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Production of Spirulina (*Arthrospira Platensis*) Using Different Culture Media

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ABSTRACT

Three experiments was conducted on roof top of agriculture faculty, Sher-e-Bangla Agricultural University (SAU), Dhaka, Bangladesh, from the period of 31st December 2018 to 30th April 2019 to study the production of spirulina (*Arthrospira platensis*) using different concentration of culture media and chemicals. Experiments were laid out in Completely Randomized Design (CRD). Zarrouks media were used in first experiment to study spirulina production. An experiment was accomplished to evaluate modify zarrouks media on spirulina production. TSP was used instead of K_2HP0_4 in this experiment. Another experiment was conducted to study the economic analysis of spirulina production with four replications. Treatments were (S_0N_0): Zarrouk's media, (S_1N_0): Addition of Spirulina, (S_0N_1): Addition of Nutrient and (S_1N_1): Spirulina + Nutrient. Data were taken on following parameter: optical density observation (%), fresh weight (g), wash weight (g), dry weight (g) and Beneficial cost ratio. In zarrouks media, fresh wt. (427.0g), wash wt. (335.0 g), dry wt. (55.41 g) was recorded. In modify zarrouks media, fresh wt., wash wt., dry wt. (258.0 g, 145.0 g, 23.5 g) were recorded respectively. In third experiment, maximum BCR (5.0%, 2.66% and 2.05%) respectively found in S_1N_1 upto three harvests. In overall observation and calculation, it can be said that zarrouks media is standard and modify media will be the potential for spirulina production in Bangladesh.

Key words: Standard medium, indoor spirulina production, rich source of protein, optical density observation, economic

Introduction

Spirulina (*Arthrospiraplatensis*) belongs to Oscillatoriaceae family, a blue-green algae and a natural food found in high alkaline aqua environments. Spirulina under a microscope looks like little spirals and grows quickly under the right conditions. Ancient civilizations grew their Spirulina in lakes and ponds. Today Spirulina producers grow Spirulina in controlled aqua environments to ensure the quality and safety of Spirulina. It is important to consider the water source and growing environment when determining the quality and safety of Spirulina. Spirulina is one of the oldest food sources because of its high nutritional value. Ancient Aztec and African civilizations understood Spirulina's health benefits and now with modern scientific research, we know Spirulina's nutritional profile. Spirulina is known to be a rich source of protein, contain essential and non-essential amino acids, gamma-linolenic acid (GLA), vitamin A (beta-carotene), B12, iron, calcium, chlorophyll, and phycocyanin. Spirulina contains a high content of protein (up to 70%), along with high amounts of essential fatty acids, essential amino acids, minerals, vitamins (especially B12), antioxidant pigments (phycobiliproteins and carotenoids) and polysaccharides (Belay *et al.,* 1993; Vonshak, 1997). Consequently, the commercial production of Spirulina has gained worldwide attention for use in human food supplements, animal feed and pharmaceuticals. In aquaculture, Spirulina is used as a feed additive to improve growth, feed efficiency, carcass quality, and physiological response to disease in several species of fish (Mustafa *et al.,* 1994).

In Bangladesh, a research study introduced a totally new advanced technology for the production of spirulina on rooftop (as indoor production system) with substituting some chemicals used in media. Zarrouk's medium has successfully served as the standard medium (SM) for Spirulina culture for many years (Zarrouk, 1966). Consequently, in this experiments SM is modified with some doses of chemical ingredients and substituted it with different treatments doses of as the cost varies almost 50%. Modified media could be possible to make by substituting some chemicals and production found almost same in quantity. The cost of nutrients is considered the second major factors influence the cost of Spirulina biomass production after labor. Many media have been developed using seawater (Faucher et al., 1979), sewage water (Saxena et al., 1982) and industrial effluents (Tanticharoen et al., 1993).

Methods and Materials

Experiment setup

Total 24 numbers of 5 Lt Capacity containers were used for the experiments. RFL food grade transparent and protective plastic made container is used. Spirulina needs sunlight so a transparent container is a good way to increase exposure.

Requirements

Temperature

Spirulina is grown in a culture medium that is made of water and fertilizers. Due to the high pH of this culture medium, Spirulina has almost no competitors. The pH level of the growing culture should be maintained at 10.5-11. This means that it is not "fussy" regarding the water it needs: drinking water, brackish water, water from a natural body of water or rainwater can be used. Almost all parasites, germs and viruses cannot survive the alkaline environment in which Spirulina strives. It is important to note that excluded from this are water containing heavy metals, as Spirulina will absorb them. Spirulina needs sunlight having 30-35^{0}C temperature

Microorganism

The cyanobacterium *S. platensis*, required amount of strain were used in the present study was obtained from the 2abiotech Laboratory, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka, Bangladesh. Normally per wet strain is required for per liter of water.

Culture media

The culture medium in which the Spirulina is grown consists of water and fertilizers. The specific 'recipe' can vary according to environment, type of Spirulina and availability of fertilizers. All 'recipes' are a variation of the Zarrouk's Media (g/L) defined in 1966 and it should be the reference for Spirulina farmers that want to experiment with the culture medium.

Table 1. List of chemicals used in Zarrouk's media preparation

Zarrouk's Media (g/L)	
Ingredients	g/l
$NaHCO_3$(Sodium bicarbonate)	16.8
$NaNO_3$ (Sodium Nitrate)	2.5
Nacl (Sodium chloride)	1.0
K_2HPO_4 (Dipottasium Hydrogen Phosphate).	0.5
k_2SO_4(Sodium Sulphate)	1.0
EDAT (Na)	0.08
$CaCl_2.H_20$ (Calcium Chloride)	0.04
$FeSO_4.7H_2o$ (Ferrous Sulphate)	0.01
$MgSO_4.7H_2o$ (Magnesium Sulphate)	0.2
Micronutrients	0.01

Table 2. List of chemicals used in Modified media preparation

Modified Media (g/L)	
Ingredients	g/l
$NaHCO_3$(Sodium bicarbonate)	16.8
$NaNO_3$ (Sodium Nitrate)	1.5
Nacl (Sodium chloride)	1.0
K_2HPO_4 (Diopttasium Hydrogen Phosphate) is substituted by TSP(Tripple super phosphate)	0.36
K_2SO_4(Sodium Sulphate)	1.0
EDAT (Na)	0.08
$CaCl_2.H_2O$ (Calcium Chloride)	0.04
$FeSO_4.7H_2O$ (Ferrous Sulphate)	0.01
$MgSO_4.7H_2O$ (Magnesium Sulphate)	0.2

Modified Media used as:

Control	S_0N_0
Addition of Nutrient	S_0N_1
Addition of Spirulina	S_1N_0
Addition of Spirulina + Nutrient	S_1N_1

Multiplication characteristics evaluation

Biomass concentration was determined almost every day by measuring the optical density at 560 nm using spectrophotometer.

Harvesting

Harvesting during morning hours is best for both nutritional and practical reasons. Harvesting is done in the following manner: Hold filtration cloth over the pool or container; Pour culture onto cloth. Cloth with a density of 30-40 microns in diameter, made of polypropylene, nylon or polyester is a good solution. This can be done using a bucket or any container, but make sure it is cleaned before and after use; The Spirulina will remain on the cloth. The culture medium will flow through the cloth and return to the bucket or container; The Spirulina on the cloth will at this point still have some residue of the culture medium. In order to bring the Spirulina to a pH level that is healthy for consumption (7pH), these residues need to be eliminated following with 20% Nacl water by washing it. To do so press the filtering cloth (with the Spirulina in it) evenly and gently. The culture medium which is transparent – will drain from the cloth. When the water draining through the filtering cloth is no longer transparent but green, stop squeezing - this means that all the culture medium has been squeezed out and the Spirulina is at a healthy pH level. The drained and pressed Spirulina should at this stage be similar in texture to paste.

Manual way drying:

In this process, spread Spirulina on net/plastic sheet - Using a finger (gloved), knife or spatula (cleaned before use), a syringe (disposable or cleaned before use) spread Spirulina on net in noodle-like stripes. A net/plastic sheet enables drying from top and bottom. This will shorten the time needed to dry and lessen the danger of contaminators (when Spirulina is harvested it is no longer protected by the high pH level of the culture medium). It is best that this net/plastic sheet should be placed inside the greenhouse or controlled room. This will further protect the Spirulina from contaminators. Approximately 24-30 hrs should be sufficient for drying.

Data Collection:

Data collection were done by following three steps of harvesting procedure like fresh weight, wash weight and dry weight . To know the production growth rate of spirulina g/liter of water as well as cost of production for it.

Result and discussion

OD (Optical density)

Optical density (OD) is one of the most important parameters in Spirulina cultivation. Measuring the OD of cell growth is useful to measure the biomass concentration. Growth estimation by optical density measurement is generally determined in a spectrophotometer. This application note describes a simple procedure to determine the biomass of Spirulina versus OD, using the Cuvette with wavelength of 56o nm. Average OD (Optical density) is sown in figure

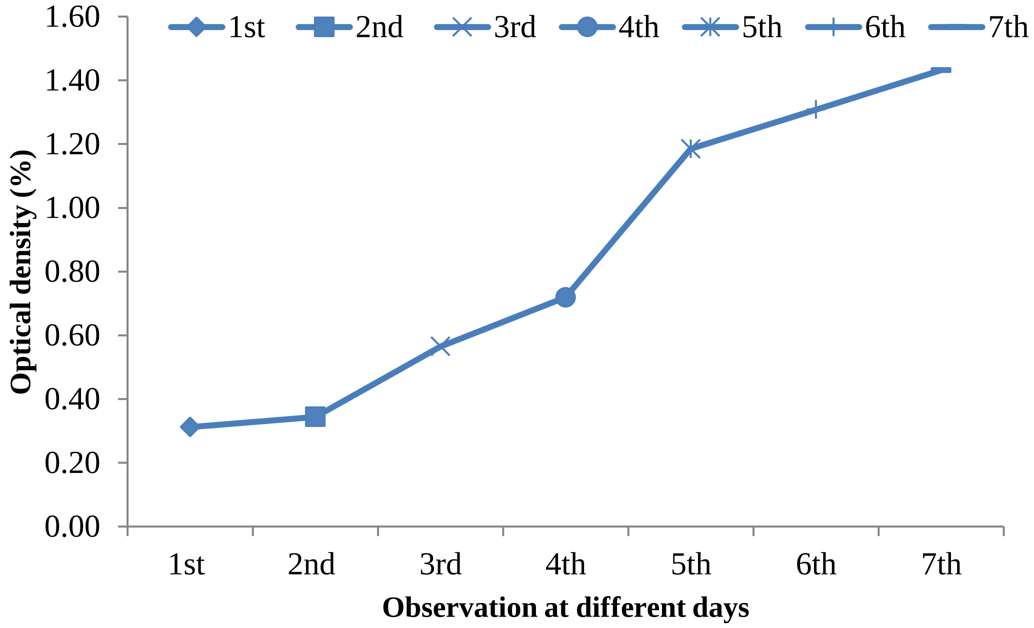

Fig.1 Optical density of spirulina growth with days

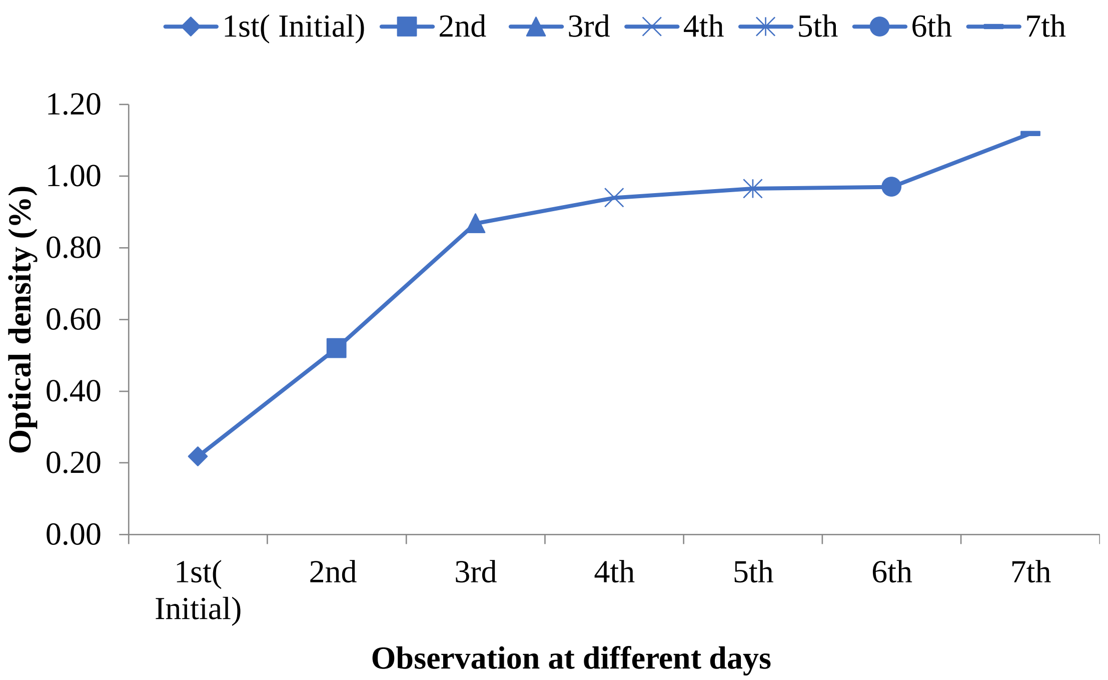

Fig.2 Optical density of spirulina growth with days

Fresh weight (g), Wash weight (g) and Dry weight (g)

Table 1. Production of spirulina g/L of water

Harvest Details of 7 days	
Fresh weight	427.0 g
wash weight	335.0 g
Dry weight	55.41 g
Total liter of water	56.0 L
Production of spirulina	0.98 g/L

Table 2.Production of spirulina g/L of water

Harvest Details of 7 days	
Fresh weight	258.0 g
wash weight	145.0 g
Dry weight	23.52 g
Total liter of water	56 L
Production of spirulina	0.42g/L

Productivity

Different treatments showed different significant of productivity like fresh weight (g) 19.73, wash weight (g) 15.03 and dry weight (g) 2.53 showed the highest productivity in S_1N_1 and the lowest was observed in S_0N_0 treatment as fresh weight(g) 9.8 , wash weight(g) 7.44, and dry weight (g) 1.25 respectively.

Economic analysis

Initial cost of set up is high and costly due to materials cost but later it become more economical to grower/producer. Input cost for materials and non-materials were recorded as per liter water used for spirulina production. Price value of spirulina and chemicals used ware considered as per available market price. The economic analysis is presented under the following heading

Gross return

Different treatments showed different values in terms of gross return .Overall the highest gross return was obtained from the S_1N_1 and the lowest gross return was obtained fromS_0N_0 during whole research work period. Gross returns calculation is done on the basic of cost calculation of media used g/l and available market value of dry product of spirulina.

Net return

In case of net return, Among all treatments , three treatments were S_1N_1, S_1N_0 and S_0N_1 showed positive net returns where S_0N_0 showed worst performance returns which is not considerable for furthermore production.

Benefit cost ratio (BCR)

The lowest benefit cost ratio was noted for S_0N_0(1.65) and the highest benefit cost ratio were observed in three different treatments S_1N_1, S_1N_0 and S_0N_1. The highest benefit cost ratio was noted (8.13) and (7.13). Therefore, it is assume that from overall observation that among four treatments S_1N_1, S_1N_0, S_0N_1 found better than the rest one of S_0N_0 treatment from the economic point of view.

Table 3 . Benefit Cost Ratio of 1st harvest details

Treatment	Final dry weight (g)	Total production cost (Tk)	Available Market Price Tk/kg	Total Price of product(TK)	Net return (Tk)	BCR
SoNo	4.87	49.14	Approx. 10000	48.7	-0.44	0.99
SoN1	8.84	16.00		88.4	77.53	5.52
S1No	8.22	59.14		88.2	47.12	1.49
S1N1	9.61	36.00		96.1	60.1	2.66

Here, S_o : No spirulina, S_1: Spirulina, N_0: No chemical, N_1: Chemicals

Table 4. Benefit Cost Ratio of 2nd harvest details

Treatment	Final dry weight (g)	Total production cost (Tk)	Available Market Price Tk/kg	Total Price of product(Tk)	Net return (Tk)	BCR
SoNo	6	49.14	Approx. 10000	60.00	10.76	1.22
SoN1	4.5	16.00		45.00	29.00	2.81
S1No	10.5	59.14		105.00	45.86	1.77
S1N1	18	36.00		180.00	144.00	5.00

Here, S_o : No spirulina, S_1: Spirulina, N_0: No chemical, N_1: Chemicals

Table 5. Benefit Cost Ratio of 3rd harvest details

Treatment	Final dry weight (g)	Total production cost (Tk)	Available Market Price Tk/kg	Total Price of product(Tk)	Net return (Tk)	BCR
SoNo	2.67	49.14	Approx. 10000	26.70	-22.44	0.54
SoN1	3.28	16.00		32.80	15.20	2.05
S1No	2.41	59.14		24.10	35.04	0.40
S1N1	3.77	36.00		37.70	-1.70	1.04

Here, S_o : No spirulina, S_1: Spirulina, N_0: No chemical, N_1: Chemicals

Conclusion

It is concluded that, Zarrouks media found standard media for spirulina production. Despite that modified media found low economic for spirulina production. K_2HPO_4 is substituted with Triple Super Phosphate (TSP) in modified media. It can be also concluded that the different treatments used under study exposed denoting variation in the studied characteristics under Bangladesh condition. According to the result, S_1N_1 materialized the best production among the four treatments. From the point of its conceivable productivity and higher marketable yield, other parameters and economic analysis, S_0N_0 has the lowest position in spirulina production.

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