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DOE Grant #: DE-FG22-93PC93212

BIOLOGICAL DETERMINANTS OF PHOTOBIOREACTOR DESIGN2nd Quarterly Report: Period - December 1, 199³~~4~~ - February 28, 1994

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OSTJ**Abstract:**

In the first quarterly report the optimal composition of light was discussed. In this report the intake rates of CO₂ are being investigated. Inlet CO₂ and O₂ concentration was varied and the growth kinetics was studied. It was found that 1% CO₂ concentration is sufficient to support growth of cells up to a concentration of 1×10^8 cells/mL in flask cultures. This experiment was repeated and the results were found to be consistent. In this report we present the results from one set of experiment.

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EFFECTS OF CARBON DIOXIDE CONCENTRATION

Introduction

Algae cells need carbon dioxide to grow autotrophically. Carbon dioxide is the sole source of carbon for the process of photosynthesis and biomass production and oxygen is liberated as a product. Thus, higher carbon dioxide concentration and lower oxygen concentration is desirable. To investigate the effect of carbon dioxide and oxygen concentrations in inlet gas stream on algal cell growth kinetics, the following experiment was designed.

Materials and Methods

Five identical flasks, each with 22 W fluorescent light, were prepared. Each flask was supplied with a different composition of inlet gas concentration. The detailed compositions are shown below.

F1 :	0.03 %	CO ₂	20 % O ₂	(air, control)
F2 :	0.03 %	CO ₂	10 % O ₂	
F3 :	5.0 %	CO ₂	10 % O ₂	(same as F4 after 98.5 hrs)
F4 :	5.0 %	CO ₂	20 % O ₂	
F5 :	0.5 %	CO ₂	10 % O ₂	
Actual concentration				
	F1:	0.06 CO ₂	20.70 O ₂	
	F2:	0.05 CO ₂	09.96 O ₂	
	F3:	4.84 CO ₂	10.21 O ₂	
	F4:	5.17 CO ₂	20.53 O ₂	
	F5:	0.52 CO ₂	10.04 O ₂	

Gas flow rate is 80 mL/min

Inoculum density around 1.0×10^5 cells/mL

Culture volume: 55 mL

Temperature : room temperature

Results and Discussion

The entire set of data is shown in Figure 1.

0.03 % CO₂ was not enough to support the cultures in shake flasks. 0.5 % CO₂ seems to be provide sufficient carbon dioxide transfer to the medium . The growth rates and cell concentrations of cultures provided with 0.5 % CO₂ are highly comparable to culture provided with 5 % CO₂. However, the lower concentration of O₂ did not help in the growth of the cultures.

The initial poor growth in F1 was either due to harmful wavelengths emitted from an old fluorescent light or lack of carbon dioxide from an old air cylinder. It began to grow like F2 after both the fluorescent light and gas cylinder had been changed.

Even though the oxygen production rate and carbon dioxide consumption rate could not be measured, due to gas analyzer constraints, the effects of inlet carbon dioxide concentration could be monitored by the growth curve. Furthermore, the higher carbon dioxide concentration apparently induced chlorophyll synthesis (page 4 of Figure 1.). The per cell chlorophyll contents were about two times higher in flasks number 3 to 5 (0.5 - 5.0 % CO₂) than those in flasks 1 and 2 (0.03 % CO₂).

It is interesting to see the pH increase was the highest in F5, which had 0.5 % CO₂ and 10 % O₂ inlet gas composition (page 1 of Figure 1.). From this graph, we can deduce two things. Firstly, higher the activity (higher cell concentration), higher is the pH as is observed on comparing F1 and F2 to other flasks. Secondly, higher the inlet carbon dioxide concentration, the more stable the pH is (we can see this from the comparison of F3 and F4 to F5). Since there are some reports that higher pH can inhibit the cell cycle via bicarbonate accumulation [1], the inlet carbon dioxide concentration should be higher than a certain value. The result of this experiment suggests that it should be higher than 0.5 %.

Final cell concentrations of each flask (total biomass) at the end of the run (211.5 hr) were as followings:

F1 :	0.03 %	CO ₂	20 % O ₂	0.09119 v/v %
F2 :	0.03 %	CO ₂	10 % O ₂	0.08682 v/v %
F3 :	5.0 %	CO ₂	10 % O ₂	0.63574 v/v %
F4 :	5.0 %	CO ₂	20 % O ₂	0.62340 v/v %
F5 :	0.5 %	CO ₂	10 % O ₂	0.49108 v/v %

This data clearly shows that 0.03 % of CO₂ is not enough to support the flask cultures. However there was a small improvement in the culture kinetics under 5.0 % CO₂ over that under 0.5 % CO₂. Based on these observations, 1 % CO₂ should suffice to support algal cultures to over 1.0×10^8 cells/mL in the flask cultures.

Conclusion

From this observation, we can conclude that about 1 % CO₂ should be enough to support the *C. vulgaris* cultures up to 10^8 cells/mL in flask cultures.

References

- [1] R. K. Mandalam and B. O. Palsson (1994) Cell Cycle Modulating Activity of Conditioned Medium from High Density *Chlorella vulgaris* Cultures. submitted to *J. Phycol.*

Figures

- Figure 1. All the analyzed data from the carbon dioxide experiment including cell concentration, average cell volume, total biomass, growth rate, doubling time, pH, average DNA, chlorophyll contents, and other statistical parameters.

Figure 1.

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