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Bangladesh J. Sci. Ind. Res. **43(3)**, 369- 376, 2008

BANGLADESH JOURNAL OF SCIENTIFIC AND INDUSTRIAL RESEARCH

E-mail: bjsir07gmail.com

Spirulina Culture in Bangladesh XII. Effects of Different Culture Media, Different Culture Vessels and Different Cultural Conditions on Coiled and Straight Filament Characteristics of *Spirulina*

Parvin Noor,* Nasima Akhtar, John Liton Munshi and Sajeda Begum

Biological Research Division, BCSIR Laboratories, Dhaka-1205, Bangladesh.

Abstract

Effects of different culture media (Bd-2, Bd-3, Bd-4 and IFP), different culture vessels (conical flasks, plastic bowls and earthen pots) and different cultural conditions (stock, pilot plant and shade culture) on coiled and straight filament characteristics of *Spirulina* were studied. Stock culture maintained under special conditions, retained coiled filament characteristics even after 17 years, while *Spirulina* filaments in pilot ponds lost their coiled characteristics. Food value of the straight filaments was acceptable and their survival capacity was more than that of coiled filaments.

Key words: *Spirulina,* Media culture, Vessels culture, Cultural condition, Filaments character.

Introduction

Protein, vitamins and iron rich, microscopic blue- green alga, *Spirulina* is commercially produced in some tropical and subtropical climatic regions of the world (Venkataraman and Becker, 1985, Henrikson, 1989, Bonnin, 1982). But its commercial production in monsoon climatic zone has been possible only in Bangladesh (Jahan *et al,* 1999). In Wardha, India, laboratory experiments were carried out in an optimized low cost culture medium, for cultivation of *S. platensis* as a nutritive food supplement for the malnourished rural families. 50% concentration of Zarrouk medium showed maximum growth. Low cost medium using 10% biogas slurry supplemented with sodium bicarbonate or carbonate as basic ingredients, has been optimized for cultivation of *S. platensis* (Kate *et al,* 1991). Domestic scale *Spirulina* production is now being conducted in the country

* Corresponding author

(Begum, *et al* 1998)). *Spirulina* stock culture, initially obtained from Madras and Mysore of India, required several years for adjustment to monsoon climate in Bangladesh. However, during adjustment to the adverse climatic conditions, the natural coiled filament characteristics of the alga were gradually lost. Number of the straight filaments increased in the culture, while that of the coiled filaments were greatly reduced in course of time. An attempt was made to culture *Spirulina* in sodium bicarbonate with rice husk ash. The initial result was encouraging, in that, *Spirulina* was found to grow in the medium containing rice husk ash in combination with common fertilizers. But *Spirulina* filaments became long when grown in the above medium for a long period (Majid, 1991). It is known that, *Spirulina* develops straight filaments when cultured on solid medium, instead of coiled filaments, may be due to adverse condition. The climatic condition in Bangladesh is definitely unfavorable for *Spirulina* culture. This probably is the reason for gradual increase in straight filaments in its commercial culture in Bangladesh. However, the straight filaments were also found to be rich in protein and iron, hence its commercial value was hardly reduced. Nevertheless, researches were conducted in BCSIR Laboratories, Dhaka, on coiled and straight filaments of *Spirulina* (Jahan *et al*, 1999), in that, effects of three different ashes (rice husk ash, coconut coir ash and banana leaf ash) on the coiled characteristics and general growth of *Spirulina* were studied. Two commercial media (IFP and Bd-1) and one domestic/rural medium (Bd-4) were supplemented with the above ashes. The media and the ashes had varied effects on the characteristics of *Spirulina*. In that, Bd-4 medium was found to be the best, followed by Bd-1. Banana leaf ash was found to be superior to the other ashes. The present study included domestic/rural media (Bd-2, Bd-3, Bd-4) with ash and one commercial medium (IFP), without any ash supplement. Effects of warm weather and cold weather, different types of culture vessels and different cultural condition were also studied.

Materials and Methods

The study was conducted in BCSIR laboratories, Dhaka, using stock culture of *Spirulina platensis*, originally obtained from MCRC at Madras, CFTRI at Mysore and Gandhigram University of India. The experiments were conducted as follows :-

Effects of different culture media on coiled and straight filament characteristics of *Spirulina* in winter (November)

The cultures were maintained in 3 rural/ domestic media: Bd-2 ($NaHCO_3$ 2g., Urea 50mg., NaCl 1g., rice husk ash 1g., H_2O 1000ml.), Bd-3 ($NaHCO_3$ 2g., Urea 50mg., NaCl 1g., gypsum 1.5 gm., H_2O 1000ml.), Bd-4 ($NaHCO_3$ 4g.,Urea 250mg., NaCl 5g., banana leaf ash 1g., H_2O 1000ml.) and commercial medium IFP ($NaHCO_3$ 16.8g.,

$NaNO_3$ 2.5g., KCl 1g., $MgSO_4$ H_2O 0.5g., $CaCl_2$ 0.04g., $FeSO_4$ $7H_2O$ 0.01g., H_2SO_4 (96%) 0.5ml., H_3PO_4 (85%) 0.2ml., EDTA 0.04g., A_8 1ml., B_3 1ml., H_2O 1000ml). The pH of Bd-2 was 9.0, Bd-3 8.5, Bd-4 9.5 and IFP 9. One litre conical flasks containing 500ml. experimental media, were inoculated with 10ml. stock culture. The flasks were shaken in the morning and evening manually. These were kept in southern veranda, exposed to natural climatic condition. Condition of *Spirulina* filaments was observed under the compound microscope everyday. Each treatment had 3 replicas.

Effects of Bd-4 and IFP media on coiled and straight filament characteristics percentage of *Spirulina* in summer (May).

Bd-4 medium was found to be superior to the other rural/domestic media (Jahan *et.al*, 1999, Begum *et. al*, 1998) hence it was included in the present trail excluding the other rural media (in the previous trail all the rural media had similar effect on *Spirulina* filaments). The microscopic observations were taken on 7th, 14th, 21st and 28th day of the culture. Cultures were kept as in the previous trail and each treatment had 3 replicas. The flasks were shaken in the morning and evening manually. The study was conducted for 28 days.

Change in percentage of coiled and straight filaments in IFP medium in different types of culture vessels in summer (May).

Only IFP medium was included in this study. The cultures were kept as in the previous trails. However, in addition to 1 liter conical flasks, 6 liter capacity plastic bowls and earthen pots were also included in this comparative study as culture vessels. 500 ml. medium was added in the conical flasks and 3 litre medium was added in 6 liter plastic bowls and earthen pots. Inoculum size of *Spirulina* was (20ml/l., O.D 3/4). Microscopic observations were made on 7th, 14th, 21st and 28th day. The conical flasks were shaken and the other were stirred manually in the morning and evening. Each treatment had 3 replicas and the study was conducted for 28 days.

Effects of different cultural conditions on coiled and straight filament characteristics of *Spirulina*.

Stock culture

Conical flasks (3 liter capacity) were filled with IFP medium, nearly up to the neck. *Spirulina* was added to the medium till the colour was light green. It was kept exposed to direct sunlight and air bubbles were passed through the culture, 24 hours, with the help of air pumps. The cultures were harvested when optimum density (O.D) of the cultures was 1. Fresh cultures were set after washing the flasks. Stock cultures have been maintained in this way for 17 years (up to date). The present study records only the microscopic observations.

Pilot plant culture

Spirulina is being cultured in concrete pilot plant ponds ($50m^2$ and $125m^2$), provided with rotary paddle wheel, in Bd-1 medium ($NaHCO_3$ 3.2g, $NaNO_3$ 0.5g, KCL 0.2g., $MgSO_4$. $2H_2O$ 0.1g, $CaCl_2$ 0.008g., $FeSO_4.7H_2O$ 0.002g., EDTA 0.008g. Urea 0.05g, TSP 0.01g, H_2SO_4 (96%) 0.1ml, H_3PO_4 (85%) 0.04ml, A_8 0.2ml, B_3 0.2ml, and H_2O 1000ml.), since 1989. The cultures are harvested and feedback added depending on the growth of Spirulina. Like the stock cultures in flasks, only microscopic observations are included in this trial. The rotary paddle wheels are run in the daytime only.

Shade culture

One liter conical flasks containing 500ml. Bd-4 medium were inoculated with 10ml. *Spirulina.* The culture flasks were kept in a shaded eastern veranda for 3 years. The flasks were shaken as mentioned before. The cultures were harvested and fresh cultures were set up using inoculum from the previous culture. In the present study also only the microscopic observations were recorded.

Results and Discussion

Table I contains the comparative chemical composition of coiled and straight filaments of *Spirulina*. Results of the present study are presented in Tables II-V. Coiled filaments were collected from stock culture (94.48% coiled) and straight filaments from cultures in Bd-4 medium (96% straight). Protein content of coiled filaments was 62.72% and that of straight 59.96%, while fat content of coiled was 0.19% and that of straight filaments 0.41%. Iron content in straight filaments was 0.068% and that of coiled filaments was 0.034%. No lead or arsenic could be detected in either of the samples. Therefore, the food values of straight filaments were also found to be acceptable.

Effects of different culture media on coiled and straight filament characteristics of *Spirulina* in winter (November).

The results of the study are presented in the Table II. Percentage of coiled filaments 99% and that of straight filaments 1% did not show any change in Bd-2, Bd-3 and Bd-4 media, from their initial percentage, in 10

Table I. Chemical composition of coiled and straight filaments of *Spirulina*, cultured in IFP and Bd-4 medium

Medium	Sample	Protein(%)	Fat(%)	Iron(%)	Lead(ppm)	Arsenic(ppm)
IFP	Coiled - 99%	62.72	0.19	0.034	Not traceable	Not traceable
Bd-4	Straight - 96%	59.96	0.41	0.068	Do	Do

Analyzed by Bangladesh Standards and Testing Institute.

Table II. Effects of different culture media on coiled and straight filament characteristics of *Spirulina* in winter (November) in conical flask

Culture media	Observation period 10 days(Average of 30 microscopeobservation)		Observation period 28 days(Average of 45 microscope observation)	
Rural / Domestic				
Bd-2	Coiled - 99%,	Straight -1%	Coiled-97%,	Straight -3%
Bd-3	Coiled - 99%,	Straight -1%	Coiled-97%,	Straight -3%
Bd-4	Coiled - 99%,	Straight -1%	Coiled-97%,	Straight -3%
IFP(Commercial)	Coiled - 98%,	Straight -2%	Coiled-95%,	Straight -5%

days. Coiled filaments slightly decreased (98%) while straight filaments slightly increased (2%) in IFP medium during the same period. After 15 days all the three rural/domestic media showed slight decrease in coiled filaments 97% and increase in straight filaments 3%, IFP medium after 15days showed 95% coiled and 5% straight filaments. Growth rate was low in winter in all the media (Akhtar, 2006).

Effects of Bd-4 and IFP media on coiled and straight filament characteristics of *Spirulina* in summer (May).

Results are resented in Table III. In summer, both IFP and Bd-4 media showed better growth of *Spirulina* (Akhtar, 2006). However, IFP was found to produce better response than Bd-4. In that, on 7th day Bd-4 had 89% coiled, 11% straight filaments while that IFP was 96% and 4% respectively.

Table III. Effects of Bd-4 and IFP media on coiled and straight filament characteristics of *Spirulina* in summer (May) in conical flask

Medium	7th day (Average of 10 observation)	14th day (Average) of 10 observation)	21th day (Average of 10 observation)	28th day (Average of 10 observation)
Bd-4	Coiled-89% Straight-11%	Coiled-80% Straight-20%	Coiled- 72% Straight-28%	Coiled-60% Straight-40%
IFP	Coiled-96% Straight-4%	Coiled-90% Straight-10%	Coiled-89% Straight-11%	Coiled-87% Straight-13%

Initial coiled filament percentage 98.

On the 14th day Bd-4 had 80% coiled and 20% straight, while IFP had 90% coiled and 10% straight. On 21st day, Bd-4 had 72% coiled and 28% straight filaments, while IFP had 89% coiled and 11% straight. On 28th day, Bd-4 had 60% coiled and 40% straight filaments, while IFP had 87% coiled and 13% straight filaments. Since IFP was found to be superior to Bd-4, it was selected for further trials, excluding Bd-4.

Change in percentage of coiled and straight filaments in IFP medium in different types of culture vessels in summer (May).

Results of the study are presented in Table IV. Cultures in conical flasks, receiving more light than plastic bowls and earthen pots, retained their coiled characteristics for longer period while cultures in plastic bowls and earthen pots showed identical response. In that, on 7th day conical flasks had 97% coiled and 3% straight filaments, while it was 82% coiled and 18% straight in plastic bowls and 88% coiled and 12% straight in earthen pots. On 14th day conical flasks had 92% coiled and 8% straight filaments, while plastic bowls had 71% coiled and 29% straight, earthen pots had 75% coiled and 25% straight filaments. On 21th day conical flasks had 89% coiled and 11% straight, while plastic bowls had 56% coiled and 44% straight and earthen pot had 57% coiled and 43% straight filaments. On 28th day conical flasks had 87% coiled and 13% straight filaments, while both plastic bowls and earthen pots contained 93% coiled and 7% straight filaments.

During the period of this study, having many cloudy and rainy days, sunlight seemed to play a significant role. In case of straight filaments, the percentage increased more in plastic bowls and earthen pots than in coni-

Table IV. Change in percentage of coiled and straight filaments in IFP medium in different types of culture vessels in summer (May).

Culture vessels	7th day (Average of 10 observation)	14th day (Average) of 10 observation)	21th day (Average of 10 observation)	28th day (Average of 10 observation)
Conical flask	Coiled-97% Straight-3%	Coiled-92% Straight-8%	Coiled- 89% Straight-11%	Coiled-87% Straight-13%
Plastic bowl	Coiled-82% Straight-18%	Coiled-71% Straight-29%	Coiled-56% Straight-44%	Coiled-7% Straight-93%
Earthen pot	Coiled-88% Straight-12%	Coiled-75% Straight-25%	Coiled-57% Straight-43%	Coiled-7% Straight-93%

Initial coiled filament percentage 98.

cal flasks, cultures of which received more sunlight.

Effects of different cultural conditions on coiled and straight filament characteristics of *Spirulina*.

Results of the study are presented in Table V. Stock cultures and pilot plant cultures had adequate sunlight while shade cultures were devoid of direct sunlight. Stock cultures in conical flasks, having the benefit of constant air bubbles and more sunlight, showed 94.48% coiled filaments and 5.52% straight filaments after a maintenance period of 17 years. Initial percentage of coiled filaments was 100 and that of straight 0%. Pilot plant culture having adequate sunlight and rotary paddle wheel, after the same maintenance period of 17 years showed only 0.38% coiled filaments and 99.62% straight filaments. Similar reports were obtained from the 3 *Spirulina* growing commercial companies of the country (Life Line International, Wonder Herbs and Nature Food Products) after about 10-12 years of commercial production (Personal communication).

Cultures grown in shade showed very low growth rate and heavy contamination in the beginning gradually the cultures adjusted, surviving for 3 years. But the growth is still very low. The cultures have no coiled filaments, all filaments turning straight.

Conclusion

Spirulina cultures obtained from warm and dry regions of India, required 2 years to adjust to the monsoon climate of Bangladesh, which is not favourable for its growth. Stock cultures maintained in IFP medium, under special conditions (not feasible for large scale production), retained their

Table V. Effects of different cultural condition on coiled and straight filaments characteristics of *Spirulina* (3 to 17 years)

Cultural condition	Maintenance period /year	Observation period	Coiled filament percentage (average of 20 observations under microscope)	Straight filamentpercentage (average of 20 observation under microscope)
Stock culture (IFP)	17	December	94.48	5.52
Pilot plant culture (Bd-1)	17	Do	0.38	99.62
Shade culture (Bd-4)	3	Do	0.00	100

coiled characteristics (94.48% coiled) even after 17 years of growth in this climate. Where as cultures in the same medium (IFP) and same container (conical flask) devoid of air bubbling system, showed reduction of coiled filaments percentage within a month (87%). Since straight filaments were found to have adequate percentage of protein and iron , their acceptance is assured from commercial point of view. It may be mentioned here that, *Spirulina* powders from BCSIR originating from 90% - 100% straight filaments were analyzed abroad and found to be acceptable.

The present study indicates that, straight filaments have better survival capacity, and under less favourable conditions coiled filaments turn straight. Further investigations are required for ascertaining this supposition.

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Received :February, 10, 2008;
Accepted : March, 30, 2008.