Karnataka J.Agric.Sci.,18 4):(1003-1006) 2005

Optimization of Various Sources of Nitrogen and Their Effect on Growth and Cell Constituents of Strains of *Spirulina platensis**

R. MANJUNATH AND G.S. GEETA

Department of Agricultural Microbiology
University of Agricultural Sciences, Dharwad - 580 005

(Received : January, 2005)

Abstract : An attempt was made to increase the biomass production of the cyanobacterium, *Spirulina platensis*, using various sources of nitrogen viz., urea, sodium nitrate and ammonium sulphate. These were used in borewell water along with other nutrients as stated. *Spirulina platensis* was inoculated at 10 per cent level. Among the nitrogen sources, urea (0.2 g/l) was found to be the best in enhancing the growth and biomass production (587.0 mg/l) followed by ammonium sulphate (557.0 mg / l). Later, the growth of *Spirulina platensis* was compared to growing in Zarrouk's medium which is supplemented with sodium nitrate (2.0 g/l), which showed biomass production of 490.0 mg per l. The results showed that the growth and biomass was increased significantly with urea and ammonium sulphate and thus they can be used as a source of nitrogen.

Introduction

The cyanobacterium *Spirulina* has already been commercially exploited in several countries as a health food and in therapeutic preparations because of its valuable cell constituents, particularly protein and vitamins (Venkataraman and Becker, 1986). The criteria of an organism for commercial exploitation requires maximum yield beside utilizing cellular components. One of the recent approaches for this is selection of efficient strains available in nature with suitable nutrient to produce maximum biomass and cell constituents. Selection of strain can give natural variant with specific increase in constituents with high value (Venkataraman, 1992). Although work on screening and selection of strains for production of particular end products like hydrogen, chemicals etc. was done on many algal form, not much attention has been paid to screening and selection of *Spirulina* strains for their nutritive value. Micro algal cultivation depends on the good quality of water since distilled water in large quantity becomes a constraint. As a result, available natural source of water need to be used, which then requires optimization of nutrients. The cultivation of the microalga *Spirulina* was done using urea as the nitrogen source by a fed batch process which showed a positive influence on growth of *Spirulina*. But, there was no effect on final chlorophyll content of culture (Danis *et al*., 2002) . Costa (2004) studied the possibility of using water from Mangeria Lagoon (Brazil) which has high level of carbonates and a high pH. Results showed that lagoon water supplemented with 1.125 mg per l of urea and 42 mg per l of sodium bicarbonate showed 2.67 fold increase in final biomass. Stanca and Popovia (1996) showed that the use of urea as source of nitrogen in *Spirulina platensis* cultivation causes an increase in biomass production as well as chlorophyll content. The main aim of work presented in this paper was to study the growth of *S. platensis* with different nitrogen sources at various concentrations.

Material and Methods

The *Spirulina* strains SM, S_4 and G1 were obtained from culture collection maintained at department of Agricultural Microbiology, University of Agricultural Sciences, Dharwad. The strain G_1 was a mutant strain (Geeta, 2001). The strains of *Spirulina platensis* were grown and maintained

* Part of the M.Sc. (Agri.) thesis submitted by the senior author to the University of Agricultural Sciences, Dharwad-580005

on the standard synthetic medium of Zarrouk (1966) containing (g/l): $NaHCO_3$, 16.8; $NaNO_3$, 2.5; K_2HPO_4, 0.5; K_2SO_4, 1.0; NaCl, 1.0; $MgSO_4.7H_2O$, 0.2; $CaCl_2.H_2O$, 0.04; $FeSO_4$; $FeSO_4$. $7H_2O$, 0.01 and micronutrients. This medium was also used to prepare the biomass for initial inoculation of each experiment. The batch culture was carried out in 250 ml Erlenmeyer flask containing 100 ml medium prepared with campus borewell water and 10 per cent initial inoculum. The cultures were maintained at 28 ± 2°C in a light chamber with Philips TL 40 W fluorescent lamps at an illuminance of 2.0 x 10_3 Lux and with a 12 h light / dark photoperiod (Tanticharoen *et al*., 1994). For the experiment, sodium nitrate ($NaNO_3$) was used at different levels *viz*., 1.0, 2.0 and 3.0 g per l. Urea levels were 0.1, 0.2 and 0.3 per l and ammonium sulphate at 0.1 , 0.2 and 0.3 g per l were added separately maintaining all other nutrients of Zarrouk's medium constant. Samples were taken after 21 days of growth to estimate following parameters. Algal biomass was estimated by the method of Richmond and Gobbelaar (1986), protein content by Lowry's method (Herbert *et al*., 1971), chlorophyll 'a' (Method of Mackinney, 1941) and phycocyanin (Bennet and Bogorad, 1971). Carotenoids were estimated as described by Jensen (1978). Analysis of water samples was done for the following parameters i.e., EC, pH, calcium and magnesium ions, carbonates and bicarbonates using standard methods (Table 1).

Table 1. Chemical properties of water samples screened for the study

Sl. No.	Sample	EC (dS/m)	pH (me/l)	Ca and Mg (me/l)	Carbo-nates (me/l)	Bicarbo nate (me/l)
1.	Malaprabha (muncipal treated water)	0.15	7.60	0.96	Absent	3.20
2.	Pond water (Kelageri pond)	3.13	7.20	5.93	Absent	12.61
3.	Borewell water (College borewell)	1.20	7.85	7.33	Absent	14.30
4.	Distilled water	0.07	7.05	0.69	Absent	0.58

Results and Discussion

When water samples were screened borewell water was found to have highest pH and bicarbonates (Table 1). The study was carried out to find out the suitable nitrogen source and its concentration for maximum production of biomass and cell constituents of *spirulina*. In the present study, among the three nitrogen sources, urea showed maximum biomass production at 0.20 g per l concentration. With G1 strain (0.58 mg/ ml), which was followed by S4 and SM starin. On dry weight basis, 12 per cent of *Spirulina* is nitrogen, derived from salts of nitrogen. Table 2 shows that higher concentration of ammonium sulphate showed low growth of *Spirulina*. Since, the ammonium ion gives up a protein (NH4 - NH3 + H^+), pH of the medium may turn to acidic which is not favourable for growth of *Spirulina*. However, for economic cultivation urea can be used. Urea, which is commercially available is almost half as expensive as sodium nitrate (Faucher *et al*., 1979). In addition, each molecule of sodium nitrate contains only one. Urea utilization as a nitrogen source provides an energetic gain due to its spontaneous hydrolysis to ammonia in the alkaline medium, which is then easily assimilated by *Spirulina* (Danis *et al*., 2002).

Protein content among the three strains varied and maximum amount of protein was

Table 2. Effect of different nitrogen sources on growth and cell constituents of strains of *Spirulina platensis*

Sl.	Treatments	Biomass (mg/ml)				Protein (mg/ml)				Chlorophyl (µg/ml)				Phycocyanin (µg/ml)				Allophycocyanin (µg/ml)				Carotenoid (µg/ml)		
No.		G_1	SM	S_4	Mean	G_1	SM	S_4	Mean	G_1	SM	S_4	Mean	G_1	SM	S_4	Mean	G_1	SM	S_4	Mean	G_1	SM	S_4
1.	Ammonium sulphate (g/l)																							
	0.1	0.23	0.08	0.06	0.12	0.15	0.12	0.13	0.13	12.03	11.37	8.27	10.55	35.00	19.00	32.00	28.66	83.0	61.0	80.0	74.66	38.00	33.00	32.00
	0.2	0.56	0.23	0.25	0.34	0.45	0.13	0.15	0.24	16.53	13.89	14.26	14.89	51.00	46.00	48.00	48.33	98.0	96.0	94.0	96.11	64.00	49.00	49.00
	0.3	0.24	0.10	0.12	0.15	0.15	0.13	0.13	0.14	14.72	12.63	12.52	13.29	36.00	30.00	33.00	33.00	84.0	76.0	81.00	80.43	51.00	34.00	33.00
	Mean	0.34	0.13	0.14	0.20	0.25	0.13	0.13	0.17	14.42	12.63	11.68	12.91	42.30	31.60	37.33	36.66	88.3	77.6	85.1	83.73	50.88	38.66	38.11
2.	Sodium nitrate (g/l)																							
	1.0	0.17	0.14	0.14	0.15	0.12	0.10	0.11	0.11	14.25	13.19	13.90	13.78	30.00	19.00	25.00	24.66	69.0	55.0	63.0	62.44	36.00	16.00	17.00
	2.0	0.49	0.31	0.44	0.41	0.41	0.30	0.39	0.37	19.75	16.94	18.38	18.35	44.00	36.00	40.00	40.00	89.0	80.0	84.0	84.33	6.00	34.00	50.00
	3.0	0.19	0.15	0.17	0.17	0.13	0.10	0.12	0.12	17.20	14.95	16.24	16.13	35.00	30.00	32.00	32.22	77.0	70.00	74.0	73.66	49.00	18.00	36.00
	Mean	0.28	0.20	0.25	0.24	0.22	0.16	0.21	0.20	17.06	15.02	16.17	16.08	36.33	28.33	32.22	32.29	78.40	68.3	73.6	73.47	50.60	22.60	34.30
3.	Ureal (g/l)																							
	1.0	0.13	0.10	0.11	0.11	0.10	0.09	0.08	0.09	15.05	13.85	14.49	14.46	31.0	17.00	23.00	23.66	72.00	53.0	61.0	62.00	36.00	16.00	17.00
	2.0	0.58	0.31	0.53	0.47	0.51	0.28	0.49	0.43	20.19	16.35	17.83	18.12	48.0	32.00	38.00	39.55	94.0	74.0	81.0	83.00	69.00	33.00	49.00
	3.0	0.17	0.11	0.15	0.14	0.14	0.18	0.12	0.14	16.97	15.48	16.86	16.43	34.0	26.00	26.00	28.66	76.00	67.0	66.0	69.60	49.00	18.00	35.00
	Mean	0.29	0.17	0.26	0.24	0.25	0.18	0.23	0.22	17.40	156.22	16.39	16.33	37.40	25.30	29.00	30.69	80.6	64.6	69.3	71.55	51.30	22.30	33.60
4.	Overall mean	0.30	.17	0.22	0.23	0.24	0.16	0.19	0.19	16.29	14.25	14.79	15.10	38.45	28.51	32.85	32.12	82.4	70.2	76.0	76.25	50.90	27.80	35.70
5.	St. medium with 2.5 g/l of $NaNO_3$	0.52	0.31	0.21	-	0.39	0.24	0.18	-	44.75	12.16	13.29	-	26.10	21.00	13.00	-	7.0	64.0	47.0	-	38.0	22.00	24.00

observed in *Spirulina platensis* G_1 strain (0.51 mg/ml) which was followed by *S. platensis* S_4 strain (0.49 mg/ml).

The chlorophyll content analysis shown in table 2 indicates that urea at 0.2 g produced maximum chlorophyll with G_1 strain (20.19 µg/ml) while with Na NO_3 at 2.0 g per l G_1 strain showed 19.75 µg per ml chlorophyll production. These facts prove the possibility of substituting sodium nitrate with urea . Besides the price of urea is lower than that of $NaNO_3$. Similar results were observed in an experiment by Danis *et al*. (2002). Phycocyanin, abliliprotein, owing to its fluorescent nature, has gained commercial importance as a marker and also as a food colourant. Among the strains phycocyanin and allophycocyanin content was significantly higher in *Spirulina platensis* G_1 strain with urea as nitrogen source at 0.2 g per l. Similarly, ammonium sulphate at 0.2 g l^{-1} has also yielded maximum phycocyanin compared to other constituents. The phycocyanin content was reported to vary with nitrogen concentration in the medium (Boussiba and Richmond, 1980). β-carotenoid, a natural pigment which acts as an antioxidant for vitamin a, has been reported to be present in higher amount in *Spirulina* compared to other vegetable source. In the present study, the carotenoid content of *S.platensis* G_1 strain with urea (0.2 g/l) as nitrogen source found to yield maximum carotenoid (69.0 µg/ ml). It was very low in SM strain. The reason for such variation may be preferential uptake of inorganic nutrients by the strains of alga from the medium.

The overall results of present study showed that *Spirulina platensis* G1 strain is more suitable for commercialization and further studies. Beside substantial yield in biomass high protein content was also observed. The facts of present study also prove the possibility of substituting the conventional nitrogen source, $NaNO_3$ with urea. Considerable variation in growth and cellular constituents of the three studied strains of *Spirulina* justify the importance of the strains.

References

BENNET, A. AND BOGORAD, L., 1971, Properties of subunits and aggregates of blue green algal biliproteins. *Biochemistry,* **10**: 3625-3634

BOUSSIBA, S. AND RICHMOND, A.E., 1980, C-phycocyanin as a storage protein in the blue green alga, *Spirulina platensis*. *Arches Microbiology*, **125** : 143-147.

HERBERT, D., PHILLIPS, P.J. AND STRANGE, R.E., 1971, Determination of total protein content in microorganisms. In : *Methods in Microbiology,* Eds. I.R. Norris and D. W. Robbins, Academic Press, New York, pp; 249-251.

JENSEN, A., 1978, Chlorophyll and carotenoids. In : *Handbook of Physiological and Biochemical Methods,* Eds. J.A. Hellebust and Craige, J.S., Cambridge University Press, pp. 59-70.

COSTA, J.A., COLLA, L.M. AND DUARTE FILHO, P., 2004, *Spirulina platensis* growth in open raceway ponds using freshwater supplemented with carbon, nitrogen and metal ions. *Bioresource Technology*, **92**: 237-241.

DANIS, E.D.G., RANGEL, CO, CARVALHO, J.C.M. AND SATO, S., 2002, An Investigation of effect of replacing nitrate by urea in the growth and production of chlorophyll by *Spirulina platensis*. *Biomass and Bioenergy*, **23**: 261-269.

FAUCHER, O., COUPAL, B. AND LEDUY, A., 1979, Utilization of seawater - urea as a culture medium for *Spirulina maxima*. *Canadian Journal of Microbiology*, **25**: 752-759.

GEETA, G.S., 2001, Isolation and characterization of mutants of *Spirulina platenssis*. *Ph.D thesis*, IARI, New Delhi.

MACKINNEY, G., 1941, Absorption of light by chlorophyll solutions. *Journal of Biological Chemistry*, **140**: 315-322.

RICHMOND, A. AND GOBBELAAR, J.U., 1986, Factors affecting the output rate of *Spirulina platinas* with reference to mass cultivation. *Biomass*, **10**: 253-264.

STANCA, D. AND POPOVIA, E., 1996, Urea as nitrogen source in modified Zarrouk's medium. *Biomass*, **41** : 25-31.

TANTICHAROEN, M., REUNGITCHACHAWALI, M., BOONG, B., VONKTAVEESU, P., VONSHANK, A. AND CHEN, Z., 1994, Optimization of λ-linolenic acid (GLA) production in *Spirulina* plantinas.

VENKATARAMAN, L.V. AND BECKER, E.W., 1986, *Biotechnology and Utilization of Algae, The Indian Experience,* Department of Science and Technology, New Delhi.

VENKATARAMAN, L. V., 1992, What next on *Spiirulina*? In : *Proceedings of ETTA, National Symposium*. Eds. Sheshadri, C.V. and Jeejibai, Madras, pp. 107-112.

ZARROUK, C., 1966, Contribution to the study of cyanophycea : Influence of various physical and chemical factors on the growth and photosynthesis of *Spirulina maxima* (in French). *Ph.D. thesis*, University of Paris.