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**[Short Note]**

# Growth and Carbon Budget of *Spirulina pacifica*, with a Special Reference to Light*

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In order to get a basic knowledge about the growth and carbon budget of *Spirulina pacifica*, the alga was cultured under different light regimes (40, 100 and 200 $\mu$mol photon $m^{-2} \cdot s^{-1}$) and its growth, organic carbon content and carbon incorporation were investigated. The alga grew faster at the intermediate level of light compared to the high and low regimes, with the maximum growth rate being 0.5 doublings per day and the end biomass density 2 g per L; it also grew well in diluted seawater. The removal of inorganic carbon and the accumulation of total organic and extracellular organic carbon increased with increased light intensity. The organic carbon content of *Spirulina pacifica* did not show significant difference among the cultures maintained under different light regimes. The mean organic carbon content was 30% (w / w) of the dry alga.

**Key Words:** blue-green alga, carbon, growth, *Spirulina pacifica*

## INTRODUCTION

The blue-green alga *Spirulina*, because of its economic values (e.g. human health foods, animal feeds and carotenoids), has been well characterized on the biochemical aspect. Phycobilisome components, lipid, fatty acid, chlorophylls, carotenoids and hydrocarbons have been estimated on quantitative and qualitative bases under variety of physical and chemical factors (Olaizola and Duerr 1990; Tedesco and Duerr 1989; Piorreck *et al.* 1984; Manabe *et al.* 1992; Xiang *et al.* 1991). Organic carbon budget is informative as one of its biochemical properties as to its economic values and carbon utilization. However, nothing has been documented on this aspect. From the viewpoint of hydrogen production, higher quota of cellular organic carbon may result in higher yield of hydrogen since the carbon reserves provide ultimately the reducing equivalents for hydrogen evolution in the dark (e.g. Aparicio *et al.* 1985). Consequently, the author investigated the growth and carbon budget of *spirulina pacifica* while grown under varied light regimes.

## MATERIALS AND METHODS

This study was carried out at the Bioresources Laboratory of Hawaii Natural Energy Institute. The axenic seed of *Spirulina pacifica* (27893) was obtained from Pacific Biomedical Research Center of University of Hawaii. The seed was inoculated and cultured in SOT culture medium (Ichimura 1979). Several generations was repeated at 40, 100 and 200 $\mu$mol photon $m^{-2} \cdot s^{-1}$ (12/12 light and dark cycle) and 25°C before initiation of the experiments. Photosynthetically active radiation (PAR) was measured with a Li-Cor quantum sensor (SPQA 1397). All transfers and samplings were conducted in a horizontal Laminar Flow Module (EACI). The cultures were agitated by magnetic stirrers. Thirty m*l* of the culture (500 m*l* in 1000 m*l* sterilized flask) was sampled each time, of which 20 m*l* was used for measuring biomass density (mg dry wt $ml^{-1}$) and 10 m*l* for organic and inorganic carbon analyses. The biomass density was assessed by filtrating the sample (10 m*l* each time) on pre-dried (95°C, 3 hrs) Whatman glass filter (GF/C), washing with distilled water, drying at 95°C for 5 hrs, cooling down in a desiccator, weighing to the nearest

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*Dedicated to the Memory of the late Dr. Isamu Umezaki (1925-1995), Professor of Kyoto University, and the late Dr. Kelton R. McKinley (1948-1995), Technical Program Administrator of Hawaii Natural Energy Institute, University of Hawaii.

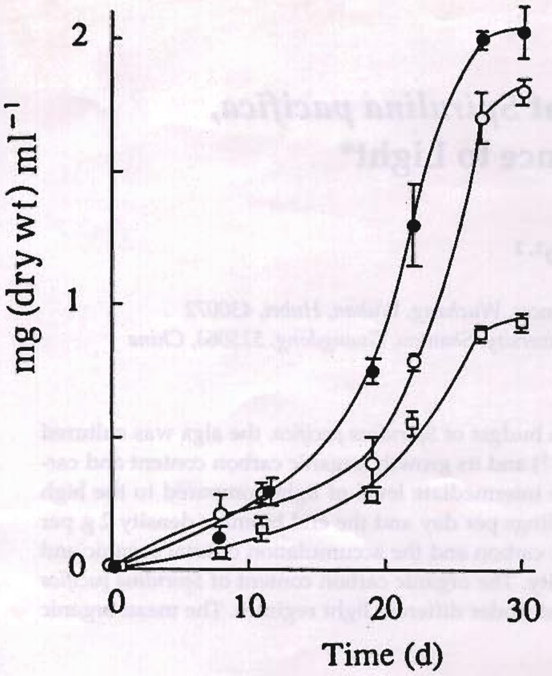

**Fig. 1.** Growth of *Spirulina pacifica* under different PARs (○, 200; ●, 100; □, 40 $\mu$mol photon $m^{-2} \cdot s^{-1}$). Means ± SD (n = 3).

0.01 mg and subtracting the weight of the blank filter. The filtered and diluted solution was used to estimate the dissolved inorganic and organic carbon before and after acidified (below pH 3) with 6 N HCl and sparged off gaseous $CO_2$ with $N_2$, respectively. The particular organic carbon of the alga was measured by acidifying 10 m*l* culture and sparging off the gaseous $CO_2$, breaking up to tiny particles by a Fisher Sonic Dismembrator, measuring the total organic carbon and subtracting the dissolved organic carbon in the medium. Organic carbon and inorganic carbon were measured by using a Shimadzu total organic carbon analyzer (TOC-5000). The amount of organic carbon as components of the medium was subtracted. Specific growth rate was calculated from the equation:

$$\mu = \frac{\log_2(D_t/D_i)}{t}$$

where $\mu$ is the specific growth rate experessed as doublings per day, $D_i$ and $D_t$ indicate biomass densities (mg m$l^{-1}$ dry wt) at the initial and after t number of days, respectively. The rates of inorganic carbon removal and incorporation were calculated as coefficients of the linear ($r^2 > 0.9$) regression of inorganic and total organic carbon concentrations versus time in the exponential phase, respectively.

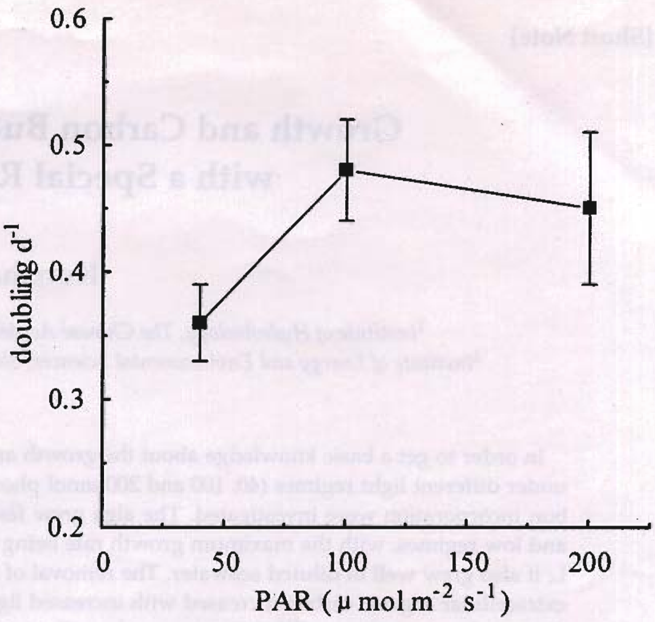

**Fig. 2.** Growth rate of *Spirulina pacifica* during the exponential phase as a function of light. Means ± SD (n = 3).

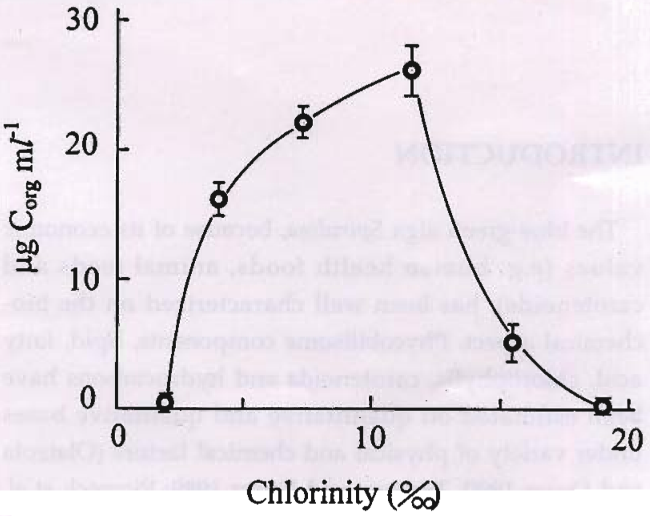

**Fig. 3.** Organic carbon accumulation in the cultures of *Spirulina pacifica* as a function of chlorinity. Seawater was diluted to obtain the various levels of chlorinity. Means ± SD (n = 3).

## RESULTS AND DISCUSSION

Growth of *Spirulina pacifica* was shown in Figure 1. Rapid growth was observed at above 100 $\mu$mol photon $m^{-2} \cdot s^{-1}$, at which the maximum biomass density reached about 2 mg m$l^{-1}$ dry weight (wt) at the end of exponential phase. Daily doubling rate at 100 $\mu$mol photon $m^{-2} \cdot s^{-1}$ differed significantly ($P < 0.05$) from and was about 30% higher compared to that at 40 $\mu$mol photon

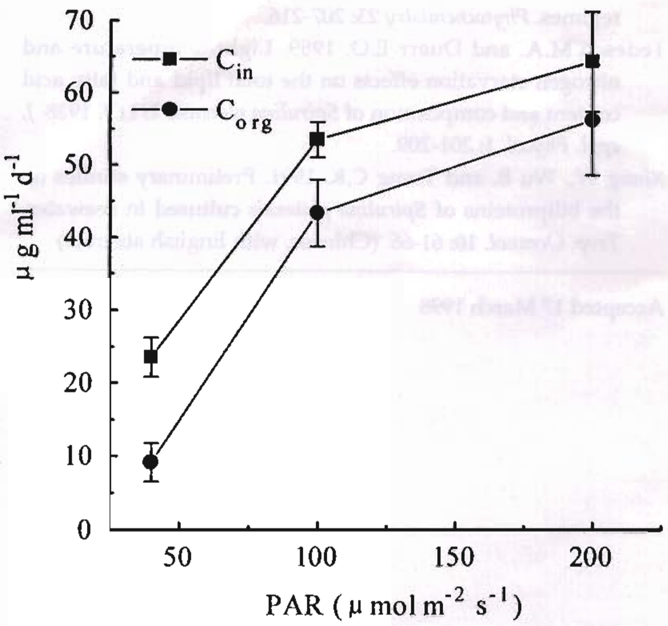

**Fig. 4.** Rates of inorganic carbon removal ($C_{in}$) and organic carbon accumulation ($C_{org}$) of *Spirulina pacifica* during the exponential phases of cultures maintained under different light regimes (PAR). Means ± SD (n = 5 to 7).

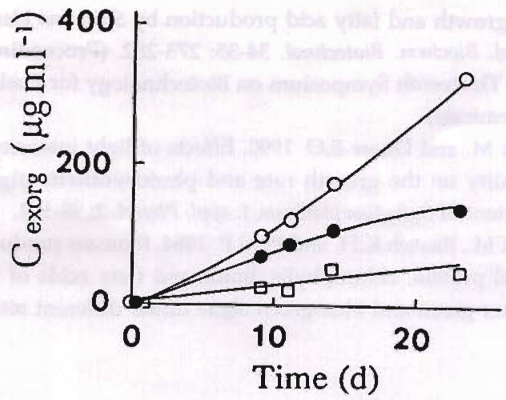

**Fig. 5.** Accumulation of extracellular organic carbon ($C_{exorg}$) in the cultures of *Spirulina pacifica*. Symbols as in Figure 1.

$m^{-2} \cdot s^{-1}$ (Fig. 1). Within the exponential phase, growth rates at 100 and 200 $\mu$mol photon $m^{-2} \cdot s^{-1}$ were identical, indicating the growth was saturated and no photoinhibition occurred (Fig. 2). However, the end biomass density was higher at 100 than 200 $\mu$mol photon $m^{-2} \cdot s^{-1}$ (Fig. 1). The alga showed best growth at 10‰ chlorinity when cultured in variously diluted seawater (Fig. 3). Both the inorganic carbon removal and total organic increased with increased light intensities (Fig. 4). The imbalance of the inorganic carbon removal over the organic carbon could increase due to the release of gaseous $CO_2$ from liquid phase to air. Photosynthetic carbon removal or incorporation were not saturated at 200 $\mu$mol photon $m^{-2} \cdot s^{-1}$. The discrepancy in light requirement between the photosynthetic carbon incorporation and growth could be related to the accumulation of extracellular organic carbon (Fig. 5). The extracellular organic carbon concentrations increased with time and were higher at higher levels of PAR, being about 25% of the total organic carbon at the end of exponential phase at the highest light level. The production of extracellular organic matters does not contribute to growth which is only proportional to the increased organic carbon as biomass. That algal growth is light-saturated at lower PAR than photosynthesis is a known phenomenon (Lünning 1981) but has not been elucidated. For *Spirulina pacifica*, such discrepancy can be attributed to the portion of organic carbon excreted in the light above the growth-saturating PAR. That is, when light was raised from growth-saturating intensity, the subsequently increased organic portion due to enhanced photosynthesis was excessive and excluded out of the cell. Such excretion of organic matter must have resulted from photorespiratory carbon oxidation. On the other hand, the values of organic carbon content were 26.3 ± 9%, 35.8 ± 11% and 28.7 ± 7% (w/w, n = 6) at 40, 100 and 200 $\mu$mol photon $m^{-2} \cdot s^{-1}$, respectively. The differences among the averages are not significant ($P > 0.20$). Disregarding the light regimes, the totally averaged mean was 30%. This implies that intracellular organic carbon was not significantly affected by culturing at different light regimes.

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