0273-2289/93/3940—0337$03.10

Yield and Quality of Cyanobacteria

Spirulina maxima in Continuous Culture in Response to Light Intensity

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ABSTRACT

Cynobacteria *Spirulina maxima* was evaluated in an effort to increase the growth rate, biomass yield in continuous cultures. The light efficiency was dependent on the light energy absorbed by the cultures. Cultures with optical densities of 0.48 and 0.49 were considered the ones in which most of the incident light energy was converted to chemical energy (mass). Low-light experiments (30 $\mu Em^{-2}s^{-1}$) were efficient in terms of mass output and absorbed light energy (calorie). Low-light cultures did not require high calories to produce the same yield as high-light ones (100 $\mu Em^{-2}s^{-1}$). In addition, the total protein percentage was higher in low-light cultures, whereas the total carbohydrate percentage was higher in high-light cultures. In conclusion, *Spirulina maxima* responds the same way as many other alga and cyanobacteria.

Index Entries: *Spirulina;* biomass; yield; light; efficiency.

INTRODUCTION

Spirulina, a filamentous alga, has been used as a model organism for outdoor cultivation of algal biomass as a source of protein and chemicals (*1–4,39*). *Spirulina* has been the subject of intense ecological and physiological studies necessary for development and improvement of large-scale applications (*5,6*). Laboratory studies have been limited to growth kinetics (*7,8*) and environmental factors (*9*). In the United State, *Spirulina* has been recently recommended (*10*) and is under investigation (*11*) for the revitalization of air, waste processing, and production of food for the

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Space program CELSS (Controlled Ecological Life Support System). Cyanobacteria occupy a unique taxonomic position, since they combine an autotrophic mode of growth that is common to eukaryotic plant cells with a metabolic system that is generally regarded as bacterial, rather than plant-like. These prokaryotic organisms thus offer the growth potential of microbial cells together with the light-harvesting capabilities of plant cells, making them ideal candidates for biosolar energy conversion to useful forms of chemicals. The advantages of this alga are easy cultivation, good yields per unit area, and good quality of its protein.

Photosynthetic efficiency is fundamentally important in both technological exploitation of photosynthesis and in deciding the molecular mechanism whereby light energy is converted to chemical energy. The efficiency of photosynthesis is simply the rate by which chemical energy is stored in organic biomass divided by the supply rate of available light energy (*12,13*). Pirt et al. (*13*) recommended that efficiencies be measured in growing cultures rather than by short-term oxygen evolution. The photosynthetic efficiency (PE) is an expression of the growth yield from the energy source, light. Growth yield (Y) is defined as the biomass formed by light absorbed, and if K is the caloric value of the biomass, we have $PE = KY$.

Increasing the efficiency of the yield of algal culturing in bioregenerative life-support system is one of the primary concerns of a CELSS. In order to design and operate such a culture system, it is necessary to understand how the macroparameters of a culture system, e.g., productivity and efficiency, are related to the physiological aspects of the algal culture. Photosynthetic organisms physiologically adapt to variations in light intensity. Their survival requires continuous adaptation to prevailing conditions of light. Photoadaptive responses have been characterized by changes in their metabolic accumulation products and pigments (*14–20*). The objective of this work was to determine the yield and chemical composition of the biomass of *Spirulina maxima* cultures incubated at two light intensities in steady state.

MATERIALS AND METHODS

Culturing

Cyanobacteria *Spirulina maxima* (UTEX LB 2342) was cultured in Zarrouk (*38*) medium. BRL's Airlift Fermenter was used for culturing the alga. It consists of a reactor vessel (2 L). The reactor was provided with white-light mercury vapor lamps. The culture were aerated with air at a flow rate of 1000 mL/min. Two peristaltic pumps were used, one for feeding the fermenter with fresh medium, and the second for removal of the overflow. The system was provided with a pH-controlling system.

The pH of the medium remained almost unchanged at 9.3–9.4 in all experiments by using 4*N* sodium hydroxide. The temperature of the culture was maintained at 35 °C. Batch cultures were kept parallel to the fermenter and used for inoculating the reactor. The alga was precultured in small bottles (250-mL capacity) containing 100 medium at 35 °C for 4 to 5 d. The precultures were set up under the same conditions of the reactor experiment.

Light Measurement

Light incident and absorbed by the culture was measured with an Li Cor Li-185 Quantum sensor probe ($U = \mu E\ m^{-2}\ s^{-1}$). Measurements of optical density within the main culture were also taken by measuring light (l) transmitted through the culture and light incident (l_0) on the culture. Log l/l_0 (optical density) correlated very well with the dry weight.

Absorbed Light by the Culture

Volume of culture (reactor) = 2000 mL
Illuminated area = 528 cm^2
(area of fermenter exposed to light irradiation)
a/v = area/vol of culture = 0.264
(ratio of illumination area [a] to culture vol [v])
la (absorbed light) = intensity of light incident on culture − intensity of light out of culture
$la \times a/v$ = absorbed light by culture

Principles of Continuous Culturing

A continuous culture is a constant-volume cell culture system in which the rate of cell growth is controlled by the dilution rate of nutrient solution. The constant volume was maintained by ensuring that the rate of culture outflow equaled the inflow rate of the fresh medium. When steady-state conditions were reached, there existed a constant cell number and biomass within the vessel, since the specific growth rate equaled the dilution rate. The fresh medium was kept refrigerated to avoid contamination. Sterile medium was pumped from a reservior into the vessel by a peristaltic pump. The outflow from the culture was collected in sterile bottles plugged with sterile cotton-wool filters. The was assumed to be in steady state when the cell concentration remained constant for at least 96 h after the initial flow rate was adjusted. In steady-state culture: Dilution rate $(D) = F/v$, where F = inflow rate (mL/h) and v is culture volume (mL). D = growth rate.

Analytical Methods

Harvesting of Cells

Cells were collected by filtration, washed with buffer solution (pH 8), diluted to known volume, and processed for further analysis.

Dry-Weight Measurements (DW)

A volume from the culture was filtered, dried for 4 h at 80°C in previously dried, preweighed filter paper, and weighed after cooling in a desiccator.

Ash-Free Dry Weight (AFDW)

After recording the dry weight, the dried cells were ashed at 500°C for 2 h. The difference between dry weight and ash weight gave the organic weight of the sample.

Chemical Analysis

The total carbohydrates and proteins were determined according to the methods of Kochert (*22*) and Lowry et al. (*23*), respectively. Total lipid of the cells was extracted and quantified according to the Bligh and Dyer method (*24*).

Productivity (g/h)

Productivity is defined as the product of dry weight (g/L) of the culture and the overflow rate (l/h).

Light Efficiency

Light efficiency was calculated using the absorbed light intensity (cal/cm^2*l*) and biomass productivity, converted from g/h to cal/h using heat of combustion. In this study, the cultures were not dense enough to absorb all the incident light on the bioreactor. Therefore, it was necessary to measure the absorbed light. This study was aimed at measuring the yield of the alga in a bioreactor, at steady state, without reference to solar energy utilization. Therefore, it was not appropriate to calculate the photosynthetically active radiation (PAR) of the absorbed light by the culture.

Growth Yield

Yields are grams of dry algae per unit illuminated surface area and per unit total culture volume.

Calculations of Light Efficiency

The absorbed light intensity (cal/h/cm) and biomass productivity converted to cal/h according to Aiba and Ogawa (*7*). Efficiencies were expressed as the ratio of cellular calories produced to those supplied by the light source.

All of the above analytical data were related to the organic weight of the algae. All tests were performed in triplicate.

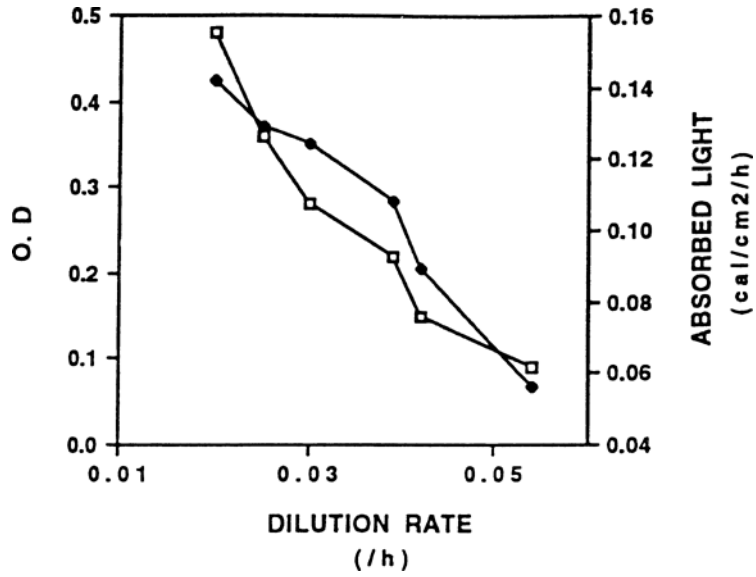

Fig. 1. Growth characteristics of *Spirulina maxima* maintained at different optical densities in steady-state cultures: optical density of the culture, absorbed light as a function of dilution rate (based on results presented in Table 1). —□— OD, —◆— absorbed light.

RESULTS AND DISCUSSION

Growth Characteristics of *Spirulina* Maintained at Different Optical Densities in Steady State (Fig. 1)

Experiments were incubated in one light intensity 30 μE $m^{-2}s^{-1}$, but at various optical densities by changing dilution rates. The results are presented in Fig. 1.

The optical density of the cultures varied with changing the dilution rates. Accordingly, the absorbed light by the cultures varied. The figure shows that the optical densities and absorbed light by the cultures increased at low dilution rates and decreased at high dilution rates. In other words, cultures that were light limited were obtained at low dilution rates.

Growth Characteristics of *Spirulina* Cultures Maintained at Two Incident Light Intensites at Steady State

Cultures of the alga were analyzed in light deficient and light sufficient by changing the dilution rate of the cultures. Two sets of cultures at

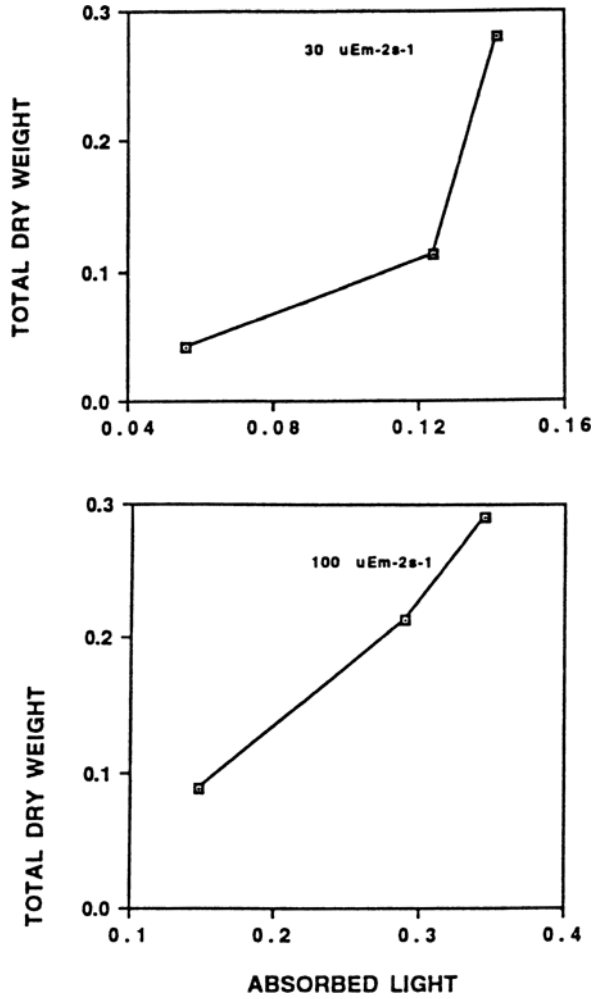

Fig. 2. Growth characteristics of *spirulina maxima* maintained in light-deficient cultures, sufficient cultures at steady state: total dry wt, as a function of absorbed light (based on results presented in Table 1).

steady state were run at two different light intensities. Each set was run at three different culture densities by changing dilution rate. One set was run at low-light irradiation (30 μE $m^{-2}s^{-1}$), and the other at high-light irradiation (100 μE $m^{-2}s^{-1}$). Results are presented in Fig. 2 and 3.

Total Dry Weight as a Function of the Absorbed Light (Fig. 2)

The total dry weight of the cells increased when the absorbed light was increased. In addition, the same yield of cells was reached in cultures exposed to low light when compared to those exposed to high light. In other words, the low-light incubated cultures were more efficient than high-light cultures.

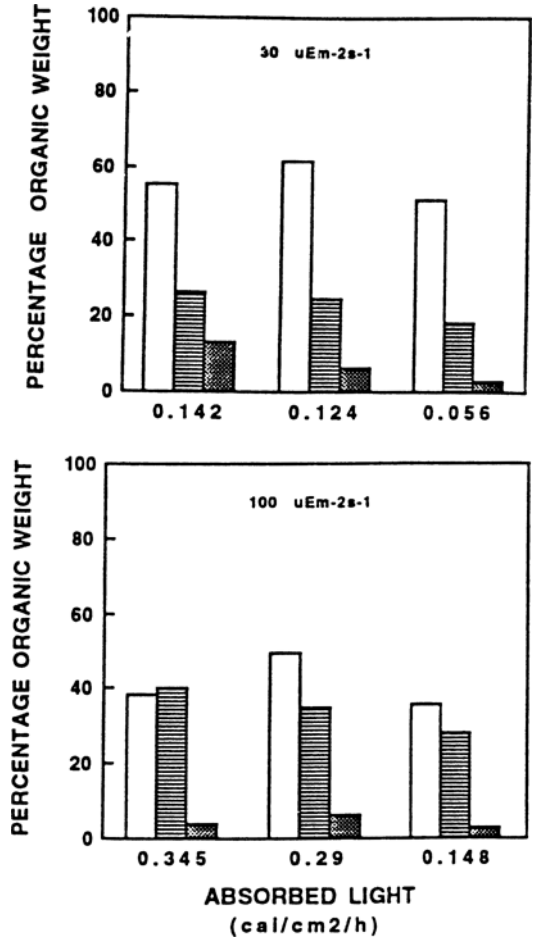

Fig. 3. Growth characteristics of *Spirulina maxima* maintained in light-deficient cultures, sufficient cultures at steady state: chemical composition of cultures as a function of absorbed light (based on results presented in Table 1). ☐ Protein, ≣ carbohydrate, ▩ lipid.

Chemical Composition of Cultures as a Function of Incident Light Irradiation (Fig. 3)

When comparing the chemical composition of both sets of cultures, the carbohydrate percentage increased with increasing light intensity (100 μE $m^{-2}s^{-1}$) at the expense of protein percent decrease, whereas the lipid percentage increased to a degree when decreasing the light intensity (30 μE $m^{-2}s^{-1}$). The protein percent was higher in low-light cultures than in high-light cultures.

Cultures (in low light or high light) with biomass output equivalent to absorbed light energy without loss have light efficiency of 13.34 and 16.24. The efficiency with respect to incident light reflects the balance be-

tween light absorption and utilization, and losses owing to the culture metabolism. In this fermenter, the maximum light utilization efficiency occurs between 0.28 g/L and 0.29 g/L dry wt. Increasing the dilution rate of the fermenter led to a decrease in the yield of the alga and light absorbed by the culture (Table 1). The total dry weight of the alga and the productivity increased at a low dilution rate (0.02g/L or 0.35g/L). Low dilution rates usually correspond to high optical density of the culture in the fermenter (Table 1). In other words, in high dense cultures (light limited), the absorbed light is equivalent to the dry weight or productivity (Fig. 2). Our results are in agreement with those previously reported dealing with continuous cultures (*12,17,25*). One of the primary limitations of most photosynthetic organisms is that they do not perform in strong light. Photoinhibition has been documented in algae (*25–27*). The total chlorophyl shows (Table 1) increase in cultures incubated at low light intensity than those in high light intensity. Like most algae (*35*), cyanobacteria respond to low light intensities with an increase in their pigmentation (*28–31*).

The total protein of the alga was higher in low light intensity (Table 1) than in high light intensity. The results agree with the previously obtained optimal relative protein accumulation occurring in *Merismopedia tennissima* when incubated at a low intensity of 20 $\mu E\ m^{-2}s^{-1}$ (*20*). Increasing light intensities to 90 $\mu E\ m^{-2}s^{-1}$ led to more carbohydrate accumulation. Similar results were reported (*26*) where reductions in the growth light level (40–6 $\mu E\ m^{-2}s^{-1}$) resulted in increasing of chlorophyll and protein per cell for green species. Photoinhibition of two strains of *Spirulina* (*27*) resulting from high light intensity caused damage of the protein of the algae. The photosynthetic oxygen evolution was lowered as well. The total carbohydrate content of the *Spirulina* increased in cultures exposed to high light intensity more than in cultures in low light (Table 1). Similar results were obtained by cyanobacteria (*20,30–34*). Algal species adapt to light-intensity variation by changing their chemical composition (*18,21*).

Spirulina maxima responds to changes in growth irradiance levels by altering the quality of yield. The protein of the algae cells increased with decreasing the light irradiation of the culture (Table 1). At low-growth irradiance levels, the photosynthetic response was associated with increased light utilization efficiencies. Increasing the light irradiation was associated with increasing the total carbohydrate of the cultures. The results of this work indicate also that nutritional quality of an algal product can be controlled during the production process to the extent that the chemical composition of that species is variable (*36*).

In conclusion, *Spirulina maxima* responds the same way as many other algae and cyanobacteria. Although this is not a surprise, it may merit publication, because it supports the current understanding of steady-state operation of a photobioreactor.

Table 1
Growth Characteristics of *Spirulina maxima* Maintained in Two Light Intensities at Steady State

OD	Dilution rate, /h	Tot. dry, DW, g/L	Productivity, g/h/L	Chlorophyll, mg/L	Incident light, cal/cm/h	Absorbed light a/v, cal/cm/h	Growth yield Y, g dw/cal	Light efficiency %[a]	% Organic wt, AFDW		
									Protein	Carbohydrate	Lipid
0.48	0.020	0.280	0.0040	5.8	0.1475	0.142	0.023	13.34	55.4	26.21	13.34
0.28	0.030	0.112	0.0034	1.97	0.1475	0.124	0.027	15.2	61.5	24.90	6.42
0.09	0.054	0.042	0.0024	1.016	0.1475	0.056	0.041	22.14	51.2	18.52	2.82
0.49	0.035	0.290	0.010	1.8	0.3947	0.345	0.029	16.24	38.5	40.09	4.3
0.28	0.048	0.214	0.0102	2.86	0.3947	0.290	0.035	20.30	49.5	34.83	6.52
0.09	0.083	0.089	0.0074	0.44	0.3947	0.148	0.050	27.00	35.7	28.31	2.73

[a] *See text* for calculations.

ACKNOWLEDGMENT

This work was financially supported by a contract from the US National Aeronautics and Space Administration (Contract No. NCC2-501).

REFERENCES

1. Grobbelarr, J. U. (1988), *S. Afric. J. Sci.* **84,** 4–5.
2. de la Noue, J. and de Pauw, N. (1988), *Biotech. Adv.* **6,** 725–770.
3. Iwamoto, H. (1989), in *Biomass Handbook,* Kitani, O. and Hall, C. W. eds. Gordon and Breach Science Publications, pp. 213–218.
4. Richmond, A. (1987), in *Miroalgal Biotechnology,* Borowitzka, M. and Borowitzka, L. eds., Cambridge Univ. Press, pp. 85–121.
5. Becker, E. W. and Venkataraman, L. V. (1984), *Biomass* **4,** 105–125.
6. Richmond A., Karg, S., Boussiba, S. (1982), *Plant and Cell Physiol.* **23,** 1411–1417.
7. Aiba, S. and Ogawa, T. (1977), *J. Gen. Microbiol.* **102,** 179–182.
8. Iehana, M. (1983), *J. Ferm. Technol.* **61,** 457–466.
9. Vonshak, A., Abeliovich, A., Boussiba, S., and Richmond, A. (1982), *Biomass.* **2,** 175–185.
10. MacElroy, R. D. and Bredt, J. (1985), in *CELSS NASA CP-2378,* MacElroy, R. C., Smeroff, D. T., Klein, H. P., eds., pp.1–9.
11. Tadros, M. G. (1990), in *CELSS 1989,* MacElroy R. D., ed., NASA and Tech. Memo 102277 pp. 217–243.
12. Pirt, S. J., Lee, Y. K., Pirt, M. W., Balyusi, H. H. W., and Bazin, M. J. (1983), *J. Chem. Tech. Biotechnol.* **33B,** 35–58.
13. Pirt, S. J., Lee, Y. K., Richmond, A. and Pirt, M. W. (1980), *J. Chem. Tech. Biotechnol.* **30,** 25–34.
14. Morris, I.)1981), *Can. Bull. Fish. Aquat. Sci.* **210,** 83–102.
15. Collins, C. D. and Boylen, C. W. (1982), *J. Phycol.* **18,** 206–211.
16. Hitchcock, G. L. (1980), *Mar. Biol.* **57,** 271–278.
17. Goldman, J. C. and Dennett, M. R. (1983), *Mar. Ecol. Prog. Ser.* **15,** 169–180.
18. Falkowski, P. G. (1984), *J. Plankton Res.* **6,** 295–307.
19. Fabregas, J., Herrero. C., Abalde, J., Cabezas, B. (1986), *J. Industr. Microbiol.* **1,** 251–257.
20. Konopka, A., Kromkamp, J., and Mur, L. R. (1987), *J. Phycol.* **23,** 70–78.
21. Tadros, M. G. Johansen, J. R. (1988), *J. Phycol.* **24,** 445–452.
22. Kochert, G. (1978), *Physiological and Biochemical Methods,* Cambridge University Press, London, pp. 189–195.
23. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951), J.B24.
24. Blight, E. G., Dyer, W. J. (1959), *Can. J. Biochem. Physiol.* **37,** 911–917.
25. Radmer, R. and Kok, B. (1977), in *Encyclopedia of Plant Physiology,* vol. 5, Trebst and Avron, eds., Springer-Verlag, Berlin, p. 125.
26. Osborne, B. A. and Raven, J. A. (1986), *Biol. Rev.* **61,** 1–61.
27. Vonshak, A., Guy, R., and Guy, M. (1988), *Archiv. Microbiol.* **150,** 445–420.
28. Van Liere, L. and Mur, L. R. (1979), *J. Gen. Microbiol.* **115,** 153–160.

29. Zevenboom, W. and Mur, L. R. (1984), *Arch. Microbiol.* **139,** 232–239.
30. Gibson, C. E. and Foy, R. H. (1983), *Br. Phycol. J.* **18,** 39–45.
31. Post, A. F., Loogman, J. G., and Mur, L. R. (1986), *J. Gen. Microbiol.* **13,** 2129–2136.
32. Kromkamp, J. C. and Mur, L. R. (1984), *FEMS Microbiol. Lett.* **25,** 105–109.
33. Fontes, A. G., Moreno, J., and Vargas, M. A. (1989), *Biotechnol. Bioeng.* **34,** 819–824.
34. Torzilla, G., Sacchi, A. R., Materassi, and Richmond, A. (1991), *J. Appl. Phycol.* **3,** 103–109.
35. Falkowski, P. G., Owens, T. G., Ley, A. C., and Mauzerall, D. C. (1981), *Pl. Physiol. Lancaster,* **68,** 969–973.
36. Taub, F. B. (1980), Elsevier/North-Holland and Biomedical-Press, pp. 707–721.
37. Myers, J. (1957), in *Encyclopedia of Chemical Technology,* Kirk, R. and Othmer, D. eds., Interscience, New York, p. 33.
38. Zarrouk, C. (1966), Influence de divers facteurs physiques sur la crossance et al photosynthesis. Thesis, University of Paris (France).
39. Matsunaga, T., Nakamura, N., Tsuzakis, N., and Takeda, H. (1988), *Apl. Microbiol. Biotechnol.* **28,** 373–376.
40. Graves, D. A. and Greenbaum, E. (1989), *Plant Physiol.* **90,** 246–250.