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FACTORS AFFECTING THE PRODUCTIVITY OF SPIRULINA PLATENSIS

BY

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Received: 23-4-1992

ABSTRACT

The optimum culture conditions for the formation of the highest values of biomass, protein and phycocyanin by strain of Spirulina platensis isolated from Gamasa, Egypt were achieved by incubating the cultures for 20 days at 35°C. under light intensity of 2000 lux. The medium used was that suggested by Zarrouk (1966) containing 0.25% (w/v) $NaNO_3$ as the sole source of nitrogen and 8.0% (w/v) sodium chloride.

INTRODUCTION

Fogg et al. (1973) reported that the pigments of the cyanobacteria are localized in definite chromatophores. The pigments include chlorophyll a, carotenes and xanthophyll; in addition to a blue pigment (c-phycocyanin) and a red pigment (c-phycoerythrin). Clement (1975) stated that Sprulina

Key words: Spirulina; phycocyanin; protein; growth

maxima is rish in carotenes and xanthophylls and in the dried thallus, carotene and xanthophyll contents were 1.4-1.6 and 1.5-2.5 g/kg, respectively. The analysis of pigments of any photosynthetic apparatus suffers from a number of difficulties. Davies (1976) recorded certain difficulties in studying the photosynthetic pigments of cyanobacteria due to their instability, as well as their sensitivity to light, heat and oxygen. Lijima et al. (1982) stated that phycocyanin may generally stimulate the immunity system and provide protection from a variety of diseases. Clement et al. (1967) stated that certain of the pharmaceutical compounds extracted from Spirulina sp. accelerated the cicatrization of wounds. In the meantime, Schwartz and Shklar (1986) have supported the use of Spirulina extracts in the treatment of certain cancers.

Spirulina platensis has been isolated from a wide range of habitats differing in their water quality, from low ionic concentration through brackish to saline (Cifferi, 1983). Spirulina strains may form, at a given time 90% blooming of the total phytoplankton biomass (Richmond, 1988).

In this study, the factors which affect the biosynthesis of biomass, proteins and phycocyanin by Spirulina platensis were investigated.

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MATERIALS AND METHODS

EXPERIMENTAL ORGANISM:

A strain of Spirulina platensis, a filamentous cyanobacterium was isolated from Gamasa City, Dakahlia Governorate, Egypt, in an area where the Nile water interferes with the sea water of the Mediterranean Sea.

Culture conditions:

The culture medium used for the cultivation and maintaining the experimental strain of Spirulina platensis was suggested by Zarrouk (1966). The components and their concentrations of this medium (mg/l) were Sodium nitrate, 2500; calcium chloride, 53; potassium monohydrogen phosphate, 500; potassium sulphate, 1000; magnesium sulphate, 200; sodium bicarbonate, 16800; sodium chloride, 1000; ferrous sulphate, 10; EDTA, 80 and one ml microelements solution. The micronutrient solution contained the following concentrations (mg/l) of different trace elements salts: $MnCl_2.\ 7H_2O$, 1810; $CuSO_4.\ 5H_2O$, 80; $Co(NO_3)_2.\ 6H_2O$, 44; $ZnSO_4.\ 7H_2O$, 220; H_3BO_3, 2860; $MoBO_3$, 15; NH_4VO3, 23; $NiSO_4.\ 7H_2O$, 47.82; $NaWO_4.2H_2O$, 17.94; $K_2(Cr_2SO_4)_4.\ 24H_2O$, 96 and $Ti\ (SO_3)_3$, 40. The pH value was adjusted at 8.3.

In order to start a culture of a cyanobacterium, a concentration of 10^6 hormogonia (the vegetative cells) per 100 ml of the culture medium was used as the stock suspension for inoculating the experimental cultures (El-Malky, 1982).

Fifteen counts were taken from the quadrats of a haemocytometer and the arithmatical mean of the cell number was calculated to give the hormogonial numbers per milliliter of suspension which was assumed to represent the recommended concentration.

The cyanobacterial cultures were grown in 250 ml conical flasks containing 100 ml of the experimental medium, each culture flask was inoculated with 1 ml of stock algal suspension. The algal cultures were incubated at 30 ± 2°C under light intensity 2000 lux for 20 days. At the end of the incubation period, the cyanobacterial growth was collected by centrifugation at 3000 rpm for 10 minutes. The cells were washed with distilled water, and recentrifuged. The residue was divided into two parts. A part was used for pigment and protein determination. The other part was used for weight determination, by drying in an oven at 80°C and weighing at intervals till constant weight.

With regard to culture age studies, the conical flasks containing 100 ml basal culture medium were inoculated with 1 ml stock suspension and then incubated at 30 ± 2°C and illuminated with different light intensities (1000, 2000 or 3000 lux) for different periods of time. At the end of each time interval, cell biomass, phycocyanin and protein production were estimated.

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With respect to temperature studies, the culture flasks were kept after inoculation with 1 ml of the cell suspension at different temperatures (5, 10, 15, 20, 25, 30, 35, 40, 45°C), ... 000 lux and pH 9.0.

The effect of the initial pH value was studied in conical flasks containing culture media adjusted at different pH value values (1.5, 3.0, 4.5, 6.0, 7.5, 9.0, 10.5, 12.0), 35°C under light intensity 2000 lux. At the end of incubation period, the different parameters were estimated.

The effect of the different nitrogen sources on the algal growth was studied by using different nitrogen salts ($NaNO_3$, NH_4Cl, urea, $(NH_4)_2SO_4$ and ammonium citrate) in different concentrations ranging from 0.05% to 0.35% (w/v).

The effect of sodium chlo... tress, was investigated by supplementing the following ... tration of NaCl into the basal culture medium: 0.0, ...0, 6.0, 8.0, 10.0, 12.0% (w/v). At the end of the incuba... ...d the different growth parameters were determined.

The protein content was ... ned using the method adopted by Lowery et al. (1951). ... ocyanin determination was carried out using method of ... and Eshel (1985). The pigment content was computed from ... following equation:

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Phycocyanin (mg/100ml) = $[(A_{618} - A_{645}) - (A_{592} - A_{645})$ 0.51] 0.15 where: A_{618} = optical density at 665 nm

A_{645} = optical density at 645 nm

RESULTS AND DISCUSSION

From Fig (1) it is clear that the optimal light intensity was 2000 lux during the different periods of incubation. Under this light intensity, maximal values of phycocyanin, protein and biomass were recorded after 20 - 24 days of incubation. The other tried light intensities were less suitable. The maximal values of proteins, pigments and biomass were achieved after 18, 20 and 22 days of incubation at 1000, 2000 and 3000 lux respectively. However, the values at 1000 were comparatively lower than those obtained at the higher light intensities.

From figures 2 - 4 and table 1, it is evident that the optimal condition affecting the production of phycocyanin, protein and biomass by the experimental organism were: incubation period of 20 days, at 35°C, pH value of 9.0, $NaNO_3$ at a concentration of 0.25% w/v and NaCl at a concentration of 8.0% w/v. Any changes in these factors induced significant reduction in the values of the biosynthesis of the studied parameters.

A number of growth conditions are known to affect the cell biomass, phycocyanin and protein of cyanobacteria. Among

these factors are temperature, pH value, salt stress, light intensity and culture age.

As mentioned above the maximal value of the mentioned parameters were attained after 20 days of incubation. However, the incubation temperature seems to depend on strain variation. Oran et al. (1979) obtained a high value of Spirulina maxima growth (3 9/1) after 30 days cultivation in batch culture. The light intensity seems to play a role in reducing the incubation period. The cyanobacterial growth, protein contents and phycocyanin production were attained after 18 days at light intensity 1000 lux, while at higher light intensities (2000 lux and 3000 lux), the maximal values of growth parameters were obtained after 20 days. From the obtained data, it is evident that the light intensity of 2000 lux was the best for achieving the optimal values of the different growth parameters studied. Light intensity seems also to be strain variation dependent. Dohler and datz (1980) reported that the growth and fatty acid contents doubled at low light intensity, while Seto et al. (1984) recorded the opposite effect at low light intensities.

The optimal temperature of the growth of Spirulina platensis, protein and phycocyanin production was 35°C, a finding that runs with results obtained by Zarrouk (1966), who recorded a maximal rate of growth of Spirulina between 35 - 40°C. Richmond et al. (1980) mentioned that in contrast

to day temperature, Spirulina can tolerate low night temperatures even below the freezing point.

Zarrouk (1966) also reported that alkalinity (pH 8.3 - 11) is mandatory for the growth of Spirulina and in the current work, the optimal pH value recorded was 9.0.

Nitrogen sources were found to affect the cyanobacterial growth, total protein and phycocyanin formation by the experimental organism. Sodium nitrate ranked the first among the tested nitrogen sources which gave high productivity of biomass, protein and phycocyanin. Nitrate was reported by Zarrouk (1966) to be the most favourable source of nitrogen by Spirulina. Parallel to our results, the same investigator reported that ammonium salts may be used at low concentrations. Urea could be used with no ill effects at pH 8.4 as long as its concentration is kept below 1.5 g/1 (Soong, 1980). Spirulina platensis could grow well at the following concentrations: 0.0, 2.0, 4.0, 6.0, and 8.0% of NaCl. The maximum values of phycocyanin, biomass and protein were achieved at 8.0% NaCl. Any change in sodium chloride concentration resulted in decreasing the studied parameters. Contrary to that Wyn-Jonen and Gorham (1983) stated that high intracellular sodium concentration are toxic to most biological system. The adaptation of cyanobacterium to high salt concentration may refer to the extrusion of sodium from the cells which coupled to the inwardly movement of the protons

(Krulwish, 1986) or to the ability of cells to build-up internal organic osmotica in order to cope with the unbalanced osmotic pressure (Hagemann et al., 1987).

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Table 1: Effect of different nitrogen sources and their concentrations on the cell mass , pigment content and protein content

Nitrogen Conc. (w/v %)	0.05	0.10	0.15	0.20	0.25	0.30	0.35
$NaNO_3$:				(mg/100 ml)			
Cell mass	13.8±1.81	27.4±4.20	38.2±3.60	45.2±5.01	52.80±6.12	39.70±3.12	28.70±2.49
Pigment content	2.74±0.41	5.42±0.10	7.64±1.71	9.43±0.89	11.02±2.01	8.41±1.20	5.74±0.08
Protein content	7.70±1.20	12.7±0.80	17.9±0.81	19.7±2.41	23.01±1.61	18.31±2.10	13.9±0.81
NH_4Cl:							
Cell mass	16.1±2.10	18.4±2.10	15.8±0.71	14.0±1.20	9.8±0.06	0.0±0.0	0.0±0.0
Pigment content	3.70±2.10	3.67±0.71	3.20±0.61	2.85±0.60	2.10±0.07	0.0±0.0	0.0±0.0
Protein content	9.82±1.40	9.80±1.31	9.61±2.00	8.5±0.71	6.7±0.81	0.0±0.0	0.0±0.0
Urea:							
Cell mass	10.0±4.0	24.5±5.1	16.8±0.8	9.4±2.1	0.0±0.0	0.0±0.0	0.0±0.0
Pigment content	1.9±0.2	4.9±0.3	3.41±0.6	1.85±0.02	0.0±0.0	0.0±0.0	0.0±0.0
Protein content	6.7±0.8	12.3±2.4	9.81±2.1	6.61±1.2	0.0±0.0	0.0±0.0	0.0±0.0
$(NH_4)_2\ SO_4$:							
Cell mass	13.8±2.8	16.1±1.47	8.0±0.12	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Pigment content	2.76±0.5	3.22±0.12	1.61±0.26	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Protein content	7.6±1.2	9.3±0.12	5.98±0.41	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Amm. Citrate:							
Cell mass	14.3±0.9	17.6±2.1	13.8±2.91	6.2±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Pigment content	2.85±0.12	3.56±0.5	2.76±0.5	1.24±0.21	0.0±0.0	0.0±0.0	0.0±0.0
Protein content	8.5±0.7	9.5±0.52	7.6±1.20	4.18±0.1	0.0±0.0	0.0±0.0	0.0±0.0

Each measurement is 3 replicated ± S E M.

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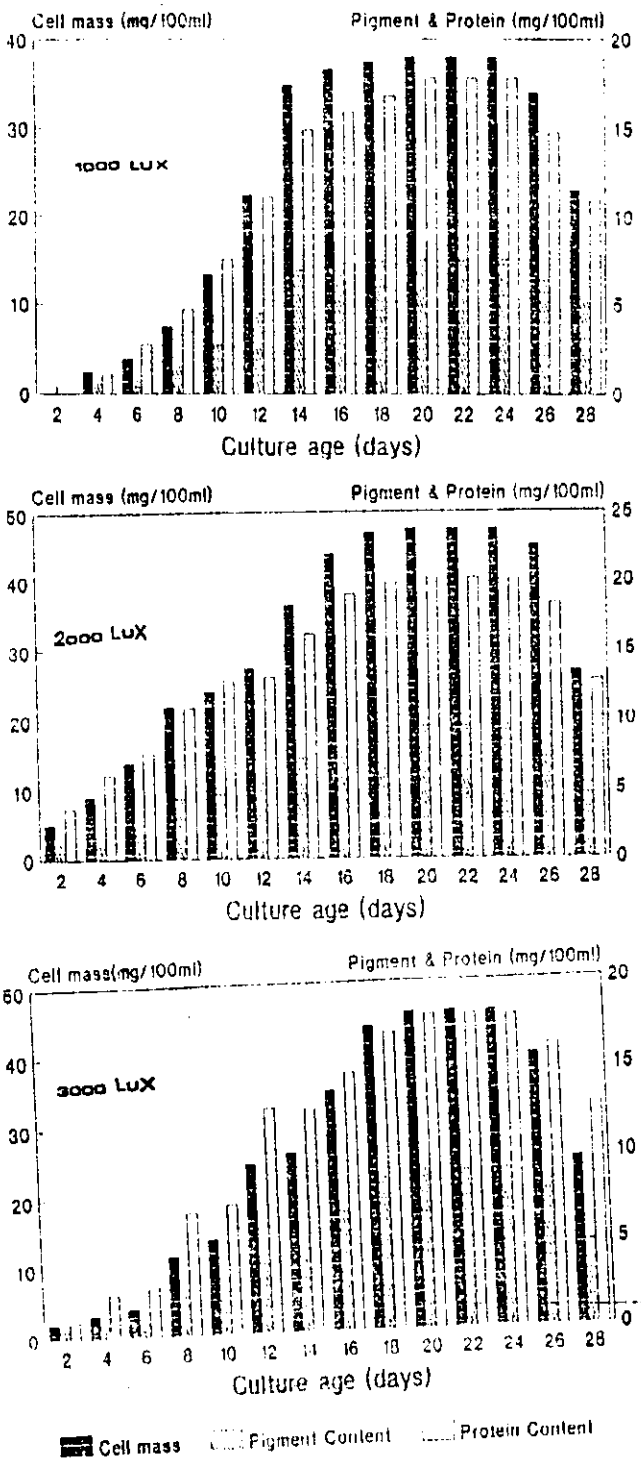

Fig. 1: Effect of culture age on phycocyanin content and cell mass under different light intensities (1000, 2000, and 3000 lux).

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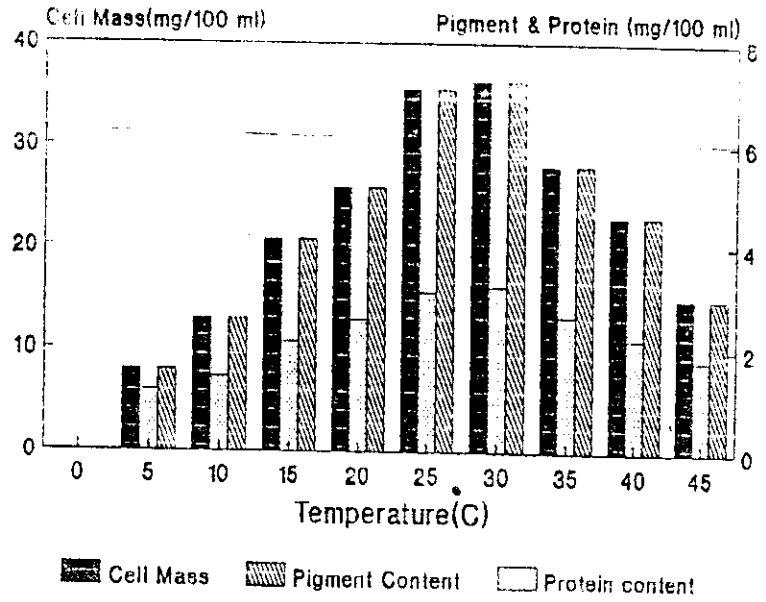

Fig. 2: Effect of temperature on phycocyanin content, protein content and cell mass.

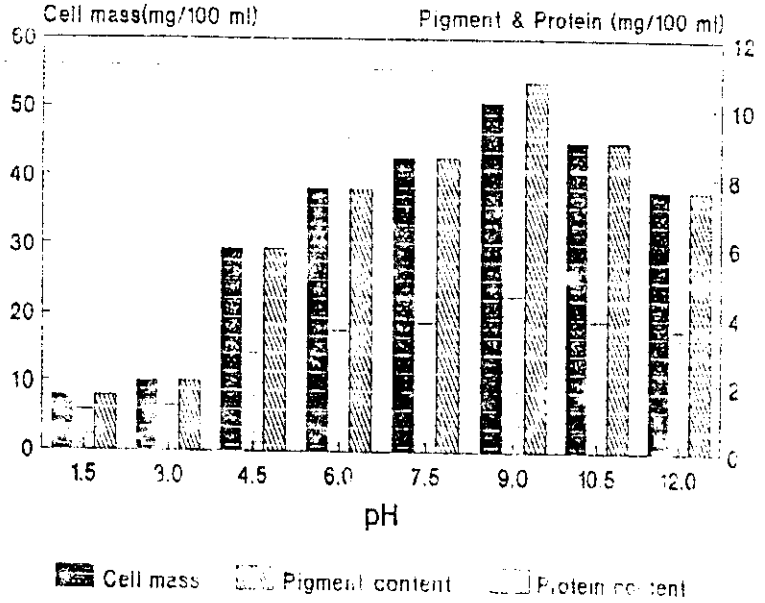

Fig. 3: Effect of pH value on phycocyanin content, protein content and cell mass.

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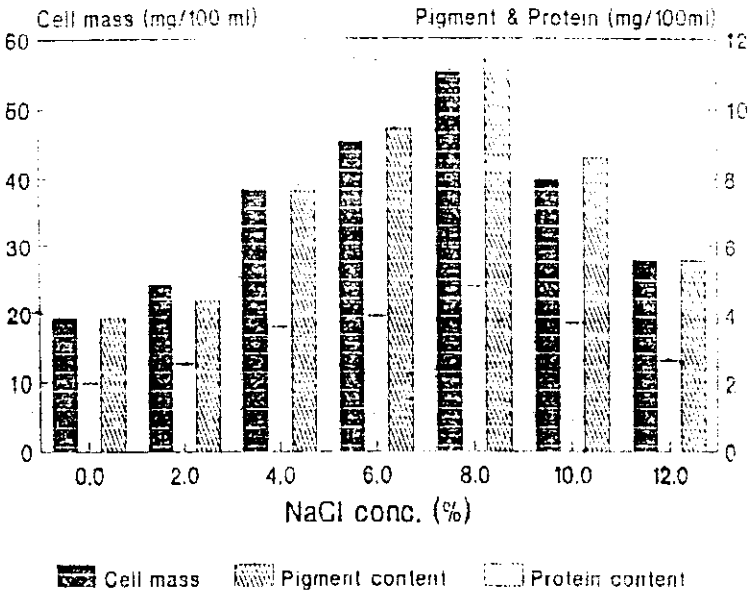

Fig. 4: Effect of NaCl stress on phycocyanin, protein content and cell mass.

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"العوامل المؤثره على انتاجية سبيرولينا بلاتنسس"

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أوضحت الدراسة التى اجريت على سلالة سبيرولينا بلاتنسس من البكتريا الزرقاء أن الظروف الزراعيه المناسبه لاعطاء أعلى انتاج من النمو والمحتوى البروتينى وكذلك محتوى صبغ الفيكوسيانين هى أن تكون فترة الحضانة عشرون يوما وشدة الأضاءة ٢٠٠٠ لاكس ودرجة الحرارة ٣٥°م باستخدام الوسط الغذائى لزاروك عام ١٩٦٦ المحتوى على ٢٥و٠٪ نترات الصوديوم كمصدر للنتروجين و ٥٠٨٪ كلوريد الصوديوم.