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Effects of Chemical Parameters on *Spirulina platensis* Biomass Production: Optimized Method for Phycocyanin Extraction*

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Abstract: The micro alga, *Spirulina* is a rich source of protein, which is used as a protein supplement for humans, chicks and also in aquaculture. Among the cultures, CS-1 registered maximum biomass production and S-20 showed highest biomass production among the local isolates. Optimum temperature of 35°C was the best for maximum biomass production of *S. platensis* cultures. Among the cultures CS-1 culture, put forth maximum biomass production at 35°C. The biomass production of all *S. platensis* cultures was maximum at pH 10.0. Among the cultures, CS-1 recorded maximum biomass at pH 10.0. *S. platensis* culture S-20 showed highest biomass production among the local isolates. *S. platensis* cultures were grown under different light wave lengths ranging from 340-700 um and observed that it grows best in red light but later on there was no significant difference between the biomass produced under red and normal white lights. *S. platensis* culture CS-1 has shown the highest chlorophyll, carotenoids and phycocyanin and protein contents. When the extracted protein was resolved on a 15% SDS-PAGE gel, the cultures have polypeptide subunits ranging from the molecular weights 20 to 95 kDa. The liquid nitrogen method was found to be the best by extraction higher quantity of phycocyanin from all *S. platensis* cultures. Among the cultures, *S. platensis* culture CS-1 recorded the highest phycocyanin content and among the local isolates SM-2 showed the highest pigment content. SDS-PAGE analysis of phycocyanin pigment revealed two characteristic bands with a molecular weights of 14.3 and 20.1 kDa approximately for α and β subunits.

Key words: *Spirulina platensis*, phycocyanin, liquid nitrogen, SDS-PAGE

INTRODUCTION

The micro algae, *Spirulina* seemed to be a good protein source (60-70%) and is comparable with the milk proteins (Shelef and Soeder, 1980); further research revealed that it is a rich source of vitamins, essential amino acids, minerals and β-carotene. It also find its immense use as anti cancer formulations, diabetes control wound treatment and to promote skin metabolism (Surekha Rani and Uma Bala, 2006). This is widely exploited in the manufacturing of beauty products such as anti-wrinkling, anti-pimple creams, facemasks and high protein shampoos. It has been commercially cultivated for its bluish green pigment, called phycocyanin, which can be used as a natural colorant for food, cosmetics etc (Liang *et al*., 2004). Though several thousand algal forms are available in nature, only a few are amenable to technology. This is based on their ability to grow in synthetic media, case of separation and stability to drying and importance of their chemical constituents and finally the cost effectiveness of the whole system (Venkatraman, 1983). In the light of the above facts, the present study was undertaken to determine the chemical parameters of *S. platensis* with response to organic and inorganic substrate.

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MATERIALS AND METHODS

Five cultures of *S. platensis* were used for the present study (CS-1, SM-2, S-10, S-20 and Sp). Three *S. platensis* cultures viz, SM-2, S-10 and S-20 were isolated from field soils of paddy breeding station and lands of TNAU, Coimbatore and the biomass production and biochemical constituents were compared with standard cultures CS-1 and SP obtained from algal laboratory, Madurai. The collected soil samples were inoculated into the sterile Zarrouk's Medium (ZM) (Hung *et al*., 2002) in 100 mL of 250 mL Erlen Mayer flasks and inundated in laboratory growth chamber. During the experimental period, the light intensity of 3000 lux with 16/8 h alternate light and dark cycles at 32°C was maintained. The flasks were examined for the algal growth periodically. They were then observed under microscope. The medium normally used for *S. platensis* cultures was Zarrouk 's medium with $NaHCO_3$ as the main carbon source.(Binaghi *et al*., 2003) Other carbon sources viz., Na_2CO_3, D-glucose, Mannitol, $(NH_4)_2CO_3$, $CaCO_3$, sucrose and urea were used as the sole carbon source. These compounds were added to the media on equimolar concentration basis. The flasks were incubated for 30 days.

To know the effect of temperature on growth, the S. *platensis* was exposed with different temperatures viz, 15, 20, 25, 30, 35 and 40°C. The incubation conditions were same as mentioned earlier. The growth of *S. platensis* cultures was tested at 6.0, 8.0, 10.0, 12.0 and 14.0 pH levels. The solution was adjusted to acidic pH with 1 N HCl and to alkaline pH was with 1 N NaOH. The incubation conditions were same as mentioned earlier. The growth of *S. platensis* was tested under different wavelengths of light sources viz., red (640-740 um), green (520-570 um) and blue (450-520 um) with white light (350-740 um) as the control (Gevorgiz and Golovnya, 2002). The different wavelengths of light were maintained using different colored electric bulbs. The incubation conditions were same as mentioned earlier and the biomass was estimated after10, 20 and 30 days after inoculation. The cells were subjected to sonication incubated a solicitor. The cultures were filtered through pre weighed Whatman No. 42 filter paper. The filter papers with the cyanobacterial cultures were oven dried at 80°C for 24 h and weighed. The biomass production was calculated based on the different incubated weight and expressed as mg dry weight per mL of the culture suspension.

The biochemical constituents were estimated at a regular interval of 10 days after inoculation up to 30 days by following the standard procedures. The total chlorophyll-a content of the *S. platensis* cultures was estimated as per the method of Tailing and Driver (1961). The amount of carotenoid pigment present in different *S. platensis* cultures was estimated by following the method of Siegelman and Kycia (1976). Four different methods (Liquidnitrogen, Freezing and thawing, Sonication and Using lyses buffer and lysozyme methods) were followed for efficient extraction of phycocyanin from *S. platensis*. The cultures were taken at a regular interval of 10 days i.e., 10, 20, 30 days after inoculation. The total protein content of the *S. platensis* cultures was estimated by following the method of Lowry *et al*. (1951). The separation of protein was done by SDS-PAGE following the method described by Maniatis *et al*. (1989).

RESULTS

The effect of carbon sources on the biomass of *S. platensis* cultures was estimated at three regular intervals, i.e., 10, 20 and 30 Days after Inoculation (DAI) and the data are presented in Table 1. Among the different carbon sources, Na_2CO_3 significantly enhanced the biomass production while the least biomass production was observed with $CaCO_3$. In general, the biomass produced by all the cultures was significantly higher when NaH_2CO_3 of the medium was replaced with Na_2CO_3 irrespective of the inoculation periods. There was also significant difference between the biomass produced when $(NH_4)_2CO_3$ and $CaCO_3$ are added to the growth medium as sole carbon sources. Maximum biomass

Table 1: Effect of carbon source on biomass production of *S. platensis* cultures

		Biomass produced (mg mL^{-1} dry wt.)		
Cultures	Carbon sources	10 DAI	20 DAI	30 DAI
CS-1	$(NH_4)_2CO_3$	4.18	8.34	12.56
	$CaCO_3$	2.67	5.41	7.98
	$NaHCO_3$	5.78	11.45	17.20
	Na_2CO_3	7.51	15.25	22.50
SM-2	$(NH_4)_2CO_3$	2.42	4.83	7.34
	$CaCO_3$	1.38	2.76	4.12
	$NaHCO_3$	3.39	6.81	10.18
	Na_2CO_3	4.74	9.52	14.28
S-10	$(NH_4)_2CO_3$	2.38	4.76	7.12
	$CaCO_3$	1.35	2.69	4.01
	$NaHCO_3$	3.30	6.81	9.98
	Na_2CO_3	4.72	9.48	14.15
S-20	$(NH_4)_2CO_3$	3.18	6.48	9.72
	$CaCO_3$	2.12	4.42	6.36
	$NaHCO_3$	4.67	9.34	13.98
	Na_2CO_3	6.42	12.86	19.25
SP	$(NH_4)_2CO_3$	4.08	8.12	12.48
	$CaCO_3$	2.59	5.17	7.75
	$NaHCO_3$	5.61	11.27	16.88
	Na_2CO_3	7.33	14.88	22.01

was recorded at 30 days after inoculation. Among the cultures, CS-1 produced the highest biomass (22.50 mg mL^{-1}) while S-10 registered a least biomass (14.15 mg mL^{-1}) at 30 DAI when Na_2CO_3 was used as a sole carbon source. The biomass produced by the cultures, SM-2 and S-10 under different carbon source was on par with each other at all three intervals. It was also found that *S. platensis* was unable to utilize the organic carbon source like D-glucose, mannitol sucrose, urea etc.

The biomass production of *S. platensis* cultures was evaluated to different temperatures, ranging from 20-40°C at three regular intervals i.e., 10, 20 and 30 days after inoculation. In general, all the cultures produced maximum biomass at 30 and 40°C (Table 2) It was noticed that when the laboratory cultures were kept at 45°C for up to 24 h, they were unable to grow but growth was resumed when the cultures were brought back to 35°C. The highest biomass (17.22 mg mL^{-1}) was recorded by CS-1 culture at 35°C on 30 DAI and the least biomass (15.98 mg mL^{-1}) was recorded in the case of S-10 at same temperature and incubation.

The biomass produced by *S. platensis* cultures was calculated at different pH levels ranging from 6.0-12.0 at three regular intervals, i.e., 10, 20 and 30 days after inoculation and the results are presented in Table 3. In general, all the cultures produced highest biomass at pH 10.0 of 30 DAI. There was no significant difference between the biomass produced at 8.0 and pH 12.0 levels. Least biomass was recorded when the pH was maintained at 6.0. Maximum biomass (16.92 mg mL^{-1}) and the minimum biomass (9.65 mg mL^{-1}) were recorded in the case of CS-1 and S-10 respectively at 30 DAI when the pH was 10.0.

The biomass produced by *S. platensis* cultures was estimated under different light wavelengths at three regular intervals i.e., 10, 20 and 30 DAI and the results are shown in Table 4. The cultures were grown under different light sources, viz; red, blue and green along with normal white light as control. In general, biomass produced by all the cultures was maximum under red light at 30 DAI and the least biomass was recorded when the cultures were grown under green light. There was no significant difference between the biomass produced by CS-1, S-20 and *S. platensis* cultures at 30 DAI under red light. Among the cultures, *S. platensis* has shown highest biomass (20.89 mg mL^{-1}) and the least biomass (13.96 mg mL^{-1}) by SP and SM-2 respectively at 30 DAI under red light. However, there is not much variation in biomass production between red and white lights.

Table 2: Effect of temperature on biomass production of *S. platensis* cultures

		Biomass produced (mg mL^{-1} dry wt.)		
Cultures	Temperature (°C)	10 DAI	20 DAI	30 DAI
CS-1	20	3.22	6.54	8.24
	25	4.21	9.26	10.43
	30	4.98	11.84	14.12
	35	6.92	12.54	17.22
	40	4.73	10.64	12.14
SM-2	20	2.68	5.34	8.01
	25	3.18	6.18	9.24
	30	4.54	9.24	13.56
	35	5.34	10.68	16.02
	40	4.32	8.75	12.98
S-10	20	2.33	4.68	7.01
	25	2.72	5.53	8.22
	30	4.14	8.52	12.76
	35	5.33	10.54	15.98
	40	4.06	8.63	12.54
S-20	20	2.74	5.48	8.21
	25	3.02	6.10	9.16
	30	4.56	8.98	13.24
	35	5.58	11.14	16.72
	40	4.42	8.83	12.98
SP	20	2.78	5.54	8.28
	25	3.10	9.32	9.28
	30	4.66	6.22	13.98
	35	5.62	12.24	16.87
	40	4.38	8.69	13.12

Table 3: Effect of pH and biomass production of *S. platensis* cultures

		Biomass produced (mg mL^{-1} dry wt.)		
Cultures	pH	10 DAI	20 DAI	30 DAI
CS-1	6.0	2.89	5.78	8.66
	8.0	4.32	8.52	12.84
	10.0	5.65	11.32	16.92
	12.0	4.21	8.53	12.72
	14.0	-	-	-
SM-2	6.0	1.40	2.81	4.21
	8.0	3.28	4.53	6.78
	10.0	3.65	7.32	10.92
	12.0	2.24	4.45	6.64
	14.0	-	-	-
S-10	6.0	1.39	2.76	4.14
	8.0	2.21	4.21	6.62
	10.0	3.41	6.43	9.65
	12.0	2.19	4.01	6.58
	14.0	-	-	-
S-20	6.0	1.71	3.51	5.14
	8.0	3.13	6.18	9.41
	10.0	4.54	9.18	13.62
	12.0	3.08	6.24	9.22
	14.0	-	-	-
SP	6.0	2.88	5.74	8.59
	8.0	3.94	8.08	12.12
	10.0	5.51	11.08	16.55
	12.0	3.89	7.96	11.94
	14.0	-	-	-

The chlorophyll-a content of *S. platensis* cultures was estimated at three regular intervals i.e., 10, 20 and 30 DAI and the data are presented in Table 5. In general increase in chlorophyll-a content of *S. platensis* cultures was observed as the inoculation period increases. Highest chlorophyll content (9.723 µg mL^{-1}) was recorded in CS-1, which is on par with a local isolate, S-20 and the least chlorophyll content (8.526 µg mL^{-1}) were observed in S-10.

Table 4: Effect of light wavelength on biomass production of *S. platensis*

		Biomass produced (mg mL^{-1} dry wt.)		
Cultures	Light wave length (λmax)	10 DAI	20 DAI	30 DAI
CS-1	Blue (450-520)	4.78	9.54	14.25
	Red (640-740)	5.59	11.38	17.02
	Green (520-570)	3.28	6.55	9.81
	White (350-740)	5.64	11.29	16.92
SM-2	Blue (450-520)	3.39	6.78	10.15
	Red (640-740)	4.67	9.32	13.96
	Green (520-570)	2.09	4.18	6.24
	White (350-740)	4.55	9.12	13.68
S-10	Blue (450-520)	3.44	6.88	10.28
	Red (640-740)	4.71	9.43	14.12
	Green (520-570)	2.49	4.95	7.42
	White (350-740)	4.66	9.31	13.94
S-20	Blue (450-520)	4.41	8.83	13.22
	Red (640-740)	6.34	12.74	18.98
	Green (520-570)	3.25	6.52	9.74
	White (350-740)	6.28	12.52	18.62
Sp	Blue (450-520)	5.32	10.68	15.74
	Red (640-740)	6.96	13.84	20.89
	Green (520-570)	2.57	5.28	7.71
	White (350-740)	6.90	13.73	20.75

Table 5: Chlorophyll-a content of *S. platensis* cultures

	Chlorophyll-a content (μg mg^{-1} dry wt.)		
Cultures	10 DAI	20 DAI	30 DAI
CS-1	3.011	5.982	9.723
SM-2	2.871	5.145	8.618
S-10	2.871	4.988	8.526
S-20	2.908	5.616	8.724
SP	3.009	6.124	9.422

Table 6: Carotenoids content of *S. platensis* cultures

	Carotenoid content (μg mg^{-1} dry wt.)		
Cultures	10 DAI	20 DAI	30 DAI
CS-1	0.917	1.876	2.813
SM-2	0.742	1.498	2.262
S-10	0.817	1.625	2.513
S-20	0.908	1.814	2.725
SP	0.912	1.851	2.775

The carotenoid content of *S. platensis* cultures was measured at three regular intervals i.e., 10, 20 and 30 days after inoculation and the results are presented in Table 6. In general, the carotenoid content increased gradually for all cultures with increase in the inoculation period. Highest carotenoids content (2.813 μg mg^{-1}) was recorded in CS-1 followed by 2.775 μg mg^{-1} in the culture SP. The least carotenoids content (2.262 μg mg^{-1}) was observed in case of SM-2 at 30 DAI.

The phycocyanin produced by *S. platensis* cultures was estimated at 10, 20 and 30 days after inoculation using different methods, viz., liquid nitrogen, freezing and thawing, sonication and lysozyme methods and the data are given in Table 7. In general, highest phycocyanin was extracted from all the cultures when liquid nitrogen method was used. Least phycocyanin was extracted when sonication method was used. Among all the cultures, CS-1 culture has shown the highest phycocyanin content (110.20 μg mg^{-1}) at 30 DAI by liquid nitrogen method followed by Sp, Sm-2, S-10 and the least phycocyanin content (65.12 μg mg^{-1}) was shown by S-20. When the pure phycocyanin pigment was resolved on 15% gel, these were two bands observed, one having the molecular weight greater than 14.3 kDa and another band giving the molecular weight greater than 20.1 kDa.

Table 7: Phycocyanin content (μg mg^{-1} dry wt) of *S. platensis* cultures

	Liquid nitrogen method			Freezing and thawing method			Sonication method			Lysozyme method		
	10	20	30	10	20	30	10	20	30	10	20	30
Cultures	(DAI)											
CS-1	36.73	71.45	110.20	33.19	62.12	101.80	25.40	34.87	82.20	32.98	61.96	101.62
SM-2	32.04	61.02	96.12	29.38	56.76	88.14	22.06	48.12	72.18	29.12	56.52	87.99
S-10	29.81	59.72	89.14	26.64	52.28	78.42	20.74	43.56	65.24	26.02	52.14	78.21
S-20	21.70	44.24	65.12	18.49	38.98	58.47	13.22	28.45	42.68	18.22	38.84	58.24
SP	33.08	66.19	99.24	29.44	59.48	89.22	24.09	50.18	78.28	29.12	89.41	89.12

Table 8: Protein content of *S. platensis* cultures

	Protein content (μg mg^{-1} dry wt)		
Cultures	10 (days)	20 (days)	30 (days)
CS-1	214.04	418.12	642.12
SM-2	192.73	399.46	598.25
S-10	187.65	391.90	593.70
S-20	204.20	401.69	612.58
SP	208.05	414.98	624.15

The total protein content of different *S. platensis* cultures was estimated at 10, 20 and 30 days after inoculation and the data are given in Table 8. In general, protein content increased as the inoculation period increased. However, the highest protein content (642.12 μg mg^{-1}) was recorded in the case of CS-1 culture and the least protein content (593.70 μg mg^{-1}) was recorded in the case of S-10 at 30 DAI. The total protein was isolated from different cultures of *S. platensis*. The results have clearly indicated that the total protein content differ widely among all the cultures with the molecular weight ranging from nearly 20 kDa to more than 95 kDa. All the cultures produced almost similar bands.

DISCUSSION

S. platensis has commercial importance due to overall nutritional qualities, especially high protein and vitamin contents, particularly B12. Various strains of *S. platensis* have been grown in various nutrient media in order to have high yield with low cost inorganic nutrients. Zarrouk's medium has almost universally been adopted for *S. platensis* before Venkatraman (1983) replaced the costly chemical of Zarronk's medium (Zm) with commercial fertilizer (NPK 15:15:15). Chandgothia and Srivastava (1994) replaced the A5 nutrient solution by 20% soil extract and 0.2% NaCl for growing *S. subsalsa*. In case of *S. labyrinthoformis*, when the 70% medium was substituted with groundnut shell ash extract (GSAE) the growth was on par with Zarrouk's medium, while the lost input was reduced by 73%. Sharma and Srivastava (1997) experimented with *S. subsalsa* and observed that BGSE added Zarronk's medium enhanced 40% density of the cultures.

In the present study, an attempt was made to grow *S. platensis* under different source of carbon, which are added to the medium on the basis of equimolar concentration. Different carbon source that are used in the experiment include ammonium carbonate, calcium carbonate, glucose, mannitol, sodium carbonate, sucrose and urea, while the growth medium with sodium bicarbonate act as a control. Maximum biomass production was observed when sodium bicarbonate in the normal Zarronk's medium was replaced with sodium carbonate, which is a cheaper chemical. This result is in agreement with Shelef and Soeder (1980), who stated that carbonate and bicarbonate source of carbon are best for the maximum biomass production of *S. platensis*. *S. platensis* has completely failed to show any growth in the medium, when $NaHCO_3$ was replaced with any organic source of carbon and within ten days after inoculation, the death of the culture was observed. This might be due to the reason that photoautotrophs cannot show any growth in the medium with organic carbon as a sole carbon source

(Ciferri, 1983) and another reason might be that the insufficient quantity of the carbon needed for specific growth of *S. platensis*, if supplied in organic form on equimolar concentration. This is in accordance with the findings of Joardan (1998) who estimated that 0.5 kg of sugar is needed to produce one kg biomass of *S. platensis*, when sugar is added as sole carbon source. He also suggested the use of cheaper sugar cane juice in large-scale cultivation. Chen and Zhang (1997) also observed maximized growth when Zarrouk's medium was supplemented with glucose (2 g L^{-1}). This confirms the fact that glucose can be used as an additional carbon source to improve the growth, but not as a sole carbon source when added on equimolar concentration basis. This is also in tune with the findings of Snoog (1980), Torre *et al*. (2003) and Binaghi *et al*. (2003) who observed that 2 kg of glucose or 3.5 kg of acetic acid are needed to produce 1.0 kg of *Chlorella* dry matter.

Temperature is undoubtedly the most fundamental factor for all living organisms, which affects all metabolic activities. The optimum temperature for growth of *S. platensis* is 30-35°C. However, in tropical regions in summer; the temperature may go beyond 40°C. The study on effect of high temperature on photochemical activity of *S. platensis* in such situations will help in optimizing its productivity. A detailed study on the response of *S. platensis* M-2 strain to temperature was performed by Torzillo and Vonshak (1994). They observed 28 and 23% of the optimum growth at the extreme 10°C minimum and 50°C maximum temperatures respectively.

In the present investigation *S. platensis* cultures were grown at different temperatures and the highest biomass was obtained when *S. platensis* was grown at 35°C. This could be due to the increased activity of metabolic enzymes at that temperature, ultimately leading to higher biomass production; this is in conformity with the findings of Vonshak (1997). There was no growth when the cultures were grown above 40°C or blow 20°C. But the culture resumes its growth when once it has been brought to the normal conditions. This might be due to some mechanism, which must have taken place before the original photosynthetic activity was reached (Vonshak, 1997).

Cyanobacteria are ubiquitous and able to grow in aside range of pH from acidic to alkaline (pH 4-12). However *S. platensis* prefers ouly alkaline pH. This assumes significance in the context of minimizing the microbial contaminations, both *in vitro* and *in vivo* conditions.

In the present study, the biomass production of *S. platensis* has been evaluated under different pH levels. Maximum growth of *S. platensis* was observed at a pH of 10.0. The reason could be attributed to optimal activity of all the enzymes needed for photosynthesis and respiration, at this pH, however, at high acidic and alkaline pH, there was a decreased activity of the photosynthetic enzyme RUBP-carboxylase and an increased activity of respiratory enzymes glucose 6-phosphate dehydrogenate and isocitrate dehydrogenate (Kaushik and Sharma, 1997), leading to the reduction in the biomass production. These are the key enzymes in providing energy, carbon skeleton and pyridine nucleotide biosyntheses. But some specific *Spirulina* strains can adapt themselves to salinity conditions, when grown in a medium containing 70 g L^{-1} Nacl (Zotina *et al*., 2000). One mechanism proposed for the ability of cyanobacteria to liquor salinity is the formation of internal osmoticum by the accumulation of inorganic ions or organic solutes like carbohydrates polyols and quaternary ammonium compounds.

Light is the most important factor affecting photosynthetic organisms. Due to the prokaryotic nature of *S. platensis*, light does not affect the differentiation and development processes. Nevertheless, *S. platensis*, like many other algal grown photoautotrophically depends on light as its main energy source. Light considered in terms of photoperiod, qnality and intensity, is of paramount importance to micro algae. It is obviously significant as energy source for photosynthesis, but the fact that light fluctuate tremendously in both space (depth and latitude) and time (daily and seasonally) suggests that light may often be a limiting factor for phytoplankton growth.

In the present study, *S. platensis* growth was tested under different wavelengths of light using a mineral medium and bicarbonate as the only carbon source. The different light sources

include red (λmax 640-740 nm), blue (λmax 450-420), green (λmax 520-570 nm) and white light (λmax 350-740 nm). It was found that the biomass production under red and white lights was significant initially, but later it was non significant. The reason could be attributed to the maximnm absorption of chlorophyll initially under red light, but after some time the red light is converted into white light, which has inhibitory effect on photosynthesis resulting in reduced biomass. Similar results were obtained by Vonshak *et al*. (1996) who started that the growth of *S. platensis* became saturated at a range of 150-200 μmol m^{-2} sec^{-1} and this is about 10-15% of the total solar radiance at 400-700 nm. This value is highly dependent on growth and correlates with the chlorophyll to biomass concentration. Almost similar results were drawn by many workers (Jones and Myres, 1963; Venkatraman, 1983).

The photo systems in cyanobacteria contain only chlorophyll a and not chlorophyll b. Cyanobacteria, though it is considered as a prokaryote, it is similar in performing photosynthesis as eukaryotes (Carr and Whitton, 1982). They contain photosynthetic pigments like chlorophyll a, phycobilin proteins and carotenoids in their filaments. Phycobilin proteins located in the phycobilinsomes may account for about 40-50% of the total protein in cyanobacteria (Goedheeri, 1976).

Carotenoids are important components of photosynthetic apparatus of vegetative cells serving as additional pigment (Giovaunoni *et al*., 1988). They protect chlorophyll molecules against photo destruction and oxidation by molecular oxygen (Krinsky, 1979). When both *S. platensis* is trichomes and isolated thylakoids were exposed to elevated temperatures only above 60°C for 10 min in the dark, there was a significant degradation and bleaching of phycocyanin and allophycocyanin, but not that of chlorophyll a (Babu *et al*., 1992). An individual chlorophyll molecule absorbs ouly two or three photons per second even under direct solar illumination. No living organism could grow if its reaction center did not have light harvesting antenna molecules excess.

In the present study the chlorophyll and carotenoid content of *S. platensis* cultures were evaluated at 10, 20 and 30 days after inoculation and both CS-1, SP cultures were found to be on par with each other and contents increased with the increase in the incubation period. Among the local isolates, S-20 has shown highest chlorophyll content and carotenoid content and was within the range of the value prescribed for *S. platensis* (Vonshak, 1997).

Phycocyanin is gaining importance as natural pigments in food, drug and cosmetic industries, as an alternative to currently used synthetic colour. The organisms of commercial importance are *S. platensis* and phormidinm (Glazer, 1981). The blue, water-soluble pigment, which may be up to 10% of the dry weight of *S. platensis*, stimulates the immune system and is also used for the treatment of ulcers and haemorrhoidal bleeding (Richmond, 1986). It is also plays a major role in immuno diagnostics apart from its use as food colour (Glazer and Stryer, 1984). Phycocyanin has been extensively studied due to their involvement in photosynthesis as major accessory pigment (Myers and Kratz, 1955; Goedheeri, 1976). The stability of this pigment even at acidic pH of 4.5 has now been exploited in food products and soft driuks (Veukatraman, 1983).

In the present investigation, four different extraction procedures were employed for better extraction of phycocyanin pigment from *S. platensis* culture. Out of these four methods, highest phycocyanin was extracted with liquid nitrogen method followed by freezing and thawing method and lysozyme method, which were on par with each other and the least phycocyanin was extracted with sonication method. The reason could probably due to the efficient breakage of cell wall with liquid nitrogen; releasing maximnm amount of the pigment and the cell wall might not be broken completely with sonication leading to reduce phycocyanin with that method. Similar results were obtained by Chen and Zang (1997) who reported that freezing at -20°C; repeated thawings for one hour followed by sonication has increased the phycocyanin content.

The extracted and purified phycocyanin pigment of *S. platensis* cultures, when run on 15% SDS-PAGE gel, typically resolved into two bands of molecular weights with 14.3 and 20.1 kDa approximately, which might correspond to the α and β subunits of the pigment. This result was in tune with Boussiba and Richmond (1980) who observed that the molecular weight of α subunits and β subunit are 20.5 and 23.5 kDa for C-phycocyanin and 18 and 20 kDa, respectively for allophycocyanin in *S. platensis*.

Algae as a source of protein have a long history. Micro algae are no more cheap protein sources. But fairly high value low volume biological material (Benemann, 1990). *S. platensis* has been the subject of a number of basic and applied investigations (Richmond, 1986). This photosynthetic microorganism can be harvested, processed and used as a natural food for milleunia. Protein Efficiency Ratio (PER) value of *S. platensis* has been reported to be higher than vegetable, cereals and soya proteins. The value of the digestibility coefficient, biological values etc. are duly marginally lower than casein. Supplementation of *S. platensis* with cereals like rice and wheat on isoproteinic levels improved the protein quality (Anasuya Devi and Venkatraman, 1983). Detailed haematological and histological tests on chronic toxicity of *S. platensis* fed rats (Patterson, 1996) revealed no abnormalities. In the seventies there were Protein Advisory Guidelines (PAG) of the united nations when micro algae were considered as a protein source and lots of efforts were made to meet the safety requirements of a Single Cell Protein (SEP).

In the present study the protein content of *S. cultures* was measured at 10, 20 and 30 days after inoculation and it were observed that the protein content has increase with the increase in inoculation period. The highest protein content was observed in case of CS-1 cultures and among the local isolates; SM-2 has shown highest protein content of nearly 60%. These results are in accordance with Bhattacharjee (1970), who stated that *S. platensis* contains 60-70% protein and about 50, 000 kg of protein per hectare could be produced annually.

When the total proteins were extracted from the whole cells of *S. platensis* and resolved on a 15% SDS-PAGE gel, it showed a wide range of polypeptide subunits, the molecular weight of which ranged from 20 to 95 kDa. It is in corroboration with the finding of Chadgothia and Srivastava (1994) who stated that the total protein content of *S. platensis* cultures varied widely when resolved on SDS-PAGE gel. The consistency in the protein bands of *S. platensis* cultures either grown in laboratory or an open condition was further reported by them.

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