

Full Length Research Paper

Influence of aeration and lighting on biomass production and protein biosynthesis in a *Spirulina* sp. isolated from an oil-polluted brackish water marsh in the Niger Delta, Nigeria

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The influence aeration and light intensity could have on biomass production and protein biosynthesis in a *Spirulina* sp. isolated from an oil-polluted brackish water marsh is examined. Biomass, proximal composition and amino acid composition obtained from aerated cultures of the organism were compared with non-aerated cultures after a 35 days incubation period. Cultures of the organism were also grown in ordinary sunlight, and artificial light intensities of ca.7.5 and 15 $\mu\text{Em}^{-2}\text{s}^{-1}$. Results showed that both aeration and exposing the cultures of the organism to artificial illumination improved biomass production and protein biosynthesis in the *Spirulina* sp. compared to sunlight illuminated and non aerated cultures. These results would be useful in optimizing growth conditions for maximum biomass and protein yields from the organism.

Key words: *Spirulina*, aeration, light intensity, biomass, protein.

INTRODUCTION

Studies of the environmental factors that affect the growth and metabolism of microorganisms such as microalgae, are necessary in that they contribute to understanding control of cellular metabolism and the optimising of yields of certain biosynthetic products. One of the factors that may limit out-door production of algal biomass grown in pond cultures, according to Vonshak et al. (1982), is daily and seasonal variations in solar radiation (light intensity). Robin (1981) has noted that the duration, intensity, and quality of light are the most important factors in the success of photosynthetic plants. The synthesis of various cell components is known to be influenced by light intensity. For instance, visible light exerts stimulatory influence on protein synthesis (Bilgrami and Verma, 1981). Takano et al (1995) noted that phycocyanin production by *Synechococcus* species, a marine blue-green alga increased three-fold when the surface light intensity at the culture vessel was increased

from 5 to 55 $\mu\text{molm}^{-2}\text{s}^{-1}$. Cell division, an aspect of cell growth, is also known to be influenced by light intensity. Sorokin and Krauss (1965) had reported that an increase in light intensity first favours cell division then, after the optimal light intensity was attained, a further increase in light intensity inhibited cell division. Dubey (2006) reported moderate light intensity in the cultivation of *Spirulina*, suggesting low light intensity at the beginning to avoid photolysis. He also noted that exposing *Spirulina* to high light intensity photolyses them.

Aeration, which could be achieved by rotators, and which provides agitation of growing cells to maintain the cells in suspension, has been described as very necessary in getting good quality and better yields of *Spirulina* species (Dubey, 2006).

Spirulina organisms are known to be useful to man in virtually all aspects of man's life, including health (Richmond, 1986a; Sasson, 1988); food (Bilgrami and Verma, 1981; Santillan, 1982; Fox, 1986; Richmond, 1986b; Babu and Rajasekaram, 1991; Ortega-Calvo et al., 1993; Mephram, 1997) and cosmetics (Shelef and Soeder, 1980; Richmond, 1986b). In this paper we demonstrate the influence of aeration and light intensity

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on biomass production and protein biosynthesis in a *Spirulina* species that was isolated from an oil-polluted brackish water marsh in the Niger delta. The results would be useful in optimising the cultivation of this organism. It is believed that the isolate would be more readily acceptable by the local community when used as food supplement or in animal feed formulations.

MATERIALS AND METHODS

Spirulina species

The *Spirulina* species used in this study was previously isolated from an oil-polluted brackish water marsh (Ogbonda et al., 2007).

Determination of the influence of aeration and light intensity

Bangladesh medium No.3 (Khatum et al., 1994) was used as the growth medium. It was composed of NaHCO_3 , 2.0 g l^{-1} ; Urea, 0.05 g l^{-1} ; NaCl, 1.0 g l^{-1} ; gypsum ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$), 1.50 g l^{-1} ; water, 1000 ml ; pH 7.0-7.5.

Six 100 ml-conical flasks each containing 50 ml of the growth medium were set up. Each flask was inoculated with 10 ml of the culture. One set of three flasks was aerated using an aquarium pump which pumped air at ca 150 bubbles per min through a drip set (plastic tubing) fitted with a regulator (Anaga and Abu, 1996). This set-up also provided agitation.

Light sources were sunlight, and artificial illumination provided from white fluorescent tubes. A growth chamber was constructed and illuminated by two 4ft-white fluorescent tubes, which were mounted in the chamber at a height of about 30 cm from the bench top. Nine 100 ml-conical flasks each containing 50 ml of the growth medium and inoculated with 10 ml of the culture were used. One set (triplicate) was incubated in natural light (sunlight), the second set (triplicate) was incubated in the growth chamber with only one of the fluorescent tubes switched on and emitted ca $7.5 \mu\text{Em}^{-2}\text{s}^{-1}$, and the third set (triplicate) was incubated with two tubes switched on and emitted ca $15 \mu\text{Em}^{-2}\text{s}^{-1}$ of light (Anaga and Abu, 1996).

Biomass analysis

Biomass concentrations in the cultures were determined by 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 determined by the micro-kjedahl method (AOAC, 1984) and the conversion factor from nitrogen to protein was 6.5. Total available carbohydrate was determined using the anthrone method (Osborne and Voogt, 1978). Crude fibre was calculated by difference.

Amino acid composition

Amino acids were estimated by paper chromatography, employing the methods of Allen et al. (1974).

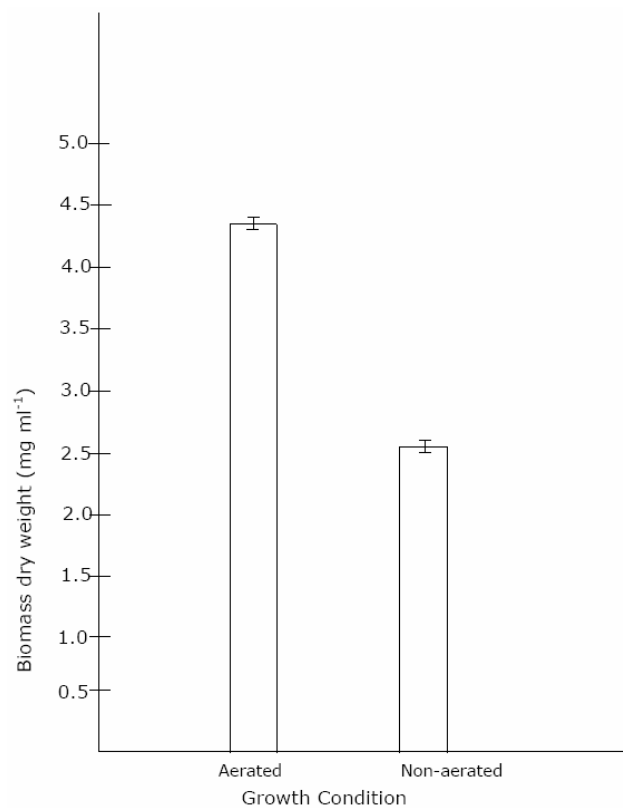


Figure 1. Influence of aeration on biomass production in *Spirulina* sp.

Statistical analysis

Analysis of variance (ANOVA) method was used to find out if significant difference existed in the amount of protein produced at the various lighting and aeration conditions

RESULTS AND DISCUSSION

Effect of agitation/aeration

This work examined the influence of aeration and light intensity on biomass production and protein biosynthesis in a *Spirulina* species. The *Spirulina* species produced higher biomass when the growth medium was bubbled with air (aerated) than when the medium was not bubbled with air (non aerated). At the end of the 35 days incubation period, biomass concentration in the aerated culture increased by 41.8% over that of the non-aerated culture (Figure 1). The amounts of protein and total amino acids synthesized by the organism when aerated and when not aerated are presented in Tables 1 and 2. A significant difference ($P = 0.05$) was found in the amounts of protein produced when the medium was aerated and when not aerated [F tabulated (5.05) is less than F calculated (49.1)]. A summary of the total protein

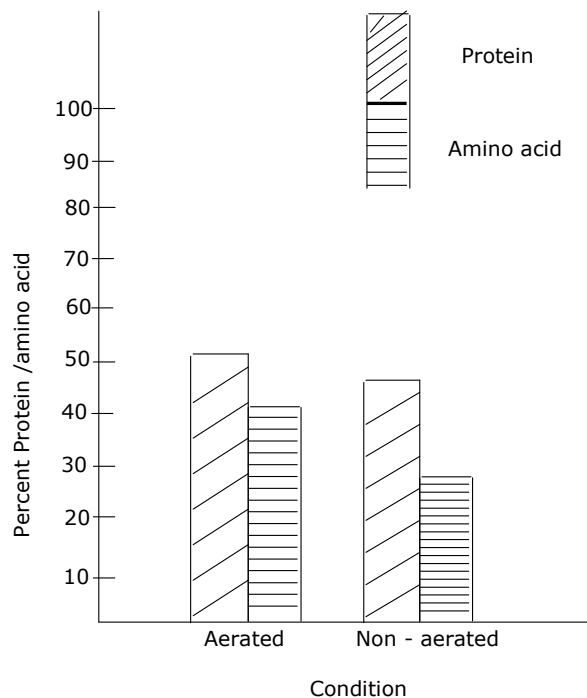


Figure 2. Total protein and amino acid concentration in *Spirulina* sp. under aerated and non aerated conditions.

and amino acids synthesized when aerated and when not aerated is presented in Figure 2. More protein and amino acids were synthesized when medium was aerated than when it was not. When the growth medium was not aerated, the amounts of protein and total amino acids synthesized declined by 15.90 and 23.0% respectively. Aeration, therefore, enhances for protein production by the organism. Aeration agitates the growth medium and this gives a homogenous distribution of the *Spirulina* filaments throughout the growth vessel for adequate exposure to illumination. It also helps to distribute oxygen concentration uniformly and removes some inhibitory substances produced such as carbon dioxide (Richmond and Vonshak, 1978; Famelart et al., 1987). Aeration is, therefore, essential for the cultivation of the *Spirulina* filaments such as *Spirulina platensis* (Richmond, 1986b). Powls (1985) and Vonshak (1987) also noted that continuous mixing of the culture medium is required to prevent cell sinking and thermal stratification, maintain even nutrient distribution, and to remove excess oxygen. When aeration is not adequate, the efficiency of energy utilization and hence, biomass production will be low (Richmond, 1987). Similarly, if growth medium is not aerated, the cell on the surface of the medium (*Spirulina* cells normally float to the surface due to the presence of air-filled vacuoles) suffer photo-inhibition, that is, they are exposed to constant (or, high) illumination which kills them, resulting in low growth or low biomass production (Vonshak and Guy, 1987).

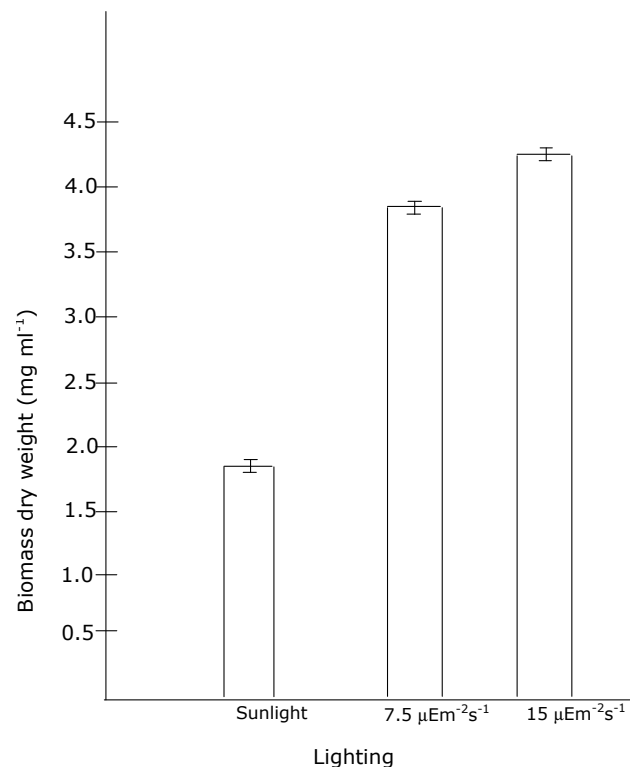


Figure 3. Influence of lighting on biomass production in *Spirulina* sp.

Effect of light intensity

The *Spirulina* sp. had better biomass production in the illuminated chamber than in ordinary sunlight. Growth (biomass production) when illuminated was about 100% greater than in ordinary sunlight and about 14.7% greater when illumination was doubled (Figure 3). The amounts of protein and total amino acids synthesized under the different lighting conditions are presented in Tables 3 and 4. A significant difference ($P = 0.05$) was found in the amounts of protein produced at the various lighting conditions [(F tabulated (3.20) is less than F calculated (42.17)]. A summary of the total protein and amino acids synthesized at the various lighting conditions is presented in Figure 4. Protein and total amino acids produced increased with increase in lighting. The increase was 29.64% (protein) and 26.25% (total amino acids) when $7.5 \mu\text{Em}^{-2}\text{s}^{-1}$ (light intensity) was used for illumination and 44.32 (protein) and 53.97% (total amino acids) when the illumination was doubled to $15 \mu\text{Em}^{-2}\text{s}^{-1}$. Thus, biomass production and protein synthesis in the *Spirulina* species was lower at ordinary sunlight condition and higher when the growth chamber was artificially illuminated. According to Robin (1981), algae, other than those recently evolved seldom function effectively today in natural environments. Also Anaga and Abu (1996)

Table 1. Proximal composition of *Spirulina* sp. grown under aerated and non-aerated conditions

Condition	Gross composition in g/100 g dry weight					
	Moisture	Ash	Carbohydrate	Lipids	Crude fibre	Crude protein
Aerated	9.66±0.81	10.71±0.53	14.21±1.30	5.33±0.43	9.11±0.61	51.46±0.88
Non-aerated	7.22±0.31	11.61±0.44	18.91±0.53	6.29±0.73	12.68±0.43	43.20±1.10

Mean of 3 determinations ± S.D.

Table 2. Amino Acid composition of *Spirulina* sp. grown under aerated and non-aerated conditions

Condition	Ser	Met+	Lys ⁺	Asp	Try ⁺	Tyr	Asn	Glu	Ile+	Cys	Gln	His ⁺	Arg	Leu	Phe ⁺	Ala	Thr ⁺	Val ⁺	Pro	Gly	Total
Aerated	2.10	1.34	1.51	1.71	-	4.10	1.88	4.78	2.98	-	3.26	1.81	4.93	1.86	1.53	3.10	1.38	1.38	1.35	1.68	43.13
Non aerated	1.45	0.98	0.63	1.44	-	3.69	1.28	4.23	2.11	-	2.78	1.48	4.18	1.33	0.91	2.41	0.67	0.67	0.86	1.19	32.90

*Did not separate; + Essential amino acids; - Not determined.

Table 3. Proximal composition of *Spirulina* species grown under different lighting conditions.

Condition	*Gross composition in g100g ⁻¹ dry weight					
	Moisture	Ash	Carbohydrate	Lipids	Crude fibre	Crude protein
Sunlight	10.19±81	12.65±0.34	21.09±1.30	7.68±0.41	9.01±0.51	39.71±0.61
7.5µEm ⁻² s ⁻¹	8.38±.40	11.61±0.81	14.71±1.10	6.59±0.88	7.46±0.80	51.48±0.83
15µEm ⁻² s ⁻¹	8.59±0.31	6.91±0.66	17.76±0.11	4.99±0.38	4.69±0.71	57.31±1.10

*Mean of 3 determination ± S.D.

Table 4. Amino acid composition of *Spirulina* sp. grown under different lighting conditions.

Lighting condition	Ser	Met ⁺	Lys ⁺	Asp	Try ⁺	Tyr	Asn	Glu	Ile ⁺	Cys.	Gln	His ⁺	Arg	Leu	Phe ⁺	Ala	Thr ⁺	Val ⁺	Pro	Gly	Total
Sunlight	1.91	0.43	0.43	1.66	-	4.43	1.35	6.71	1.29	-	2.33	0.77	6.33	0.83	0.45	4.12	1.89	2.33	0.44	1.66	39.69
7.5 µEm ⁻² s ⁻¹	2.31	1.28	1.28	2.91	-	4.98	1.86	6.33	1.88	-	2.89	1.10	6.81	1.28	0.81	4.93	2.56	2.68	0.90	2.39	50.11
15 µEm ⁻² s ⁻¹	3.89	1.88	3.41	3.82	-	5.61	2.01	7.11	2.31	-	3.41	1.63	7.01	1.78	1.33	5.22	2.98	3.02	1.28	3.41	61.11

* Did not separate; - Not determined; + Essential amino acids.

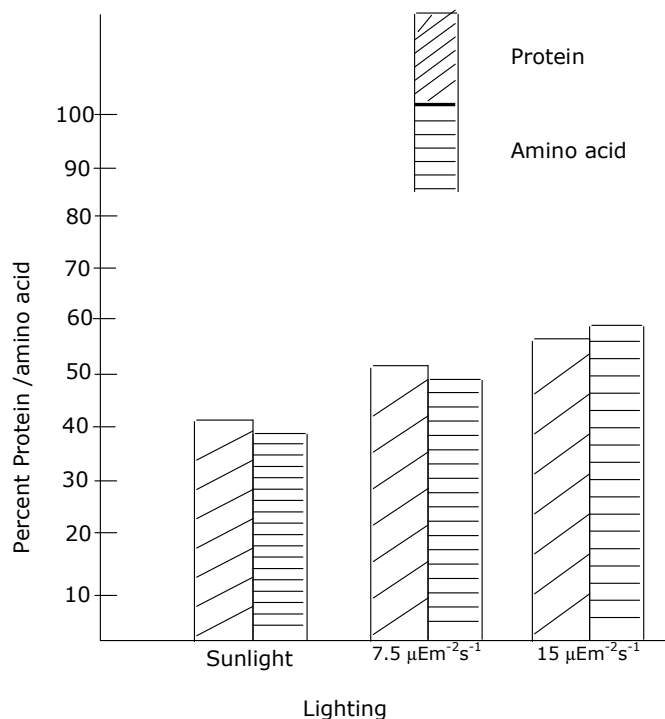


Figure 4. Total protein and amino acid concentration in *Spirulina* sp. under different lighting regimes.

have observed that artificial illumination resulted in higher biomass production in *Spirulina* species.

In conclusion, the result of this investigation shows that agitation (by aeration) and adequate lighting are very important factors in biomass production and protein biosynthesis in the *Spirulina* species isolated from an oil-polluted flame pit. In controlled cultivation of this organism adequate aeration, agitation and lighting are necessary for increased yield of cell mass and cell protein.

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