SPIRULINA *(Spirulina platensis)* PRODUCTION IN DIFFERENT PHOTOBIOREACTORS ON ROOFTOP

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Most surely in the creation of the heavens and the earth and the alternation of the night and the day there are signs for men who understand.

(Surah Al Zumar 3:190)

DEDICATED TO-

MY BELOVED PARENTS

To whom I owe every fiber of my being

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*This is to certify that the thesis entitled "**SPIRULINA (Spirulina platensis) PRODUCTION IN DIFFERENT PHOTOBIOREACTORS ON ROOFTOP**" submitted to the Department of Horticulture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE IN HORTICULTURE**, embodies the result of a piece of bonafide research work carried out by **OSMAN GANI**, Registration No. **13-05392** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

I further certify that any help or source of information, received during the course of this investigation has been duly acknowledged.

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- Author

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ABSTRACT

An experiment was accomplished on the rooftop of agricultural faculty of Sher-e-Bangla Agricultural University during the period from July to September, 2018 to screen some Photobioreactors for finding out more economically convenient and easily available one for spirulina production in Bangladesh. Four types of photobioreactors *viz*. Rectangular shaped 5L photobioreactor (PBR_1), Cuboidal shaped 3L Photobioreactor (PBR_2), Cylindrical shaped 15L Photobioreactor(PBR_3), Rectangular shaped 15L Photobioreactor (PBR_4) ware used in this experiment arranged in a Completely Randomized Design (CRD) with three replications. Fifteen days of production was carried out in the selected photobioreactors to fully determine the performance of the PBR_s where culture condition were kept the same and data on different growth and yield parameters were taken throughout the experiment to which all the PBR_S showed significant variations. Among photobioreactors, minimum doubling time (3.41 days), maximum productivity (0.90 $gL^{-1}day^{-1}$) and maximum marketable yield (3.34 Kg/1000L) were found in PBR_4 while maximum doubling time (10.63 days) and minimum productivity (0.40 $gL^{-1}day^{-1}$) and minimum marketable yield (1.64 Kg/1000L) in PBR_2. These findings may be a source of valuable information for adopting Rectangular shaped 15L Photobioreactor (PBR_4) as a promising photobioreactor for spirulina cultivation in Bangladesh.

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ABBREVIATIONS AND ACCORONYMS

AEZ	=	Agro-ecological Zone
Agric.	=	Agricultural
ANOVA	=	Analysis of Variance
BARI	=	Bangladesh Agricultural Research Institute
Biol.	=	Biology
CV	=	Coefficient Variance
DAI	=	Days after Inoculation
EPB	=	Export Promotion Bureau
et al.	=	And others
GDP	=	Gross Domestic Product
Hort.	=	Horticulture
i.e.	=	That is
J.	=	Journal
LSD	=	Least Significance difference
mm	=	Millimeter
CRD	=	Completely Randomized Design
Res.	=	Research
SAU	=	Sher-e-Bangla Agricultural University
Sci.	=	Science
Technol.	=	Technology
Viz.	=	Namely
μ	=	Specific growth rate
OD	=	Optical density
PBR_S	=	Photobioreactors

CHAPTER I

INTRODUCTION

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INTRODUCTION

Spirulina (*Spirulina platensis*) belongs to the family Spirulinaceae is a free floating filamentous microalgae belonging to the class Cyanobacteria (Komárek and Hauer 2009). It has two genera namely Spirulina and Arthrospira with characteristic photosynthetic ability (Sapp, 2005). Spirulina was primarily classified in plant kingdom but was later placed in the bacteria kingdom based on new understandings on the genetics, biochemical properties and physiology (Vonshak, 1997).

It's native to Central Africa, Mexico, some parts of Asia and America. Spirulina naturally grows on high salt alkaline water reservoirs found across these areas (Vonshak, 1997; Gershwin and Belay, 2007). Spirulina was first known to the modern world by a European scientific mission conducted in Chad. Spirulina or "Dihe" as the Chadians call it has been found growing in the alkaline lagoons scattered all around Chad. Chadians have been taking it as food for several centuries (Abdulqader *et al*., 2000). Later studies show that Mayans, Toltecs, Kanembus dursing the Aztec civilization had incorporated spirulina in their diet 400 years ago (Gershwin and Belay, 2007). There are a large number of Spirulina species; among them three species of spirulina namely *Spirulina platensis*, *Spirulina maxima* and *Spirulina fusiformis* are intensively investigated as these are edible and has high nutritional and medicinal properties (Vonshak, 1997; Gershwin and Belay, 2007; Khan *et al*., 2005; Karkos *et al*., 2008).

Spirulina mainly grows in brackish water conditions with the correct chemical balance. It grows well between pH 8-11. Spirulina grows substantially in 20^0C temperature but the growth of spirulina thrives between 35^0C-37^0C. The growth is seriously hampered if the temperature is above 38^0C. Spirulina growth is also detrimental in low temperature. It requires ample sunlight to thrive as it an autonomous organism but 30% of sunlight is perfect for the growth and development.

It is mainly found in the market as powder, capsules or as tablets. Early studies conducted on spirulina were based basically on the nutritional properties of spirulina as it's a highly nutritious food with loads of vitamins and minerals. According to USDA Food Composition Database, Dried spirulina contains 60-70% protein, 24% carbohydrates, 8% fat (Khan *et al*., 2005, Campanella *et al.*, 2002). It's a complete protein source meaning it has all the essential amino acids. It's also exceptionally high in macro micro nutrients and essential fatty acids like Alpha- linoleic acid, Gamma-linoleic acid (Colla *et al*., 2003, Golmakani *et al*., 2012), stearidonic acid, eicosapentaenoic acid arachidonic acid etc. (Tokusoglu and Onal, 2003). Spirulina is long held as a highly nutritious food for some decades. In 1974, World Health Organization described Spirulina as "an interesting food for multiple reasons, rich in iron and protein, and is able to be administered to children without any risk", considering it "a very suitable food (IIMSAM, 2010). The United Nations established the intergovernmental institution for the use of Micro-algae spirulina against malnutrition (IIMSAM) in 2003 (Habib *et al*., 2008). In the late 1980s and early 90s, both NASA and European Space Agency proposed spirulina as one the primary foods to be cultivated during long term space missions (Riley, 2014, European Space Agency, 2005).

Spirulina is an ecologically sound, nutrient rich super food that is grown all around the world as a dietary supplement. Spirulina is considered as the "food of the future" that will effectively tackle the existing malnutrition problem. (Jamal uddin *et al.*, 2018*)* So, it is high time to give most priority to produce human grade spirulina for consumption. Spirulina is a phototrophic organism that's why it is mainly cultivated in different photobioreactors such as, open tanks, closed plastic tank, open race pond, Mud Pot, Polythene bag also includes Vertical, Horizontal and Flat Plane photobioreactor. So the main barriers of quality spirulina production are contamination, P^H maintenance, Growth rate, productivity and photosynthetic efficiency and the costly growth chamber.

A photobioreactor is a bioreactor that utilizes a light source to cultivate phototrophic microorganisms. These organisms use photosynthesis to generate biomass from light

and carbon dioxide and include plants, mosses, macro algae, cyanobacteria and purple bacteria.

There are alternative technologies to optimize the photobioreactors productivity and its applications on the reactor geometry. Based on the literature it was possible to verify that there are different types of photobioreactors (Table 1), but the two types of geometry more current are: the photobioreactors plats (flat geometry, used preferably in industrial production) and photobioreactors tubular (cylindrical geometry, laboratory use). For cylindrical photobioreactors, there are various possible configurations (Tredici *et al*., 2004):

- a large tube and forming a vertical column;
- two tubes of different diameters arranged one inside the other forming an annular chamber;
- a tube placed in the soil and diameter moderate but significant length arranged in a serpentine;
- a tube of small diameter and large length wound helically around a turn;
- several small diameter tubes arranged in parallel and vertical.

The cylindrical photobioreactors are widely used because their design is simple and easy to scale for large volumes of several hundred liters (Tsygankov, 2001).

Based on the literature, it is also possible to verify the most favorable geometry and conditions necessary to obtain an efficient productivity.

Table 1: Productivity obtained by different types of photobioreactor

Type of Photobioreactor	**Productivity, g dry spirulina/ l/day**
Cone shaped photobioreactor (Watanabe & Hall, 1996)	0.51
Spiral form of a tubular photobioreactor (Carlozzi and Torzillo, 1996)	1.06 (turbulent regime)
Vertical column photobioreactor (Marty, 1997)	With 304 W/m²: 0.79
Gazosiphon photobioreactor with internal loop (Vernerey, 2000)	0.9 to1.0
Helical tubular photobioreactor (Travieso,2001)	0.40
Mini tanks (Pelizer *et al.*, 2003)	0.67 to 0.76
Open raceway ponds (Radmann *et al*., 2007)	0.028 to 0.046

An overview of photosynthetic efficiencies obtained with different reactors, locations and micro algal species; 1.5% for open raceway pond, 3% for horizontal tubular photobioreactors and 5% for flat panel photobioreactors (Norsker *et al*., 2011). For the selection of a photobioreactor for large scale as well as small scale production, knowledge on the actual productivity and photosynthetic efficiency decontaminating as well as other factors that are related to the quality production of spirulina of different photobioreactor designs is required.

As already mentioned previously, spirulina is commercially available, being produced through a commercial process, plant-scale cultivation, leaving as final product under the form of tablets, capsules, powders, drinks etc. The application of this method not only requires considerable investments, which results in high price of the product, but also leads to the loss of some nutrients from spirulina, after drying process. Sometimes, due to drying process, some ingredients were deteriorated, resulting in a terrible flavor (Li *et al*., 2004), whereas in fresh product it does not happen.

As an alternative to the problem, it was proposed a reactor design to allow the cultivation and consumption of fresh spirulina at home, avoiding the damage to public health caused by the counterfeiting of food (Li *et al*., 2004). This need has already been taken into account by Li *et al*., (2004), but the reactor was not implemented on a commercial level. However, in industrial and laboratory level the implementation of a reactor for the *Spirulina platensis* production was well suited.

The design of this reactor would enable the consumer to cultivate and consume fresh *Spirulina spp*., at home, and it would change high-grade nourishing health foods into daily foods that everyone and every family could afford (Li *et al*., 2004)

Considering the above circumstances, the study was undertaken with following characteristics:

i. To compare different photobioreactors on the basis of growth rate, productivity, and yield of spirulina, and to find out more convenient one.
ii. To screen out the suitable and a cost effective photobioreactor as “Proto type” for commercial production in Bangladesh so that anyone can adopt this technology.

CHAPTER II

REVIEW OF LITERATURE

CHAPTER II
REVIEW OF LITERATURE

Bangladesh is basically a poverty ridden, highly populous and malnourished country. The agriculture, despite making a huge leap forward, it just isn't compatible enough to ensure total nutritional security in Bangladesh. Malnutrition plagues the majority of people, especially in children, adolescents and women. In addition, it causes individual tragedies such as maternal and child mortality, premature delivery, elevated risks of heart diseases and diabetes. Not only does it risk health, malnutrition also cuts a heavy figure in the national future as it results in lost productivity and reduced intellectual and learning capacity. In a country where the diet is dominated by cereal crops (about 70%) a diversified nutrient source is a must. Securing nutrition should be the first priority and this is where spirulina comes in. Presence of some essential nutrients required by human beings makes spirulina a modern day super food. Taking spirulina as a food supplement will ensure nutrition security of Bangladesh. Spirulina appears to have considerable potential for sustainable financial development, especially as a small scale crop for nutritional enhancement. But the problem for spirulina cultivation is to find out sustainable and cost effective production chamber. With this view in mind, the present research work was conducted to screen out suitable photobioreactor used as production unit through optical density, productivity and yield characterization. Some important research works related to production of spirulina in different photobioreactors used as growth chamber that have been conducted so far have been presented here according to year in a descending order.

Saranraj *et al.* (2013) reported that, *Spirulina platensis* was cultivated in the conical flasks containing Zarrouk's medium alone [SP_1] and Zarrouk's medium with three different concentrations (0.5 g/l [SP_2], 1.0 g/l [SP_3] and 1.5 g/l [SP_4] of LFA supplementation. Among the four different supplementations used, SP_4 which contained 441.5 g Lignite fly ash in one liter of Zarrouk's medium highly induced the growth and protein content of *Spirulina platensis* when compared to other supplementations. The optical density of *Spirulina platensis* was followed by SP_3 (0.237 mg/ml) and SP_2 (0.169 mg/ml), high in SP_4 (0.759) followed by SP_3 (0.667) and SP_2 (0.578). The least optical density was recorded in SP_1 in SP_1 (0.121 mg/ml).

Sukumaran *et al.* (2018) was conducted an experiment the prospect of *Arthrospira platensis* cultivation in newly designed medium with commercial or industrial grade fertilizers under real environment. Consequently, growth and yield of *A. platensis* was investigated under outdoor condition using modified Kosaric medium (MKM). The growth and yield of *A. platensis* was significantly higher ($p < 0.05$) in MKM in terms of optical density with 2.541 ABS, biomass dry weight with 1.30 g L^{-1}, chlorophyll a content with 12.96 mg L^{-1}, productivity with 0.141 g L^{-1} d^{-1} and specific growth rate with 0.253 μ d^{-1} compared to SKM in eight days of cultivation period.

Oncel and Vardar sukan (2007) were conducted an experiment to evaluate the performance of two photobioreactors such as internal loop airlift and bubble column photobioreactors where airlift PBRs give higher dry biomass weight (2.27g/l), chlorophyll-a value (24.3mg/l) and higher growth rate (0.47 day^{-1}) while in bubble column, it was observed that dry biomass (1.87 g/l), chlorophyll-a (21.6mg/l) and growth rate was (0.37 day^{-1}) respectively.

Reichert *et al*. (2006) studied that the cultivation of photosynthetic microorganisms such as the cyanobacterium *Spirulina platensis* has been studied by researchers in many countries because these organisms can produce products with industrial potential. We studied the specific growth rate (μ_x, day^{-1}) and productivity (P_x, in mg/L/day of *Spirulina platensis* biomass, dry weight basis) of two *S. platensis* strains (LEB-52 and Paracas) growing in aerated semi continuous culture in two-liter Erlenmeyer flasks for 90 days (2160 h) at 30°C under 2500 lux of illumination in a 12 h photoperiod. Independent of the *S. platensis* strain used we found that low biomass concentrations (0.50 g/L) and high renewal rates (50% v/v) resulted in a high specific growth rate ($\mu_x = 0.111$ day^{-1}) and high productivity ($P_x = 42.3$ mg/L/day).

Elias *et al.* (2002) were documented improvement for the mass-scale culture of microalgae with the use of sophisticated closed systems. The proposed photobioreactor in this study is a combination of helical parts receiving strong light with a main culture vessel mixed and degassed by airlift. A first experimental trial was carried out with the filamentous cyanobacterium, *Spirulina platensis* achieving maximal volumetric productivity of 1.2 $gl^{-1}d^{-1}$ comparable with the maximal reported

values by other closed culture designs for the same organism. The proposed system is under optimization of its engineering elements in order to comprise a sound modular solution for commercial production of microalgae of economic interest.

Suphi *et al.* (2006) conducted a laboratory scale integrated type of photobioreactor was operated for spirulina production where the integrated unit consisted of tank unit for main production site and a helical coil unit. Maximum dry weight was reached 1.88 gL^{-1}on days 17 and specific growth rate reached 0.221 day^{-1}.

Shimamatsu (2004) studied the mass production of *Spirulina* in the open pond system. They used modified aeration technique produce high mass in ambient temperature and decrease some metals in the medium to obtain more yield, production cost was minimized cost was minimized by improved harvesting and filtration methods.

Reichert *et al.* (2006) studied the specific growth rate and productivity of two *Spirulina platensis* strains. *Spirulina platensis* strain used was found that low concentration (0.50g/L) and high renewal rates (50% v/v) resulted in high specific growth rate and productivity. These values are two to four times higher than those obtained in simple batch cultivation and indicate that the semi continuous cultivation of *Spirulina platensis* is viable.

Vree *et al*. (2015) conducted an experiment where it was observed in case of vertical photobioreactors, higher areal productivities and photosynthetic efficiencies, 19–24 g m^{-2} day^{-1} and 2.4–4.2%, respectively, were found in comparison to the horizontal systems; 12–15 gm^{-2} day^{-1} and 1.5–1.8%. The higher ground areal productivity in the vertical systems could be explained by light dilution in combination with a higher light capture. In the raceway pond low productivities were obtained, due to the long optical path in this system. Areal productivities in all systems increased with increasing photon flux densities up to a photon flux density of 30 mol m^{-2} day^{-1}. Photosynthetic efficiencies remained constant in all systems with increasing photon flux densities. The highest photosynthetic efficiencies obtained were; 4.2% for the vertical tubular photobioreactor, 3.8% for the flat panel reactor, 1.8% for the horizontal tubular reactor, and 1.5% for the open raceway pond.

Zou and Richmond (1999) carried out an experiment that showed the effect of light-path length (i.e. reactor width or thickness) of flat plate glass reactors on outdoor production of eicosapentaenoic acid (EPA) and cell mass of *Nannochloropsis sp.* was tested, using a range of light-paths from 1.3 to 17.0 cm. Volumetric productivity of cell mass and optimal, as well as maximal cell density which represents the highest sustainable cell density under the experimental conditions, decreased with increase in light-path. Daily areal output rate (g dry weight $m^{-2}day^{-1}$) increased with increased light-path, in contrast with results obtained in similar reactors with spirulina cultures, in which areal output rates increased when the light-path was reduced. Maximal areal productivity of *Nannochloropsis sp.* (12.8 and 22.4 g ash-free dry weight per day per m2 of irradiated reactor surfaces, in winter and summer, respectively), reflecting maximal efficiency in light utilization, was obtained with the long light-paths, i.e. 10.4 and 17.0 cm. Increasing the light path from 1.3 to 17.0 cm resulted in an increase in areal EPA productivity, from 66.7 to 278.2 $mgm^{-2}day^{-1}$ in winter and from 232.1 to 515.7 $mgm^{-2}day^{-1}$ in summer. This enhancement in areal productivity of EPA stems from increased productivity of cell mass which was associated with the increase in light path.

Bosma *et al.* (2007) conducted an experiment where volumetric productivities in the bubble column were predicted and compared with experimental volumetric productivities. The light integration model over-estimated productivity, while the model in which we assumed no light integration under-estimated productivity. Light integration occurred partly (47%) during the period investigated. The average observed biomass yield on light was $0.60g.mol^{-1}$. The model of partly light integration predicted an average biomass yield on light of $0.57gmol^{-1}$ and predicted that productivity could have been increased by 19% if culture temperature would have been maintained at 24^{o} C.

Tredici *et al.* (2004) showed that the VAP photobioreactor has proven to be well suited to the outdoor mass cultivation of cyanobacteria, allowing operation at high cell concentrations (4–7 $gliter^{-1}$) and achieving high biomass productivity even in winter. The high surface-to-volume ratio ($80m^{-1}$), its flexible orientation with respect to the sun's rays, effective mixing and O_2 removal through air bubbling and a good control of

environmental and nutritional conditions seem to be the major advantages of the system.

Carlozzi and Torzillo (1996) accomplished an experiment with tubular (single tube and traditional loop) to flat and column and again to tubular photobioreactors (coil and loops) where it has improved micro algal yield. It increased from 25.0 $gm^{-2}d^{-1}$ in 1986 (using a traditional loop set down on the ground) to 47.7 $gm^{-2}d^{-1}$ in 2003, when results of a new tubular undulating row photobioreactor (TURP) were reported. This very high TURP productivity was attributed to a light dilution growth-strategy using *Arthrospira platensis*; the photic ratio (R_f) ranged from 3 to 6.

Torzillo *et al*. (1986) investigated an experiment on the outdoor mass culture of *Spirulina platensis* and *S. maxima* in closed tubular photobioreactors are reported. On average, under the climatic conditions of central Italy, the annual yield of biomass obtained from the closed culture units was equivalent to 33 t dry weight $ha^{-1}year^{-1}$. In the same climatic conditions, the yield of the same organisms grown in open ponds was about 18 t $ha^{-1}year^{-1}$. This considerable difference is due primarily to better temperature conditions in the closed culture system.

Doucha and Livansky (2009) conducted an experiment where they have previously estimated the productivity and photosynthetic efficiency of the microalga *Chlorella sp.* grown in an outdoor open thin-layer photobioreactor under climate conditions typical of the Middle European region, The mean values found for Trebon (49°N), Czech Republic, as an average of several sunny summer cultivation periods in July, were: net areal productivity, P_{net}=38.2 g dry weight (DW) $m^{-2}day^{-1}$; net volumetric productivity, P_{vol},=4.3 g algal DW $L^{-1}day^{-1}$, photosynthetic efficiency (based on PAR),η_{net} =7.05%. The peak values were: P_{net} about 50 g (DW) $m^{-2}day^{-1}$, η_{net} about 9%. Algal growth rate was practically linear up to high biomass densities (40–50 g DW L^{-1}, corresponding to an areal density of 240–300 g DW m^{-2}), at which point the culture was harvested.

Richmond *et al*. (1990) carried out an experiment in open pond. This work represents an attempt to assess the relative contribution of the factors limiting productivity of *Spirulina platensis* in open raceways throughout the year. Temperature of the culture during daylight exerted the predominant effect on productivity and elevating the

temperature resulted in a significant rise in productivity even in summer. Photo inhibition had a decisive role in summer in determining productivity of spirulina in open raceways in that growth almost ceased after mid-day. Contamination by other microorganisms, particularly *S. minor* and *Chlorella* sp. was estimated to reduce the net biomass yield by at least 15 to 20%, but measures to curtail the establishment of these species in the raceway have been devised. They found 20-25 g fresh weight per liter water.

Mitchell *et al.* (2007) conducted an experiment where they found under controlled conditions the maximum specific growth rate (μ_{max}) was 0.102 day^{-1}, the biomass doubling time (td) was 6.8 d, the maximum dry biomass concentration (X_{max}) was 1.94 g L day^{-1} and the maximum productivity (P_{max}) was 0.059 g L^{-1} day^{-1}, while the corresponding values in the greenhouse experiments were μ_{max} = 0.322 day^{-1}, d_t = 2.2 d, X_{max} = 1.73 g L^{-1} and P_{max} = 0.112 g L^{-1} day^{-1}. Under controlled conditions the highest values for these parameters occurred when X_0 = 0.15 g L^{-1}, while in the greenhouse X_0= 0.4gL^{-1} produced the highest values.

Kumar *et al.* (2011) studied that growth analysis of cultures grown at different temperatures showed significant difference ($P < 0.05$) in growth pattern. Maximum biomass concentration (as dry weight) i.e. 0.73 gL^{-1} was observed at temperature 35°C and least i.e. 0.26 gL^{-1} was found at temperature 20°C. During the growth of cultures, a wide range of temperature tolerance from 20°C to 40°C was observed. But at 45°C, the growth was almost negligible (data not recorded). The maximum growth rate i.e. 0.091 doubling day-1 was observed at 35°C, but with further increase in temperature reduction in growth rate was observed. At, 40°C culture showed 0.041 doubling day^{-1} which is almost half as compared to growth rate at 35°C.

Indian Ocean Commission conducted a research and found that spirulina is essentially a plant which grows in water; the technical inputs to set up a spiurlina farm are quite basic. On average 1 sq. m of water will produce 10 grams of spirulina, so to produce 1000 grams of spirulina (or 1kg) you require 100 sq. meters of pond roughly a pond 5 meters wide by 20 meters long. A pilot pond 500sq. m. would be approximately 6 meters wide by about 85 m in length.

Feng *et al*. (2011) carried out an experiment to evaluate optical density, specific growth rate, doubling time, dry weight and productivity of *Chlorella zofingiensis* in flat plate photobioreactors. He recorded highest optical density (0.500) at 550nm, specific growth rate (0.994 day^{-1}), doubling times minimum (0.697 day), maximum dry weight (1.587 gL^{-1}) and maximum productivity was (58 $mgL^{-1}day^{-1}$).

CHAPTER III

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

This chapter demonstrates information regarding methodology that was exploited in accomplishment of the experiment. It encompasses a brief outline of location of experiment, climate condition and material used for the experiment. It also flourishes the treatments of the experiment, data recording procedure and data analysis along with a report general practice adopted during the experiment.

3.1 Experimental site

The experiment was accomplished on rooftop of Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, during the period from July 2018 to October 2018. Location of the site is 23074' N latitude and 90035' E longitudes with an elevation of 8 meter from sea level (UNDP - FAO, 1988) in Agro-Ecological Zone of Madhupur Tract (AEZ No. 28).

3.2 Growth factors

Spirulina production plants for mass cultivation are to be done in areas with suitable climatic conditions, particularly with the sunshine throughout the year. It is difficult to have an ideal growth due to different environmental factors like solar radiation, rain, wind, temperature fluctuation, etc.

3. 3 Liquid media

The liquid used for the production of spirulina is a solution of mineral salts in water. This liquid has to supply the spirulina with all the chemical elements it needs. The pH of the culture media (i.e. its level of alkalinity) should be between 8.0 and 11.

There are various recipes for culture medium of spirulina. The one shown here is one of the most “friendly”, it is the best for ensuring an easy culture, even if it is far from being the cheapest (for composition, Table 1).

3.4 Climatic conditions for spirulina production

3.4.1 Temperature

Temperature in the range of 25-35°C even if the outside temperature as 38°C was most ideally suited for getting maximum yield of spirulina. Below 20°C, growth is practically nil but spirulina does not die. Temperature above 35°C leads to bleaching of cultures. Partial shading provided a culture temperature of about 30°C even if the outside temperature was 38°C. Rafiqul Islam *et al*. (2003) reported that, the maximum specific growth rate of 0.141 was found at 32^0C for *Spirulina platensis s*and that of 0.144 was found at 37^0C for *Spirulina fusiformis*. Maximum biomass production of 2.4 g /land chlorophyll a production of 16.6 mg/l were observed at 32^0C for *Spirulina platensis*. Maximum biomass production of 2.3 g/l and chlorophyll - a production of 14.2 mg/l were observed at 37^0C for *Spirulina fusiformis*. Colla *et al.* (2004) found that, temperature was the most important factor and that the greatest amount of gamma-linolenic acid (GLA) was obtained at 30 ºC, the fatty acid profile of the spirulina cultivated showing that (in order of abundance) palmitic, linolenic and linoleic acids were most prevalent.

3.4.2 Light

Spirulina required light intensities during its growth phase. The optimal light intensity was between 20 and 30 K lux. Subramanian and Jeejibai (1992) reported on the effect of different light quality on growth, protein and pigment synthesis of *Spirulina fusiformis.*

3.5 Water quality

The characteristics of water quality contributed in the algal mass production. It had dual influence, firstly by affecting the solubility of nutrients added in the medium and also selective accumulation of certain heavy metals by algae during the growth phase

3.6 Inoculums' size

Tasneem Fatma (1990) reported that synchronous growth of *Spirulina platensis* was failed to grow both in liquid and solid media at its higher dilution. It was observed that minimum cell population is necessary to initiate and sustain spirulina cultures.

3.7 Agitation

Agitation of algal cultures had the advantages of uniform distribution of CO_2 and prevention of thermal stratification. Many agitation devices had been reported which range from motor driven paddles, pumps, gravity flow, air light systems and manual agitation. Dubey (2006) found that, 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

3.8 Research Materials

Producing spirulina algae from a strain is, for most, the easiest and rapid technique to propagate spirulina. Fresh, healthy and disease free spirulina were used as experimental materials in the present study.

3.8.1 Source of materials

Spirulina strain was collected from "Energia Bangladesh Limited".

3.9 Treatment of the Experiment

The experiment was carried out to select cost effective, sustainable photobioreactors on rooftop condition for growth and yield attributes. The experiment was single factor. It was as follows-

Factor: Photobioreactors

In experiment, four different photobioreactors (Plate 1) were used. The photobioreactors has been characterized in (Appendix VII). The experiment consisted a single factor.

These were:

i. Photobioreactors-01, (PBR_1)
ii. Photobioreactors-02, (PBR_2)
iii. Photobioreactors-03, (PBR_3)
iv. Photobioreactors-04, (PBR_4)

3.10 Design and layout of the experiment

Experiment was propelled in completely randomized design (CRD) with three replications. To maintain equal volume, different numbers of photobioreactors was used

in each replication. For the first photobioreactor, three of 5L photobioreactors was used for each replication. In case of second photobioreactor, five of 3L photobioreactors, for third and fourth photobioreactor, one of 15L photobioreactor was used for each replication. The experiment comprises total thirty pots (Plate 2). Solution with spirulina strain was poured in each pot, enclosing the pot by cover.

3.11 Materials and Chemicals for spirulina production

Materials: Materials which was required for spirulina cultivation as follows (Plate 3):

a. Air pump
b. Silicon tube
c. Drilling machine
d. Connector
e. Harvesting net
f. Crusher/blender
g. Photobioreactors
h. Syringe
i. PVC pipe

Chemicals: Chemicals which was needed was showed in (Plate 4)

3.12 Protocol for spirulina production

A series of steps have carried out for successful spirulina production as follows (Plate 5):

3.12.1 Installation of the photobioreactors

Thirty photobioreactors were vertically placed on an iron made bench. Thirty food grade silicon tubes were coming out from a central PVC pipe which was directly connected with a motor (RESUN ACO 006). The silicon tubes were linked to photobioreactors for supplying air. The motor connected with electric line performed two services at a time one was air circulation and another was agitation. The air circulation was continued at one our interval automatically by using timer (Plate 5a).

3.12.2 Chlorination of photobioreactor

The Chlorination of photobioreactor was carried out @.02g/L $H_2O$with bubbling for one day (Plate 5b).

3.12.3 Dechlorination of photobioreactor

The dechlorination of photobioreactor was done Ascorbic acid =.04g/L with bubbling for one day (Plate 5c).

3.12.4 Culture media

3.12.5 Media preparation

The culture medium was prepared by making four types of solution separately in four 1000 ml Erlenmeyer flasks for making 180 liters of culture medium (Plate 5d). This volume was diluted with filtrated water to obtain the initial intended concentration. The initial concentration of each solution is obtained using the dry biomass weight method. Then the culture medium was prepared in a 500L plastic container by pouring the solution sequentially for 180 liters of culture medium.

Table 1. List of the quantities of chemical needed to prepare 180 liters' culture

Items	Chemicals required (g/L)
$NaHCO_3$	16.8
$NaNO_3$	2.5
TSP	0.36
NaCl	1
$MgSO_4$	0.2
$FeSO_4$	0.01
EDTA	0.08
K_2SO_4	1
Bleaching powder	0.02
Ascorbic acid	0.04

3.12.6 Strain inoculation in photobioreactors

Choose a spirulina strain containing a high proportion of coiled filaments (less than 25 % straight filaments, if possible none at all), easy to harvest, and containing at least 1 % of gamma-linolenic acid (GLA) based on dry weight. Concentrated spirulina seed culture can be obtained either from the floating layer of an unagitated culture, or by

rediluting a freshly filtered biomass (beware of lumps). A concentration of up to .03 g spirulina (dry) or .85g spirulina (fresh weight) per liter is permissible if storage and transportation last less than a week's time, and provided the seed culture be aerated at least two times a day. If aeration can be continuous, the concentration may be up to 10 g/l (weights of spirulina always refer to contained dry matter). The media was inoculated with .75g/l fresh weight basis (Plate 5e).

3.12.7 Intercultural operations

Apart from harvesting and feeding, a spirulina culture requires some attention in order to be kept in good condition. Agitation is a requisite. Continuous agitation however is not required. Agitation was carried out by a motor (RESUNACO 006). One third of full sun will saturate the photosynthetic capacity of spirulina, but shading is not required except to reduce the consumption of water (evaporation) or the temperature (< 38°C) or the pH (< 11.3). The temperature will practically never be too high, but the pH may soon become too high if insufficient carbon is supplied. The depth of culture must be kept between 10 and 20 cm. Accumulation of "white skins" and foam may float in the afternoon when the temperature of the culture goes above 35°C. These are not harmful as they will go back to the bottom again during the night, but their appearance is unpleasant and interferes light transmission during day time. They can be removed using a net. If the concentration of spirulina is too low, the culture may be invaded by chlorella (a unicellular, edible alga). It also interferes light transmission. So they can be removed using a net (Plate 5f). Usual pathogenic bacteria do not survive the high pH (> 9.7) of a spirulina culture in production; however, a microbiological assay of the product should be made also at least once a week. Contaminations most generally occur during or after harvesting. The color of the culture should be deep green. If it turns yellowish, this may be due to either a lack of nitrogen or an excess of light (photolysis) or of ammonia (excess of urea). In the latter two cases recovery is generally possible within two weeks while resting the culture under shading.

3.12.8 Harvesting

When the spirulina is in good condition, separating it from the water ("harvesting") is an easy operation (Plate 5g).

The best time for harvesting is early morning for various reasons:

- The cool temperature makes the work easier,

- More sunshine hours will be available to dry the product,

- The % proteins in the spirulina is highest in the morning.

There are basically two steps in harvesting:

- filtration to obtain a "biomass" containing about 10 % dry matter (1 liter = 100 g dry) and 50 % residual culture medium,

- removal of the residual culture medium to obtain the "fresh spirulina biomass", ready to be consumed or dried, containing about 20 % dry matter and practically no residual culture medium.

3.12.8.1 Harvest Index

Spirulina is harvested based on two methods.

a. Measuring Optical density (OD) by using spectrophotometer
   When spectrophotometer indicates that OD become above 1 then spirulina biomass is ready to harvest
b. Using “Secchi disk" reading
   The "Secchi disk" is a self-made instrument: a piece of white plastic fixed at the tip of a graduated rod. In case of "Secchi disk", after dipping it vertically into the spirulina culture up to 3cm if the white piece cannot see then it ready to harvest.

3.12.9 Washing and filtering

Washing was done by tap water containing 20% of NaCl that reduced the excess amount of and other chemicals. Filtration was simply accomplished by passing the culture through a fine weave cloth, using gravity as the driving force. Synthetic fiber cloth (especially polyamide or polyester) with a mesh size of about 30 to 50 microns is the preferred filtering medium. Supporting the filtration cloth by a fine net will accelerate somewhat the filtration and protect the cloth against rupturing, but a simple bag made from the cloth works well also. Here I used 80 microns filtering bag. The filtration is accelerated by gently moving or scraping the filter. When most of the water has filtered through, the biomass will often agglomerate into a "ball" under the motion, leaving the cloth clean (this desirable condition happens mostly when the biomass is richer in

spiraled forms and the culture medium is clean). Otherwise it may then be necessary to scrape it out from the cloth (Plate 5h).

3.12.10 Pressing

The final dewatering was accomplished by pressing the biomass enclosed in a piece of filtration cloth plus a strong cotton cloth, either by hand or in any kind of press (Plate 5i).

3.12.11 Drying

The industrial type of spirulina dryer is the spray drier which flash dries fine droplets at very high temperature and yields an extremely fine powder of low apparent density. This type is outside the reach of artisanal producers. Freeze drying is the best way of drying but far too expensive and complicated.

Sun drying is the most popular among small producers, but requires a few precautions. Direct sun drying must be very quick, otherwise the chlorophyll will be destroyed and the dry product will appear bluish. Whatever the source of heat, the biomass to be dried must be thin enough to dry before it starts fermenting. A very simple technique was applied to dry spirulina. Firstly, spirulina pest was poured in a syringe and pressed it. Finally, spirulina came out from the syringe very thin spiral rod shaped diameter of 1 to 2 mm (Plate 5j). The total duration of the drying should not exceed a few hours, preferably 2 hours.

3.12.12 Crushing

Crushing was accomplished by blender to fine powder. The dry chips or rods are usually converted to powder by grinding in order to increase their apparent density (Plate 5k).

5.12.13 Storage

The best storage is in heat sealed, aluminized plastic bags. It can be stored in glassed made bottle (Plate 5l).

3.13 Data collection on different parameters

Observations of experimental data were extracted from each pot of every replication. Data were recorded respectively on which parameters observation as follows- (Plate 6).

a) Growth parameter

1. Optical density (OD)
2. Specific growth rate (μ, d^{-1})
3. Doubling time (t_d, day)
4. Productivity (P, $gL^{-1}d^{-1}$)

b) Yield related parameter

1. Fresh weight (g/L)
2. Dry weight (g/L)
3. Dry weight (kg/1000L)
4. Marketable yield/ inoculation (Kg/1000L)

3.13.1 Optical density

The efficiency of algae biomass growth was measured due to optical density, defined as the absorption of visible radiation at 560 nm through the spectrophotometer and mean was calculated (Plate 6a).

3.13.2 Specific growth rate

The specific growth rate (SGR, μ/day) of cultured microalgae was calculated by the following equation (Clesceri *et al*., 1989):

$SGR\ (\mu/day) = \ln (X_1 - X_2)/ t_2 - t_1$

Where,

X_1 = Biomass concentration at the end of selected time interval,

X_2 = Biomass concentration at the beginning of selected time interval,

And $t_2 - t_1$= Elapsed time between selected time in day.

3.13.3 Doubling time

The biomass doubling time (td, d) was calculated using natural logarithms (ln) as

$t_d = \ln 2/ \mu_{max}$ (Bailey and Ollis, 1986).

3.13.4 Productivity

The maximum productivity (P_{max}, $gL^{-1}d^{-1}$) calculated from the equation,

$P = (X_t - X_0)/ (t - t_0)$,

Where,

X_t is the biomass concentration (gL^{-1}) at time t (d)

And X_0 is the initial biomass concentration (gL^{-1}) at t_0 (Schmidell *et al*., 2001).

3.13.5 Fresh weight

After harvesting, the fresh biomass of spirulina was calculated by using electric balance (Plate 6b).

3.13.6 Dry weight

After drying the fresh biomass lost its moisture then the dry weight was taken by using electric balance (Plate 6c).

3.14 Estimation of physio-chemical properties of culture media

P^H of the culture media was measured by electric pH meter (Jenwey Model 3032). Temperature and light intensity of the culture media were recorded by using a Celsius thermometer and lux meter, respectively (Plate 6 d, 6f).

3.15. Statistical Analysis

Collected data were tabulated and analyzed in accordance with the objectives of the study using MSTAT-C computer package programme and difference between treatments was assessed by Least Significant Difference (LSD) test at 5% level of significance (Gomez and Gomez, 1989). Mean for every data was calculated and analysis variance in each character of every treatments was acted upon by F- test (variance ratio).

3.16 Economic analysis

The cost of production was analyzed in order to find out the most economical solution of production unit means photobioreactor. All the input cost including cost of production is in (Table 5, 6). The current market price of spirulina was considered for the cost and return.

The benefit cost ratio(BCR) was calculated as follows:

$$\text{Benefit cost ratio (BCR)} = \frac{\text{Gross return per 1000L}}{\text{Total cost of production per 1000L}}$$

Photobioreactor-01

Photobioreactor-02

Photobioreactor-03

Photobioreactor-04

Plate 1: Pictorial view of different photobioreactors

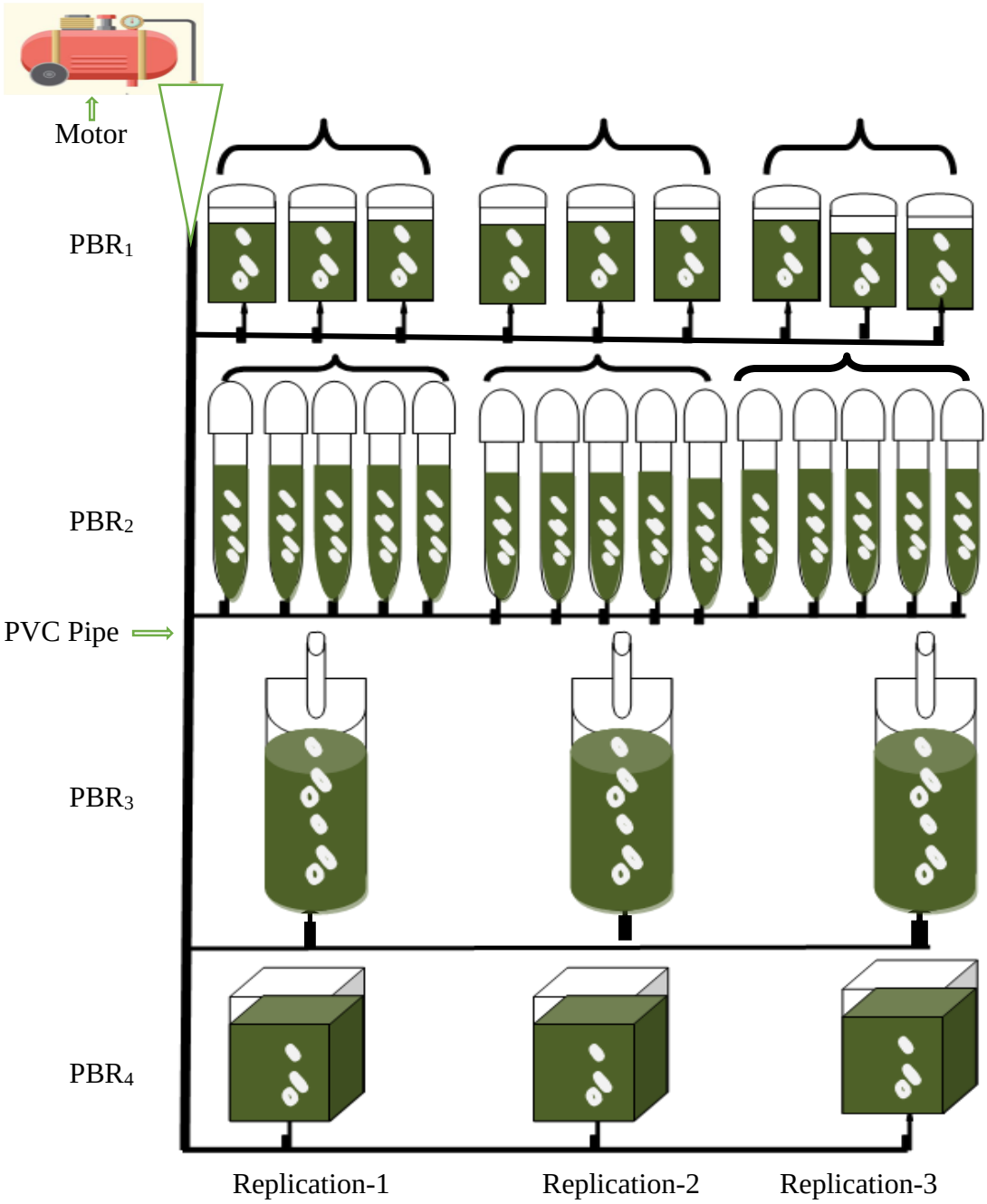
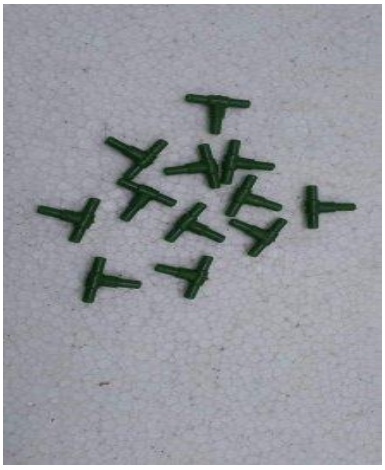


Plate 2: Schematic view of design and layout of the experiment

a. Air pump

b. Drilling machine

c. Silicon tube



d. Connector

e. Harvesting net

f. Blender

g. Photobioreactor

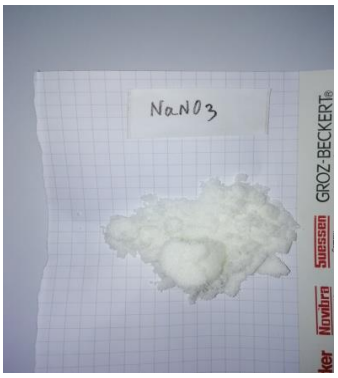
h. Syringe

i. PVC pipe

Plate 3: Pictorial view of different materials

$NaHCO_3$

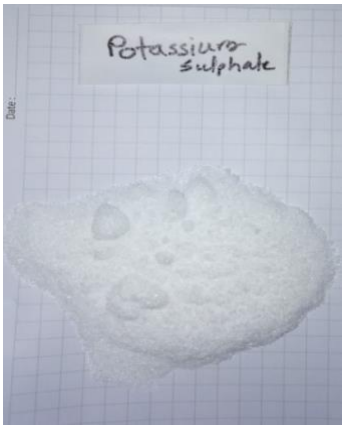
TSP



$NaNO_3$

NaCl

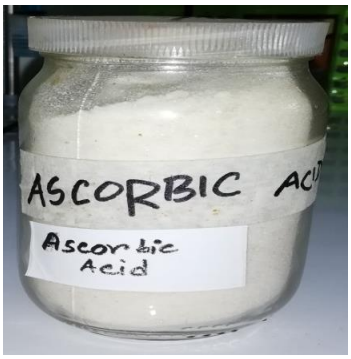
$MgSO_4$



K_2SO_4

Na-EDTA

$FeSO_4.7H_2O$

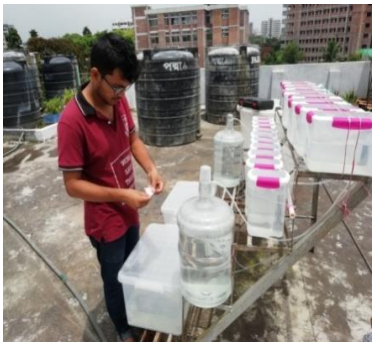


Ascorbic acid

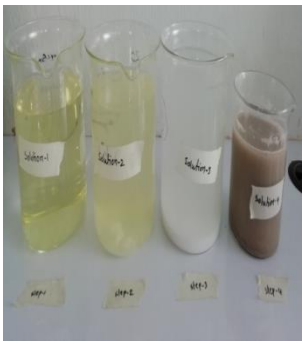
Bleaching powder

Plate 4: Pictorial view of different necessary chemicals

a. Installation



b. Chlorination and dechlorination

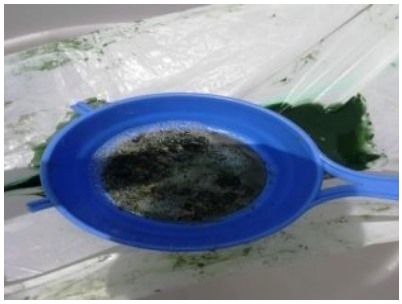


c. Media preparation

d. Inoculation

e. Culturing in photobioreactors

f. Deforming



g. Harvesting

h. Washing

i. Pressing

j. Drying

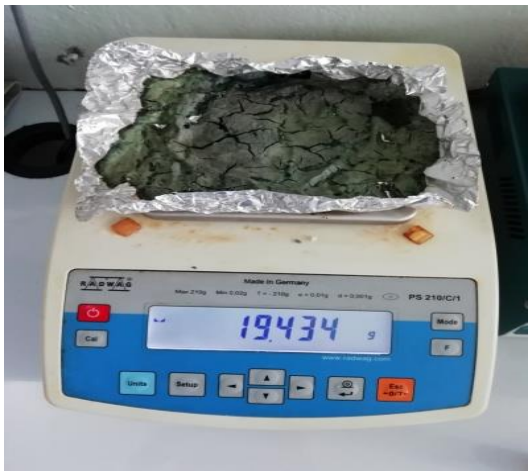
k. Crushing

l. Storage

Plate 5: Pictorial view of sequential activities was carried out for Spirulina production

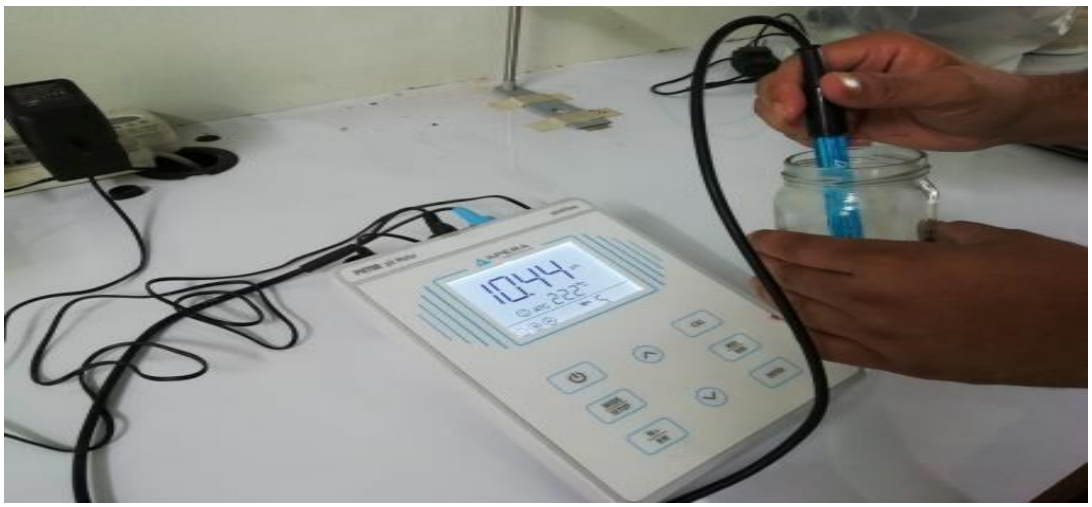
a. OD measurement

b. Fresh weight



c. Dry weight

d. Light intensity measurement



f. P^H measurement

Plate 6: Pictorial Presentation of data collection

CHAPTER IV

RESULTS AND DISCUSSION

CHAPTER IV
RESULTS AND DISCUSSION

The aim of this research works is to screen out suitable photobioreactor for commercial production in Bangladesh by studying their growth, productivity and yield capacity. Variation in the studied parameters was observed among the photobioreactors due to their different light interception, photo inhibition, productive capacity and other algae competition. This contrasting attributes were presented and discussed in this chapter. Some of the characters were presented in tables and some presented in figure for easier comprehension of the findings. A summary of analysis of variances in respect of all parameters was presented in the appendices. Results have been presented, discussed and possible interpretation was presented under the following heads:

4.1 Parameter

4.1.1 Optical Density (OD)

Optical density is the most important growth parameter in spirulina which is positively correlated with yield and the growing conditions significantly influenced this trait. Significant variation was observed among the different photobioreactors in case of optical density at maturity (Appendix I). The highest Optical Density (OD) was observed in PBR_4 (1.56) followed by PBR_1 (1.31) and the lowest OD was observed in PBR_2 (0.96) (Figure 1.). Light interception, foaming, environmental factors and competition of other algae plays an important role to regulate Optical Density (OD) along with it's over all performances. Similar trend of results was also observed by Saranraj *et al.* (2013). Effective Recycling of Lignite Fly Ash for the Laboratory Cultivation of Blue Green Algae, *Spirulina platensis*. Sukumaran *et al.* (2018) also observed variation in optical density. Feng *et al.* (2011) showed variation in optical density also observed in flat plate photobioreactors outdoors.

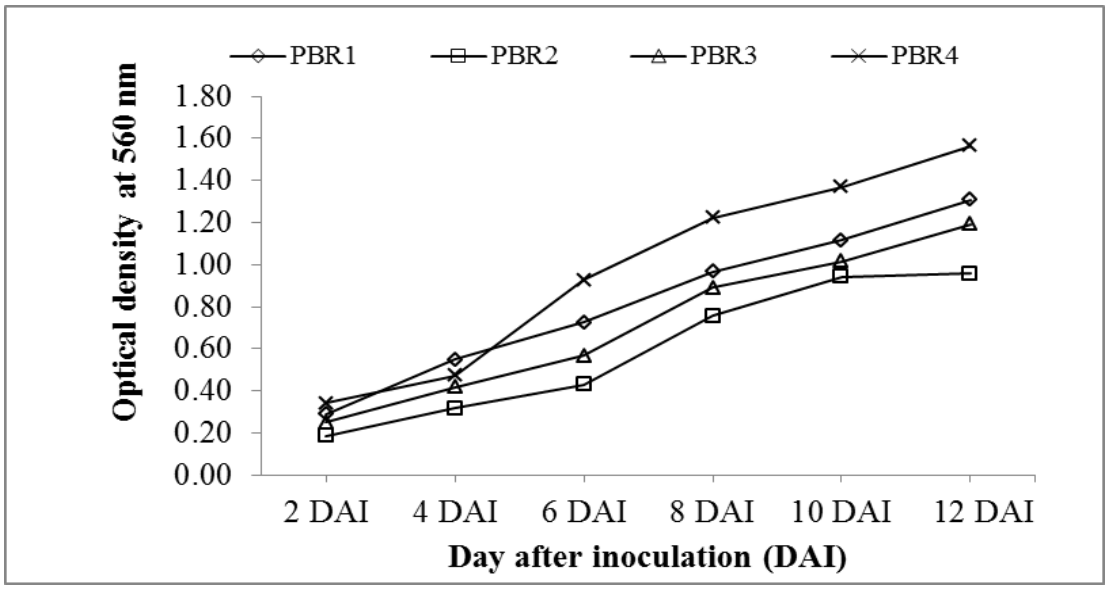


Figure 1: Performance of different photobioreactors on optical density at different days after inoculation of spirulina

4.1.2 Specific Growth rate (%)

Growth rate showed significant variation in different photobioreactors used under study (Appendix V). Highest average growth rate was observed in PBR_4 (2.48) and the lowest was observed in PBR_2 (0.977) (Figure 2.). *Chlorella zofingiensis* shows variations in growth rate observed by Feng *et al.* (2011) during the evaluation of growth, productivity and yield in flat plate photobioreactors outdoors. Variation was also observed in airlift PBR_s by Oncel and Vardar sukan (2007). Reichert *et al.* (2006) also showed difference in two-liter Erlenmeyer flasks experiment. Suphi *et al. (*2006) found variation in specific growth rate in integrated type of photobioreactor. There is a relatively positive co-relation between specific growth rate and yield.

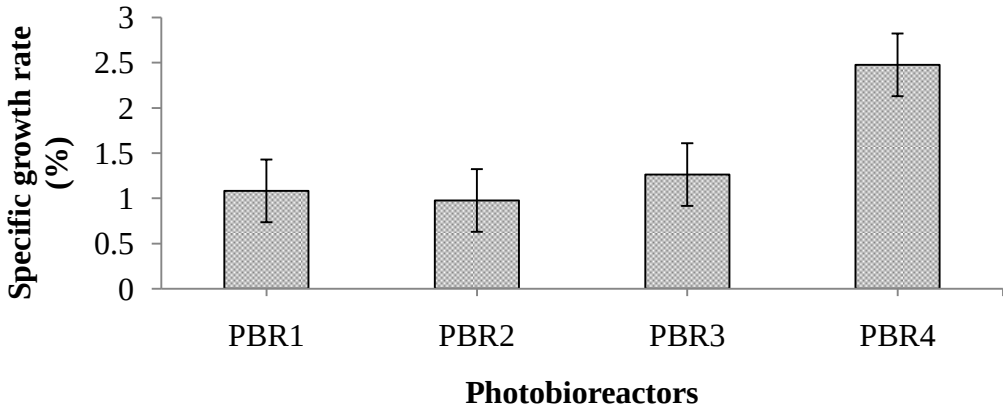


Figure 2: Performance of different photobioreactors on specific growth rate

4.1.4 Doubling time

Doubling time showed significant inequality in different photobioreactors under study (Appendix V). Minimum days required for doubling was observed in PBR_4 (3.41) and maximum days of doubling time was observed by PBR_1 (10.63) followed by PBR_2 (8.02) (Figure 3.). Mitchell *et al.* (2007) also showed similar variation in case of cultivation of spirulina in close and open bioreactor. He found variation in case of doubling time. Light interception plays an important role doubling time towards the total productivity of spirulina.

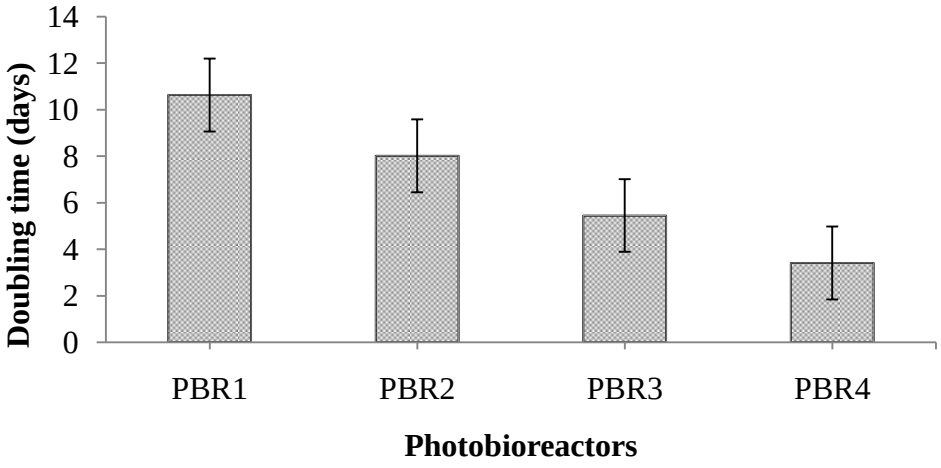


Figure 3: Performance of different photobioreactors on doubling time

4.1.4 Fresh weight

Fresh weight varied significantly among the different photobioreactors under study (Appendix II). Harvested fresh biomass at three times and every times it varied significantly in different photobioreactors. The highest biomass content collected on fresh weight basis was observed in PBR_1 (14.44 g/l), (12.44 g/l) and (9.013 g/l) whereas the lowest weight was observed in PBR_2 (5.403 g/l), (5.087 g/l) and (3.97 g/l) successively (Table 2). Doucha and Livansky (2009) observed similar variation in his study on open pond algae production. Richmond *et al*. (1990) also found the similar kind of variation on biomass production. Sheehan *et al*. (1998) found similar variation in case of biomass.

Table 2. Performance of different photobioreactors on fresh biomass weight**

Photobioreactors*	1st Fresh weight (g/l)		2nd Fresh weight (g/l)		3rd Fresh weight (g/l)	
PBR_1	9.75	b	8.08	b	6.98	ab
PBR_2	5.40	d	5.09	d	3.97	c
PBR_3	7.07	c	6.17	c	5.05	bc
PBR_4	14.44	a	12.64	a	9.01	a
CV%	8.24%		6.7%		10.32%	
LSD (0.05)	1.51		1.07		1.29	

* Here, PBR_1=Rectangular shaped 5L photobioreactor, PBR_2= Cuboidal shaped 3L Photobioreactor, PBR_3= Cylindrical shaped 15L Photobioreactor, PBR_4= Rectangular shaped 15L Photobioreactor

**In a column, means having similar letter (s) are statistically identical and those having dissimilar letter(s) differ significantly as per 0.05 level of probability.

4.1.5 Dry weight (g/l)

Significant variation was found in case of dry weight among different photobioreactors under study (Appendix III). Highest amount of dry weight was observed in PBR_4 (1.19 g/l), (1.22 g/l) and (0.91 g/l) and the lowest was observed in PBR_2 (0.53 g/l), (0.44 g/l) and (0.40 g/l) successively (Table 3). Variation in dry weight was also observed in a laboratory scale integrated type of photobioreactor by Suphi *et al.* (2006). Mitchell *et al.* (2007) also show similar variation in case of cultivation of spirulina in close and open bioreactor.

Table 3. Performance of different photobioreactors on Dry weight**

Photobioreactors*	1st dry weight (g/l)		2nd dry weight (g/l)		3rd dry weight (g/l)	
PBR_1	0.72	b	0.82	b	0.77	b
PBR_2	0.53	c	0.44	d	0.40	d
PBR_3	0.65	bc	0.61	c	0.63	c
PBR_4	1.19	a	1.22	a	0.91	a
CV%	10.06%		7.00%		10.2%	
LSD (0.05)	0.155		0.109		0.126	

* Here, PBR_1=Rectangular shaped 5L photobioreactor, PBR_2= Cuboidal shaped 3L Photobioreactor, PBR_3= Cylindrical shaped 15L Photobioreactor, PBR_4= Rectangular shaped 15L Photobioreactor

**In a column, means having similar letter (s) are statistically identical and those having dissimilar letter(s) differ significantly as per 0.05 level of probability.

4.1.7 Productivity

Different photobioreactors displayed significant non-uniformity in case of productivity (Appendix IV). PBR_4 showed the highest productivity (0.90), (0.87) and (0.91) and the lowest was observed in PBR_2 (0.41), (0.40) and (0.40) successively at each three harvest (Table 2). Similar variation in productivity was observed by Reichert *et al.* (2006) in two-liter Erlenmeyer flasks). Elias *et al.* (1997) also documented difference in productivity in case of different photobioreactors. Variation in productivity is controlled by photobioreactor quality. Presence of higher productivity of spirulina in photobioreactors is criteria for selection of photobioreactor.

Table 4. Performance of different photobioreactors on Productivity **

Photobioreactors*	1st Productivity ($gL^{-1}day^{-1}$)		2nd Productivity ($gL^{-1}day^{-1}$)		3rd Productivity ($gL^{-1}day^{-1}$)	
PBR_1	0.53	b	0.64	b	0.77	b
PBR_2	0.41	c	0.40	c	0.40	c
PBR_3	0.55	b	0.51	bc	0.63	b
PBR_4	0.90	a	0.87	a	0.91	a
CV%	9.79%		15.41%		8.66%	
LSD (0.05)	0.109		0.190		0.089	

* Here, PBR_1=Rectangular shaped 5L photobioreactor, PBR_2= Cuboidal shaped 3L Photobioreactor, PBR_3=Cylindrical shaped 15L Photobioreactor, PBR_4= Rectangular shaped 15L Photobioreactor

**In a column, means having similar letter (s) are statistically identical and those having dissimilar letter (s) differ significantly as per 0.05 level of probability.

4.1.8 Marketable yield/Inoculation (kg/1000L)

Variation in number of marketable yield was observed among the different photobioreactors used for spirulina cultivation (Appendix V). The highest amount of marketable yield was found in PBR_4 (3.34kg) and the lowest was observed in PBR_1 (1.64kg) (Figure 4.). Similar result was recorded by a report by Indian Ocean Commission (2016).

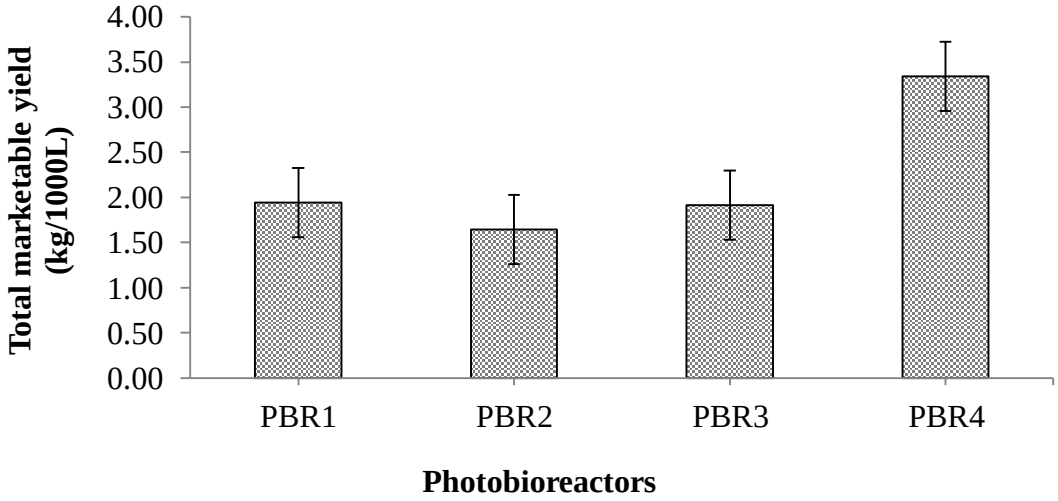


Figure 4: Performance of different photobioreactors on total marketable yield

4.2 Economic analysis

Input cost for materials and non materials were recorded as per 1000L inoculation. Price of spirulina was considered as per market rate. The economic analysis is presented under the following heading-

4.2.1 Gross return

Different photobioreactors showed different values in terms of gross return (Table 5). The highest gross return (Tk.66800) was obtained from the PBR_4 and the lowest gross return (Tk.32800) was obtained from PBR_2 in first month.

4.2.2 Net return

In case of net return, only PBR_4 showed positive net returns but others showed losses in first month (Table 5) but the losses could be minimized with the consecutive month's production because there is no cost in next two consecutive returns in case of small scale culture (Table 6).

4.2.3 Benefit cost ratio

The highest benefit cost ratio was noted (1.82) from PBR_4 and the lowest benefit cost ratio was observed in PBR_2 (0.64) in case of first month (Table 5). The highest benefit cost ratio was noted (5.06) from PBR_4 and the lowest benefit cost ratio was observed

in PBR_2 (2.49) in case of 2nd month (Table 6). Therefore, it is apparent that PBR_4 was better than the rest of the photobioreactors from the economic point of view as well.

Table 5: Cost and return of spirulina grown in different photobioreactor for 1st month

Photobioreactors	Marketable yield (Kg/1000L)	Gross return (Tk)	Total production cost (Tk)	Net return (Tk)	BCR
PBR_1	1.9	38800	49486	-10686	0.78
PBR_2	1.6	32800	51386	-18586	0.64
PBR_3	1.9	38200	44686	-6486	0.85
PBR_4	3.3	66800	36686	30114	1.82

Here, sign (-) means loss. PBR_1=Rectangular shaped 5L photobioreactor, PBR_2= Cuboidal shaped 3L Photobioreactor, PBR_3=Cylindrical shaped 15L Photobioreactor, PBR_4= Rectangular shaped 15L Photobioreactor

Table 6: Cost and return of spirulina grown in different photobioreactor for 2nd month and so on (Approximate data calculation)

Photobioreactors	Marketable yield (Kg/1000L)	Gross return (Tk)	Total production cost (Tk)	Net return (Tk)	BCR
PBR_1	1.9	38800	13186	25614	2.94
PBR_2	1.6	32800	13186	19614	2.49
PBR_3	1.9	38200	13186	25014	2.9
PBR_4	3.3	66800	13186	53614	5.06

Here, PBR_1=Rectangular shaped 5L photobioreactor, PBR_2= Cuboidal shaped 3L Photobioreactor, PBR_3= Cylindrical shaped 15L Photobioreactor, PBR_4= Rectangular shaped 15L Photobioreactor

CHAPTER V

SUMMARY AND CONCLUSION

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5.1 Summary

Spirulina (*Spirulina platensis*) belongs to the family Arthrospira is a free floating filamentous microalgae belonging to the class Cyanobacteria. Spirulina was a food source for the Aztecs and other Mesoamericans until the 16th century; the harvest from Lake Texcoco in Mexico and subsequent sale as cakes were described by one of Cortés' soldiers . The Aztecs called it "tecuitlatl". It is also known as Super food, Dihe. Spirulina is an ecologically sound, nutrient rich super food that is grown all around the world as a dietary supplement. Spirulina is considered as the “food of the future” that will effectively tackle the existing malnutrition problem. Spirulina is a phototrophic organism that’s why it is mainly cultivated in different photobioreactors such as, open tanks, closed plastic tank, open race pond, mud pot; polythene bag also includes Vertical, Horizontal and Flat Plane photobioreactor. So the main barriers of quality spirulina production are contamination, P^H maintenance, growth rate, productivity and photosynthetic efficiency and the costly growth chamber. Bangladesh has a climate that is particularly favorable for production of spirulina which can enable us to exploit its high demand as dietary food supplement and boost a new processed food industry that has recently started its expansion.

For screening out suitable cost effective photobioreactor for adoption in production of spirulina, an experiment was accomplished on the rooftop of agricultural faculty of Sher-e-Bangla Agricultural University during the period of July to September, 2018 to screen some Photobioreactors for finding out more economically convenient and easily available one for spirulina production in Bangladesh. Four types of photobioreactors *viz*. PBR_1= Rectangular shaped 5L photobioreactor, PBR_2= Cuboidal shaped 3L Photobioreactor, PBR_3= Cylindrical shaped 15L Photobioreactor, PBR_4= Rectangular shaped 15L Photobioreactor was used in this experiment arranged in a Completely Randomized Design with three replications. All the collected data to the relevant parameters were arranged accordingly and analyzed to evaluate the

performance of different photobioreactors for adoption in Bangladesh. The findings of the experiment are summarized in this segment.

In case of the various parameters studied, significant variations were observed among the different photobioreactors under study.

The highest optical density (OD) was observed in PBR_4 (1.56) followed by PBR_1 (1.31) and the lowest OD was observed in PBR_2 (.96).

Highest average specific growth rate was observed in PBR_4 (2.48%) and the lowest was observed in PBR_2 (0.977%).

The highest biomass content collected on fresh weight basis was observed in PBR_1 (14.44 g/l), (12.44g/l) and (9.013g/l) whereas the lowest weight was observed in PBR_2 (5.403g/l), (5.087g/l) and (3.97g/l) from one inoculation successively.

In case of dry weight, highest amount of dry weight was observed in PBR_4 (1.19 g/l) (1.22 g/l) and (0.91 g/l) and the lowest was observed in PBR_2 (0.53 g/l), (0.44 g/l) and (0.40 g/l) from one inoculation successively.

In case of productivity, PBR_4 showed the highest productivity ($0.90gL^{-1}day^{-1}$), ($0.87gL^{-1}day^{-1}$) and ($0.91gL^{-1}day^{-1}$) and the lowest productivity was observed in PBR_2 ($.41gL^{-1}day^{-1}$), ($0.40\ gL^{-1}day^{-1}$) and ($0.40\ gL^{-1}day^{-1}$) from one inoculation successively.

Highest amount of marketable yield was found in PBR_4 (3.34 kg) and the lowest was observed in PBR_2 (1.64 kg).

From economic analysis, significant variation was observed in case of benefit cost ratio for first and consecutive months in respect of different photobioreactors. Maximum benefit cost ratio was found in PBR_4 (1.82) whereas minimum in PBR_2 (0.64) followed by other two types of photobioreactors in first month and so on.

5.2 Conclusion

From the result and discussion, it can be concluded that the different photobioreactors used under study showed significant variation in the studied characteristics under Bangladesh condition. According to the result, PBR_4 appeared to be the best cultivation unit among the 4 types of photobioreactors under study. From the point of its potential productivity and higher marketable yield including quick doubling times, other parameters and economic analysis, PBR_4 has the promise to acquire a core position in the commercial spirulina production market in a very short time. Through the present study, the studied photobioreactors were evaluated based on the different parameters and performance as a base line for the researchers.

Suggestions

i) Further experiment regarding its sustainability could be done.
ii) New photobioreactor development through conventional and technical method could be done.

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APPENDICES

APPENDICES

Appendix I. Analysis of variance of the data on Optical density at 560nm DAI of spirulina

Source	Degrees of Freedom (df)	Mean Square for Optical density at 560nm					
		2 DAI	4 DAI	6DAI	8 DAI	10 DAI	12 DAI
Factor A (Photobioreactors)	3	0.013 *	0.029 *	0.029 *	0.116*	0.104*	0.191*
Error	6	0.001	0.001	0.006	0.002	0.001	.010

*: Significant at 0.05 level of significance

Appendix II. Analysis of variance of the data on Fresh biomass weight at different harvest of spirulina

Source	Degrees of Freedom (df)	Mean Square of		
		1st Fresh weight (g/l)	2nd Fresh weight (g/l)	3rd Fresh weight (g/l)
Factor A (Photobioreactors)	3	46.697*	33.404*	14.810*
Error	6	0.571	0.287	0.416

*Significant at 0.05 level of significance

Appendix III. Analysis of variance of the data on Dry weight at different harvest of spirulina

Source	Degrees of Freedom (df)	Mean Square of		
		1st Dry weight (g/l)	2nd Dry weight (g/l)	3rd Dry weight (g/l)
Factor A (Photobioreactor)	3	0.255*	0.312*	0.308*
Error	6	0.006	0.003	0.006

*Significant at 0.05 level of significance

Appendix IV. Analysis of variance of the data on Productivity at different harvest of spirulina

Source	Degrees of Freedom (df)	Mean Square of		
		1st Productivity ($gL^{-1}day^{-1}$)	2nd Productivity ($gL^{-1}day^{-1}$)	3rd Productivity ($gL^{-1}day^{-1}$)
Factor A (Photobioreactor)	3	0.131*	0.119*	0.122*
Error	6	0.003	0.009	0.002

*Significant at 0.05 level of significance

Appendix V. Analysis of variance of the data on Growth rate, Doubling time and Total marketable yield of spirulina

Source	Degrees of Freedom (df)	Mean Square of		
		Growth rate %	Doubling time (day^{-1})	Total marketable yield (Kg/1000L)
Factor A (Photobioreactor)	3	1.447*	29.450*	0.316*
Error	6	0.018	0.054	0.008

*Significant at 0.05 level of significance

Appendix VI. Production cost of spirulina /1000L for first month (contd.)

1.Input Cost (TK)				
1.A Non Material Cost (TK)				
Photobioreactors	**Chemical cost**	**Electricity bill**	**Miscellaneous cost**	**Sub total cost 1(A)**
PBR_1	11686	500	1000	13186
PBR_2	11686	500	1000	13186
PBR_3	11686	500	1000	13186
PBR_4	11686	500	1000	13186

Here, * Here, PBR_1=Rectangular shaped 5L photobioreactor, PBR_2= Cuboidal shaped 3L Photobioreactor, PBR_3= Cylindrical shaped 15L Photobioreactor, PBR_4= Rectangular shaped 15L Photobioreactor

Appendix VII. Production cost of spirulina /1000L for first month

1.Input Cost (TK.)						
2. **B Material Cost (TK.)**						
Photobioreactors	**Motor**	**Silicon tube**	**Harvesting net**	**Photobioreactor**	**Sub total cost 1(B)**	**Total input cost 1(A)+1(B)**
PBR_1	5500	4000	300	25500	35300	49486
PBR_2	5500	3500	300	27900	37200	51386
PBR_3	5500	4200	300	20500	30500	44686
PBR_4	4200	3000	300	15000	22500	36686

Here, * Here, PBR_1=Rectangular shaped 5L photobioreactor, PBR_2= Cuboidal shaped 3L Photobioreactor, PBR_3= Cylindrical shaped 15L Photobioreactor, PBR_4= Rectangular shaped 15L Photobioreactor

Appendix VIII. Production cost of spirulina /1000L for consecutive month until damage of photobioreactor

1.Input Cost (TK)				
1.A Non Material Cost (TK)				
Photobioreactors	**Chemical cost**	**Electricity bill**	**Miscellaneous cost**	**Sub total cost 1(A)**
PBR_1	11686	500	1000	13186
PBR_2	11686	500	1000	13186
PBR_3	11686	500	1000	13186
PBR_4	11686	500	1000	13186

Here, * Here, PBR_1=Rectangular shaped 5L photobioreactor, PBR_2= Cuboidal shaped 3L Photobioreactor, PBR_3= Cylindrical shaped 15L Photobioreactor, PBR_4= Rectangular shaped 15L Photobioreactor

Appendix IX. Characterization of different Photobioreactors

Characters	Characterization of different Photobioreactors Photobioreactors			
	PBR_1	PBR_2	PBR_3	PBR_4
Shape	Rectangular	Cuboidal	Cylindrical	Rectangular
Length(inch)	9	5	none	18
Breadth(inch)	7	5	none	13
Height(inch)	6	10	18	12
Diameter(inch)	none	6	10	none
Thickness(mm)	1.2	1.3	1.6	.8
Volume(Each)L	5	3	15	15
Total number/Replication	3	5	1	1