A CELSS SIMULATOR TO EVALUATE MARINE ALGAE FOR SPACE RESEARCH

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ABSTRACT

For long-term manned space missions a regenerative life-support system is necessary to convert the waste products of respiration into usable oxygen. Closed Ecological Life-Support Systems (CELSS) represent a natural and efficient means to meet the gas exchange requirements for human survival. This paper presents the results of a preliminary screening of local species of marine algae for suitability in a CELSS system. For this purpose four photobioreactors were constructed. A photobioreactor is a system designed to culture photosynthetic cells. At present only a few species of algae are used regularly in CELSS research. It is likely that new varieties of algae can contribute to CELSS research, either by direct participation or through gene transfer of desirable characteristics. The purpose of this research was to determine the performance of a polyculture of marine algae under CELSS conditions. The species that performed well were identified and compared with *Chlorella Vulgaris* on the basis of observed maximum specific growth rate.

INTRODUCTION

In order to determine the performance of a marine algae polyculture under CELSS conditions, a series of experiments were conducted using Hawaiian cell cultures from Keehi Lagoon at Sand Island on the island of Oahu. Cultures were either taken directly from the ocean or were obtained from an experimental program that takes cell cultures from Keehi lagoon and provides pH and nutrients to encourage the dominance of *Chaetoceros Sp.* in the cell culture.

The maximum specific growth rate is used to evaluate the experimental culture because it is a means to compare how quickly Carbon Dioxide is being metabolized and fixed as cell mass. In a bioregenerative life support system this could equate to better bioreactor performance and a smaller sized bioreactor to meet the oxygen requirements of a crew of astronauts. In these experiments,the general performance of a seawater culture is examined in the first trial and compared with the performance of a culture with a vigorously growing population of *Chaetoceros Sp.*

Three 14-day trials were conducted in order to observe the growth and population compositions over time. The first trial used seawater from Keehi lagoon as a source of the marine polyculture.

For the second trial the marine polyculture from Keehi lagoon was compared to a marine polyculture from Keehi lagoon that had been provided conditions to select for *Chaetoceros Sp.* This trial was to compare the performance of *Chaetoceros Sp.* against the performance of a wild culture from the same location..

The third trial was initially intended to compare two pure cultures of marine algae against the results of the second trial. However pure culture samples could not be obtained from local researchers due to state regulations. Instead the growth of the *Chaetoceros Sp.* enhanced culture strain that performed well in the second trial was further studied.

To observe how long a maximum growth rate could be propagated the seed culture was grown in two bioreactors. After two days the culture was propagated to another bioreactor. Every two days a new culture was propagated to simulate culture harvesting and for the purpose of perpetuating the maximum specific growth rate of *Chaetoceros Sp.*

METHODS

In order to determine the maximum specific growth rate of a marine polyculture, four airlift photobioreactors were constructed. The photobioreactors were sized at 1/10 the volume of a 30L photobioreactor used to support a human being for 30 days by the Russian space program[1].

. The four photobioreactors were designed to contain a three-liter algae cell polyculture for the purpose of screening species of marine algae for space research. One photobioreactor is equipped with instrumentation to monitor Carbon Dioxide uptake. Two other photobioreactors are run at identical conditions to verify the results. The fourth photobioreactor was fed a 1% CO_2 mixture to simulate high Carbon Dioxide atmospheric conditions for CELSS operation. Three bioreactors received 500cc/min of air input. A fourth bioreactor received 500cc/min of 1% CO_2 enriched atmosphere.

CO_2 was chosen to be the growth limiting substrate. pH was measured using commercial probes interfaced with a Campbell 21X micrologger by a custom high impedance amplifier. This was so that the difference in ion concentrations inside and outside of the pH probe could be measured as a voltage difference between -5000 and 5000mV. The temperature, pH and CO_2 concentrations were measured every minute and the average values were recorded by the micrologger every 15 minutes. Data points for temperature, pH and CO_2 concentrations were recorded every 15 minutes and plotted over the course of the experiment.

The experiment was housed in a growth shed outfitted with 40-40watt cool white fluorescent tubes to provide light on an 18-hour daylight cycle. The room was equipped with an air-conditioning system to dissipate the heat generated by the lights. A commercial nutrient media was provided to meet the nitrogen, phosphorous and microelement requirements of the algae. The addition of nutrient solutions and withdrawal of culture samples was accomplished via a septum. The concentration and species composition of the culture is determined by withdrawing a culture sample and taking cell counts using a hemacytameter.

For identification purposes local researchers were consulted. Dr. Greg Paterson was helpful in identifying the composition of algae species present in the latter stages of culture development. Dr. Ed Laws and David Hashimoto have been conducting research on the genus *Chaetoceros* for some time and were quite knowledgeable regarding the nutrient requirements and successful culturing techniques for this genus.

A single-species cell culture fol[illegible] [illegible]wth curve. Initially, there was a lag phase where cells adapted t[illegible] [illegible] this phase there was very little growth where the specific growth [illegible]) was ($\mu \cong 0$). Then growth started in the acceleration phase [illegible] achieved its maximum rate ($\mu = \mu max$). This phase continued [illegible] was exhausted or there was a build-up of inhibition causing pro[illegible] ceased to grow and only maintained itself ($\mu = 0$). This was usually [illegible] phase where cells lost viability and lysed ($\mu < 0$)[2].

During each trial the composition [illegible] went through several stages. *Chaetoceros Sp.* composed the initial [illegible] fast-growing and free-floating diatom. Two to four days after the [illegible] *Chaetoceros Sp.* the population would crash. During this period [illegible] could be observed. The most commonly observed ones were penn[illegible] genus *Phaeodactyl.* and green algae from the genus *Teracelmis.* [illegible]

The other species of algae observed [illegible] cell concentrations of the initial *Chaetoceros Sp.* bloom,and popula[illegible] algae observed in the cell counts declined as time progressed up until [illegible]. During the later parts of each trial a complex biofilm developed on [illegible] and could not be readily counted.

Professor Greg Patterson observed a [illegible] complex biofilm under a light microscope and tentatively identified the species [illegible] found within the trial 2 culture after fourteen days.

The bulk of the biofilm was compromised [illegible] filamentous blue-green algae possibly of the genus *Oscillatoria*. Round green [illegible] also visible that exhibited four divisions per cell when reproducing. This [illegible] believed to be of the genus *Gliacytis*. Diatoms of the genus *Navicula* were [illegible]. No *Chaetoceros Sp.* was observed in the complex biofilm. This is not surprising because the population of *Chaetoceros Sp.* appeared to crash after about seven days of culture growth. While *Chaetoceros* populations were unstable within the marine polyculture used in this project pure cultures of *Chaetoceros* have been kept viable for many years[3].

For trial 1. cell counts began after seven days of bioreactor operation. Unfortunately most culture blooms began about day four, and the initial significant cell growth data was not recorded. The third unit, which had a CO_2 enriched atmosphere expressed a delayed bloom which was recorded by cell counts and reached a maximum recorded concentration of about $8.0*10^5$ cells/ml.

Trial 2 cell counts were initiated at the first day of operation and continued throughout the trial. A large bloom of *Chaetoceros Sp.* was recorded almost immediately from the *Chaetoceros Sp.* favored culture. The maximum recorded concentration of cells was $3.6*10^6$ cells ml.

For trial 3 two cultures of *Chaetoceros* dominant cultures from the Sand Island research facility were started. After two days a third culture was propagated using one liter of cell culture from the unit with highest cell density. At two-day intervals a new culture was initiated using the most vigorously blooming bioreactor recent culture available. This procedure was intended to continue the *Chaetoceros* dominant initial

algae blooms as long as possible by reducing cell density, reducing the concentrations of metabolic waste products and providing new nutrient substrate. After about ten days the cell populations declined overall,and only a few secondary species of algae could be identified via cell counts. The maximum recorded concentration of cells was $1.3*10^6$ cells/ml.

The fastest growth rate recorded was between the second and third day of trial two in a bioreactor seeded with *Chaetoceros sp.* favored culture from Sand Island. In a 24-hour period The cell count jumped from a concentration of $3.6*10^5$ cells/ml to $3.61*10^6$ cells/ml. Measurements of culture pH, ambient temperature and carbon dioxide levels from the bioreactor headspace showed a direct correlation between these three values. Over a 24 –hour cycle the lowest pH values and CO_2 concentrations occurred during the coldest part of the night.

CONCLUSION

The direct relationship between pH, temperature and CO_2 levels is believed to be due to the increased solubility of Carbon Dioxide at lower temperatures and not cellular metabolic activity. The increased amount of Carbon Dioxide in solution would acidify the culture resulting in the observed decrease in pH.

The composition of cells in the four bioreactors was initially dominated by *Chaetoceros Sp.* After seven to nine days,filamentous algae that grew in complex biofilms superceded the *Chaetoceros.*

The maximum observed specific growth rate was of *Chaetoceros Sp.* and involved an increase in cell density from $3.6*10^5$ cells/ml to $3.61*10^6$ cells/ml in 24-hours. This amount of growth indicates a cell doubling time of 7.5 hours.

In comparison,*Chlorella vulgaris*, a freshwater algae species typically used in CELSS research has a doubling time of 9 hours[4]. *Chlorella vulgaris* is prized for its fast growth rates. Based on the specific growth rates observed in the *Chaetoceros sp.* cultures, it is apparent that some species of marine algae demonstrate an observed maximum specific growth rate at least as good. and perhaps better than that of algae currently used in CELSS research.

Chaetoceros Sp. also has significant nutritional value and has been used as a food source in the Aquaculture industry. It is concluded that this species exhibits a fast growth rate and nutritional characteristics of significant value to CELSS research and should be considered as a candidate for further research and for possible inclusion in regenerative life-support system applications.

Problems encountered during the experiment-included inability to gain access to quality microscopes on weekends and holidays and degradation of culture samples taken on weekends for later viewing. Within the polyculture,zooplankton could be observed on occasion and their predation may have suppressed autotrophic cell growth. Further testing with reliable access to quality equipment and using pure culture samples for further experiments would reduce experimental uncertainty in further species evaluations.

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