

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

Gideon O. Abu*, Kemka H. Ogbonda and Rebecca E. Aminigo

Department of Microbiology, University of Port Harcourt. P. M. B 5323 Port Harcourt, Nigeria.

Accepted 19 October, 2007

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 PO_4^{3-} , 2.0 g/L HCO_3^- , 25 mg/L NO_3^{2-} , 1.0% Cl^- and light intensity 15 $\mu\text{Em}^{-2}\text{s}^{-1}$. 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 (Grobelaar 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-

*Corresponding author. E-mail: gideonabu1@yahoo.com.

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 an 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 (CaSO₄), 0.30 mg l⁻¹; phosphate (K₂HPO₄), 0.008 mg l⁻¹; nitrate (NaNO₃), 25 mg l⁻¹, salinity (NaCl), 1.0% (10 g l⁻¹); bicarbonate (NaHCO₃), 2.0 g l⁻¹; and light intensity, 5 μEm⁻²s⁻¹; aeration. The Bangladesh medium No. 3 (Khatun et al., 1994) with the following composition was used as the normal growth medium NaHCO₃, 2.0 g l⁻¹; Urea, 0.05 g l⁻¹; NaCl, 1.0 g l⁻¹; gypsum (CaSO₄.2H₂O), 1.5 g l⁻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:

$$\text{Crude fibre} = 100 - (\% \text{ protein} + \% \text{ TAC} + \% \text{ moisture} + \% \text{ fat} + \% \text{ ash})$$

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 $F_{\text{tabulated}} (5.05)$ is less than $F_{\text{calculated}} (8.07)$. Tables 1, 2, 3 shows certain discernible features in growth (biomass produced) by the *Spirulina*. First, biomass pro-

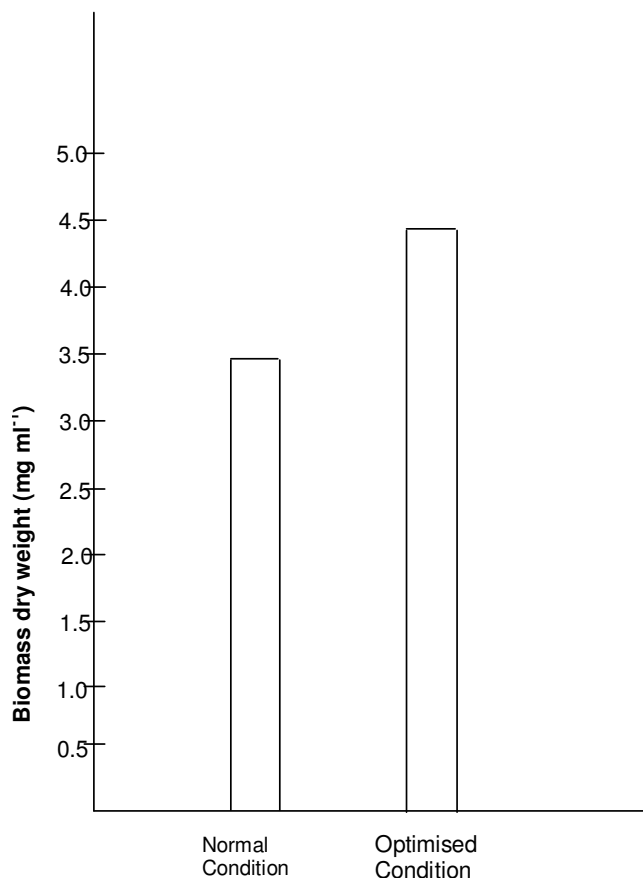


Figure 1. Biomass concentration in *Spirulina* sp. under normal and optimised conditions.

duced under certain optimal conditions was higher than biomass produced under the overall optimised 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%), PO_4^{3-} (0.008 mg l⁻¹), SO_4^{2-} (0.30 mg l⁻¹), and HCO_3^- (2.0 g l⁻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

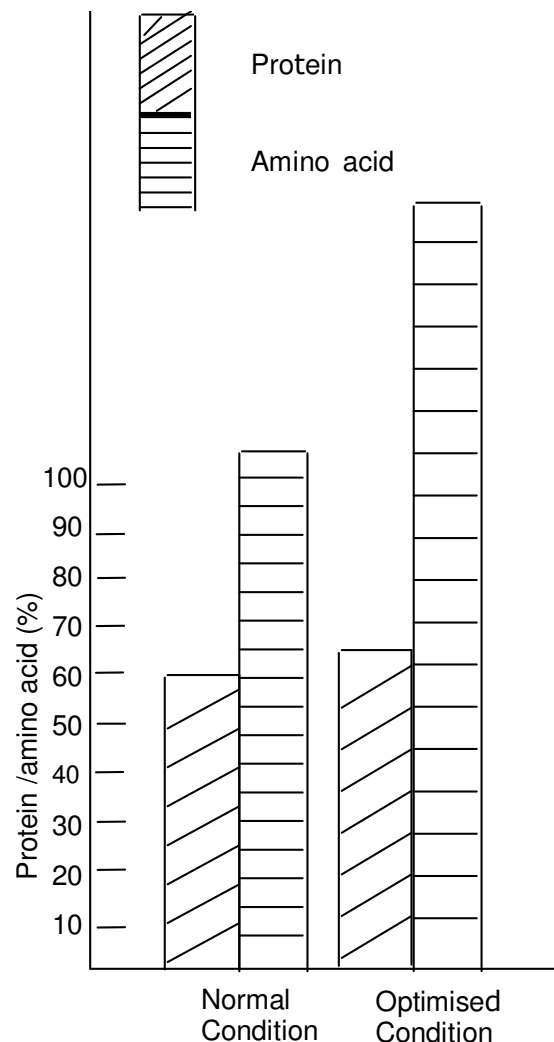


Figure 2. Protein and total amino acid concentration under normal and optimised conditions in *Spirulina* sp.

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 (HCO_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 NO_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
Optimised	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%	P ₀₄ ³⁻ 0.008mg/l ⁻¹	SO ₄ ²⁻ 0.30mg/l ⁻¹	NO ₃ ²⁻ 25mg/l ⁻¹	HCO ₃ ⁻ 2.0gl ⁻¹	Aeration 3.80	Light intensity 3.70
			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°C	pH 9.0	Salinity, 2.0gl ⁻¹	P ₀₄ ³⁻ 0.008mg/l ⁻¹	SO ₄ ²⁻ 0.30mg/l ⁻¹	NO ₃ ⁻ 25gl ⁻¹	HCO 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.

REFERENCES

Abeliovich A, Shilo M (1972). Photooxidative death in blue green algae. *J. Bacteriol.* 111: 682-689.
 Allen SE, Grimshaw HM, Parkinson JA, Quarmby C (1974). *Chemical Analysis of Ecological Materials*. Blackwell Scientific Publication, Oxford, pp. 413-422.
 Anaga A, Abu GO (1996). A laboratory- scale cultivation of *Chlorella* and *Spirulina* using waste effluent from a fertilizer company in Nigeria. *Biores. Technol.* 58: 93-95.
 Association of Official Analytical Chemists (AOAC), (1984). *Official Methods of Analysis*. 14th ed. Washington, DC, USA, pp. 635-678.
 Cost JAV, Cozz KI, Oliveria I, Magagin G (2001). Different nitrogen source and growth response of *Spirulina platensis* microenvironments, *World J. Microbial. Biotechnol.* 17: 439-

442.
 Egan H, Kirk RS, Sawyer R (1981). *Pearson's Chemical Analysis of Foods*, 8th ed. Churchill Livingstone, London pp. 7-34.
 Grobbelaar JU, Soeder CJ (1985). Respiration losses in green algae cultivated in raceways ponds. *J. Plankton Res.* 7: 497.
 Khatun R, Hossain MM, Begum SMS, Majid FZ (1994). *Spirulina* culture in Bangladesh V. Development of simple, inexpensive culture media suitable for rural or domestic level cultivation of *Spirulina* in Bangladesh. *J. Sci. Ind. Res.* 29: 163-166.
 McDaniel HR, Middlebrook JB, Bowmann RO (1962). Isolation of pure cultures of algae from contaminated cultures. *Appl. Microbiol.*, 10: 223.
 Ogbonda KH, Aminigo RE, Abu GO (2007). Influence of temperature and pH on biomass production and protein

- biosynthesis in a putative *Spirulina* species. *Bioresour. Technol.* 98: 2207-2211.
- Osborne DR, Voogt P (1978). The analysis of nutrients in food. Academic Press, London. pp. 107-155.
- Rafiqul IM, Jalal KCA, Alam MZ (2005). Environmental factors for optimisation of *Spirulina* biomass in laboratory culture. *Biotechnology.* 4: 19-22.
- Richmond A (1986). *Spirulina*. In Borowitzka, M. and Borowitzka, L. (eds). *Microalgal Biotechnology*. Cambridge University Press, London and New York, p.118.
- Sasson A (1988). *Biotechnologies and Development*. UNESCO. TCAR. Netherlands, p.12.
- Sorokin C, Krauss RW (1965). The dependence of cell division in *Chlorella* on temperature and light intensity. *Am. J. Bot.* 52: 331-339.
- Vonshak A, Abeliovich A, Boussiba S, Arad S, Richmond A (1982). Production of *Spirulina* biomass: effects of environmental factors and population density. *Biomass.* 2: 175-185.