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Full Length Research Paper

Optimization studies of biomass production and protein biosynthesis in a *Spirulina* sp. isolated from an oil-polluted flame pit in the Niger Delta

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Using a modification of the Bangladesh 3 medium, dose response analysis was carried out to evaluate the effect of certain parameters on biomass production and protein biosynthesis in a *Spirulina* sp. isolated from an oil-polluted flame pit. A set of conditions described as optimized was compared with another set of conditions described as normal. The optimized parameters include pH 9.0, temperature 30°C, 0.30 mg/L SO_4^{-2}, 0.008 mg/L $P0_4^{3-}$, 2.0 g/L $HC0_3^-$, 25 mg/L $N0_3^{2-}$, 1.0% Cl^- and light intensity 15 µEm^{-2}s^{-1}. Agitation was achieved through aeration using an aquarium pump to supply air at *ca* 150 bubbles min^{-1}. Biomass and protein produced were significantly (P = 0.05) higher in the optimized than in the normal condition; that is, biomass and protein produced in optimized condition were 22.7 and 25.8%, respectively, higher than those produced in normal growth condition. The total amino acid concentration in optimized condition was 58.7% higher than in normal conditions. These results show that cultivating the *Spirulina* species at these optimized environmental conditions could significantly improve biomass yield and protein synthesis in the organism.

Key words: Optimal parameters, optimized condition, normal condition, biomass, protein biosynthesis, environmental factors, *Spirulina.*

INTRODUCTION

Certain physical, environmental and nutritional factors are required for growth (biomass production) and metabolism (including protein biosynthesis) in *Spirulina* species (Ogbonda et al., 2007). The optimum requirement is the condition that supports best biomass production and protein biosynthesis. It is usually a small range instead of a particular point.

Spirulina has economic importance due to its nutritional value (Cost et al., 2001; Rafiqul et al., 2005) and is also important in the area of healthcare (Richmond, 1986; Sasson, 1988).

An attempt to predict the performance of an alga under optimal growth conditions is necessary when one is considering large-scale production of the organism for industrial purpose. Such information is basic, also, to the design and operation of culture apparatus. Algae, including *Spirulina* adapt to varying environmental conditions. One difficulty would, therefore, be in the maintenance of optimal conditions for best biomass production and protein synthesis. For instance, prolonged exposure to high light intensity may cause photo oxidative death (Sorokin and Krauss, 1965; Abeliovich and Shilo, 1972). Outdoor mass cultivation is not illuminated during the night. Dark respiration losses in outdoor culture of *Spirulina* have been estimated to be up to 35% of the total biomass produced during the day (Grobbelaar and Soeder, 1985; Rafiqul et al., 2005). Different algae (and different *Spirulina* species) have different optima. Outdoor cultures of *Spirulina* face a lot of limitations all of which have not yet been elucidated. In the present study, we set out to investigate how a combination of a set of parameters namely temperature, light intensity, pH, sali-

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Table 1. Proximal composition of *Spirulina* sp. grown under normal and optimised conditions.

Condition	Gross composition in g100g^{-1} dry weight					
	Moisture	**Ash**	**Carbohydrate**	**Lipids**	**Crude fibre**	**Crude protein**
Normal	10.12±0.60	12.48±0.33	21.89±1.10	7.29±0.16	9.49±0.38	38.61±1.80
Optimised	6.58±0.50	5.11±0.71	14.11±0.61	5.81±0.33	4.41±0.55	64.38±1.20

Mean of 3 determinations ± S.D

nity, mineral nutrients and agitation affect maximum production of biomass and protein in a *Spirulina* sp. that was isolated from an oil-polluted flame pit.

MATERIALS AND METHODS

The organism

The *Spirulina* species used in this study was previously isolated from and oil-polluted brackish water marsh in the Niger Delta region of Nigeria (Ogbonda et al., 2007).

Growth conditions

The *Spirulina* isolate was grown under the following parameters as optimal conditions, they are namely temperature, 30°C; pH, 9.0; sulphate ($CaS0_4$), 0.30 mgl^{-1}; phosphate (K_2HP0_4), 0.008 mgl-1; nitrate ($NaN0_3$), 25 mgl^{-1}, salinity (NaCl), 1.0% (10 gl^{-1}); bicarbonate ($NaHC0_3$), 2.0 gl^{-1}; and light intensity, 5 $\mu Em^{-2}s^{-1}$; aeration. The Bangladesh medium No. 3 (Khatum et al., 1994) with the following composition was used as the normal growth medium $NaHC0_3$, 2.0 gl^{-1}; Urea, 0.05 gl^{-1}; NaCl, 1.0 gl^{-1}; gypsum ($CaS0_4.2H_20$), 1.5 gl^{-1}; water, 1000 ml; pH, 7.0-7.5.

In the growth experiment three 100 ml conical flasks were used. Each contained 50 ml of the optimised medium that is medium containing optimal values of the various factors selected. Each flask was inoculated with 10 ml of the pure culture of the organism. Inoculated flasks were incubated under artificial light provided in a growth chamber illuminated by two 4 ft, white fluorescent tubes which gave an estimated light output (intensity) of 15 μEm^2s^{-1} (Anaga and Abu, 1996). The cultures were aerated using an aerator, which pumped air at *ca* 150 bubbles per minute through a drip set (plastic tubing) fitted with a regulator (Anaga and Abu, 1996). A control was set up, also in triplicate, which contained the various components in their normal concentration that is as present in the Bangladesh medium. These were not aerated and incubation was in sunlight. In both experimental (optimised condition) and control (normal condition) incubation period was 35 days (based on previous experimentation, unpublished data).

Biomass analysis

Biomass concentrations in the cultures were determined through the cell dry weight by the method of Vonshak et al. (1982).

Proximal composition

Moisture and ash were determined by standard methods (AOAC, 1984). Crude fat was determined by the soxhlet extraction method of Egan et al. (1981). Crude protein was estimated by the micro-kjedahl method (AOAC, 1984) and the conversion factor from nitrogen to protein was 6.5. Total available carbohydrate (TAC) was determined using the Anthrone method (Osborne and Voogt, 1978). Crude fibre was calculated by difference, thus:

Crude fibre = 100 – (% protein + % TAC +% moisture + %fat +% ash)

Amino acid composition

In estimating the protein and amino acid contents of the organism, it was necessary to deal with cultures free from contaminating bacteria. The *Spirulina* cultures were freed of bacterial contamination by a method involving treatment with a mixture of detergent and phenol (McDaniel et al., 1962).

Two millilitres of klin concentrate detergent solution (manufactured by P.T. Sayap Mas Utana, Indonesia), were mixed with 8 ml of 1% phenol solution and the mixture shaken vigorously. This mixture was added to centrifuge-packed cells in a test tube, shaken for 20 s and allowed to stand for 15 min. It was centrifuged for 5 min and the supernatant discarded. Ten milliliters (10 ml) of distilled water was added to the cells and the tube was shaken vigorously to disperse the cells. After centrifuging for another 5 min, the supernatant was discarded and the residue (*Spirulina* filaments) used for proximal and amino acid analysis. The amino acids were estimated by paper chromatography, employing the methods of Allen et al. (1974).

Statistical analysis

Analysis of variance (ANOVA) method was used to find out if significant difference existed in the amount of protein produced in normal and optimised conditions.

RESULTS AND DISCUSSION

The *Spirulina* species produced higher biomass and protein in optimised than in normal medium (Figures 1 and 2). Biomass and protein produced in optimised conditions were 22.7 and 25.8%, respectively, higher in optimised condition than in normal condition. The total amino acid concentration in optimised condition was 56.7% higher than in normal condition (Figure 2). A significant difference (P = 0.05) was found in the amounts of protein produced under the two conditions that is Ftabulated (5.05) is less than F calculated (8.07). Tables 1, 2, 3 shows certain discernible features in growth (biomass produced) by the *Spirulina.* First, biomass pro-

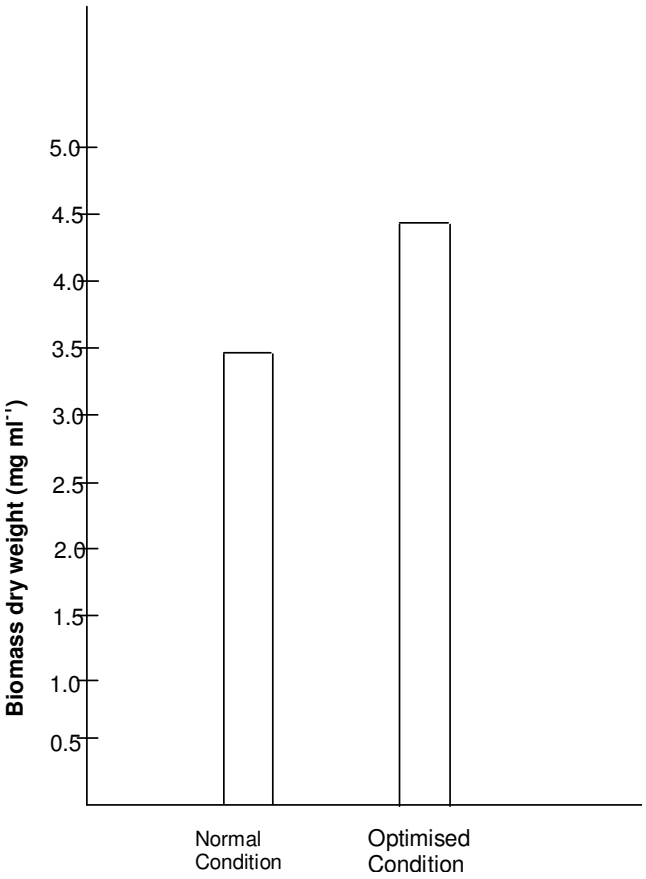

Figure 1. Biomass concentration in *Spirulina* sp. under normal and optimised conditions.

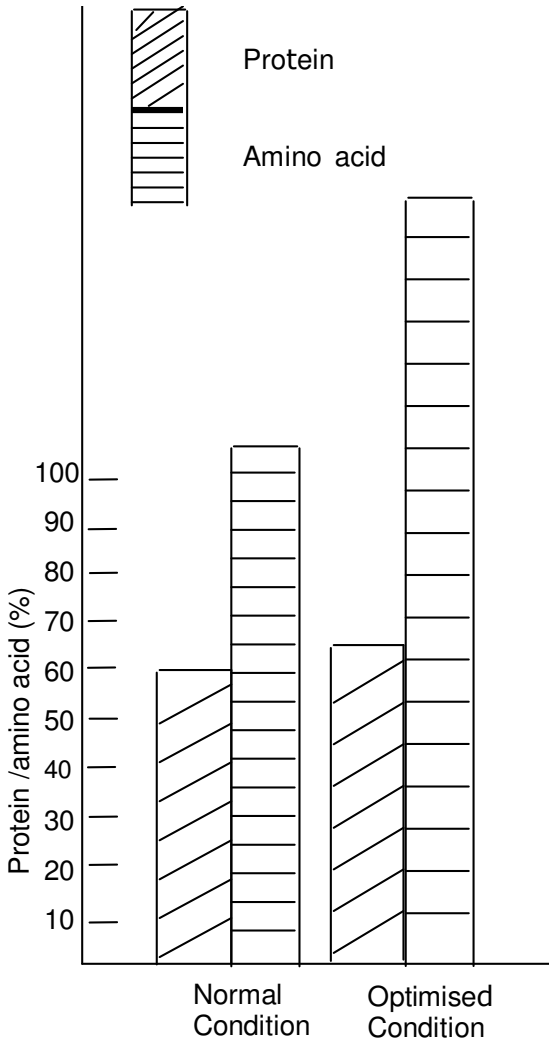

Figure 2. Protein and total amino acid concentration under normal and optimised conditions in *Spirulina* sp.

duced under certain optimal conditions was higher than biomass produced under the overall optmised condition. Such optimal conditions were temperature, pH, temperature and pH combined, and light intensity. Biomass produced at optimal levels of the respective parameters was higher than in the optimised condition due to interaction occurring when the various factors were combined in the optimised condition. The parameters which at optimal levels caused greater biomass production than was obtained in the optimised conditions are the critical factors for biomass production in the *Spirulina*. Thus, temperature and pH (individually and in combination) and light intensity are the critical factors for biomass production. Secondly, biomass production at certain optimal conditions (evaluated individually) was lower than biomass production in normal condition. Such optimal conditions were salinity (1.0%), $P0_4^{3-}$ (0.008 mgl^{-1}), $S0_4^{2-}$ (0.30 mgl^{-1}), and $HC0_3^-$ (2.0 gl^{-1}). These optimal conditions which had lower growth (biomass production) than was obtained in the normal condition are the non-critical factors for biomass production in the organism. Their concentrations are not critical for the normal growth of the organism. However, these factors were found to be essential for protein synthesis in the organism, probably because sodium, sulphur, and phosphorus are necessary for the activity of certain enzymes involved in protein synthesis, formation of co-enzymes and adenosine phosphates and biosynthesis of sulphur-containing proteins respectively. Bicarbonate ($HC0_3^-$) furnishes the carbon skeleton needed for biomass including proteins. Thirdly, biomass production at certain optimal levels was lower than was obtained in the optimised condition, but higher than in the normal condition. Such optimal conditions included $N0_3^{2-}$ and aeration. These conditions are also considered as critical for biomass production and protein synthesis in the organism. The protein and total amino acids synthesized when the conditions were optimised were greater than the amounts synthesized at each of the various optimal conditions (Table 4). Simi-

Table 2. Amino acid composition of *Spirulina* sp. grown under normal media conditions and when the conditions were optimized.

Condition																				
Amino acid composition (g/16gN)																				
	Ser	Met+	Lys+	Asp	Try+*	Tyr	Asn	Glu	Ile+	Cys.	Gln	His+	Arg	Leu	Phe+	Ala	Thr+	Val+	Pro	Gly	Total
Normal	1.88	0.68	1.22	1.54	-	4.18	1.18	5.34	1.78	-	3.18	0.81	5.89	0.77	0.38	3.89	1.66	2.18	0.45	1.71	38.72
Optmised	3.91	2.11	4.71	2.94	-	7.80	4.28	8.41	7.61	-	4.98	3.21	9.38	3.36	2.37	7.91	5.48	7.12	4.81	5.21	95.52

* Did not separate, not determined; + essential amino acids.

Table 3. Growth (mg/ml, cell dry mass) of *Spirulina* sp. under the respective culture parameters in the laboratory.

Condition	Temp 30°C	pH 9.0	Temperature and pH				Salinity 1.0%	$P0_4^{3-}$ 0.008mgl^{-1}	$SO4^{2-}$ 0.30mgl^{-1}	NO_3^{2-} 25mgl^{-1}	$HC0_3^-$ 2.0gl^{-1}	Aeration	Light intensity
			35°C,8.5	30°C, 9.0	30°C, 9.5	30°C, 10.0							
Growth (mgml^{-1})	4.90	4.90	3.6	4.6	4.1	3.9	2.70	3.30	3.20	3.60	3.10	3.80	3.70

Table 4. Protein and total amino acid concentrations in the respective optimal parameters of growth of *Spirulina* sp. in the laboratory

	Parameter								
	Temp 30^{0}C	pH 9.0	Salinity, 2.0gl^{-1}	$P0_4^{3-}$ 0.008mgl^{-1}	SO_4^{2-} 0.30mgl^{-1}	$N0_3^-$ 25gl^{-1}	$HC0_3^-$ 2.0gl^{-1}	Aeration	Light intensity 15µEm^{-2}s^{-1}
Crude protein (g100g^{-1}DM)	46.39	48.23	54.30	58.21	55.30	61.50	39.61	51.46	57.31
Total Amino acid g/16gN)	76.09	78.74	75.43	100.62	67.96	91.07	40.71	43.13	61.11

larly, the protein and total amino acids synthesized at each of the various optimal conditions were higher than those synthesized in the normal condition. The *Spirulina* species, therefore, grew best and synthesized the highest amounts of protein when certain environmental factors were optimised.

In conclusion, optimal growth conditions for obtaining increased yields of the *Spirulina* species isolated from the oil-polluted brackish water marsh in the Niger delta were established under laboratory conditions. This further enhances the potential of this isolate in algal biotechnology.

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