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GROWTH AND PHYCOCYANIN FORMATION OF *SPIRULINA PLATENSIS* IN PHOTOHETEROTROPHIC CULTURE

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SUMMARY

Glucose and acetate enhanced cell growth and phycocyanin production of *S. platensis*. The highest specific growth rate, cell concentration and phycocyanin production were respectively 0.62 d^{-1}, 2.66 g/l and 322 mg/l on glucose and 0.52 d^{-1}, 1.81 g/l and 246 mg/l on acetate. The specific growth rate of the alga on 2.5 g glucose/l was markedly increased with increasing light intensity up to 2 klux. Further increasing light intensity to 4 klux only resulted in a very slight increase in specific growth rate. At a light intensity above 4 klux, photoinhibition occurred. Light favoured phycocyanin formation. The highest phycocyanin content was obtained at a light intensity of 4 klux. When the light intensity decreased to 2 klux or less, the optimal glucose concentration for biomass production shifted from 2.5 g/l to 5.0 g/l.

INTRODUCTION

Spirulina platensis has long been grown photoautotrophically in open ponds for production of health food because of its high content of protein and other nutritional elements, in which cell concentrations were low (typically 0.4 - 0.8 g/l) (Richmond, 1988). More recently, much attention has been drawn to the potential employment of *S. platensis* for production of high value chemicals such as phycocyanins, carotenoids and γ-linolenic acid since it is found that this organism can accumulate large quantities of these products (Olaizola and Duerr, 1990; Cohen *et al.*, 1993; Borowitzka, 1994). Phycocyanins are the major photosynthetic accessory pigments which can be used as nutrients for both humans and animals, as natural dyes for food and cosmetics and as pharmaceuticals (Belay *et al.*, 1993; Borowitzka, 1994). It is well-known that pure and high cell density culture is a prerequisite for successful commercial production of high value products since the cost for down-stream processing can be greatly reduced. Supplement of soluble carbon substrates to the culture media in a sterilised bioreactor may provide a feasible means for achieving this purpose. *S. platensis* can utilize organic carbon substrates for its growth. Ogawa and Terui (1970) reported that 50 % (w/v) ^{14}C glucose in the medium was converted into cell carbon of *S. platensis*. More recently, Marquez *et al.* (1993) found that the growth of *S. platensis* on glucose supplemented medium was much better than that under photoautotrophic conditions. In a photoheterotrophic culture, carbon substrate and light intensity are the two most important factors influencing growth and cellular composition of the microalga. However, this has not been systematically investigated. This paper reports the effects of carbon substrate and light

intensity on the growth and phycocyanin formation of *S. platensis* in photoheterotrophic culture.

MATERIALS AND METHODS

Organism and Cultures *Spirulina platensis* UTEX 1926 (University of Texas Culture Collection) was grown on Zarouk medium consisting of (per l) 1 g NaCl, 2.5 g $NaNO_3$, 1 g K_2SO_4, 0.2 g $MgSO_4 \cdot 7H_2O$, 16.8 g $NaHCO_3$, 0.5 g K_2HPO_4, 0.222 g $ZnSO_4 \cdot 7H_2O$, 2.86 g H_3BO_3, 1.81 g $MnCl_2 \cdot 4H_2O$, 80 mg EDTA (Na), 10 mg $FeSO_4 \cdot 7H_2O$, 40 mg CaCl, 79 mg $CuSO_4 \cdot 5H_2O$, 22.96 mg NH_4VO_3, 15 mg MoO_3, 96 mg $K_2Cr_2(SO_4)_4 \cdot 24H_2O$, 47.85 mg $NiSO_4 \cdot 7H_2O$, 17.94 mg $Na_2WO_4 \cdot 2H_2O$, 43.98 mg $Co(NO_3)_2 \cdot 6H_2O$ and 40 mg $Ti_2(SO_4)_3$, supplemented with or without acetate (CH_3COONa) and glucose. The alga was grown in a 250 ml Erlenmeyer flask containing 100 ml nutrient medium at 30 °C under continuous illumination and shaking (120 rpm). The initial culture pH was adjusted to 9.5, and the pH was found to vary only slightly during cultivation.

Analyses The cell concentration in the culture fluids was determined turbidimetrically at 560 nm. The cell dry weight concentration was determined by drying the cells at 80 °C in a vacuum oven until constant weight. Glucose and acetate concentrations in the culture fluids were determined by HPLC according to the methods described by Chen and Johns (1991, 1994). Phycocyanin was extracted from the algal cells and determined according to the method reported by Bossiba and Richmond (1979).

RESULTS AND DISCUSSION

Effect of carbon substrate *S. platensis* was grown under continuous illumination (4 klux). The glucose and acetate concentrations in the medium varied from 0 to 10 g/l and from 0 to 5 g/l respectively, while the bicarbonate concentration was 16.8 g/l which was used as the control. As can be seen from Figs 1 and 2, the specific growth rate and biomass concentration were significantly enhanced by the addition of glucose and acetate. The highest specific growth rate (0.62 d^{-1}) and the highest cell concentration (2.66 g/l) were found at an initial glucose concentration of 2.5 g/l. Similarly, the culture containing acetate exhibited the highest specific growth rate (0.52 d^{-1}) and the highest cell concentration (1.81 g/l) at an intermediate acetate concentration of 2 g/l. At relatively low glucose (0.5 g/l) and acetate (1 g/l) concentrations, the specific growth rates and cell concentrations were only slightly higher than those of the control (i.e. 0.35 d^{-1} and 1.45 g/l).

These results indicated that *S. platensis* could use glucose and acetate as its carbon sources. Acetate has not been reported to be used for growing *S. platensis*. The highest cell concentration (2.68 g/l) obtained was slightly higher than that of another *S. platensis* strain (NIES-39) on 2.0 g/l glucose in photoheterotrophic culture in which the highest cell concentration was 2.52 g/l (Marquez *et al.*, 1993). It was found that glucose was superior to acetate as carbon source. Under the experimental conditions, glucose could result in 20% higher specific growth rate and 50% higher cell dry weight concentration than acetate. The poorer growth in the acetate supplemented culture is probably due to the inhibitory effect by acetate as the acetate concentration supporting the best growth was 2.0 g/l compared to 2.5 g/l glucose in the glucose supplemented culture. The inhibitory effect by acetate on cell growth had previously been reported with a green microalga, *Chlamydomonas reinhardtii* (Chen and Johns, 1994; Chen and Johns, 1996).

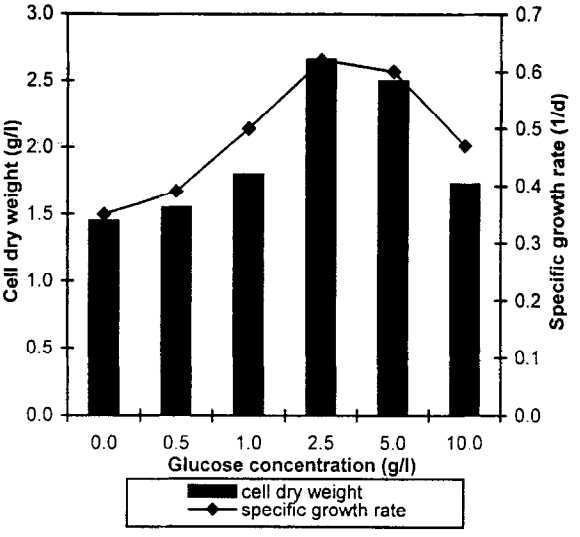

Fig 1. Effect of glucose concentration on the growth of *S. platensis*.

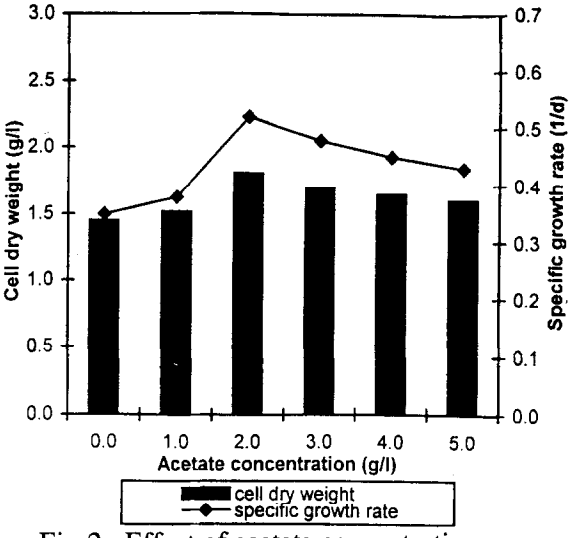

Fig 2. Effect of acetate concentration on the growth of *S. platensis*.

The effect of carbon sources on phycocyanin content as well as the cellular growth is summarised in Fig. 3. The optimal concentrations of glucose (2.5 g/l) and acetate (2.0 g/l) were used for comparison. As shown in Fig. 3, the phycocyanin content per g dry biomass was similar for all carbon sources being approximately 130 $\pm$ 10 mg/g, although very slightly higher phycocyanin content (per cell dry weight) was found in the lower cell density culture. Since phycocyanin is a major photosynthetic accessory pigment (Bogoarad, 1975), the slightly higher phycocyanin content in the lower cell density culture than in the higher cell density culture is understandable as the former attains more light energy per cell. Nevertheless, if the cell concentrations and specific growth rates are taken into consideration, the glucose-enriched culture is still the best since it produced much more phycocyanin (i.e. 322 mg/l, 200 mg/ld) than both the acetate-enriched culture (i.e. 246 mg/l, 128 mg/ld) and the control (i.e. 199 mg/l, 70 mg/ld).

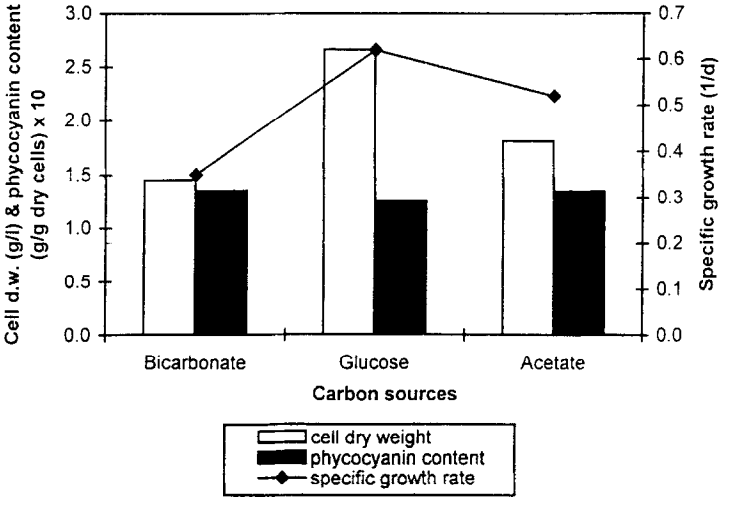

Fig 3. Effect of carbon source on the growth and phycocyanin content of *S. platensis*.

Effect of light intensity The effect of light intensity on specific growth rate of the microalga on 2.5 g/l glucose in photoheterotrophic culture is presented in Fig.4. The optimal range of light intensities was between 2 klux and 4 klux, in which a relatively stable maximum specific growth rate of approximately 0.62 d^{-1} was obtained. The specific growth rate dropped rapidly at light intensities below 2 klux. This was probably due to light limitation. In contrast, higher light intensities (> 4 klux) might result in photoinhibition. At a light intensity of 5 klux, no growth was observed (Fig. 4).

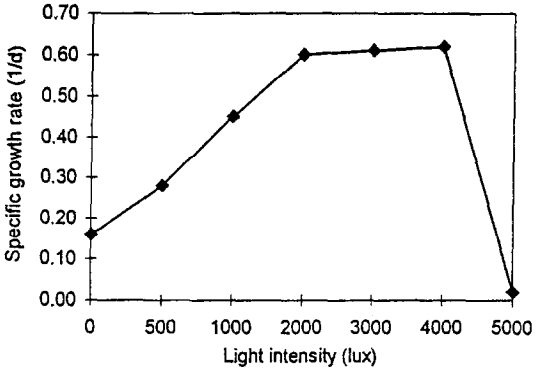

Fig 4. Effect of light intensity on specific growth rate of *S. platensis* on 2.5 g glucose/l.

Photoinhibition has been intensively investigated in microalgae in photoautotrophic cultures (Neale, 1987; Vonshak and Guy, 1992). However, to the best of our knowledge, no information is available regarding the effect of light intensity on photoheterotrophic growth of *S. platensis*. The deteriorative effect of photoinhibition might be relieved by lowering the culture temperature as suggested in photosynthetic systems (Vonshak *et al.*, 1994), since two systems, photosynthesis and oxidative metabolism of glucose, had been demonstrated in photoheterotrophic cultures of *S. platensis* and *Haematococcus pluvialis* (Kobayashi *et al.*, 1992; Marquez *et al.*, 1993). On the other hand, the inhibitory effect of light might be relieved at a higher cell density culture since light penetration could be reduced. However, this was not investigated in the present study.

The effect of light intensity on cell dry weight concentration of the alga grown on various glucose concentrations is summarized in Table 1.

Table 1. Effect of light intensity on cell dry weight concentration of *S. platensis* (UTEX 1926) grown on various glucose concentrations at 30 °C.

	Cell Dry Weight Concentration (g/l)				
Light intensity (klux)	0.0 g/l glucose	0.5 g/l glucose	1 g/l glucose	2.5 g/l glucose	5g /l glucose
0.5	0.295	0.672	0.739	1.424	1.466
2.0	0.909	1.092	1.355	2.377	2.447
4.0	1.420	1.516	1.795	2.657	2.456

For all the cultures, cell concentrations were enhanced with increasing light intensities. The highest cell concentration obtained was approximately 2.66 g/l corresponding to the culture with a glucose concentration of 2.5 g/l and a light intensity of 4 klux. This cell concentration was nearly twice as much as that in the photoautotrophic culture. It is noticeable that when the light intensity decreased to 2 klux or less, the corresponding optimal glucose concentration changed from 2.5 g/l to 5.0 g/l. This phenomenon might be explained by the metabolism mechanism. In photoheterotrophic culture, photosynthesis and oxidative glucose metabolism exist simultaneously (Marquez *et al.*, 1993). At a relatively high light intensity (i.e. 4 klux), photosynthesis might dominate. At low light intensities (i.e. 2 klux or 0.5 klux), the oxidative glucose metabolism component became significant.

Results of the effect of light intensity on phycocyanin content of *S. platensis* grown on 2.5 g/l glucose at 30 °C are plotted in Fig. 5. The phycocyanin content decreased with decreasing light intensity. The highest phycocyanin content was found at a light intensity of 4 klux. At light intensities of 2 klux and 0.5 klux, the phycocyanin content decreased respectively to approximately 80 % and 77 % of that at 4 klux. It is clear that light has an important role in affecting phycocyanin formation since phycocyanin is an important photosynthetic accessory pigment (Bogoarad, 1975).

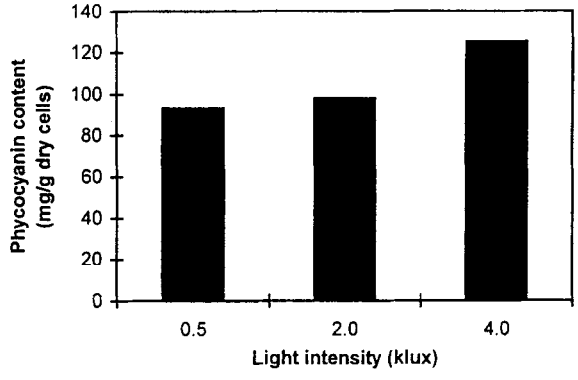

Fig 5. Effect of light intensity on phycocyanin content of *S. platensis* on 2.5 g glucose/l.

These results suggest that photoheterotrophic growth may be a feasible mode to obtain high cell density and high phycocyanin productivity. Light intensity can be so stepwisely increased with increasing carbon concentration that the only limiting factor will be carbon substrate which can be fed to the culture to extend the exponential phase and thus result in a high cell density and productivity. The stepwise increase in light intensity would also favour the phycocyanin formation. Research is continuing in our laboratory to investigate the possibility of using fed-batch culture to obtain high cell densities and high phycocyanin productivities of the microalga.

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