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## Evaluation of lab scale cultivation to assess the growth performance of *Spirulina platensis* using different substrates

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### Abstract

*Spirulina* has been so popular in the present world's context due to its high nutritional value. Due to its primitive and good quality protein, vitamins, essential fatty acids contents, antioxidant pigments, antimicrobial activity, and anticancer properties *Spirulina* which is a fast-growing cyanobacteria have been used as a possible alternative source of protein for the fish culture. As it is known that, the biomass of *Spirulina* is nutritionally rich in protein it may be used as a splendid alternative to fish protein to reduce the cost of feed. It has its tremendous attributes to replenish the required amount of protein dwindling the excessive cost as 70% of the total operating costs belongs to the feed supplement in terms of whole culture period. The study was conducted on the feasibility of *Spirulina* culture assessing its growth pattern through lab scale culture. The experiment was carried out to evaluate the lab scale cultivation of *Spirulina* by using different substrates having different concentration of Fish Meal Medium (FMM) each with the three replications T<sub>1</sub> (15% FMM), T<sub>2</sub> (20% FMM), T<sub>3</sub> (25% FMM) & T<sub>4</sub> (Kosaric medium), respectively. The growth rate of *S. platensis* was significantly higher in T<sub>4</sub> ( $p < 0.05$ ) than the other treatments (T<sub>1</sub>, T<sub>2</sub> & T<sub>3</sub>). It might be due to the availability of more nutrients in KM than various concentrations of FMM. On the other hand, the growth rate was higher in concentration of 15% FMM than other concentrations of FMM. The physico-chemical parameters i.e., temperature 28.00-29.39 °C, pH: 9.33-9.44, dissolved oxygen (DO): 5.97-6.58mg l<sup>-1</sup>, measuring the voltage between a pH sensitive glass electrode (MVPH): 129.70-149.47, total dissolved solid (TDS): 1025.00-3862.00, electric conductivity (EC): 1899.17-5726.67, hectopascal pressure unit (hpa %): 1009.50-1017.33 were observed in the optimum level.

**Keywords:** Culture of *Spirulina*, Fish Meal Medium (FMM), Kosaric Medium (KM)

### 1. Introduction

The utilization of microalgae and their nutritional value have long been acquainted in various fields of research i.e., as feed additive in aquaculture feed formulation, alternative source of protein, pigments. Within the assembly of valuable substances, they are utilized in human and animal nutrition (e.g., fatty acids, pigments) [1]. There are lots of biologically active substances and alternative unconventional sources of protein and consequently, they are going to be used as dietary feed supplements for animals [5]. Microalgae are a good source of nutrients and the application of microalgae as food, feed, drugs, pigments, source of chemical constituents, fuels, hormones is well known [4]. As food microalgae are used since about 2,000 years ago in China. For thousands of years although microalgae are mentioned because of the source of highly enriched nutrients [2]. To develop microalgal biotechnology it began only within the middle of the past century [1]. Depending upon the source, *Spirulina* contains unusually high amounts of protein, between 55 and 70% by dry weight [7]. Though with reduced amounts of methionine, cystine, and lysine, as compared to plain proteins it is an entire protein containing all the essential amino acids. It's however, superior to all or any standard plant protein, like that from legumes [8]. As a complementary dietary ingredient for fish, shrimp and poultry *Spirulina* has been used of feed and increasingly as a vitamin supplement and protein to aquafeeds [5]. As a partial substitute of imported forage China is using this micro-alga to plug the expansion, immunity and viability of shrimp.

To utilize the *Spirulina* as aquaculture feed additives in Japan there has also been comprehensive research in this arena. To utilize CO<sub>2</sub> dissolved in seawater as a nutrient source *Spirulina* may be a primitive organism originating some 3.5 billion years ago that has established the power of *Spirulina* as it could be susceptible as a photosynthesizing cyanophyte (blue-green algae) that grows vigorously in strong sunshine under highly alkaline conditions and high temperatures. Among other plant sources like dry soybeans (35%), peanuts (25%) or grains (8-10%), *Spirulina* has predominant quality of protein content (59-65%), which is quite other commonly used. The special value of *Spirulina* is that it's readily digested because of the absence of cellulose in its cell walls as it is the case for eukaryotic green microalgae like *Chlorella*, *Ankistrodesmus*, *Selenastrum*, *Scenedesmus*; as its protein is digested and assimilated [9]. The composition of spirulina powder consists of 60% protein, 20% carbohydrate, 5% fats, 7% minerals and 3-6% moisture, making it a low-fat, low calorie, cholesterol-free source of protein as a biochemical compound [1].

With the event of aquaculture, the requirements of bulk feed materials and substituents like soybean flour, organic and other resources are constantly rising and costs are increasing every year. Therefore, the research for seek new sources of raw materials has been a crucial attention. As a replacement source of feed material microalgae has many advantages which make it become superior to other cyanobacteria species (source of protein, fatty acids, vitamins, etc.). Thus, the benefits of microalgae appear increasingly in aquaculture industry. Therefore, the utilization of microalgae as feed additives is more broadly getting emphasized to use in aquaculture. As a critical think about promoting normal growth and sustaining fish health in aquaculture operations proper nutrition has been recognized [3]. Diets particularly animal and plant-based may be a factor that has significantly contributed to the huge expansion of fish farming. The *S. platensis* were cultured in various concentrations of fish meal medium, the ultimate goal for producing a cheap alternative source of protein has not yet been achieved, and the cost of production is still higher than that of conventional and non-

conventional sources of protein. So, it is needed to indicate a culture media for *S. platensis* to spare the high cost of inorganic media. So, attempt has been made to find out any inexpensive organic media containing high amount of protein for *Spirulina* culture. Therefore, the present work has been undertaken to study the growth performance of *S. platensis* in various concentrations of fish meal medium and kosaric medium to assess the comparative feasibility of the lab scale cultivation.

## 2. Materials and Methods

### 2.1 Strain procurement

In this study, the strain was procured from the Dept. of Aquaculture laboratory, Bangladesh Agricultural University, Mymensingh, Bangladesh. Inoculum of *S. platensis* was done having the purpose of pure stock culture.

### 2.2 Strain maintenance

For *Spirulina* cultivation, Fish Meal Medium (FMM) and Kosaric Medium (KM) were used in the present study. Care was taken that the pH of medium is 9.5 after autoclaving. To achieve this, pH of the medium was adjusted to 8.5 which resulted in a pH of 9.5, after autoclaving and "recovery" of the medium. Growth and maintenance of culture was done at 30±2 °C.

### 2.3 Experimental site

The experiment has been conducted in the laboratory of the Nutrition Division of Bangladesh Fisheries Research Institute, Freshwater Station, Mymensingh. The experimental procedure was handled with care so that any external intervention cannot interfere with or hinder the cultivation system.

### 2.4 Experimental design of *S. platensis* culture

The two types of media viz. Fish Meal Medium (FMM) and Kosaric Medium (KM) were used for the culture of *S. platensis*. Under this experiment, FMM and KM treatments were performed each of 3 replications. For both the culture media 12 days culture period was maintained.



**Fig 1:** Setup for *Spirulina* culture

## 2.5 Preparation of FMM and KM for *S. platensis* culture

Fish meal was dried in an oven at 50 °C for overnight after collecting from local market of Mymensingh. For complete drying, these fish meals were dried under sun for another seven days. Then Haman Dista was used to make powder of the dried fish meal. For getting fine and homogenous particle of fish meal, it was sieved through a sieve. Then it was kept in a 5-liter capacity reagent bottle and added 4-liter distilled water. Aeration was provided for 22 days for preparing Fish Meal Medium (FMM) and then the media were collected from reagent bottle and sterilized at 120 °C for 15 minutes with moist heat autoclave. About 600 ml distilled water was added into the conical flask having capacity 1.0 L volume and then prepared Fish Meal Medium with the addition of 15, 20 and 25% each with three replications. After then the cooling of the prepared media was mixed well.

Stock solutions of different chemical ingredients of KM were prepared with distilled water. The KM was prepared by taking required amount of solution from each stock and then kept in 1.0 L conical flask and volume was made up to 600ml

marked. Then mixing, sterilization and cooling of the prepared media were done as followed the same procedure during the FMM preparation. Collection of *S. platensis* under Order Volvocales, Class-Chlorophyceae was collected from the laboratory of the Department of Aquaculture, BAU, Mymensingh. For purity, the pure stock culture maintenance, the stock culture of *S. platensis* was maintained in the laboratory in Kosaric medium (KM). Growth of *S. platensis* was monitored and it was then observed under microscope to confirm its purity.

*S. platensis* were inoculated (Optical density at 620 nm. 0.41) into the 15, 20 and 25% concentration of Fish Meal Medium, respectively to produce a culture containing of *S. platensis*. Similarly, same inocula were given in KM. Using electric aerator these cultured bottles were continuously aerated. At every alternative day samplings were taken from each bottle to observe *S. platensis* cell density, chlorophyll a, water quality parameters of culture media. All the glassware's used in the experiment were sterilized by dry heat in an oven at 70 °C.

**Table 1:** Composition of Kosaric medium (Modified after Zarrouk, 1996) for *Spirulina platensis* culture

Sl. No.	Chemicals/compounds	Concentration in stock solution g/L
1.	NaHCO <sub>3</sub>	9.0
2.	K <sub>2</sub> HPO <sub>4</sub>	0.250
3.	NaNO <sub>3</sub>	1.250
4.	K <sub>2</sub> SO <sub>4</sub>	0.50
5.	NaCl	0.50
6.	MgSO <sub>4</sub> .7H <sub>2</sub> O	0.10
7.	CaCl <sub>2</sub>	0.02
8.	FeSO <sub>4</sub> .2H <sub>2</sub> O	0.005
9.	A <sub>5</sub> micronutrient solution <sup>a</sup>	0.5 ml/L
	a) A <sub>5</sub> micronutrient solution	G/L
	i) H <sub>3</sub> BO <sub>4</sub>	2.86
	ii) MnCl <sub>2</sub> .4H <sub>2</sub> O	1.81
	iii) ZnSO <sub>4</sub> .7H <sub>2</sub> O	0.22
	iv) CuSO <sub>4</sub> .5H <sub>2</sub> O	0.08
	v) MoO <sub>3</sub>	0.01
	vi) CoCl <sub>2</sub> .6H <sub>2</sub> O	0.01

## 2.6 Estimation of *S. platensis* cell weight (g/L)

For cell weight 10 ml of *S. platensis* sample from each Treatment was taken and filtered with an electric filtration unit using filter papers and shifted to the oven at 105 °C for 24 hours. The samples were then transferred to the desiccator for cooling and weight was measured using an electric balance. The weight of the paper filter was taken prior to filtering.

## 2.7 Data analysis

Collected sample data were recorded in MS Excel 2010. Statistical analysis was done to evaluate the effect of the four treatments on the growth of *S. platensis* were significant or not. ANOVA test was performed to test the significance of difference among different concentration of FMM and KM. The entire statistical test was conducted by using SPSS (Statistical Package for Social science) version 16. The graph was prepared by using both MS Excel and SPSS.

## 3. Results and Discussion

### 3.1 Physico-chemical characteristics

The physico chemical parameters. i.e., temperature, pH, dissolved oxygen (DO), measuring the voltage between a pH sensitive glass electrode (MVPH), total dissolved solid (TDS), electric conductivity (EC), hectopascal pressure unit

(hpa %) and salinity were recorded. The temperature was ranged 28.00-29.39°C, pH: 9.33-9.44, dissolved oxygen (DO): 5.97-6.58mg<sup>l</sup><sup>-1</sup>, measuring the voltage between a pH sensitive glass electrode (MVPH): 129.70-149.47, total dissolved solid (TDS): 1025.00-3862.00, electric conductivity (EC): 1899.17-5726.67, hectopascal pressure unit (hpa %): 1009.50-1017.33. Light intensity is an important physical factor for the growth of microalgae. The best growth of *S. platensis* is found at light intensity of 2110 and 2120 lux/m<sup>2</sup>/s in FMM and KM, respectively (Figure-1). It was found that <sup>[13]</sup> the growth of *S. platensis* was saturated at levels of 25-30 Klux/m<sup>2</sup>/s. This variation might be occurred due to the difference of strain of species and composition of nutrient in different media.

For normal growth of microalgae, dissolved oxygen is one of the most important chemicals, parameter. During the period of experiment, the dissolved oxygen was found to range from 5.86 to 6.58 mg/L. The fluctuation in dissolved oxygen value might be due to alteration rate of photosynthesis in the culture media. It was reported <sup>[6, 10]</sup> that high oxygen level results in reduced growth rate.

The pH of the medium is one of the most important chemical factors in culturing *Spirulina*. Maintaining pH of over 9.5 is mandatory in *Spirulina* cultures in order to avoid contamination by other algae. In contrast, at present study the pH range was found at 9.33 to 9.44 which is suitable for the

growth of *Spirulina*. The maximum cell weight followed at pH value 9.13 and 9.42 in FMM and KM, respectively. Temperature is the most important physical factor for the growth of all living organisms. More or less similar temperature was recorded during the culture period. The

maximum cell growth at 28 °C in 0.533 g/L at 15% of concentration of FMM and 0.689 g/L at 28.16 °C for KM respectively. It was reported that<sup>[12]</sup> the optimal temperature for photosynthesis of *Spirulina* strain was marked in between 28-29.39 °C which is more or less similar to the present study.

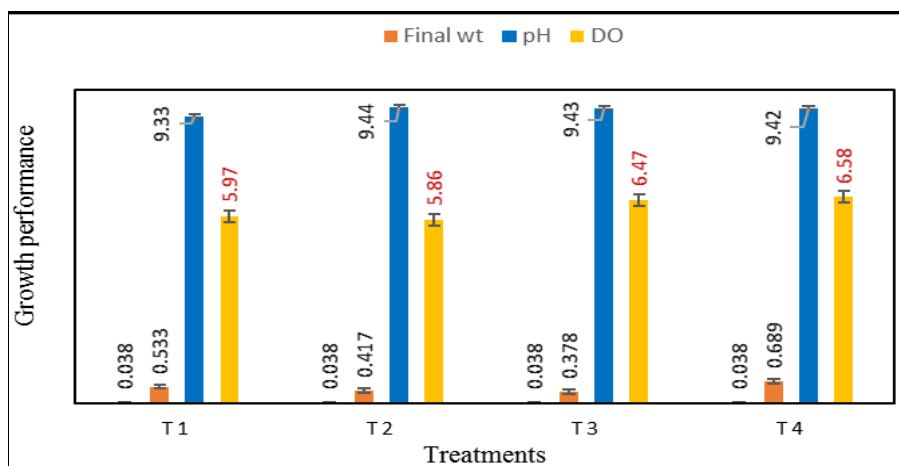
**Table 2:** Physico-chemical parameters during experimental period

Observed parameters	Treatments			
	T-1 (15% FMM)	T-2 (20% FMM)	T-3 (25% FMM)	T-4 (KM)
pH	9.33±0.05	9.44±0.02	9.43±0.01	9.42±0.13
Temperature	28.00±0.08	29.39±0.37	28.47±0.40	28.16±0.84
DO	5.97±0.10	5.86±0.22	6.47±0.35	6.58±2.03
MVPH	129.70±4.62	143.18±11.59	149.47±0.43	147.13 ±5.75
TDS	1070.67±72.60	1101.00±90.80	1025.00±103.11	3862.00±1554.52
EC	1899.17±146.37	2218±208.88	2011.33±193.41	5226.67±515.89
hpa%	1009.50±0.50	1010.33±1.04	1010.17±1.26	1017.33±2.08
Salinity	0.60±0.08	0.88±0.32	0.64±0.20	4.32±1.86

**3.2 Cell weight of *S. platensis***

The range of cell weight of *S. platensis* in different concentrations of FM medium viz., in concentration of 15% was 0.038 to 0.533 g/L; in concentration of 20% was 0.038 to 0.417 g/L; in concentration of 25% was 0.038 to 0.378 g/L and in KM was 0.038 to 0.689 g/L (Table-3). The growth of cell was varied in different media and different concentrations. This variation might be due to different nutrient composition and concentrations of different media.

The growth rate of *S. platensis* was significantly higher in KM (p<0.05) than various concentrations of FMM which might be due to the availability of more nutrients in KM than various concentrations of FMM (Figure-2,3). On the other hand, the growth rate was higher in concentration of 15% FMM than other concentrations of FMM. It might be due to the favorable growth parameter (pH, temperature, DO) and also suitable amount of nutrients in FMM (15%) than other concentrations of FMM (Table-3, Figure-3).



**Fig 2:** Growth performance of *S. platensis* based on parameters

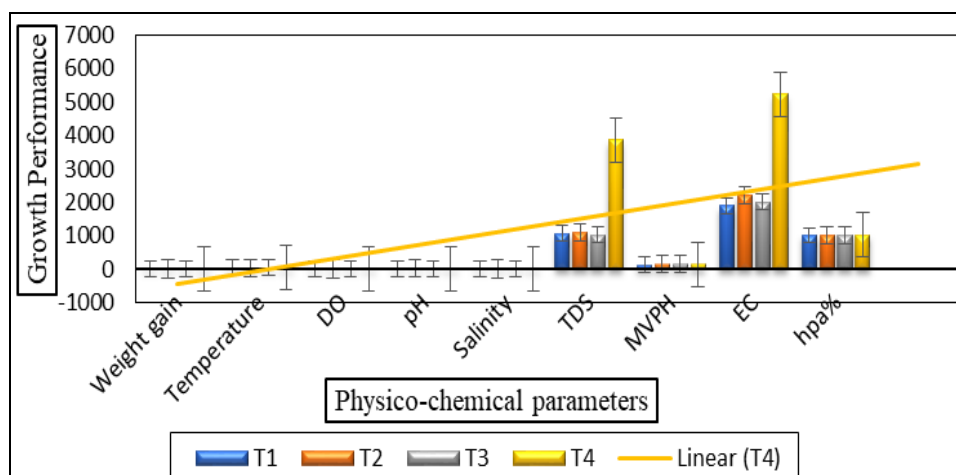
The KM shows comparatively higher growth than various concentrations of FMM. Among the different concentration of FMM, T1 (15%) given the better production ((Figure-2,3).

The growth parameter (pH, temperature, DO) and also suitable amount of nutrients may be the cause for the mentioned variation (Table-2,3).

**Table 3:** Growth parameters at various concentrations of FMM and KM

FMM (15%)		FMM (20%)		FMM (25%)		KM	
Initial weight (g/l)	Final Weight (g/l)	Initial weight (g/l)	Final Weight (g/l)	Initial weight (g/l)	Final Weight (g/l)	Initial weight (g/l)	Final Weight (g/l)
0.038	0.533	0.038	0.417	0.038	0.378	0.038	0.689
Avg. wt		Avg. wt		Avg. wt		Avg. wt	
0.286 (g/l)		0.228		0.208		0.364	
Parameters relationship with the growth of <i>S. Platensis</i>							
pH	DO	pH	DO	pH	DO	pH	DO
9.33±0.05	5.97±0.10	9.44±0.02	5.86±0.22	9.43±0.01	6.47±0.35	9.42±0.13	6.58±2.03





**Fig 3:** Growth performance trends of *S. platensis* depending on physico-chemical parameters

### 3.3 Aeration effect on different nitrogen sources

The spirulina species reveals a better result with maximum dry weight when aeration is done in the experimental condition. Maximum chlorophyll content was seen in aeration when done with an aquarium pump. Hence the chlorophyll content in spirulina species enhances due to the continuous aeration. Aeration agitates the culture medium and gives the *Spirulina* filaments a homogeneous distribution throughout the cultivation system for adequate exposure to illumination. It also contributes to the uniform distribution of oxygen concentrations and eliminates certain inhibitory substances for example CO<sub>2</sub>. Therefore, Aeration is a basic necessity for the cultivation of *Spirulina platensis*. It should also be noted that to avoid thermal stratification and settling of the cells constant mixing of the culture medium is important prior and during the experiment. It is also essential to retain uniform distribution of nutrients and remove surplus oxygen. If aeration is not sufficient, the production of biomass and efficiency of energy use is low.

### 4. Conclusion

Under this experiment, the comparative growth rate of *Spirulina* was observed both in kosaric medium and different concentration of fish meal medium. *Spirulina* was cultivated to evaluate lab scale cultivation by using different substrates and *Spirulina* grows well in all the four substrates; with the highest growth performance in Kosaric medium (0.689 g/l). The experimental data implied that *Spirulina* when cultivated on varying concentrations of substrate supplementation shows better yield and maximum growth was found in T<sub>4</sub>. It indicates that, the different concentration of fish meal medium (15%, 20%, 25%) has potential to increase the growth rate of *Spirulina*. The higher production obtained from the culture unit of kosaric medium (T<sub>4</sub>) with significantly higher production ( $p < 0.05$ ) in comparison other concentration of fish meal medium (FMM). However, it might be suggested that more research and insights is needed to account cost-benefit analysis for evaluating the grow-out potential of *Spirulina* in lab-based cultivation.

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