonstrates the ability of the system to recover from such mishaps. This ability is one the advantages it has over other bioregenerative life support systems. Later fluctuations in the value of P_{max} are attributed to nutrient limitations. Contamination by heterotrophic microorganisms may also play a part.

Values of the half-saturation constant, K, for *Chlorella,* not illustrated, rose from 150 μmol mol^{-1} to 225 μmol mol^{-1} over the first month, as the culture established itself. Thereafter, K remained relatively stable between 200 and 225 μmol mol^{-1}. Increases came at 45, 65, and 95 days. The first two of these correspond to sharp declines in P_{max} and may be related. The increase at 95 days may be related to increased contamination.

The values of K for cf. *Gloeocapsa* behaved similarly. However, in this case K began at about 225 μmol mol^{-1} and rose to over 300 μmol mol^{-1}. It declined during the period of recovery after complete desiccation.

Discussion

The overall features of the CO_2 response curves are in keeping with a photosynthetic system based on C_3 metabolism (see Eckart, 1996). Differences in the actual values of the Michaelis-Menton parameters can probably be attributed to differences in growth form. For example, cf. *Gloeocapsa* forms sheaths while *Chlorella* does not. The sheath may impede the diffusion of CO_2 to the cell and, thereby, raise the value of K.

Of more concern to the project at hand are the implications for the design of an air regeneration system. At the peak values of P_{max} recorded, 90 to 100 μmol m^{-2} min^{-1}, and assuming an average human output of 18 millimoles per minute (NASA Aerospace Medicine Advisory Committee, 1992), 180 to 200 square meters of tubes would be required for each member of the crew. However, in this set of experiments, the photon flux was fixed at 60 μmol m^{-2} s^{-1}. My previous measurements (Nienow, in press) indicate that light response curves for *Chlorella* and cf. *Gloeocapsa* in this system can also be approximated by Michaelis-Menton curves, this time with a half-saturation constant of 100 μmol m^{-2} s^{-1} (PPF) for *Chlorella* and 40 μmol m^{-2} s^{-1} (PPF) for cf. *Gloeocapsa*. If we could achieve saturating photon fluxes as well as saturating CO_2 concentrations, the total area of tubes required would be reduced by a factor of 2.7 for *Chlorella* and 1.7 for cf. *Gloeocapsa*. This would still leave the minimal area at 66 square meters. I am currently investigating changes in the nutrient regime as a way to increase efficiency.

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1a. UTEX 259--*Chlorella vulgaris*

1b. VSU 104--cf. *Gloeocapsa*

Figure 1. Micrographs of the test strains.

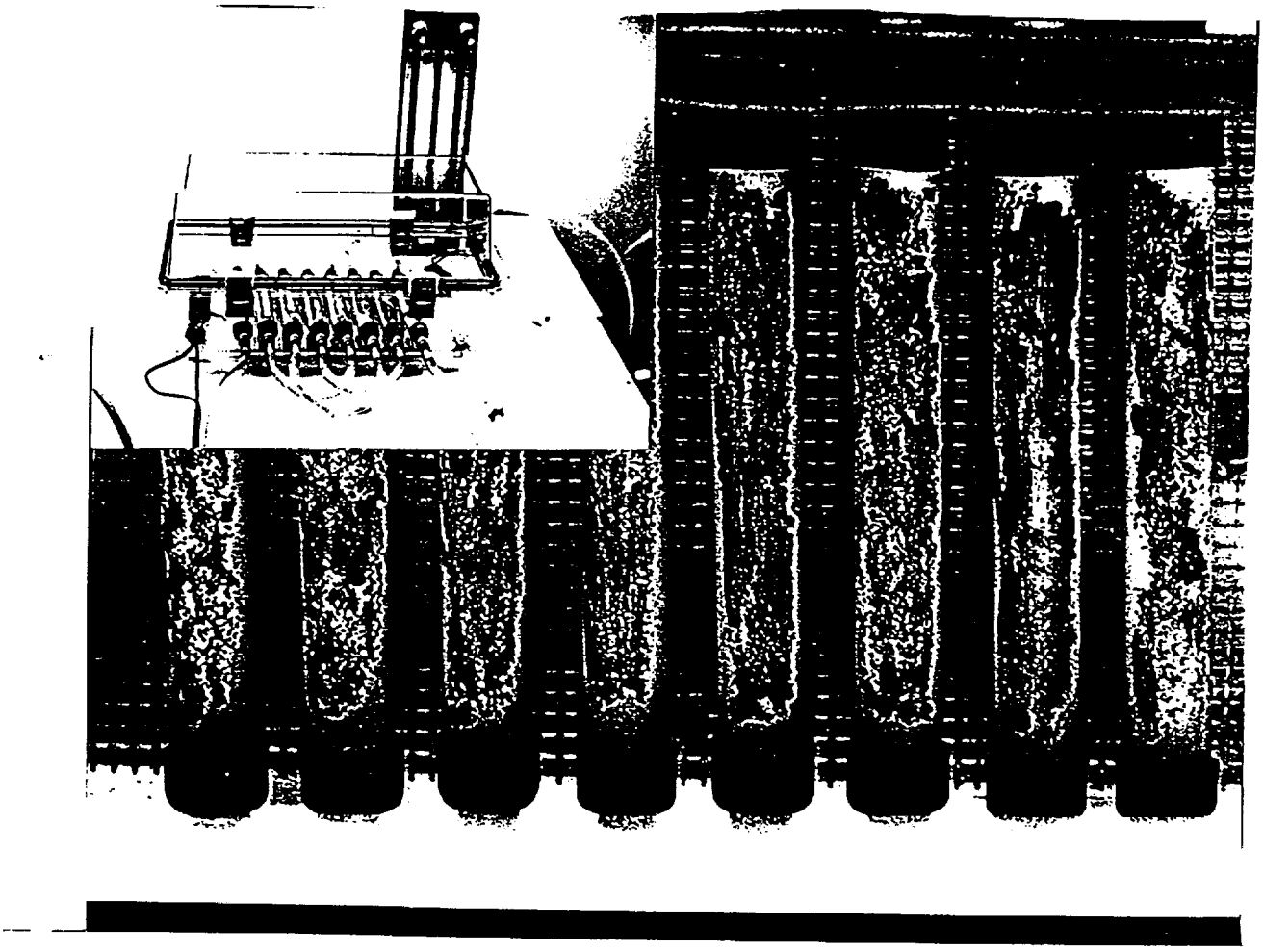
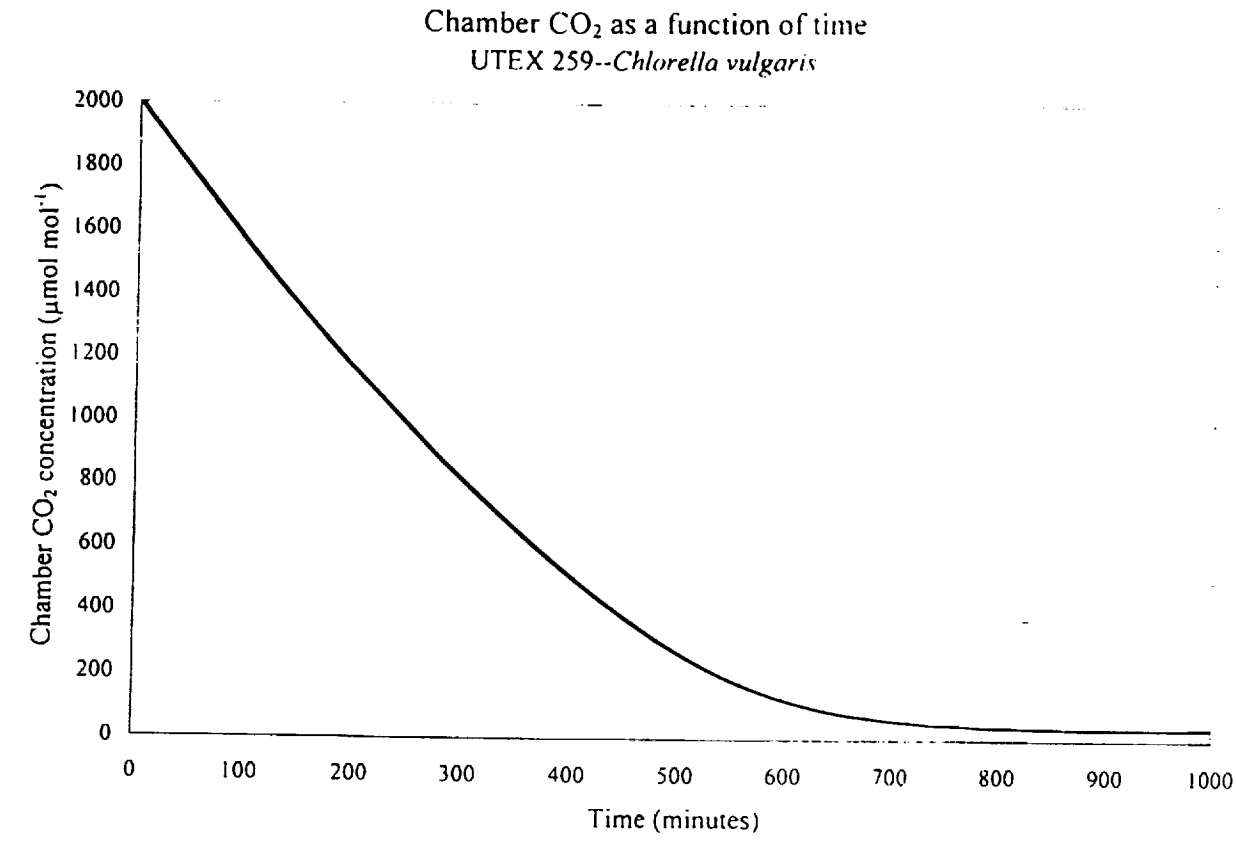


Figure 2. The test chamber. a. Overview of the chamber indicating the arrangement of nutrient lines and air lines. b. Close-up of a set of tubes inoculated with UTEX 259--*Chlorella vulgaris*, twelve days after inoculation.

Figure 3.


Chamber CO_2 as a function of time
UTEX 259--*Chlorella vulgaris*
Chamber CO_2 concentration (µmol mol^{-1})
2000
1800
1600
1400
1200
1000
800
600
400
200
0
0
100
200
300
400
500
600
700
800
900
1000
Time (minutes)

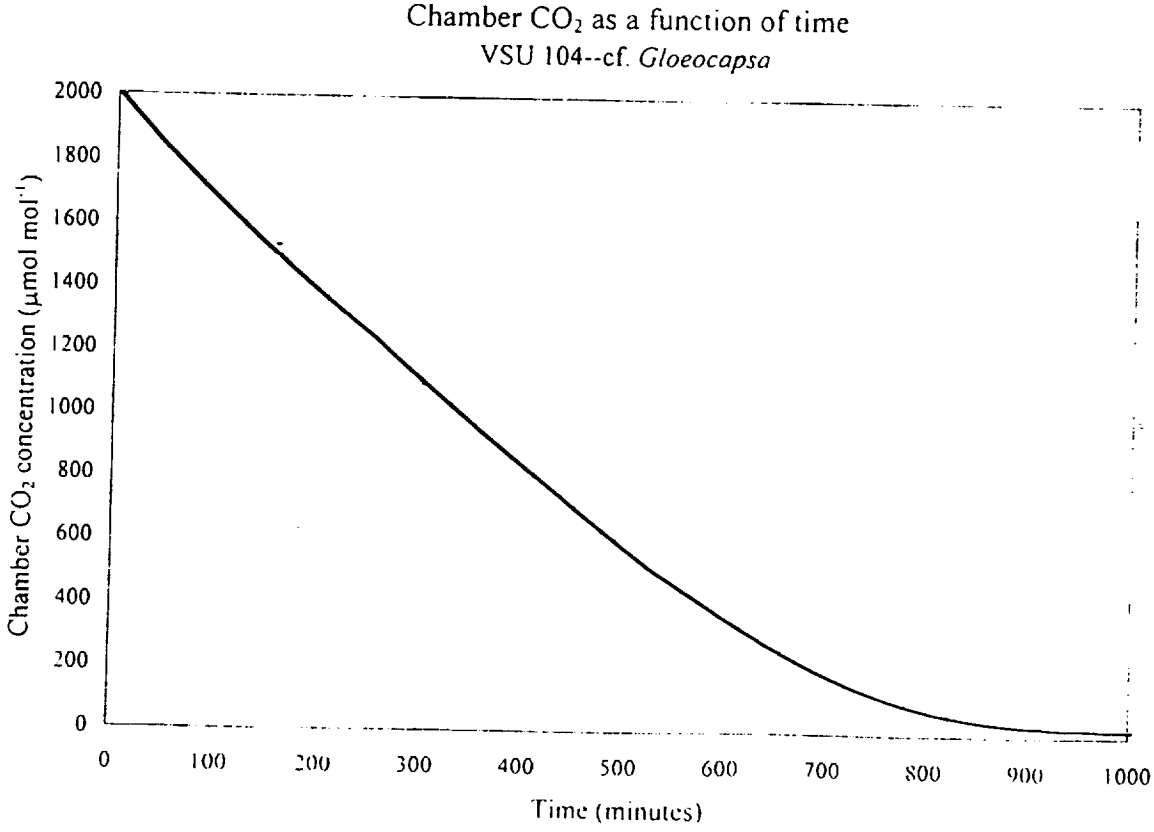
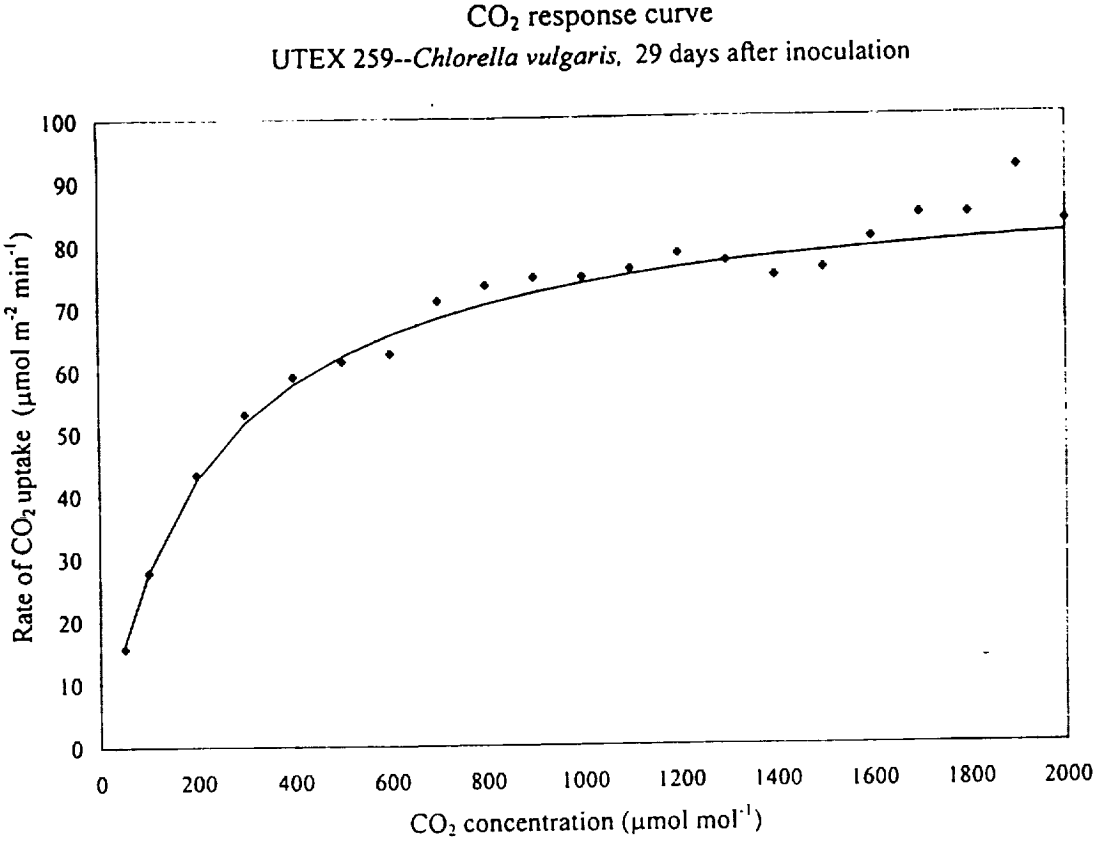

Chamber CO_2 as a function of time
VSU 104--cf. *Gloeocapsa*
Chamber CO_2 concentration (µmol mol^{-1})
2000
1800
1600
1400
1200
1000
800
600
400
200
0
0
100
200
300
400
500
600
700
800
900
1000
Time (minutes)

Figure 4


CO_2 response curve
UTEX 259--*Chlorella vulgaris*, 29 days after inoculation
Rate of CO_2 uptake (μmol m^{-2} min^{-1})
0
10
20
30
40
50
60
70
80
90
100
0
200
400
600
800
1000
1200
1400
1600
1800
2000
CO_2 concentration (μmol mol^{-1})

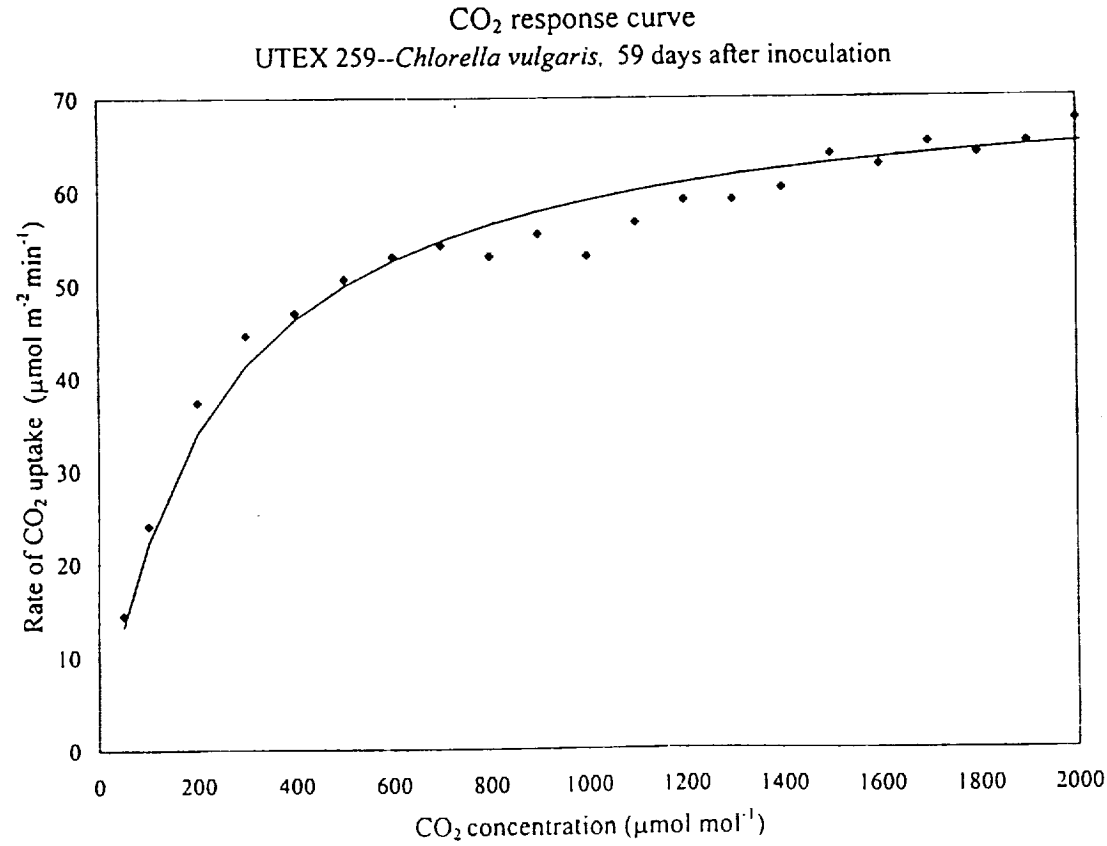
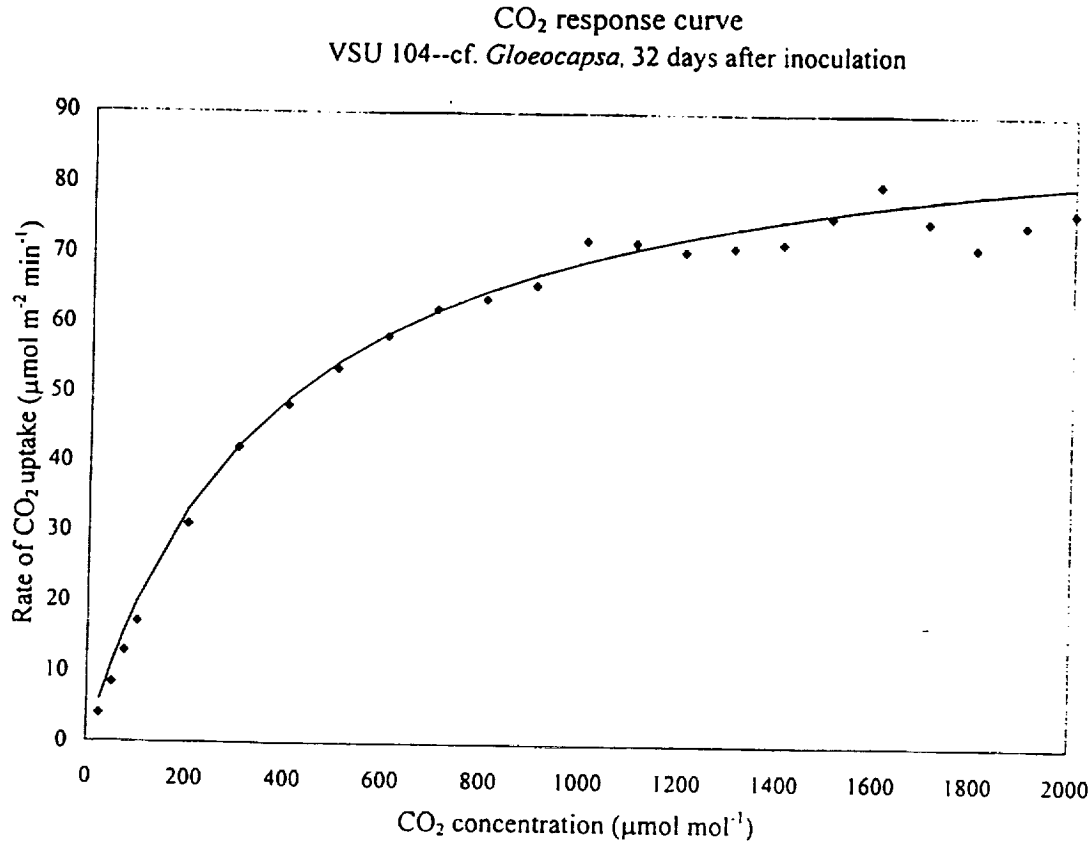

CO_2 response curve
UTEX 259--*Chlorella vulgaris*, 59 days after inoculation
Rate of CO_2 uptake (μmol m^{-2} min^{-1})
0
10
20
30
40
50
60
70
0
200
400
600
800
1000
1200
1400
1600
1800
2000
CO_2 concentration (μmol mol^{-1})

Figure 4 (continued).



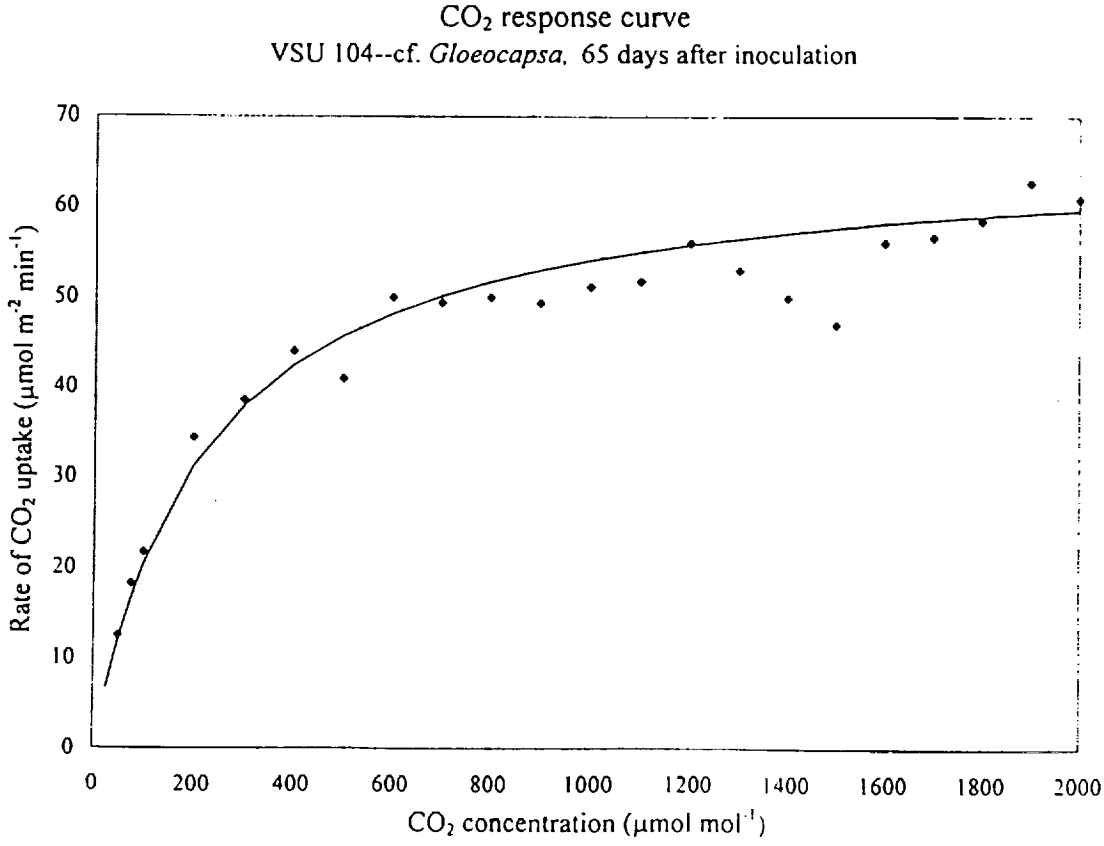
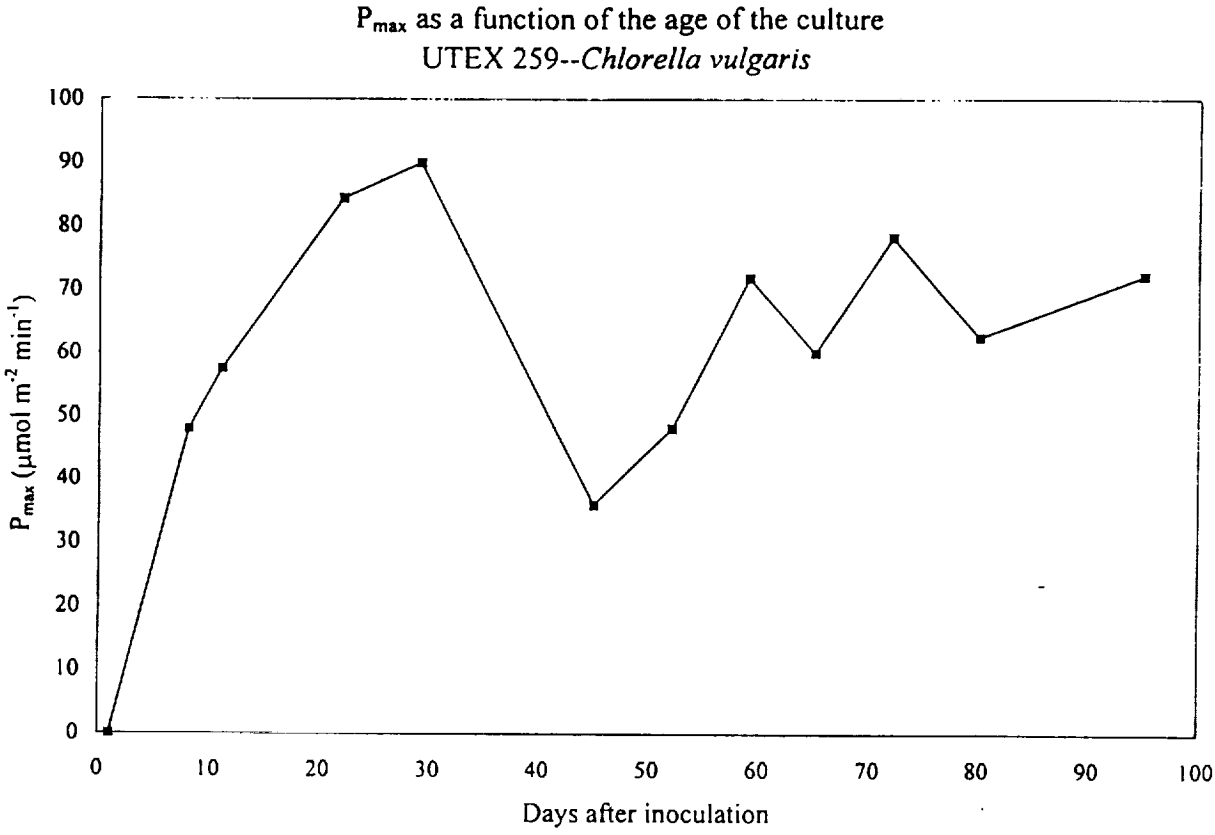
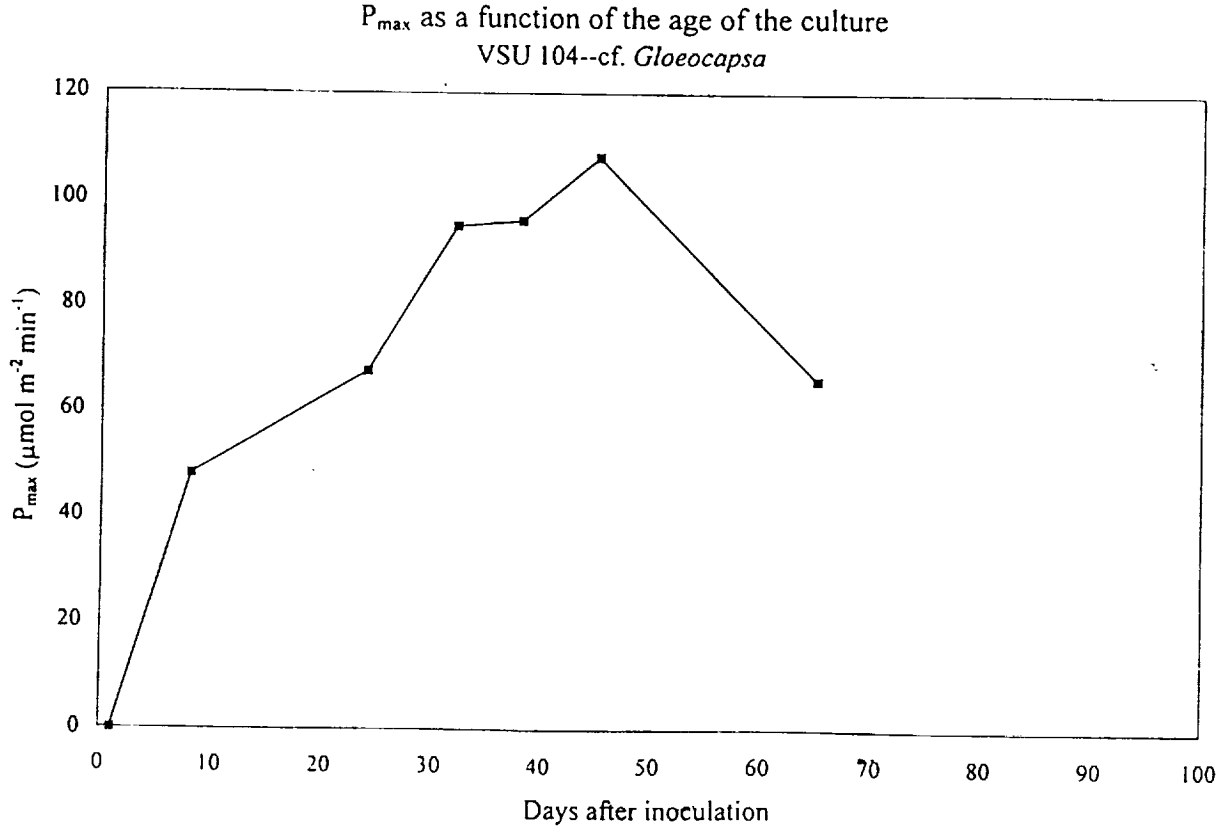


Figure 5.


P_{max} as a function of the age of the culture
UTEX 259--*Chlorella vulgaris*
P_{max} (μmol m^{-2} min^{-1})
0
10
20
30
40
50
60
70
80
90
100
0
10
20
30
40
50
60
70
80
90
100
Days after inoculation


P_{max} as a function of the age of the culture
VSU 104--cf. *Gloeocapsa*
P_{max} (μmol m^{-2} min^{-1})
0
20
40
60
80
100
120
0
10
20
30
40
50
60
70
80
90
100
Days after inoculation

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13. ABSTRACT *(Maximum 200 words)*

The potential of an air-regeneration system based on the growth of unicellular algae on the surface of porous ceramic tubes was evaluated. The system is fairly robust with respect to environmental conditions and is capable of maintaining algal cultures for up to 365 days. Under standard conditions (50-66 μmol m^{-2} s^{-1} (PPF), 450 μmol mol of CO_2), mature tubes can remove CO_2 at a rate of up to 90 μmol m^{-2} min^{-1}. Under these conditions, approximately 200 square meters of area would be required for each member of the crew. However, the rate of uptake increases with both photon flux and CO_2 concentration in accordance with Michaelis-Menton dynamics. An extrapolation to conditions of saturating light and carbon dioxide indicates that the area required can be reduced by a factor of at least 2.5.

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