

***Spirulina* Culture in Bangladesh XI**

Selection of a Culture Medium, Suitable for Culturing a Local Strain of *Spirulina*

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Abstract

Large scale *Spirulina* culture is possible in Bangladesh inspite of unpredictable climatic condition. Effect of different culture media on the growth of a local strain of *Spirulina* (*Spirulina platensis*) was studied. Bangladesh medium (Bd₁) was found to be more favourable for the growth of the alga. All together three culture media were included in this study.

Introduction

Spirulina is a microscopic unbranched, filamentous blue-green alga, rich in protein, vitamins especially vitamin B₁₂ and pro-vitamin A (beta-carotene), iron, essential amino acids, minerals and essential fatty acids like gamma linolenic acid (GLA).

Spirulina has a long history of use as food. There are reports that *Spirulina* was used traditionally by Mexicans during Aztec civilization for over 1000 years. Its use as a food by the natives in the lake Chad area has also been documented by the French phycologist Dangeard. Its safety for human food has since been established through various toxicological studies sponsored by the United Nations Industrial Development Organization (UNIDO).

It is found as an unialgal culture in some alkaline lakes with a very high pH, sometimes reaching 11. Such a pH prohibits the growth of most other algae. Now it is produced commercially and sold as a food supplement in health stores around the world. Up to very recently, the interest in *Spirulina* was mainly in its nutritive value. Currently, some people are looking into the possible therapeutic effects of *Spirulina*. Pre-clinical and clinical studies suggest several therapeutic effects of *Spirulina* ranging from reduction of cholesterol and cancer to enhancing the immune system, increasing intestinal lactobacillus, reducing nephrotoxicity, heavy metals and drugs and radiation protection.¹

Spirulina is available in ditches, ponds, lake etc. in Bangladesh in combination with other algae. A local strain of *Spirulina* in pure form was collected from a pond near the airport road, Uttara, Dhaka. Several culture media were reported to be used by various authors for *Spirulina* culture.²⁻⁴ In Biological Research Division, BCSIR Labs., Dhaka, *Spirulina* was cultured at pilot plant scale for over 12 years.⁵⁻⁷ Bangladesh medium I (Bd₁) was developed in our laboratory for commercial production of *Spirulina* in Bangladesh.⁸ Another medium was developed in the same Laboratory for domestic scale culture of *Spirulina* in Bangladesh.⁹ The present study was undertaken with a view to select a culture medium suitable for culturing the local strain of *Spirulina*.

Materials and Methods

The study was conducted using a local strain of *Spirulina*, collected from a pond near Airport Road, Uttara, Dhaka. Three media were included in this study, Bd₁, Bd₄ and IFP. Bd₁ medium was also used in the control sets of this study. It is used for *Spirulina* production in the pilot plant ponds at BCSIR. The commercial strain of *Spirulina* was used in the control sets. For the preliminary trial, 20 flasks of 1 litre capacity were used. Graduated pipettes were used to dispose the inocula and graduated measuring cylinders were used for adding 400 ml. of different cul-

ture media to each set, consisting of 5 flasks. These were inoculated with 20 ml of *Spirulina* (local strain, commercial strain in control only). The culture flasks were kept in the southern veranda in day time but at night these were exposed to total darkness. The optical density (OD) of the culture was recorded by a Spectrophotometer for maintenance of the growth record. pH of the culture was recorded by a pH meter. Temperature of the culture was recorded by a centigrade thermometer. The condition of *Spirulina* culture was observed under a compound microscope (X 80), once a week. The experiment was conducted for one month. After that the cultures were transferred to plastic bowls of 20 litres capacity (3 bowls in each set), for continuation of the comparative study at larger scale. This experiment was conducted for two months. Temperature, pH, optical density (OD) and microscopic observations were recorded twice a month. On the basis of this study, a medium was selected (Bd₁). After that, the local strain of *Spirulina* was cultured in Bd₁ medium in a cement basin (174 cm X 24 cm) for six months. Finally the *Spirulina* culture was transferred to a cement pond (2 sq. meter) for growth study at a pilot plant scale for one year.

Results and Discussion

Results of the study are presented in Tables I to IV.

The following culture media were used in this study

Particulars	Medium No. 1 Bd ₁	Medium No. 2 Bd ₄	Medium No. 3 IFP
NaHCO ₃	3.2 g.	4 g.	16.8 g.
NaNO ₃	0.5 g.		2.5 g.
KCl	0.2 g.		1.0 g.
MgSO ₄ , 2H ₂ O	0.1 g.		0.5 g.
CaCl ₂	0.008 g.		0.04 g.
FeSO ₄ , 7H ₂ O	0.002 g.		0.01 g.
H ₂ SO ₄ (96 %)	0.1 ml.		0.5 ml.
H ₃ PO ₄ (85 %)	0.4 ml.		0.2 ml.
EDTA	0.008 g.		0.04 g.
NaCl		4.0 g.	
Urea		2.5 g.	
Water	1000 ml.	1000 ml.	1000 ml.
pH	8.6	8.3	8.8

Effect of three different culture media on a local strain of *Spirulina* is presented in Table I.

Among seven different culture media, used by different authors for *Spirulina* culture three were included in this study.^{2-4, 8-9} In the preliminary comparative study, a local strain of *Spirulina* was cultured in three different media (Bd₁, Bd₄ and IFP) for one month. A commercial strain of *Spirulina*, cultured in Bd₁ medium, was used as control. Initially growth of the local strain was equally good in all the three media (Bd₁, Bd₄ and IFP). However, after one month, condition and colour of local strain of *Spirulina* was better in Bd₁ than Bd₄ and IFP media (Table I). Contamination was very few.

In continuation of the study, the local strain of *Spirulina* and the control were cultured in

plastic bowls, in the above media for another two months. Results of the study are presented in Table II.

In this study, the local strain of *Spirulina* showed better response to Bd₁ medium in comparison to the others, Bd₄ and IFP.

To ensure the growth of the local strain of *Spirulina* in Bd₁ medium, the culture was continued in larger scale for six months.

Spirulina was harvested once a month. Feed back was added to the medium after each harvest. Results of the study are presented in Table III.

During the six months trial, the local strain of *Spirulina* were successfully grown in Bd₁ medium.

Table I. Effect of three different culture media on a local strain of *Spirulina*, grown in conical flask, for one month.

Date	Culture medium	Temp. ° C	pH	Optical density (OD)	Condition of <i>Spirulina</i>	Contamination
30-06-02	Bd ₁	29 ^o C	8.7	0.33	Filaments very good and healthy, light blue-green	No contamination
	Bd ₄	29 ^o C	8.4	0.33	Filaments very good and healthy, light blue-green	No contamination
	IFP	29 ^o C	8.9	0.32	Filaments very good and healthy, light blue-green	No contamination
	Control	29 ^o C	8.7	0.31	Filaments very good and healthy, light blue-green	No contamination
06-07-02	Bd ₁	29 ^o C	9.26	0.31	Filaments very good and healthy, light blue-green	Paramecium very few
	Bd ₄	29 ^o C	8.96	0.18	Filaments in broken condition, light blue-green	No contamination
	IFP	29 ^o C	9.26	0.26	Some filaments in good condition, some in broken condition, light blue-green	Paramecium very few
	Control	29 ^o C	9.14	0.20	Filaments in very good condition, light blue-green	Zooplankton very few
12-07-02	Bd ₁	30 ^o C	8.90	0.13	Filaments very good and healthy, light blue-green	Paramecium very few
	Bd ₄	30 ^o C	8.58	0.11	Filaments broken into small pieces, very light blue-green	No contamination
	IFP	30 ^o C	9.10	0.25	Filaments good and healthy, blue-green	No contamination
	Control	30 ^o C	8.86	0.17	Filaments good and healthy, blue-green	Paramecium and Phacus very few
18-07-02	Bd ₁	28 ^o C	8.25	0.64	Filaments very good and healthy, blue-green	No contamination
	Bd ₄	28 ^o C	8.20	0.65	Filaments good and healthy, light blue-green.	No contamination
	IFP	28 ^o C	8.20	0.58	Filaments good and healthy, blue-green	No contamination
	Control	28 ^o C	8.50	1.01	Filaments good and healthy, light blue-green	No contamination

Table I. continued

Date	Culture medium	Temp. ° C	pH	Optical density (OD)	Condition of <i>Spirulina</i>	Contamination
24-07-02	Bd ₁	27° C	9.30	0.28	Filaments very good and healthy, a few filaments in broken condition, blue-green	Paramecium very few
	Bd ₄	27° C	9.22	0.26	Most of the filaments broken into pieces, light blue-green.	No contamination
	IFP	27° C	9.50	0.32	50% of the filaments in broken condition, light blue-green	No contamination
	Control	27° C	9.28	0.81	Filaments in good condition., a few in broken condition, blue-green	Protozoa a few
30-07-02	Bd ₁	28° C	9.46	0.34	Filaments good and healthy, blue-green	No contamination
	Bd ₄	28° C	--	--	All filaments destroyed, brown	No contamination
	IFP	28° C	9.75	0.09	Most of the filaments in good condition, blue-green	No contamination
	Control	28° C	9.60	0.36	Filaments in good condition, light blue-green	No contamination

* Average of 5 flasks

Table II. Effect of three different culture media on a local strain of *Spirulina*, grown in plastic bowls for two months.

Date	Culture medium	Temp. ° C	pH	Optical density (OD)	Condition of <i>Spirulina</i>	Contamination
01-08-02	Bd ₁	26° C	9.5	0.60	Very good and healthy, blue-green colour	Nil
	Bd ₄	26° C	9.4	0.60	Very good and healthy, blue-green colour	Nil
	IFP	26° C	9.4	0.60	Very good and healthy, blue-green colour	Nil
	Control	26° C	9.5	0.60	Very good and healthy, blue-green colour	Nil

Table II. continued

Date	Culture medium	Temp. ° C	pH	Optical density (OD)	Condition of <i>Spirulina</i>	Contamination
17-08-02	Bd ₁	25 ⁰ C	9.5	0.78	Very good and healthy, blue-green colour	Nil
	Bd ₄	25 ⁰ C	9.1	0.65	Good, a few filaments in broken condition, light blue-green colour	Low
	IFP	25 ⁰ C	9.4	0.70	Very good and healthy, blue-green colour	Low
	Control	25 ⁰ C	9.5	0.75	Very good and healthy, blue-green colour	Nil
31-08-02	Bd ₁	30 ⁰ C	9.4	0.85	Very good and healthy, blue-green colour	Low
	Bd ₄	26 ⁰ C	9.0	0.68	Good, a few filaments in broken condition, light blue-green colour	Low
	IFP	26 ⁰ C	9.3	0.77	Good and healthy, blue-green colour	Low
	Control	26 ⁰ C	9.5	0.80	Very good and healthy, blue-green colour	Low
15-09-02	Bd ₁	26 ⁰ C	9.5	1.00	Very good and healthy, blue-green colour	Nil
	Bd ₄	26 ⁰ C	9.2	0.70	Filaments in broken condition, light blue-green colour	Low
	IFP	26 ⁰ C	9.4	0.80	Good, a few filaments in broken condition, light blue-green colour	Nil
	Control	26 ⁰ C	9.7	0.90	Good and healthy, blue-green colour	Nil
30-09-02	Bd ₁	26 ⁰ C	9.3	1.00	Very good and healthy, blue-green colour	Nil
	Bd ₄	26 ⁰ C	9.2	0.70	Filaments in broken condition, light blue-green colour	Nil
	IFP	26 ⁰ C	9.3	0.82	Good, a few filaments in broken condition, light blue-green colour	Nil
	Control	26 ⁰ C	9.4	0.95	Good and healthy, blue-green colour	Nil

* Average of 3 bowls

Table III. Effect of Bd₁ medium on the growth of local strain of *Spirulina* in cement basin, for six months

Date	Temp. ° C	pH	Optical density (OD)	Condition of <i>Spirulina</i>	Yield of <i>Spirulina</i> (dry wt. in g.)
01-10-02	26 ^o C	9.3	0.57	Very good and healthy, blue-green colour	--
01-11-02	25 ^o C	9.0	0.50	Very good and healthy, blue-green colour	13.5
02-12-02	21 ^o C	7.0	0.65	Good and healthy, light blue-green colour	18.5
01-01-03	18 ^o C	10	0.70	Good and healthy, light blue-green colour	11.0
01-02-03	19 ^o C	9.1	0.75	Very good and healthy, blue-green colour	16.0
01-03-03	20 ^o C	9.0	1.00	Very good and healthy, blue-green colour	30.0

In a further extensive study, the local strain was cultured in Bd₁ medium at a larger scale for one whole year (pilot plant pond).

The *Spirulina* was harvested once a month and feed back was added in the culture medium after each harvest. Data representing the

Table IV. Effect of Bd₁ medium on the local strain of *Spirulina*, grown in pilot pond for one year

Date	Temp. ° C	pH	Optical density (OD)	Condition of <i>Spirulina</i>	Yield of <i>Spirulina</i> (dry wt. in g.)
02-03-03	20 ^o C	9.5	0.60	Very good and healthy, blue-green colour	--
02-04-03	20 ^o C	10	0.85	Very good and healthy, blue-green colour	17.0
02-05-03	28 ^o C	10	1.0	Good and healthy, blue-green colour, very few zooplankton present	43.0
02-06-03	29 ^o C	10	0.9	Good and healthy, blue-green colour	31.2
02-07-03	26 ^o C	10	0.75	Good and healthy, blue-green colour, a few protozoa present	16.5
02-08-03	28 ^o C	9.0	0.85	Good and healthy, blue-green colour	30.0
02-09-03	31 ^o C	9.0	1.0	Very good and healthy, blue-green colour	41.5
02-10-03	23 ^o C	9.1	0.90	Good and healthy, blue-green colour	30.5
02-11-03	29 ^o C	9.2	0.65	Good and healthy, blue-green colour, very few zooplankton present	18.5
02-12-03	23 ^o C	9.3	0.55	Good and healthy, blue-green colour	7.5
02-01-04	20 ^o C	9.5	0.60	Good and healthy, blue-green colour	11.5
02-02-04	21 ^o C	9.8	0.65	Good and healthy, blue-green colour	30.5

growth of *Spirulina* during the year are presented in Table IV.

Table IV reveals the fact that, the local *Spirulina* strain, included in this study, grew well in Bd₁ medium all throughout the year. The growth did not totally stop in any month during the study. Bd₁ medium supported good growth of local strain of *Spirulina*, retaining its normal blue-green colour.

Conclusion

The local strain of *Spirulina* (*Spirulina platensis*) included in this study was found to be suitable for culturing all throughout the year in Bd₁ medium in Bangladesh.

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