FORMULATION A LOW COST MEDIUM FOR COMMERCIAL SPIRULINA *(Spirulina platensis)* PRODUCTION

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DECEMBER, 2021

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A Thesis
Submitted to Faculty of Agriculture,
Sher-e-Bangla Agricultural University, Dhaka,
In partial fulfillment of the requirements
For the degree of

MASTER OF SCIENCE (MS)
IN
HORTICULTURE

SEMESTER: JULY-DECEMBER, 2021

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He (Allah) created the heavens and earth in truth. He wraps the night over the day and wraps the day over the night and has subjected the sun and the moon, each running (its course) for a specified term. Unquestionably, He is the Exalted in Might, the Perpetual Forgiver.

Surah Az-Zumar Ayat 5 (39:5 Quran)

DEDICATED TO
MY BELOVED PARENTS

Department of Horticulture
Sher-e-**B**angla **A**gricultural **U**niversity
Sher-e-**B**angla **N**agar, **D**haka-1207

Memo No. Dated:

CERTIFICATE

This is to certify that the thesis entitled ***"FORMULATION A LOW COST MEDIUM FOR COMMERCIAL SPIRULINA (Spirulina platensis) PRODUCTION"*** *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 authentic research work carried out by* ***IMRAN SAKIB,*** *Registration No.* ***19-10170*** *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.

Dated: December, 2021
Dhaka, Bangladesh

Prof. Dr. A.F.M. Jamal Uddin
Supervisor

ACKNOWLEDGEMENTS

Author is prostrated before Almighty Allah, most merciful and beneficent, for giving the strength and courage to successfully complete the research work.

This thesis owes its existence to the help, support and inspiration of several people. Firstly, he would like to express his sincere appreciation and gratitude to his supervisor, ***Prof. Dr. A.F.M. Jamal Uddin*** *for his guidance and constant encouragement during his research.*

He also indebted to his co-supervisor ***Prof. Dr. Khaleda Khatun*** *and all the teachers of* ***Department of Horticulture, Sher-e-Bangla Agricultural University****, who have been a constant source of encouragement and enthusiasm, not only during this thesis work but also during the two years of his Master's program.*

His deepest gratitude goes to his family for their unflagging love and unconditional support throughout his life and his studies. It made his live the most unique, magic and carefree childhood that had made him who he is now.

The author is deeply indebted to Rakibuzzaman Mony, Anil Mahato, Imam Hossain, Asmaul Husna, Raisa Islam and Dina Akter for their kind help and support which can never be forgotten.

His final words are thank you to all of the Horticultural Innovation Lab. Bd., members who supported him throughout the joyful and hard times of his works. It is you who make the bad times good and the good times unforgettable.

- Author

FORMULATION A LOW COST MEDIUM FOR COMMERCIAL SPIRULINA *(SPIRULINA PLATENSIS)* PRODUCTION

BY

IMRAN SAKIB

ABSTRACT

The experiment was conducted on the rooftop of Academic Building, Sher-e-Bangla Agricultural University, Dhaka during November 2019 to March 2020 to formulate a low cost medium for commercial Spirulina production. Four treatments were used in this experiment viz. T_1= (EcoSolv water + Zarrouk's media), T_2= (EcoSolv water + Modified media), T_3= (Freshwater + Zarrouk's media), T_4= (Freshwater + Modified media). Freshwater + Zarrouk's media (T_3) was used standard culture media (Control) and other treatments were compared with this. These experiments were carried up to 3^{rd} cycles of cultivation and in 2^{nd} and 3^{rd} cycles of cultivation used 50% nutrients media with reuse the water. In modified media $Ca_2(PO_4)_2$ (Triple Super Phosphate) @ 0.4 g/l was used instead of K_2HPO_4 (Dipotassium Hydrogen Phosphate) and Citric acid @ 0.8 g/l was used instead of EDTA. The experiment was outlined in the Completely Randomized Design with four replications. Significant variation was found among the treatments. Among the four treatments, highest optical density (2.38, 2.18 and 2.16), cell productivity (0.26, 0.24 and 0.24 g/l/day) and dry biomass (2.22, 2.08 and 2.04 g/l)) was found in T_1 treatments in 1^{st}, 2^{nd} and 3^{rd} cycles of cultivation where second highest optical density (2.09, 1.83 and 1.95), cell productivity (0.23, 0.20 and 0.22 g/l/day) and dry biomass (1.94, 1.91 and 1.76 g/l) was observed in T_2 treatment in 1^{st}, 2^{nd} and 3^{rd} cycles of cultivation and lowest optical density (1.73) in T_3, (1.55 and 1.66) in T_4 treatment, cell productivity (0.19 g/l/day) in T_3 ,(0.17 and 0.18 g/l/day) in T_4 treatment, and dry biomass (1.68 g/l) in T_3, (1.42 and 1.46 g/l) in T_4 treatment in 1^{st}, 2^{nd} and 3^{rd} cycles of cultivation. Among these treatments T_1 showed the highest result followed by T_2 treatment. Considering benefit cost ratio the highest benefit cost ratio (2.34, 4.54 and 4.19) was estimated in T_2 treatment in 1^{st}, 2^{nd} and 3^{rd} cycles of cultivation and the lowest benefit cost ratio (1.35, 2.82 and 2.75) was noted in T_3 treatment in 1^{st}, 2^{nd} and 3^{rd} cycles of cultivation . In every cycle of cultivation T_2 treatment showed the highest benefit cost ratio although T_1 treatment gave the highest yield. So, EcoSolv water with Modified media is more cost effective in compare to Freshwater with Zarrouk's media.

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ABBREVIATION AND ACRONYM

Agric.	=	Agricultural
Agron.	=	Agronomy
AEZ	=	Agro-ecological Zone
ANOVA	=	Analysis of Variance
BADC	=	Bangladesh Agricultural Development Corporation
BCR	=	Benefit Cost Ratio
BCSIR	=	Bangladesh Council of Scientific and Industrial Research
Biol.	=	Biology
Chem.	=	Chemistry
Coeff	=	Coefficient
CV	=	Coefficient of variance
DAT	=	Days after transplanting
Deve.	=	Development
DW	=	Dry weight
DRAM	=	Digested Rotten Apple Medium
Eff.	=	Effective
et al.	=	And others
Ex.	=	Experiment
EZ	=	EcoSolv water + Zarrouk's Media
EM	=	EcoSolve. Water + Modified Media
FAO	=	Food and Agriculture Organization
FW	=	Fresh weight
FZ	=	Freshwater + Zarrouk's Media
FM	=	Freshwater + Modified Media
g	=	Gram
Hort.	=	Horticulture
i.e.	=	That is
Init.	=	Initial
Int.	=	International

Kg	=	Kilogram
KM	=	Kosaric Medium
L	=	Liter
LSD	=	Least Significance Difference
Manag.	=	Management
MOP	=	Muriate of Potash
MKM	=	Modified Kosaric Medium
nm	=	Nanometer
OD	=	Optical density
pH	=	Power of Hydrogen
Rad.	=	Radiation
CRD	=	Completely Randomized Design
Res.	=	Research
Sci.	=	Science
spp	=	Species
SSP	=	Single super phosphate
SKM	=	Standard Kosaric Medium
SAU	=	Sher-e-Bangla Agricultural University
μ	=	Specific growth rate
Tech.	=	Technology
Tk.	=	Taka
UNESCO	=	United Nations Educational, Scientific and Cultural Organization
Viz.	=	Namely
WW	=	Wash weight

CHAPTER I

INTRODUCTION

Spirulina (*Spirulina platensis*) belongs to the family Spirulinaceae is a free-floating filamentous microalga belonging to the class Cyanobacteria (Komarek and Hauer, 2009). It is an exceptionally simple extract of blue-green algae. These blue-green algae were used as food from Aztec civilization.

In 1940 it is rediscovered by French phycologist Dangeard. In 1967 *Spirulina* was established as a 'wonderful future food source' in the International Association of Applied Microbiology (Sassano, 2010). USDA Food Composition Database reports that dried spirulina contains 60-70% protein, 24% carbohydrates, and 8% fat (Khan *et al.*, 2005., Campanella *et al.*, 2002). It is a complete protein source that contains all the essential amino acids. Additionally, it is an excellent anti-inflammatory and antioxidant. This can reduce blood pressure, lose weight, control diabetes, and lower cholesterol (Huang *et al.*, 2018). This food is composed of 60% highly digestible vegetable protein as well as micronutrients and essential fatty acids, such as Alpha-linolenic acid and Gamma-linolenic acid (which people who are breastfed lack) (Colla *et al.*, 2003). Spirulina is considered as the "food for the future" that will effectively tackle the existing malnutrition problem (Uddin *et al.*, 2018*).* That's why it is called a superfood.

In parts of the world with tropical or subtropical climates, Spirulina is produced commercially as a protective food with a high protein and vitamin content (Venkataraman and Becker, 1985, Henrikson, 1989). Bangladesh is the only place where it can be produced commercially in the monsoon climatic zone (Jahan *et al*, 1999). Presently, Spirulina is mostly produced by three different systems. In the first stage of this system, natural lakes are harvested, followed by outdoor pond cultivation systems, such as open ponds or covered greenhouses (advanced pond cultivation system), and finally newly developed open enclosed systems, such as transparent tubes, photo-bioreactors, and micro-farms. Now-a-day's Spirulina is cultivated on the

rooftop in the different photobioreactor in city area's which ensure more safe production and minimizes land use.

But the main hindrance of quality Spirulina production is contamination, pH maintenance, growth rate, productivity, and photosynthetic efficiency with the cost of growth chamber and high-cost production media. Safe Spirulina production depends on what environmental conditions surrounding the farm. Moreover, the growth of Spirulina and the composition of the biomass produced are affected by a number of factors, the most important of which are the availability of nutrients, the temperature, a pH value between 9.5 and 11, and the availability of light, which can do away with contamination by most algae during cultivation. Production of Spirulina with reduced costs is necessary when considering large-scale cultivation for industrial purposes.

Globally, Spirulina is mainly produced by Zarrouk's media. This media is composed of different chemical compositions. Some of these components of the zarrouk's media are very costly like Di-potassium hydrogen phosphate (K_2HPO_4) and EDTA. In this study, we used modified media to compare zarrouk's media for cost and yield evaluation. In this regard, TSP ($Ca_2(PO_4)_2$) instead of Di-potassium hydrogen phosphate (K_2HPO_4) and Citric acid instead of EDTA can be used to evaluate benefit cost ratio and growth performance.

In culture media preparation, freshwater is usually used. This can potentially lead to contamination due to different toxins present. Because of this, chlorine must be used to purify the water and it incurs an extra cost.

In this study we used EcoSolv water. Ecosolv water enhancement is an in-line water-saving device that improves the penetration rate of water. It works to change the water molecules into very small water molecule clusters, each made up of six symmetrical organized molecules. This hexagonal cluster is recognized by the cell as "bio-friendly" due to its hexagonal structure and inability to transport toxins and easily enters the passageways in plant and animal cell membranes. It makes water more bio-accessible which makes the water more productive by increasing its bioavailability to plant cells. It delivers more oxygen and nutrients to the plant while reducing scaling, harmful salts, and total dissolved solids (TDS). The increase in nutrient solubility will

create healthier plants and greater yields. In addition, the device has cleaning/de-scaling properties that will remove blockages in irrigation systems, and made-up within the drip nozzles and sprinkler heads. The main attractive feature of the device is that it is a single unit, approximately one meter long, and is simply attached to the irrigation line after the filters. This product does not require electricity or maintenance, does not erode or oxidize, and once installed provides a lifetime of benefits to the end-user.

As a phototrophic organism, spirulina is generally cultivated in photobioreactors like horizontal, vertical, and flat planar photobioreactors. A photobioreactor is a bioreactor that utilizes a light source to cultivate phototrophic microorganisms (Uddin *et al.*, 2020). Photobioreactor enables the consumer to cultivate and consume fresh Spirulina, minimizes the loss of nutrients after the drying process, and leads to high-grade nourishing health foods (Li *et al*., 2004). Photobioreactors prevent outer contamination and maintain the quality of Spirulina.

Therefore, the objectives of the research are –

1. To evaluate the suitable media for growth and productivity of Spirulina.
2. To determine the cost effective media for commercial Spirulina production.

CHAPTER II

REVIEW OF LITERATURE

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), B_{12}, iron, calcium, chlorophyll. Now-a-day's Spirulina are cultivated all over the world. For Spirulina cultivation zarrouk's media is standard but it is possible to production with modified zarrouk's media that reduces production cost. Therefore, information available regarding spirulina production with zarrouk's media and modified zarrouk's media has been reviewed and presented in this section.

Caturwati and Setyati (2020) had an experiment "Optimation of Spirulina sp. Growth in Walne Media with Variation of Urea and $NaHCO_3$ Supplements". This paper represent that urea and $NaHCO_3$ can be used as additional nutrients sources of nitrogen and carbon to *Spirulina* sp. cultivation. Deficiency of nitrogen causing the cell's enzymes change that shown through decreased lipid and chlorophyll synthesis. While deficiency of carbon can affect the growth rate. In this research, the growth rate of *Spirulina* sp. is analyzed using Optical Density (OD) method. The growth rate calculation is used to measure the growth of microalgae cells shown in the growth curve. This was a laboratory-scale method using CRD with 4 treatments and 5 replications namely treatment A addition of 0.36 g/500 ml urea without addition of NaHCO3, treatment B addition of 0.043 g/500 ml $NaHCO_3$ without addition of urea, treatment C addition of 0.36 g/500 ml urea and 0.043 g/500 ml $NaHCO_3$, and control without addition of urea or $NaHCO_3$. The results indicated that addition of urea and $NaHCO_3$ didn't affect to OD and *Spirulina* sp. growth rate. The highest growth rate was treatment A with 0.00906/day of growth rate followed by treatment C which has 0.00865/day of growth rate. Treatment B and control treatment (K) showed a low

growth rate. The maximum OD value obtained in treatment C was 0.674 cells/ml on the 10^{th} day.

Celekli *et al.* (2009) studied the effect of Modeling of biomass production by Spirulina platensis as function of phosphate concentrations and pH regimes. This paper represents the influence of phosphate concentrations (0.25, 0.5, 0.75, and 1.0 g L^{-1}) for pH regimes (9.5, 10.0, and 10.5) on the biomass production by Spirulina platensis. The best condition for cell growth (3.099 g L^{-1}) was found at 0.5 g L^{-1} phosphate and pH value of 10.0. Cultivation time, phosphate, and pH caused to increase significantly ($p < 0.01$) in biomass production by *S. platensis*. Lag time was observed up to 4 h. After then, biomass production increased sharply ($p < 0.01$) from 0.020 g L^{-1} to 2.063, 2.213, 1.532, and 0.797 g L^{-1} at 0.25, 0.5, 0.75, and 1.0 g L^{-1} phosphate values, respectively. Modified Gompertz model could be regarded as sufficient to describe the biomass production by S. platensis with high determination coefficients and low sum of square value indicated that. Biological parameters for biomass production were successfully predicted by modified Gompertz model.

Danesi *et al*. (2002) investigate the effect of replacing nitrate by urea in the growth and production of chlorophyll by Spirulina platensis. In this work, the cultivation of the microalga was done using urea as the nitrogen source by a fed-batch process. The addition of urea was done in four different modes: intermittent addition every 24 or 48 h, continuous addition by exponentially increasing the added mass, and continuous addition by using a constant mass rate. The experiments were carried out at three different temperatures: 27◦C; 30◦C and 33◦C and at a constant light intensity of 3:5 klx. The results showed a positive influence of urea in the growth of Spirulina but no effect on the final chlorophyll content of the cultures. Best results were obtained by continuous urea addition in exponentially increasing amount, at 30◦C.

Dineshkumar *et al*. (2016) had an experiment to Cultivation of *Spirulina platensis* in different selective media. This experiments were carried out to assess the optimum culture conditions for the growth of *S.platensis* in different medium *viz*, Zarrouk medium, BG11 medium, Conway medium, F/2 medium and Sea water. Growth analyses and dry weight were monitored for 30 days on daily basis. pH was found to be ranged from 9.1 to 11.0 in Zarrouk medium, 8.9 to 9.2 Conway in F/2 medium and

8.57 to 8.68 Seawater medium. Room temperature was maintained at 30 ± 2 °C under 12/12 hour light-dark cycles, light illuminated (4500 lux) and Spectrophotometer used at 550nm, 680nm for two days interval. Dry weight (DW) was gradually increased along with the age of culture and 1.86 dw/L was achieved in Zarrouk medium. The *S. platensis* inoculated in Conway and F/2 medium were survived but well the growth was not flourished, achieved the maximum dry weight of 0.52dw/L on 21^{th} day of cultivation. Seawater fortified with different amount of $NaHCO_3$ and $NaNO_3$ did not show any significant impact on *Spirulina* growth. However, results of the present investigation could be considered for commercial cultivation of *Spirulina* using Natural medium.

Faucher *et al*. (1979) had an experiment "Utilization of Seawater-Urea as a Culture Medium for *Spirulina maxima*". This paper indicates the possibilities of utilization of seawater enriched with urea as the culture medium for a blue-green alga, Spirulina maxima, were investigated. Pretreatment by precipitation with NaHCO3 and (or) Na_2CO_3 was found essential to remove the excess amounts of Ca^{2+} and Mg^{2+} present in seawater prior to cultivation. A culture medium as good as the synthetic medium reported in the literature for the growth of S. maxima was obtained after treating seawater with $NaHCO_3$ (19.2 g/L) at pH 9.2 and 35 degrees C for 2 h, filtering to remove precipitates, and enriching with K_2HPO_4 (0.5 g/L), $NaNO_3$ (3.0 g/L), and $FeSO_4$ (0.01 g/L). The same results were obtained by substituting a small amount (0.2 g/L or less) of either crystalline or polymerized urea for the NaNO3 in the above medium. Growth of S. maxima was inhibited at higher concentration of urea in the culture medium. The inhibition effect was due to the partial decomposition of urea into ammonia in alkali medium. Tests conducted on the 130-L cultivation open pond also confirmed that the seawater-urea medium supports growth of S. maxima as well as the best known synthetic medium.

Gami *et al.* (2011) conducted an experiment "Cultivation of *Spirulina* species in different liquid media". This paper represent *Spirulina* is one of the most explored cyanobacteria. Since ancient time it is being used as source of protein *Spirulina* sp. NCIM – 5421 was cultivated in different liquid medium like; synthetic medium (SM), fertilizer medium (FM) and seawater medium (SM). Dry weight and pH were monitored for 30 days on daily basis. pH was found in range from 9.1 to 10.4 in SM,

9.0 to 10.1 in FM and 8.51 to 8.55 in SM. Gradually increase in dry weight (dw) was noticed along with the age of culture, 1.84 dw/L & 1.81 dw/L was achieved in SM and FM respectively. *Spirulina* inoculated in SM was survived but growth was not flourished, achieving maximum dry weight of 0.28 dw/L on 18th day of cultivation. Natural seawater fortified with different amount of $NaHCO_3$ and $NaNO_3$ did not shown significant impact on *Spirulina* growth. However results of present investigation could be consider for commercial cultivation of *Spirulina* using seawater.

Gubbuk (2021) investigate that "First Expression of EcoSolv Water Unit in Banana Cultivation under Subtropical Condition". The objective of the the study is to evaluate the use of the EcoSolv water unit in open field banana cultivation under subtropical conditions. The experiment was carried out in the Province of Gazipasa, Antalya, Turkey. Dwarf Cavendish cultivar was used as the experimental material and drip irrigation was used. The effect of the Ecosolv water unit was examined on yield and quality of the open field banana cultivation. The experimental results showed that the Ecosolv water unit increased the yield and quality compared to the control. Average annual yield with EcoSolv water was 13% higher than control (44.91 t/ha compared with 50.85 t/ha). Furthermore, the most important quality parameter of finger wewight was higher than control.

Jung *et al*. (2019) stated that Spirulina platensis, a multicelluar, photosynthetic prokaryote (algae) contains a high amount of proteins, vitamins and minerals superior to many foods as e.g. soybeans. Thus, Spirulina platensis was recognized as nutritious food by the United Nations World Food Conference. Due to the high amount of nutritive ingredients Spirulina has a long history as dietary supplement. In addition, spirulina platensis is also efficiently used as forage with known effects on flesh, egg and plumage color, milk yield and fertility. The versatile utilization of the alga can be explained on the one hand with the nutrient levels and on the other hand with recognized effects as anti-viral, anti-bacterial, anti-oxidant, anti-diabetic, anti-cancer and anti-inflammatory substance. Therefore, this alga is named as "superfood". Beyond, these algae convert carbon dioxide into organic substances and produce oxygen during their growth in alkaline and saline water thereby not wasting fresh water allowing the production in barren areas. Despite this diverse use of Spirulina

platensis due to its beneficial properties, many basic mechanisms on a molecular and cellular level are not well understood and should be explored in future studies.

Khatun *et al.* (2006) conducted a research at Biological Research Division, BCSIR Laboratories, Dhaka, Bangladesh, for a six month period 30.06.2002 to 31.12.2002 to find out the effect of different culture media on growth of Spirulina (*Spirulina platensis*). All together three cultural media used; Media No.1 Bd_1. Media No.2 Bd_4 and Media No.3 IFP. Bangladesh medium (Bd_1) was found to be more favorable for the growth of the Spirulina. Three culture media namely Bangladesh medium 1 (Bd_1), Bangladesh medium 2 (Bd_4) and Media number 3 IFP were used in this research. Initially growth of the local strain was equally good in all the three media (Bd_1, Bd_4 and IFP). However, after one month, condition and color of local strain of Spirulina was better in Bd_1 than Bd_4 and IFP media. In continuation of the study, the local strain of Spirulina and the control were cultured in plastic bowls, in the above media for another two months. In this study, the local strain of Spirulina showed better response to Bd_1 medium in comparison to the others, Bd_4 and IFP. To ensure the growth of the local strain of Spirulina in Bd_1 medium, the culture was continued in larger scale for six months. Spirulina was harvested once a month. Feedback was added to the medium after each harvest. During the six months trial, the local strains of Spirulina were successfully grown in Bd_1 medium.

Kim *et al*. (2007) studied different factors indicating culture status of two *Spirulina platensis* strains were monitored in a batch mode cultivation for 36 days. Changing mode in all factors showed a common turning point, indicating shift of cell or culture status. Mean biomass productivity was highly sustained until day 22, chlorophyll *a* concentration peaked on day 22, pH value was >12 on day 22, coil number was abruptly shortened on day 22, and floating activity was sustained at greater than 79% after day 22, indicating that day 22 is a criterion reflecting phase-transfer in cell physiology in a batch culture system. Many of these changes may have been caused by increased pH, suggesting that pH control is essential for mass production of *S. platensis*. Fluctuations in floating activity were likely induced by the number of cellular gas vacuoles. Consequently, coil number per trichome and floating activity of *S. platensis* could readily act as simple indicators for determination of culture status or harvesting time of cells.

Kumar *et al.* (2011) “Growth and Biopigment Accumulation of Cyanobacterium *Spirulina* platensis at Different Light Intensities and Temperature”. In order to find out optimum culture condition for algal growth, the effect of light irradiance and temperature on growth rate, biomass composition and pigment production of *Spirulina platensis* were studied in axenic batch cultures. Growth kinetics of cultures showed a wide range of temperature tolerance from 20 °C to 40 °C. Maximum growth rate, cell production with maximum accumulation of chlorophyll and phycobilliproteins were found at temperature 35 °C and 2,000 lux light intensity. But with further increase in temperature and light intensity, reduction in growth rate was observed. Carotenoid content was found maximum at 3,500 lux. Improvement in the carotenoid content with increase in light intensity is an adaptive mechanism of cyanobacterium *S.platensis* for photoprotection, could be a good basis for the exploitation of microalgae as a source of bio pigments.

Mashor *et al.* (2016) conducted a research on “Different Nitrogen Sources Effects on the Growth and Productivity of Spirulina Grown In Outdoor Conditions” at Faculty of Agriculture, University Putra Malaysia (UPM). This paper presents the effects of different nitrogen sources on the productivity of *A. platensis* . Cultivation of *A. platensis* was conducted 7 days for first cultivation followed by 10 days of second cultivation with three replications each respectively. In this experiment, *A. platensis* was grown in four different culture treatments. 30L of freshwater was mixed with enrichment solutions in high-density polyethylene (HDPE) bags (60cm x 90cm) special manufactured with the addition of ultra violet (UV) protection. Inoculum size of 10% was used to initiate *A. platensis* culture with optical density of 0.2471. Thirty liters of Spirulina was grown in tied up transparent plastic bags photobioreactor supplied with gentle aeration and placed in open outdoor conditions. Physical water parameter and environmental conditions were monitored. A combination of agriculture chemicals such as urea, ammonium sulphate, ammonium nitrate with definite concentration were tested on Spirulina growth. Results have shown that Spirulina productions are affected by the weather conditions. The production of Spirulina during the second cultivation cycle (dry conditions) has yielded higher growth rate with media supplemented of T_1, ammonium nitrate as the nitrogen source.

Madkaur *et al.* (2012) conducted a research at National Institute of Oceanography and Fisheries, Inland Waters, Egypt. This study aimed to provide a cost effective medium to large scale production of *Spirulina platensis*. This intention was implemented by substituting all the nutrients present in Zarrouk's medium (SM) with cheaper and locally available commercial fertilizers and chemicals. The Reduced Cost medium contained single super phosphate (SSP), commercial sodium bicarbonate, Muriate of potash (MOP) and crude sea-salt, (Syahat salt). Four grades of nitrogen concentrations representing 10%, 20%, 30% and 40% of SM nitrogen concentration (29.42 mM-N) were taken from ammonium nitrate (Treatments 1–4) or urea (Treatments 5–8) respectively, for testing. The alga was grown for 33 days at 30 ± 2 _C, pH 9, 30 lEm2 s_1 irradiance. The growth characteristics (maximum biomass X_m, cell productivity P_x, specific growth rate lm and chlorophyll concentration), and biochemical composition (proteins, carbohydrates and lipids) of the alga grown in these media were compared with that cultivated in SM. Significant differences in the growth parameters and biochemical composition were observed for the different nitrogen sources and concentrations. The results revealed that S. platensis could utilize ammonium nitrate most efficiently and that growth was enhanced with increasing the concentrations of ammonium nitrate giving maximum biomass at 0.353 g/L (Treatment 3). Further increasing the concentration limited growth. The growth parameters in urea showed a significant decrease associated with increasing urea concentrations. The maximum biomass, chlorophyll and protein yield (0.813± 0.018 mg/L, 0.0685± 0.0024l g/L and 52.62%, respectively) were recorded using Treatment 3 which was comparable with that of SM (0.840± 0.008 mg/L, 0.0701± 0.0089l g/L and 52.95%, respectively). The results indicated that the newly prepared medium can be used profitably for large-scale mass production of protein-rich spirulina and yield performance were similar with cost effective to Zarrouk's medium.

Michael *et al.* (2019) conducted a research "Biomass and nutritive value of Spirulina (*Arthrospira fusiformis*) cultivated in a cost-effective medium" at the Department of Botany, University of Dar es Salaam. The experiment was carried out for 28 days in the growth chamber. This study assessed the biomass, proximate composition, and other useful compounds in Spirulina (*Arthrospira fusiformis*) produced with a cost-effective culture medium (LCMA), and the results were compared with those from a

standard Zarrouk medium grown spirulina. The LCMA medium was formulated by using a commercial NPK 10-20-20 fertilizer as a source of the three major nutrients for spirulina growth, and other three ingredients from Zarrouk medium. The experiment was conducted for 28 days in the glass aquaria under indoor conditions. Standard analytical methods were applied for the determination of proximate composition, chlorophyll, minerals, and vitamins in the spirulina biomass. The low-cost medium termed as LCMA was formulated by mixing four ingredients. All the ingredients for the LCMA except the trace element solution, are of commercial grade and locally available. The major elements (nitrogen, phosphorus, and potassium) for spirulina growth in the LCMA medium were from NPK 10-20-20 complex fertilizer, a common and well-known fertilizer for growing crops. NPK 10-20-20 is granular and water-soluble, composing of 10% of ammoniacal nitrogen (NH_3-N), 20% phosphorus penta-oxide (P_2O_5), and 20% potassium oxide (K_2O), with trace amount of sulfur. Sodium bicarbonate was added in spirulina medium as a source of carbon while sodium chloride offered the chloride for ideal salinity of the medium. The micronutrients are needed for proper growth of spirulina. NPK 10-20-20 is cost-effective and easily accessed in the shops of agricultural inputs, whereas 1 kg costs only 2000 Tanzanian Shillings (≈ US $ 1).

The LCMA medium showed the best growth conditions by accumulating higher chlorophyll content (0.99 ± 0.02%) and dry weight (0.75 ± 0.01 g/100 ml) as well as attaining higher optical density (2.06 at day 15) earlier than the Zarrouk medium. The results of the proximate analysis for spirulina cultured in the LCMA medium were of good quality, with the protein contributing more than 50% of its dry matter. It was further noticed that the LCMA was an ideal medium for optimization of vitamins and some minerals since it recorded a significant amount of most of the analyzed vitamins together with the minerals sodium and potassium compared with the Zarrouk medium. It is suggested that LCMA medium could be used as the alternative and cheap medium for maximization of biomass and production of useful biochemical compounds in spirulina species.

According to McGregor (2021) EcoSolv technology has developed a unique way of reducing the surface tension of water that runs through the water-enhancement device. This is done through a combination of flow design and a very specific frequency

emitted by rare earth salts embedded within the device. By reducing surface tension, irrigated water can effectively infiltrate through soils in a more uniform manner, increasing not only the availability of moisture to the root zone, but also, the availability of macro and micronutrients. Hence, EcoSolve advise all end-users to immediately reduce their fertilizer applications by a minimum of 10% to avoid the 'burning' of crops. The device also has cleaning/de-scaling properties, removing blockages in irrigation systems and build-up within mains and end lines, including the drip nozzles and sprinkler heads. One of the best features of the device is that it is a single unit, approximately one meter long and is simply attached onto the irrigation line after the filters. It has no moving parts, it doesn't erode, oxidize or require any electricity or maintenance and once installed provides a lifetime of benefits to the end-user.

Markou *et al*. (2012) Conducted an experiment "Effects of phosphorus concentration and light intensity on the biomass composition of Arthrospira (Spirulina) platensis". This paper presents the effects of various phosphorus concentrations (10, 50, 250 and 500 mg l^{-1} K_2HPO_4) on the biomass production and composition of Arthrospira (Spirulina) platensis in relation to light intensity (24, 42 and 60 lE m^{-2} s^{-1}). The maximum biomass production was 3,592 ± 392 mg l^{-1} and this was observed in 250 mg l^{-1} K_2HPO_4 at 60 lE m^{-2} s^{-1} light intensity after 32 days of cultivation. A maximum specific growth rate (max) of 0.55 d^{-1} was obtained in 500 mg l^{-1} K_2HPO_4 at 60 lE m^{-2} s^{-1}. The protein, lipid and chlorophyll contents of the biomass varied from 33.59 to 60.57 %, 5.34 to 13.33 % and 0.78 to 2.00 %, respectively. The most significant finding was that phosphorus limitation (10 mg l^{-1} K_2HPO_4) caused a drastic increase of the carbohydrate content (59.64 %). The effect of phosphorus limitation on the carbohydrate content was independent of the light intensity. The accumulated carbohydrates are proposed to be used as substrate for biofuel generation via one of the appropriate biomass energy conversion technologies. Also, it was observed that phosphorus removal is a function of biomass density, phosphorus concentration and light intensity.

Mia *et al*. (2019) investigate "A Study on Growth Performance of Spirulina Platensis in Different Concentrations of Rotten Apple as a Carbon Source". This experiment was conducted on culture and growth performance of *Spirulina platensis* in various

concentrations of rotten apple medium (RAM) and Kosaric Medium (KM). The observation was conducted for three months from March to May at the Live Food Culture Laboratory, Department of Aquaculture, Faculty of Fisheries, Bangladesh Agricultural University. Culture of S. platensis was performed in 1.0L glass flasks in three different media such as 2.5, 5.0 and 10% and KM with three replications under fluorescent light in light : dark (12 hr : 12 hr) condition of a period of 14 days. Growth performances of *S. platensis* varied from one medium to another. The initial cell weight of *S. platensis* was 0.0023 mg/L and a maximum cell weight of 12.44 mg/L was found in KM and 10.468 mg/L in RAM on 10th day of culture. It was also observed that, the initial chlorophyll a content of S. platensis was 0.0015 mg/L which was attained at a highest content of 10.54 mg/L in KM and 12.35 mg/L in RAM on 10th day of culture. A decreasing trend of cell weight was observed from 10th day of culture. The growth of *S. platensis* was significantly ($p<0.05$) better in 5.0% Digested Rotten Apple Medium (DRAM) than other concentrations 2.5% DRAM and 10% DRAM. From the results obtained in the present study, it was summarized that the growth of *S. platensis* was better in the concentrations of 5.0% DRAM than other concentrations of RAM. Thus, the concentration of 5.0% DRAM is most suitable for *S. platensis* culture compare with standard KM. These media are easily available and most inexpensive in contrast of Bangladesh. So digested rotten apple can be used for commercially and economically viable mass culture of *S. platensis*.

Kumar and Kumar (2021) conducted an experiment "Production of *Spirulina platensis* in different media". This paper reported that *Spirulina platensis* was cultured in various medium as; Standard Zarrouk's Media (SZM), Modified Zarrouk's Media (MZM) and Tap Water Media (TWM). Dehydrated weight and pH was observed for 26 days on every day. In Standard Zarrouk's Media pH was noticed from 9.2 to 10.6, in MZM 9.0 to 10.4 and in TWM 7.40 to 7.45. Gradually increase in dry weight (dw) was observed with length of time of culture, 1.84 dw/L & 1.74 dw/L was attained in SZM and MZM correspondingly. S. platensis inoculated in TWM was live but growth was not increased, achieving higher dehydrated mass of 0.28 dw/L on 17-21 day of cultivation. Tap water prepared with various quantities of basic salts didn't exposed important change on S. platensis growth. Growth was influenced by mutually temperature and light in the experimentation and the effect of temperature was better to light in the experimental time.

Nor *et al.* (2016) conducted an experiment on "The effect of different nitrogen sources on continuous growth of *Arthrospira platensis* in simple floating photobiorector design in outdoor conditions" at University Putra Malaysia. The aim of this study is to find out whether *Spirulina* can be cultured in simple photobioreactor floating in water body. *A. platensis* was cultivated in simple water based floating photobioreactor and land based tank using different composition of nitrogen sources (Urea and Ammonium Nitrate). Experiment was conducted in outdoor conditions to assess the respond of different nitrogen sources on the cell density, dry biomass and total chlorophyll of *Spirulina* gained in simple photobioreactors in actual variable culture conditions. Results showed significantly higher biomass dry weight (g L^{-1}) with ammonium nitrate treated Spirulina under dry weather conditions for land based tank and water based photobioreactor at 3.026±0.058 and 4.687±0.154 respectively. Overall, productivity (g L^{-1} d^{-1}) and specific growth rate (μ d^{-1}) of Spirulina was highest with ammonium nitrate than urea for every cycles and photobioreactors under different weather patterns (wet, dry and mix). With current price of ammonium nitrate cheaper than urea suggesting that Spirulina can be cultured at lower cost in variable weather conditions such as Malaysia.

Pandey *et al.* (2010) had an experiment on "Standardization of pH and Light Intensity for the Biomass Production of *Spirulina platensis"***.** They reported that the cyanobacterium *Spirulina platensis* is an attractive alternative source of the pigment chlorophyll, which is used as a natural color in food, cosmetic, and pharmaceutical products. In the present investigation, the influence of light intensity and pH for *Spirulina platensis* growth, protein and chl a content were examined. In the present investigation the production of *Spirulina platensis* was optimized in terms of biomass and metabolites. The dry weight of *Spirulina platensis* was 0.91g/500ml and protein and Chlorophyll *a* content were 64.3% and 13.2mg/gm respectively at pH 9. At 5 Klux light intensity the dry weight of *Spirulina platensis* was 0.85g/500ml while protein content and Chlorophyll *a* were 64.3% and 9.8mg/gm respectively.

Reichert *et al*. (2006) studied the "Semicontinuous Cultivation of the Cyanobacterium *Spirulina platensis* in a Closed Photobioreactor". They studied the specific growth rate (μx, day^{-1}) and productivity (Px, in mg/L/day of *Spirulina platensis* biomass, dry weight basis) of two *S. platensis* strains (LEB-52 and Paracas) growing in aerated

semicontinuous 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 (Px = 42.3 mg/L/day). These values are two to four times higher than those obtained in simple batch cultivation and indicate that the semicontinuous cultivation of *S. platensis* is viable.

Saeid and Chojnacka (2016) conducted an experiment on "Evaluation of Growth Yield of *Spirulina maxima* in Photobioreactors". The paper deals with the evaluation of the parameters for the cultivation of *Spirulina maxima* in two reactors (large-laboratory scale (LL) and semi-technical scale (ST)), whose illuminated areas in respect of the illuminated volume are different, and with the operating costs. We evaluated the growth yield coefficients for *Spirulina maxima* cultures. In the LL, the following factors were identified: YO_2/X – 65.5; YX/CO_2 – 0.0806;YX/P_2O_5 – 0.0082, while in the ST: YO_2/X – 583; YX/CO_2 – 0.017; YX/P_2O_5 – 0.0023. Although the reactor in the ST was equipped with many devices that should have improved the efficiency of cultivation, the obtained result was lower compared to the culture conducted in the LL. It was proved that it was possible to perform the cultivation of *Spirulina maxima* under temperate climate conditions in simply constructed, low cost reactors.

Sassano *et al.* (2010) conducted an experiment "Evaluation of the Composition of Continuously Cultivated *Arthrospira (Spirulina) platensis* Using Ammonium Chloride as Nitrogen Source". This work is focused on the influence of dilution rate ($0.08 \leq D \leq 0.32$ d^{-1}) on the continuous cultivation and biomass composition of Arthrospira (Spirulina) platensis using three different concentrations of ammonium chloride (cNo = 1.0, 5.0 and 10 mol m^{-3}) as nitrogen source. At cNo = 1.0 and 5.0 mol m^{-3} the biomass protein content was an increasing function of D, whereas, when using cNo = 10 mol m^{-3}, the highest protein content (72.5%) was obtained at D = 0.12 d^{-1}. An overall evaluation of the process showed that biomass protein content increased with the rate of nitrogen supply (D cNo) up to 72.5% at D cNo = 1.20 mol m^{-3} d^{-1}. Biomass lipid content was an increasing function of D only when the nitrogen source was the limiting factor for the growth (D cNo $\leq$ 0.32 mol m^{-3} d^{-1}),

which occurred solely with cNo = 1.0 mol m^{-3}. Under such conditions, A. platensis reduced its nitrogen reserve in the form of proteins, while maintaining almost unvaried its lipid content. The latter was affected only when the concentration of nitrogen was extremely low (cNo = 1.0 mol m^{-3}). The most abundant fatty acids were the palmitic (45.8 ± 5.20%) and the γ-linolenic (20.1 ± 2.00%) ones. No significant alteration in the profiles either of saturated or unsaturated fatty acids was observed with cNo ≤ 5.0 mol m−3, prevailing those with 16 and 18 carbons.

Widianingsih *et al.* (2008) conducted a research which aimed to determine nutrient content in Spirulina platensis culture in walne, echnicaland control media. The result showed that highest density and highest value of the protein, carbohydrate, water, ash and lipid of dry biomass were collected from Walne media. The protein, carbohydrate and lipid content of S. piantesis in Walne media were 50,05±0,53; 15,48+0,47; and 0,51±0,12% respectively. Whereas, in the technical media, the protein carbohydrate and lipid content of *S. platensis* are as follows: 16,23±0,4; 12,57±0,22; and 18±0,03%. It suggest due to different nutrient content of culture media.

Sancbez *et al.* (2003) had an investigate on *Spirulina Platensis*. This paper stated *Spirulina* is a photosynthetic, filamentous, helical-shaped, multicellular and green-blue microalga. The two most important species of which are *Spirulina maxima* and *Spirulina platensis.* For these microorganisms cell division occurs by binary fission. Since this material contains chlorophyll *a*, Jike higher plants, botanists classify it as a microalgae belonging to *Cyanophyceae* class; but according to bacteriologists it is a bacteria dueto its prokaryotic structure. Before Columbus, Mexicans (Aztecs) exploited this microorganism as human food; presently, African tribes (Kanembu) use it for the same purpose. Its chemical composition includes proteins (55%-70%), carbohydrates (15%-25%), essential fatty acids (18%), vitamins, minerals and pigments like carotenes, chlorophyll *a* and phycocyanin. The last one is used in food and cosmetic industries. *Spirulina* is considered as an excellent food, lacking toxicity and having corrective properties against viral attacks, anemia, tumor growth and malnutrition. It has been reported in literature that the use of these microalgae as animal food supplement implies enhancement of the yellow coloration of skin and eggs yolk in poultry and flamingos, growth acceleration, sexual maturation and increase of fertility in cattle.

According to Saranraj and Sivasakhti (2014) *Spirulina* can play an important role in human and animal nutrition, environmental protection through wastewater recycling and energy conservation. The present review was focused on the various characteristics of *Spirulina platensis*. *Spirulina* is rich in proteins (60-70%), vitamins and minerals used as protein supplement in diets of undernourished poor children in developing countries. One gram of *Spirulina* protein is equivalent to one kilogram of assorted vegetables. The amino acid composition of *Spirulina* protein ranks among the best in the plant world, more than that of soya bean. The mass cultivation of *Spirulina* is achieved both in fresh water and waste water. *Spirulina* grown in clean waters and under strictly controlled conditions could be used for human nutrition. The micro alga grown in waste water is used as animal feed and provide a source of the fine chemicals and fuels. The waste water system is highly applicable in populated countries like India where wastes are generated in high quantities and pose environmental problem. The present review focused the following topics: *Spirulina platensis*, Isolation and occurrence of *Spirulina platensis*, newly formulated media for *Spirulina* cultivation, Phycocyanin and Medicinal properties of *Spirulina platensis*.

Shinta *et al*. (2021) investigate the effect of Citric Acid and EDTA as chelating agents in phytoremediation of heavy metal in polluted soil: a review. This paper represent the application of metal chelating agents in phytoremediation has been shown to increase plant efficiency for heavy metal uptake in phytoextraction significantly. EDTA is a famous chelating agent used in phytoextraction. However, future use of EDTA is likely to be limited to ex-situ conditions where leachate control can be achieved, so there is limitations to its use that need to be studied. So that many phytoremediation studies have been carried out on organic chelating agents that are not expected to be harmful to the environment, one of which is Citric Acid. The purpose of this review is to compare commonly chelating agents, namely: EDTA as synthetic and Citric Acid as a natural matter for phytoremediation in polluted soils. This review also discusses the ability of Citric Acid and EDTA on phytoremediation, their effect on soil physiology and soil microbiology, advantages and disadvantages of each on the prospects of phytoremediation. EDTA can increase phytoextraction better than Citric Acid but can increase the risk of groundwater pollution because EDTA is difficult to

degrade by the environment. In contrast, Citric Acid has been shown to increase phytoextraction, phytostabilization and harmless to the environment.

Soni *et al.* (2019) conducted an experiment on "Comparative study on the growth performance of Spirulina platensis on modifying culture media" at Energy Centre, MANIT, Bhopal, India. This paper presents a novel experimental approach to maximize biomass yield, minimize evaporation rate and respiration losses in a laboratory scale closed reactor and open pond system. Lab scale open pond and closed reactor system were designed for spirulina cultivation under dry climatic conditions at Bhopal, India. Zarrouk media was used as standard and modified organic media was prepared by changing the nitrogen source. Temperature and other input parameters were maintained. Aeration was done manually in an open pond, and the air pump was used in the case of a closed reactor system. Biomass yield obtained from an open pond system was 11.34 g/l, and 12.28 g/l in the closed reactor system. Doubling time was also less in the closed reactor in comparison with the open pond system. Urea seems to be a promising alternative source of low-cost nitrogen for Spirulina cultures. From the experimental results, it is concluded that modified organic media and closed reactor system could be used for better biomass yield.

Sukumaran *et al.* (2018) conducted an experiment "Formulation of Cost-effective Medium Using Urea as a Nitrogen Source for *Arthrospira platensis* Cultivation under Real Environment". This research about *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) which was designed by substituting the major laboratory chemicals in standard Kosaric medium (SKM) with commercial grade baking soda, sea salt, urea, phosphoric acid, potassium hydroxide and Epsom salt. Urea as an alternative nitrogen resource to sodium nitrate was pulse-fed throughout the cultivation period. The algal growth was measured through optical density, biomass dry weight and chlorophyll *a* content. The algal yield was determined by calculating its productivity and specific growth rate. 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. The present finding showed the potential of MKM in lowering the medium cost up to 97% compared to SKM without compromising the algal yield under natural condition with proper cultivation techniques such as preadaptation and fed batch addition of urea in the late evening.

Torzillo *et al.* (1991) conducted an experiment "Effect of temperature on yield and night biomass loss in *Spirulina platensis* grown outdoors in tubular photobioreactors". This outdoor experiments carried out in Florence, Italy (latitude 43.8° N, longitude 11.3 ° E), using tubular photobioreactors have shown that in summer the average net productivity of a *Spirulina platensis* culture grown at the optimal temperature of 35 C was superior by 23 % to that observed in a culture grown at 25 C. The rates of night biomass loss were higher in the culture grown at 25 C (average 7.6 % of total dry weight) than in the one grown at 35 C (average 5%). Night biomass loss depended on the temperature and light irradiance at which the cultures were grown, since these factors influenced the biomass composition. A net increase in carbohydrate synthesis occurred when the culture was grown at a low biomass concentration under high light irradiance or at the suboptimal temperature of 25 C. Excess carbohydrate synthesized during the day was only partially utilized for night protein synthesis.

Uddin *et al.* (2021) had an experiment on "Subsequent Addition of Spirulina Inoculum and Growth Nutrient in Culture Media under Closed System Spirulina Cultivation". This paper represents the influence of subsequent application of spirulina inoculum and nutrient medium on growth and yield of spirulina. The experiment conducted with four treatments, *viz*., recycled water after harvest (T0), addition of spirulina inoculum (T_1), addition of subsequent nutrient (T_2) and addition of both inoculum and nutrient (T_3) following completely randomized design (CRD) with four replications. Data on different growth and yield parameters showed significant variations among them. Subsequent application of both spirulina inoculum and nutrient (T_3) exhibit significant differences with other studied treatment and influenced to increase the yield (241.6%) over control. On the other, addition of subsequent nutrient media (T_2) revealed (147.0%) higher yield over T0. Addition of inoculum had no impact on pH but application of nutrient medium influenced pH which ensured optimum growth and maximize the BCR (3.49). So, the effectiveness

of all the treatment, addition of nutrient at each after harvest would be the effective for commercial spirulina production.

Uddin *et al*. (2020) conducted an experiment on "Comparative Growth Analysis of *Spirulina Platensis* Using Urea as a Nitrogen Substitute for $NaNO_3$". To study the growth performance of microalgae *Spirulina platensis* with the application of urea as a substitute for $NaNO_3$. Spirulina was allowed to culture in standard Zarrouk's Media (T_0) and three concentrations of Modified Zarrouk's Media containing different amounts of urea solution: T_1: 0.60 g/L (0.01M); T_2: 1.20 g/L (0.02M) and T_3: 1.80 g/L (0.03M) substitute for $NaNO_3$. The growth rate of *Spirulina platensis* was found to vary in different concentrations of urea solutions. The basic concentration of 4mg/L of inoculum gained maximum cell weight (607 mg/L) in standard Zarrouk's Media (T_0) and was significantly ($p>0.05$) higher than the maximum cell weight (454 mg/L) found in Modified Zarrouk's Medium (MZM) containing 0.60 g/L of urea solution (T_1); 1.20 g/L of urea solution (T_2) and 1.80 g/L of urea solution (T_3) respectively on the 12^{th} day of the culture period. Similar trend was also observed in the case of chlorophyll a content and optical density (OD) measurement of *Spirulina platensis* as well. T_1 showed the best result in the given growth parameters such as specific growth rate (0.086), OD (0.66 mg/L) and chlorophyll a content (7.133 mg/L) in comparison with treatments utilizing the urea solution viz. T_1, T_2 and T_3. These findings may be an effective edition for a cheap but high quality Spirulina production system in Bangladesh by using urea as the readily available nitrogen source.

Uddin *et al*. (2020) had an experiment "Spirulina (*Spirulina Platensis*) Production in Different Photobioreactors on Rooftop". This experiment was accomplished on the rooftop of Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh 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) were used in this experiment following Completely Randomized Design (CRD) with three replications. Fifteen days of production was carried out in the selected photobioreactors to determine the performance of the PBRs where culture condition were kept the same

and data on different growth and yield parameters were taken throughout the experiment to which all the PBRs showed significant variations. Among photobioreactors, growth doubling require (3.41 days), maximum productivity (0.90 $gL^{-1}day^{-1}$) and the highest marketable yield (3.34 kg/kl) were found in PBR_4 while maximum doubling time (10.63 days) with lower productivity ($0.40gL^{-1}day^{-1}$) and minimum marketable yield (1.64 kg/kl) in PBR_2. So, 15L rectangular shaped Photobioreactor will be the promising photobioreactor for spirulina cultivation in Bangladesh.

According to Uddin *et al.* (2018) 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. The introduction and scope for the cultivation of Spirulina is immense in Bangladesh as rural people and urban rooftop owners can easily install the culture system in their buildings as it is a fully automated system and requires very little power to operate. Spirulina definitely ensures daily nutritional demands for the rural poor and will also improve the socio-economic status of the rural people by bringing them under integrated Spirulina production. Commercial production of Spirulina and creating demand in local and international market is a very possible aspect in Bangladesh. This ambition can easily be achieved if proper guidance, support and management from the Government and Non-Governmental Organizations are assured. Spirulina will open a door of opportunities in Bangladesh if proper infrastructure is followed.

CHAPTER III

MATERIALS AND METHODS

This chapter contains a brief description of location of the experimental site, climatic condition, materials used for the experiment, treatments and design of the experiment, production methodology, data collection procedure, statistical and economic analysis etc. which are presented as following headings.

3.1 Experimental site

This experiment was conducted on the Rooftop of Faculty of Agriculture at Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka, Bangladesh during the period from November 2019 to March 2020.

3.2 Climate

The experimental area has a tropical wet and dry climatic condition, distinct monsoonal season, with an annual temperature of 25 °C (77 °F) and monthly means varying between 18 °C (64 °F) in January and 29 °C (84 °F) in August. Nearly 80% of the annual average rainfall of 1,854 mm occurs during the monsoon season which lasts from May until the end of September. Information regarding average monthly maximum and minimum temperature, rainfall, relative humidity and soil temperature as recorded by the Bangladesh Meteorological Department (climate division) Agargaon, Dhaka, during the period of study.

3.3 Experimental materials

A. Laboratory Unit

1. Spectrophotometer
2. pH meter
3. Micro pipette
4. Test-tube
5. Test-tube rack
6. Flask
7. Cuvette

Spectrophotometer

Micro pipette

Test-tube rack

8. Soaking paper
9. Litmus paper
10. Disposable syringe (25 ml)
11. Measuring beaker
12. Measuring cylinder
13. Microscope for close inspection of the culture

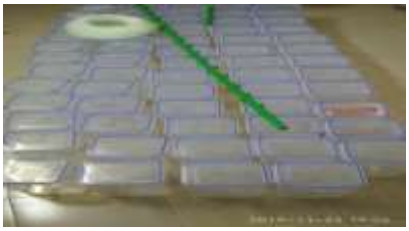

Electric motor


Filtration net

B. Production Unit

1. Iron frame rack (2 rack with 4 stair both side)
2. Transparent plastic container (4 L Bengal Techno. Food grade plastic container)
3. Measuring tape
4. Drill machine
5. Connector
6. Agitation pipe
7. Electric motor (1 hp) for agitation device
8. Filtration net (350 mesh)
9. Gloves
10. Measuring balance
11. Thermometer (For temperature records)
12. Light meter


Connector


Transparent plastic container

3.4 Experimental Design and Treatment

It is a single factorial experiment with four treatments. This experiment was conducted in Completely Randomized Design (CRD) with four replications.

The four different treatments are:

T_1 =	EcoSolv water	+	Zarrouk's Media (EZ Media)
T_2 =	EcoSolv water	+	Modified Media (EM Media)
T_3 =	Freshwater	+	Zarrouk's Media (FZ Media)
T_4 =	Freshwater	+	Modified Media (FM Media)

Those 4 treatments, Freshwater + Zarrouk's Media (T_3) treatment is considered as standard media and all other 3 treatments (T_1, T_2 and T_4) are compare to T_3 treatment. 224 L water was used to prepare culture media. In this experiment we used two iron frame rack each rack has four stairs. In every stair 4 L four transparent plastic

container was placed and each container contain 3.5 L culture media. In this experiment in first rack we used 56 L EZ (T_1) media in 16 containers in four stairs and the opposite side was 56 L FZ (T_3) media. In second rack we used 56 L EM (T_2) media and the opposite side was 56 L FM (T_4) Media.

3.5 Media

3.5.1 Zarrouk's Media

In 1966, Zarrouk developed a medium which has successfully used as the standard medium for *Spirulina* production for many years.

Table 1. List of Chemicals Used in Zarrouk's Media Preparation

Reagent	Amount (g/l)
$NaHCO_3$ (Sodium Bicarbonate)	16.8
$NaNO_3$ (Sodium Nitrate)	2.5
NaCl (Sodium Chloride)	1
K_2HPO_4 (Dipotassium Hydrogen Phosphate)	0.5
K_2SO_4 (Potassium Sulphate)	1
$CaCl_2.H_2O$ (Calcium Chloride)	0.04
$FeSO_4.7H_2O$ (Ferrous Sulphate)	0.01
$MgSO_4.7H_2O$ (Magnesium Sulphate)	0.2
EDTA	0.8

3.5.2 Modified Media

Besides Zarrouk's media many media have been developed for spirulina culture using different nitrogen source (Madkaur, *et al.,* 2012 , Soni, *et al.,* 2019). In this experiment we applied a modified media using $Ca_2(PO_4)_2$ (Triple Super Phosphate) @ 0.4 g/l instead of K_2HPO_4 (Dipotassium Hydrogen Phosphate) and Citric acid @ 0.8 g/l instead of EDTA. Which are cost effective than Zarrouk's media. EDTA and Citric acid both are used as chelating agent. Shinta *et al.* (2021) stated that EDTA has a high chelating ability for phytoextraction but is difficult to degrade by the environment. While Citric Acid is best for phytostabilization, it is also suitable for phytoextraction. The other advantages of Citric Acid are less harmful to the environment (biodegradable). Celekli *et al*. (2009) used as different phosphate concentrations to Spirulina production.

Table 2. List of Chemicals Used in Modified Media Preparation

Reagent	Amount (g/l)
$NaHCO_3$ (Sodium Bicarbonate)	16.8
$NaNO_3$ (Sodium Nitrate)	2.5
NaCl (Sodium Chloride)	1.0
$Ca_2(PO_4)_2$ (Triple Super Phosphate)	0.4
K_2SO_4 (Potassium Sulphate)	1.0
$CaCl_2.H_2O$ (Calcium Chloride)	0.04
$FeSO_4.7H_2O$ (Ferrous Sulphate)	0.01
$MgSO_4.7H_2O$ (Magnesium Sulphate)	0.20
Citric Acid	0.80

3.6 Water

3.6.1 Freshwater

Running tape water was used as freshwater. Freshwater used after chlorination to remove impurities. For chlorination of water added 0.04 g bleaching powder ($Ca(OCl)_2$) per liter water. After chlorination add 0.02 g Ascorbic acid per liter water.

3.6.2 EcoSolv Water

In this experiment we used EcoSolv water enhancement. EcoSolv water was collected from Horticultural field at Sher-e-bangla Agricultural University, Dhaka. It is a water saving device that improves the penetration rate of water. EcoSolv water enhancement helps shorten the duration of irrigation by at least 10%. It reduces soil salinity; it releases accumulated mineral deposits in the pipes of the irrigation system. Treated water will loosen and minimize mineral deposits in soil. EcoSolv water enhancement introduces a swirling effect to water entering the pipes. The swirling effect will wash away any scaling or organic debris which has accumulated within the system. It improves the wettability of water, enhancing the penetration rate of water to soil by reducing its surface tension. It will enable the same area of field to be watered with less water due to the improved spreading ability of water. It is bio-directional. It is hexagonal in structure because it has six sided molecules. Hexagonal water has a 109.50 angle, a wider angle which creates a 3 dimensional pattern where each water molecules serves as the donor and the acceptor of 2 electrons. According to to Final

Report: EcoSolv Technologies–IIRR (Kharif 2020 & Rabi 2020-21) EcoSolv water increases yield up to 18%.

3.7 Mother Culture

Spirulina strain (*Spirulina platensis*) (Mother culture) was collected from Horticulture Innovation Lab Bd., Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka, Bangladesh. The culture medium in which spirulina grown is consists of water and chemical. In this study we used 1g spirulina strain per liter water.

3.8 Installation of the experiment

At first we rinsed the plastic food grade containers with distilled water. Then the containers were set up carefully within 4 iron rack. Each rack contains 16 containers and each stair contains 4 containers. After the containers set up a 5 feet PVC pipe are installed within the air pump for agitation. 64 silicon pipes were connected to 64 food grade containers from the main PVC pipe for agitation. Then we were prepared culture media for different treatment. This experiment was carried up to 3 cycles of cultivation. After 1^{st} harvest in 2^{nd} and 3^{rd} cycle of cultivation we used 50% nutrients media with the same water.

3.8.1 Culture Media Preparation for T_1 Treatment

To prepared culture media for T_1 treatment EcoSolv water and Zarrouk's media were used and 1g mother culture (Spirulina strain) was used for per liter of water. This experiment was set-up in first rack with 56 L EZ media to 16 containers in four stairs with agitation support by electric motor (1 hp). pH level of the growing culture should be maintained at 9.7 to 10.4.

3.8.2 Culture Media Preparation for T_2 Treatment

EcoSolv water and Modified media were used to prepare culture media and 1g mother culture was added per liter of water. This experiment was set-up in second rack with 56 L EM media to 16 containers in four stairs with agitation support by electric motor (1 hp). pH level of the growing culture should be maintained at 9.6 to 10.2.

3.8.3 Culture Media Preparation for T_3 Treatment

Freshwater and Zarrouk's media were used to prepare culture media and 1g mother culture was used per liter water. This experiment was set-up in the opposite side of the first rack and used 56 L FZ media to 16 containers in four stairs with agitation support by electric motor (1 hp). pH level of the growing culture should be maintained at 9.7 to 10.4.

3.8.4 Culture media Preparation for T_4 Treatment

Freshwater and Modified media were used to prepare culture media and added 1g spirulina strain per liter water. This experiment was set-up in the opposite side of the second rack with 56 L FM media to 16 containers in four stairs with agitation support by electric motor (1 hp). pH level of the growing culture should be maintained at 9.6 to 10.2.

3.9 Aeration System

A proper aeration of spirulina is vital to meet its CO_2 requirement, and also aeration prevents the algae from settling at the bottom of the tank and forming a layer**.** Mechanical stirring or air pumps can be used for aeration. Stirrers should not rotate faster than 20 rpm when used for aeration. If rpm is increased cells may break (Soni *et.al*., 2019). In this experiment aeration is done by air pumps (1hp).

3.10 Harvesting

After 5 to 6 day's when optical density of biomass 1.0 or 1.5 it was ready to harvest. During harvesting we used filtration net (500 meshes) and filter bag (0.02 mm). Mature spirulina containers were hold up on filtration net and filter the spirulina biomass through filter bag from culture media. Filtration net was used to remove oscillatoria from spirulina. After filtration the raw spirulina were put in a clean container and added 10g NaCl per kg raw spirulina to remove unwanted impurities.

3.11 Drying

Dried spirulina are able to reserve several months in air tight container. Drying was done both manually and mechanically. In this experiment we carried this drying process manually.

3.11.1 Manual Drying

In this process at first a clean plastic sheet was placed on the table. After that washed Spirulina was pour into a disposal syringe, then the syringe was pressed over the plastic sheet it makes the Spirulina noodles like shape. These noodles like structure were placed in a room before running fan. It takes to complete dry within 24 to 48 hours. During drying we avoid sun light because radiation of sun light deteriorates the quality.

3.12 Parameters of the Experiment

Data were collected from these following parameters:

1. Optical Density (OD)
2. Productivity
3. Light Intensity
4. Temperature
5. pH
6. Fresh weight (g), Wash weight (g), Dry weight (g), Weight of Oscillatoria (g)

3.12.1 Optical Density (OD)

Spirulina cultivation involves the measurement of the optical density (OD) of cell growth, which can be used to determine the biomass concentration. Optical density was measured through spectrophotometer (model U-2001, Hitachi, Tokyo, Japan) at 570 nm wavelength. An initial blank reading was taken to avoid an error. After the blank reading, test-tube with spirulina samples was placed in the measuring chamber. The sample was diluted properly before it was placed in the measuring chamber in order to prevent deposition of a layer. Two to three repeated measurements were taken for each sample.

3.12.2 Productivity

Productivity was calculated using the following equation, $P_x = (X_m - X_i)T_c^{-1}$

Where,

P_x = Productivity ($gL^{-1}day^{-1}$)

X_i = Initial biomass concentration (gL^{-1})

X_m= Maximum biomass concentration (gL^{-1})

T_c = Cultivation time related to the maximum biomass concentration (days) (Danesi *et al.*, 2011)

3.12.3 Light Intensity

Light meter was used to measure light intensity by using a conversion factor of 12 $\mu Em^{-2}s^{-1}klx^{-1}$ (Sassano *et al.* 2010). Light is essential for spirulina growth and development. During this experiments period average light intensity range 1000 lux to 1500 lux were captured by Light meter. For spirulina production the best range of light intensity is 1500 lux to 4500 lux (Sukenik *et al.,* 1991).

3.12.4 Temperature

Cultures of *Spirulina platensis* were grown at temperatures between 20°C and 45°C (at 5°C intervals). During this experiment temperature was captured 20°C to 25°C by thermometer. Growth kinetics showed a wide range of temperature tolerance, ranging from 20 °C to 40 °C. The highest growth rate was observed at a temperature of 35 °C, with the highest chlorophyll and phytobiliprotein accumulation. (Kumar *et al.*, 2011).

3.12.5 pH

Optimum temperature for Spirulina production is 8.5 to 10.5 (Soni, *et al.*, 2019). In this study we maintained pH level 9.6 to 10.4 and pH level was measured regularly by pH meter.

3.12.6 Biomass Weight

3.12.6.1 Fresh Weight (g)

After harvesting raw spirulina called fresh spirulina. Fresh weight was taken in gram (g) in digital balance.

3.12.6.2 Wash Weight (g)

After harvest fresh spirulina was washed by 10% NaCl solution. Wash weight was taken in gram (g) in digital balance.

3.12.6.3 Dry Weight (g)

After washing fresh Spirulina in NaCl solution then it is placed for drying. Drying are done both manually and mechanically. Dry weight was taken in gram (g) in digital balance.

3.12.6.4 Weight of Oscillatoria (g)

Oscillatoria, a ubiquitous group of bacteria found in freshwater systems worldwide, cause illness and even death in humans and animals. Oscillatoria is a weed in Spirulina culture. Cultural media that are less prone to Oscillations are more productive. Weight of Oscillatoria was taken in gram (g) in digital balance.

3.13 Statistical Analysis

Data on the different attributes were statistically analyzed using Statistix-10 computer software, and the differences between varieties and treatments were assessed with the Least Significant Difference (LSD) test at 5% significance level.

3.14 Economic Analysis

To find the most economical approach to production, the cost of production was analyzed. The cost and return calculations were based on current market prices for spirulina biomass and chemicals.

Benefit cos ratio was calculated from following this formula,

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

(a)

(b)

(c)

(d)

(e)

(f)

Plate 1: Pictorial presentation of experimental setup (a) Iron stand, (b) Air pump setup, (c and d) Drilling container and PVC pipe, (e and f) Container setup.

(a)

(b)

(c)

(d)

(e)

(f)

Plate 2: Pictorial presentation of culture media preparation and Installation (a and b) Chemical of Zarrouk's media and Modified media, (c and d) Chemical measurement and mixing, (e) Mother culture mixing on culture media, (f) Experiment setup.

(a)

(b)

(c)

(d)

(e)

(f)

Plate 3: Pictorial presentation of data collection and harvesting (a) Installation of experiment, (b and c) Data collection, (d and e) Harvesting net setup and harvesting, (f) Fresh harvested Spirulina

(a)

(b)

(c)

(d)

(e)

(f)

Plate 4: Pictorial presentation of drying and processing of Spirulina (a and b) Manual drying, (c) Fresh dried Spirulina, (d and e) Grinding and filtered of Spirulina, (f) Spirulina powder.

CHAPTER IV

RESULTS AND DISCUSSIONS

The aim of the experiment is to initiate advanced spirulina production with zarrouks media and low cost modified media to determine the suitable cost effective media for both small scale and commercial production. In this experiment, we used different culture media and water bodies. Results of the research work are reported in this chapter. Throughout this chapter, tables and figures were emphasized to enhance their parallel and dissimilar characteristics through explanation, comprehension, and perception. An analysis of variances for all parameters has been summarized in the appendix. Results have been presented, discussed and possible interpretations are given under the following headings.

4.1 Optical Density (OD)

Significant variation was observed among the treatment in case of optical density at maturity (Appendix I). The highest optical density (2.38) was recorded from T_1 treatment followed by T_2 (2.09) and the lowest value (1.72) was observed in T_3 treatment (Fig.1) at 8 days after installation in 1^{st} cycle of cultivation. In 9^{th} day optical density start decreases gradually. According to Caturwati *et al.* (2020) the highest optical density was observed in 6^{th}, 7^{th} and 10^{th} days after cultivation from different treatment. This experiment was conducted on different culture media and water bodies which causes the variation of nutrients status in the growth media. Thus, this is in accordance with the statement of Widianingsih (2008) that differences in cell density are caused by differences in nutrient content in growth media.

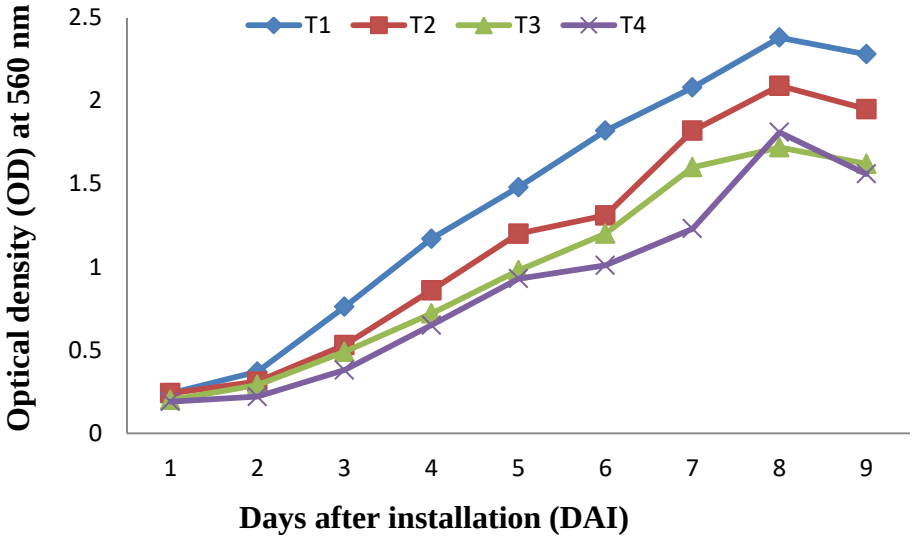


Fig.1. Optical density at different days after installation in 1st cycle of cultivation

(*Here, T_1 = EcoSolv water + Zarrouk's media, T_2 = EcoSolv water + Modified media, T_3 = Freshwater + Zarrouk's media, T_4 = Freshwater + Modified media)

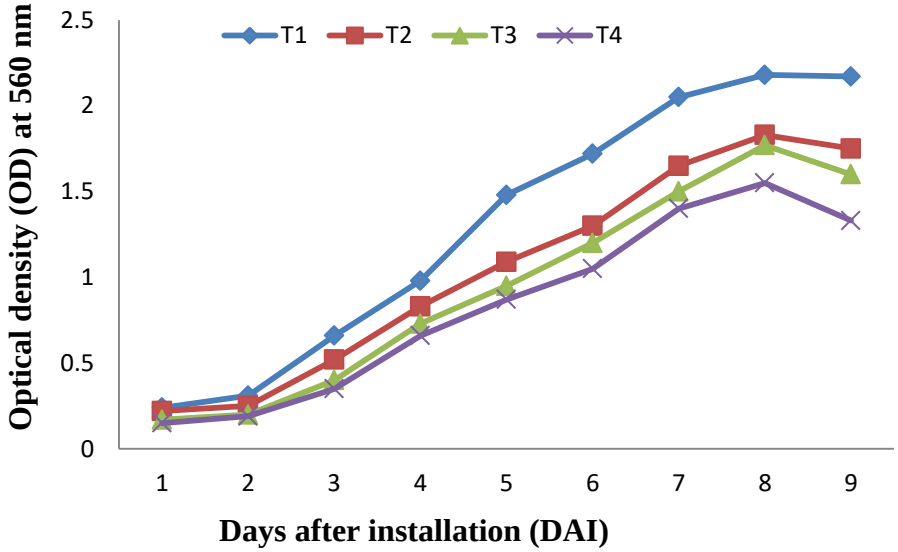


Fig.2. Optical density at different days after installation in 2nd cycle of cultivation

(*Here, T_1 = EcoSolv water + Zarrouk's media, T_2 = EcoSolv water + Modified media, T_3 = Freshwater + Zarrouk's media, T_4 = Freshwater + Modified media)

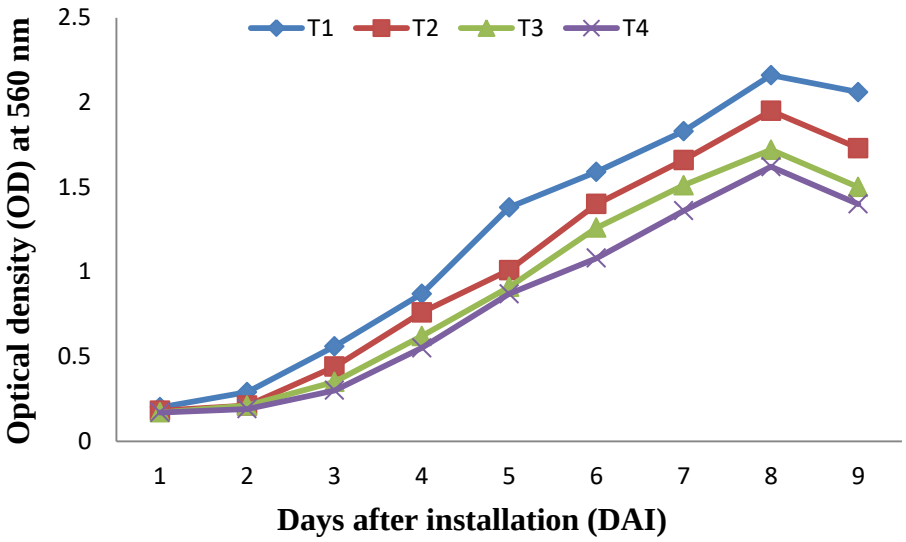


Fig.3. Optical density at different days after installation in 3rd cycle of cultivation

(*Here, T_1 = EcoSolv water + Zarrouk's media, T_2 = EcoSolv water + Modified media, T_3 = Freshwater + Zarrouk's media, T_4 = Freshwater + Modified media)

Significant difference was observed among different treatment in 2nd cycle of cultivation (Appendix II) and 3rd cycle of cultivation (Appendix III). After first harvest in 2nd cycle of cultivation the highest optical density (2.18) was observed in T_1 treatment followed by T_2 (1.83) and lowest value (1.55) was observed in T_4 treatment (Fig.2) at 8th days after cultivation where in 1st cycle of cultivation the lowest value was found in T_3 treatment. In (Fig.3) the highest value of optical density (2.16) was observed in T_1 treatment followed by T_2 (1.95) and lowest value (1.62) was observed in T_4 treatment. In compare to 1st cycle of cultivation (Fig.1) optical density was comparatively low in 2nd and 3rd cycle of cultivation (Fig.2, Fig.3).

4.2 Cell Productivity

Cell productivity was significantly influenced by different treatments. Cell productivity calculated based on optical density. The highest cell productivity (0.26 g/l/day) was measured in T_1 treatment followed by T_2 (0.23 g/l/day) and the lowest cell productivity (0.19 g/l/day) was observed in T_3 treatment (Fig.4) in first cycle of cultivation.

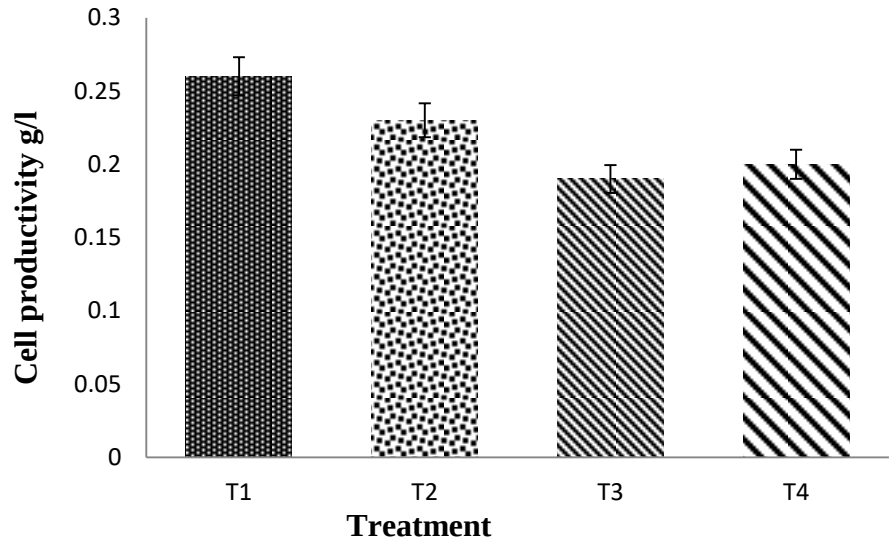


Fig.4. Cell productivity of Spirulina on different treatment in 1st cycle of cultivation

(*Here, T_1 = EcoSolv water + Zarrouk's media, T_2 = EcoSolv water + Modified media, T_3 = Freshwater + Zarrouk's media, T_4 = Freshwater + Modified media)

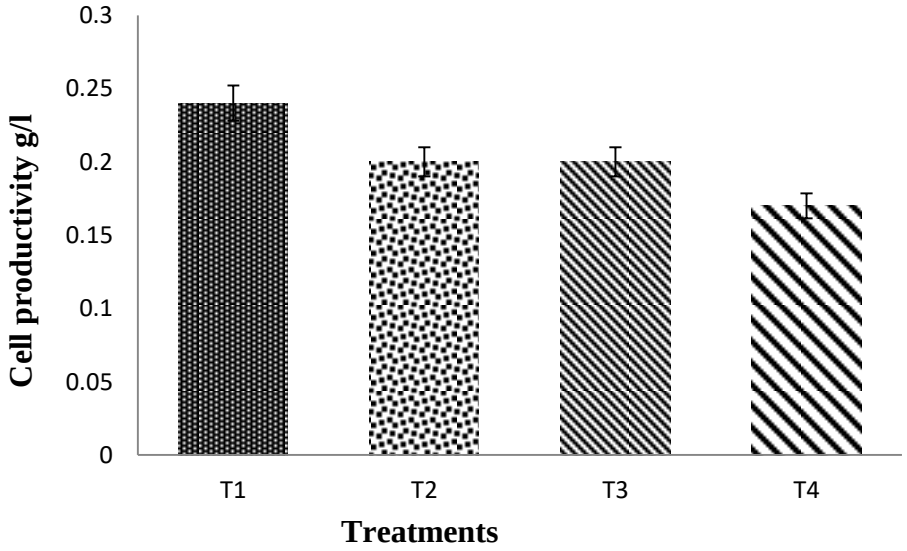


Fig.5. Cell productivity of Spirulina on different treatments in 2nd cycle of cultivation

(*Here, T_1 = EcoSolv water + Zarrouk's media, T_2 = EcoSolv water + Modified media, T_3 = Freshwater + Zarrouk's media, T_4 = Freshwater + Modified media)

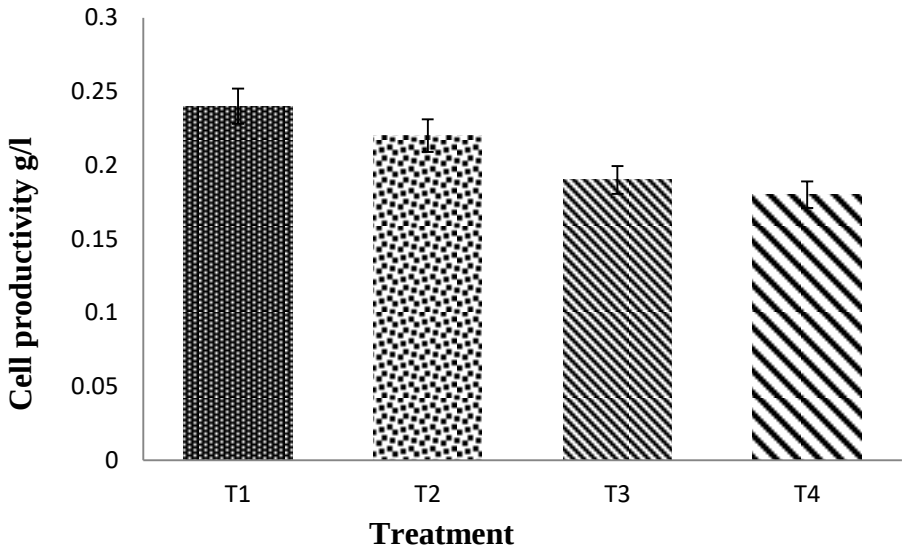


Fig.6. Cell productivity of Spirulina on different treatments in 3rd cycle of cultivation (*Here, T_1 = EcoSolv water + Zarrouk's media, T_2 = EcoSolv water + Modified media, T_3 = Freshwater + Zarrouk's media, T_4 = Freshwater + Modified media)

Significant variation was observed on cell productivity in 2nd and 3rd cycle of cultivation. In 2nd cycle of cultivation the highest value of cell productivity (0.24 g/l/day) was observed in T_1 treatment and the lowest value (0.17 g/l/day) was observed in T_4 treatment where T_2 and T_3 treatment give similar result (0.20 g/l/day) (Fig.5). In 3rd cycle of cultivation highest value of cell productivity (0.24 g/l/day) was observed in T_1 treatment followed by T_2 (0.22 g/l/day) and lowest value of productivity (0.18 g/l/day) was observed in T_4 treatment.

4.3. Effect of Different Treatments on pH Value

pH level was significantly different from media to media in 1st ,2nd and 3rd cycle of cultivation (Appendix IV,V,VI). In first cycle of cultivation pH level was higher in T_1 and T_3 treatments than T_2 and T_4 treatments (Fig.1). Initial pH level of T_1 (9.73), T_2 (9.65), T_3 (9.69) and T_4 (9.63) which remain same up to 2 days after cultivation and then gradually increases up to 6th days after cultivation. The highest pH value was observed in T_1 (10.37) at 6th day followed by T_3 (10.33) where T_2 (10.16) and T_4 (10.15). After 6 days the pH value was decreased gradually. In T_1 and T_3 treatments contained same nutrients media (Zarrouk's media) with different water bodies and also in T_2 and T_4 treatments used same nutrients media (Modified media) with different water bodies. Probably nutrients media affect pH level that's why T_1, T_3 and

T_2, T_4 treatments give nearly similar pH value (Fig.1). Similar result was observed by (Uddin *et al*., 2021 and Gami *et al*., 2011). According to Belkin and Boussiba (1991) optimum growth of Spirulina was observed in pH range 9 to 10 and minimum growth was observed in pH 7.0. At pH 11.5 growth rate decreases gradually.

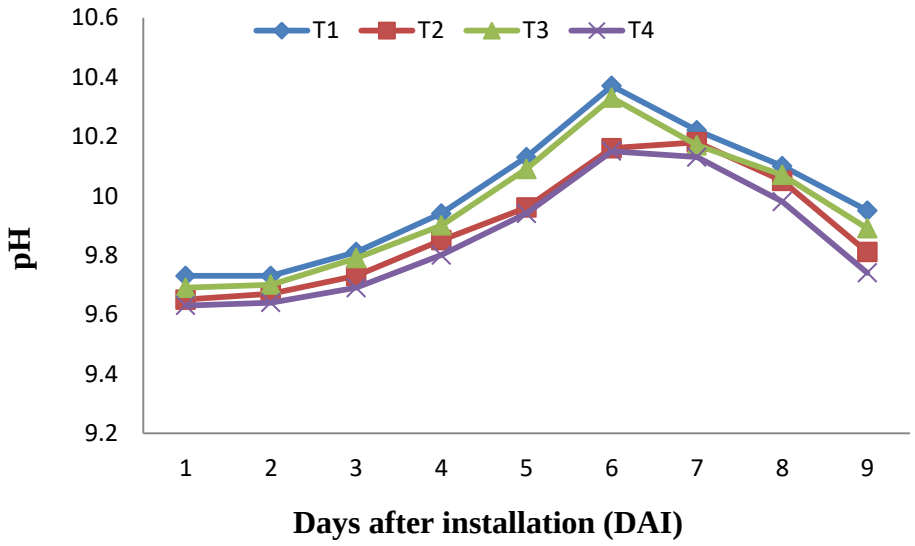


Fig.7. pH level on different treatments in 1st cycle of cultivation

(*Here, T_1 = EcoSolv water + Zarrouk's media, T_2 = EcoSolv water + Modified media, T_3 = Freshwater + Zarrouk's media, T_4 = Freshwater + Modified media)

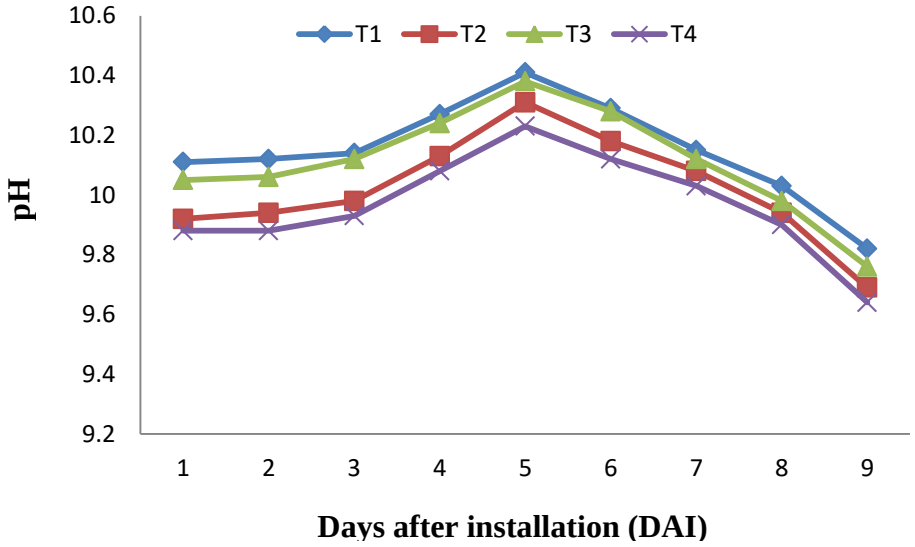


Fig.8. pH level on different treatments in 2nd cycle of cultivation

(*Here, T_1 = EcoSolv water + Zarrouk's media, T_2 = EcoSolv water + Modified media, T_3 = Freshwater + Zarrouk's media, T_4 = Freshwater + Modified media)

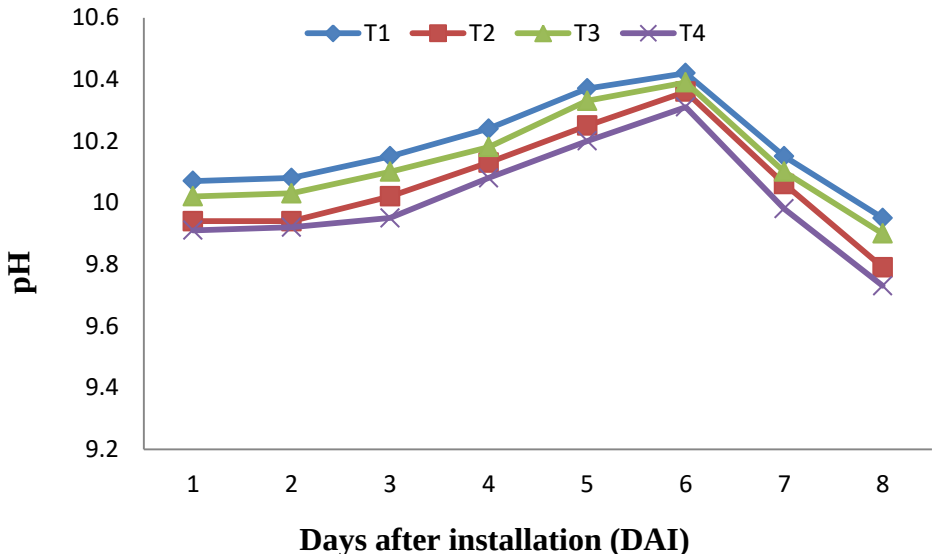


Fig.9. pH level on different treatments in 3rd cycle of cultivation

(*Here, T_1 = EcoSolv water + Zarrouk's media, T_2 = EcoSolv water + Modified media, T_3 = Freshwater + Zarrouk's media, T_4 = Freshwater + Modified media)

In 2nd and 3rd cycle of cultivation (Fig.8 and Fig.9) pH values were different from media to media in the same pattern of Fig.7. During 2nd cycle and 3rd cycle of cultivation initial pH of T_1 and T_3 was started above 10 (Fig.8 and Fig.9) where in 1st cycle of cultivation initial pH of T_1 and T_3 was above 9. Due to high initial pH 2nd cycle and 3rd cycle of cultivation was comparatively less productive than 1st cycle of cultivation. According to Belkin and Boussiba (1991) when the pH of the cultivation medium rises, it inhibits *S. platesnsis* growth, so pH control is critical for improving biomass yield. Spirulina species are capable of maintaining a constant pH inside their bodies. Schlesinger *et al*. (1996) stated that an external pH of more than 10.5 caused the slow collapse of internal pH homeostasis, especially when sodium was depleted.

4.4. Effect of Different Treatments on Biomass Weight

Biomass weight like fresh weight, wash weight and dry weight were significantly different from treatment to treatment (Appendix VII, VIII and IX). In 1st cycle of cultivation (Table 3) the highest fresh weight (127.05 g) and dry weight (31.10 g) was observed in T_1 treatment followed by fresh weight (121.05 g) and dry weight (27.20 g) in T_2 treatment. The lowest fresh weight was observed in T_3 (102.82 g) and T_4 (105.45 g) which are statistically similar in result (Table 3). The lowest dry weight

was observed in T_3 (23.52 g) and T_4 (24.27 g) (Table 3). The highest Oscillatoria was observed in T_3 (12.65 g) and T_4 showed intermediate result (8.92 g), where T_1 and T_2 showed statistically similar in result (Table 3). Among the 4 treatments EcoSolv water and Zarrouk's media (T_1) showed the highest result followed by EcoSolv water and Modified media (T_2). According to Final Report: EcoSolv Technologies–IIRR (Kharif 2020 & Rabi 2020-21) Use of EcoSolv equipment and EcoAgra product significantly enhanced the growth parameters and grain yield (up to 18%).The both treatments were found promising in enhancing water productivity. Uddin *et al*. (2020) stated that Zarrouk's medium is the standard media and give higher yield than modified media.

Table 3. Performance of different treatments on fresh weight, wash weight, dry weight and weight of Oscillatoria in 1st cycle of cultivation

Treatments	Fresh weight (g)	Wash weight (g)	Dry weight (g)	Weight of Oscillatoria (g)
T_1	127.05 a	103.75 a	31.10 a	6.87 c
T_2	121.05 b	98.63 b	27.20 b	6.40 c
T_3	102.82 c	87.65 d	23.52 c	12.65 a
T_4	105.45 c	92.60 c	24.27 bc	8.92 b
CV %	2.20	2.21	7.69	6.52
$LSD_{(0.05)}$	4.00	3.38	3.26	0.91

*Here, T_1 = EcoSolv water + Zarrouk's media, T_2 = EcoSolv water + Modified media, T_3 = Freshwater + Zarrouk's media, T_4 = Freshwater + Modified media
**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.

Table 4. Performance of different treatments on fresh weight, wash weight, dry weight and weight of Oscillatoria in 2nd cycle of cultivation

Treatments	Fresh weight (g)	Wash weight (g)	Dry weight (g)	Weight of Oscillatoria (g)
T_1	121.65 a	98.07 a	29.12 a	8.85 c
T_2	115.70 b	95.17 b	26.85 b	9.50 bc
T_3	102.60 c	91.27 c	23.32 c	10.15 ab
T_4	97.70 d	88.77 d	19.92 d	11.30 a
CV %	1.94	1.05	4.65	7.89
$LSD_{(0.05)}$	3.39	1.57	1.84	1.25

*Here, T_1 = EcoSolv water + Zarrouk's media, T_2 = EcoSolv water + Modified media, T_3 = Freshwater + Zarrouk's media, T_4 = Freshwater + Modified media
**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.

In 2nd cycle of cultivation the highest fresh weight was observed in T_1 (121.65 g) followed by T_2 (115.70 g) and the lowest fresh weight was observed in T_4 (97.70 g) and T_3 showed intermediate result (Table 4). In case of dry weight the highest result was found in T_1 (29.12 g) followed by T_2 (26.85 g) and the lowest result was found in T_4 (19.92 g) where T_3 showed intermediate result. The highest Oscillatoria was observed in T_4 (11.30 g) and the lowest result was observed in T_1 (8.85 g), where T_2 and T_3 was statistically similar in result (Table 4).

Table 5. Performance of different treatments on fresh weight, wash weight, dry weight and weight of Oscillatoria in 3rd cycle of cultivation

Treatments	Fresh weight (g)	Wash weight (g)	Dry weight (g)	Weight of Oscillatoria (g)
T_1	122.58 a	97.65 a	28.60 a	9.70 d
T_2	113.35 b	94.65 b	24.65 b	10.82 c
T_3	100.90 c	89.65 c	22.77 c	11.45 b
T_4	96.55 d	88.20 c	20.50 d	13.02 a
CV %	1.29	1.09	2.33	3.27
$LSD_{(0.05)}$	2.22	1.60	0.90	0.59

*Here, T_1 = EcoSolv water + Zarrouk's media, T_2 = EcoSolv water + Modified media, T_3 = Freshwater + Zarrouk's media, T_4 = Freshwater + Modified media
**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

In 3rd cycle of cultivation the highest fresh weight was observed in T_1 (122.58 g) followed by T_2 (113.35 g) and the lowest fresh weight was observed in T_4 (96.55 g) and T_3 showed intermediate result (Table 5). In case of dry weight the highest result was found in T_1 (28.60 g) followed by T_2 (24.65 g) and the lowest result was found in T_4 (20.50 g) where T_3 showed intermediate result. The highest Oscillatoria was observed in T_4 (13.02 g) and the lowest result was observed in T_1 (9.70 g), where T_2 and T_3 showed statistically similar in result (Table 5).

In 1st, 2nd and 3rd cycle of cultivation (Table 3, 4, 5) it was observed that Spirulina yield was vice-versa to Oscillatoria present. So, it is suggested that higher amount of Oscillatoria growth inhibit Spirulina production.

4.5 Total Dry Weight (g)

Total cultivation was done in 224 liter culture media and each treatment was cultured in 56 liter culture media at 9 days after installation harvesting was done. In 1st cycle of cultivation the highest dry weight (124.4 g or 2.22 g/l) was observed in T_1 treatment followed by T_2 (108.8 g or 1.94 g/l) and the lowest dry weight was found in T_3 (94.1 g or 1.68 g/l) treatment where T_4 (97.4 g or 1.73g/l) showed intermediate result. During 2nd and 3rd cycles of cultivation the highest dry weight (116.5 g or 2.08 g/l and 114.4 g or 2.04 g/l) was observed in T_1 treatment followed by T_2 (107.4 g or 1.91 g/l and 98.6 g or 1.76 g/l) and the lowest dry weight (79.7 g or 1.42 g/l and 82 g or 1.46 g/l) was found in T_4 treatment, where T_3 (93.3 g or 1.66 g/l and 91.1 g or 1.62 g/l) showed intermediate in result. All 3 cycles T_1 showed the highest result. Dineshkumar *et al*. (2016) found 1.86 g/l dry weight in Zarrouk's medium.

4.6 Economic Analysis

From installation to harvesting, all costs relating to motor, tank, box stand, silicon pipes, nutrient medium, equipment, electricity, and manpower were calculated and converted into cost per 1000 L volume (Appendix X, XI, XII, XIII). However, all the input costs were capital costs. Consequently, in this study all costs were omitted because they had already been used and will be used in the future. Thus, for a 1000 liter volume, only electricity, labor costs, nutrients, and inoculum costs were calculated. Spirulina inoculum and nutrient price as well as dry weight of spirulina biomass were determined by market price (Table 6, 7 and 8). A detailed economic analysis follows under the following headings:

Table 6. Cost and return of spirulina cultivation as influenced by different treatments in 1st cycle of cultivation

Treatment	Total Cost/1000 L (Tk.)	Yield/1000 L (Kg)	Gross return (10000 Tk./Kg)	Net Return (Tk.)	Beneficial Cost Ratio (BCR)
T_1	11570	2.22	22200	10630	1.91
T_2	8200	1.92	19200	11000	2.34
T_3	12365	1.68	16800	4435	1.35
T_4	8995	1.74	17400	8405	1.93

*Here, T_1 = EcoSolv water + Zarrouk's media, T_2 = EcoSolv water + Modified media, T_3 = Freshwater + Zarrouk's media, T_4 = Freshwater + Modified media

Table 7. Cost and return of spirulina cultivation as influenced by different treatments in 2nd cycle of cultivation

Treatment	Total Cost/1000 L (Tk.)	Yield/1000 L (Kg)	Gross return (10000 Tk./Kg)	Net Return (Tk.)	Beneficial Cost Ratio (BCR)
T_1	5885	2.08	20800	14915	3.53
T_2	4200	1.91	19100	14900	4.54
T_3	5885	1.66	16600	10715	2.82
T_4	4200	1.42	14200	10000	3.38

*Here, T_1 = EcoSolv water + Zarrouk's media, T_2 = EcoSolv water + Modified media, T_3 = Freshwater + Zarrouk's media, T_4 = Freshwater + Modified media

Table 8. Cost and return of spirulina cultivation as influenced by different treatments in 3rd cycle of cultivation

Treatment	Total Cost/1000 L (Tk.)	Yield/1000 L (Kg)	Gross return (10000 Tk./Kg)	Net Return (Tk.)	Beneficial Cost Ratio (BCR)
T_1	5885	2.04	20400	14515	3.46
T_2	4200	1.76	17600	13400	4.19
T_3	5885	1.62	16200	10315	2.75
T_4	4200	1.46	14600	10400	3.47

*Here, T_1 = EcoSolv water + Zarrouk's media, T_2 = EcoSolv water + Modified media, T_3 = Freshwater + Zarrouk's media, T_4 = Freshwater + Modified media

Ascorbic acid and bleaching powder were used in the 1st cycle of cultivation to purify freshwater, where EcoSolv water is not required to be purified. During the 2nd and 3rd cycles of cultivation, the same water was used with 50% of the culture media, which means that the freshwater is not required to be purified in the 2nd and 3rd cycles. This reduces the input cost.

4.7 Gross Return

The highest gross return was estimated (22200 Tk., 20800 Tk. and 20400 Tk.) in T_1 treatment followed by T_2 (19200 Tk., 19100 Tk. and 17600 Tk.) in 1st, 2nd and 3rd cycle of cultivation (Table 6, 7, 8). Where the lowest gross return was calculated (16800 Tk.) in T_3 treatment in 1st cycle of cultivation and the lowest gross return for 2nd and 3rd cycles of cultivation was calculated in T_4 treatments (14200 Tk. and 14600 Tk.) (Table 6, 7, 8).

4.8 Net Return

The highest net return was estimated (11000 Tk.) in T_2 treatment (Table 6) in 1st cycle of cultivation. In 2nd and 3rd cycles of cultivation highest net return was calculated (14915 Tk. and 14515 Tk.) in T_1 treatment (Table 7 and 8). Where the lowest net return was calculated T_3 (4435 Tk.) in 1st cycle of cultivation (Table 6), T_4 (10000 Tk.) in 2nd cycle of cultivation (Table 7), T_4 (10315 Tk.) in 3rd cycle of cultivation (Table 8).

4.9 Benefit Cost Ratio (BCR)

The highest benefit cost ratio was observed in T_2 treatment (2.34, 4.54 and 4.19) in 1st, 2nd and 3rd cycles of cultivation (Table 6, 7 and 8) and the lowest benefit cost ratio was noted in T_1 (1.91) in 1st cycle of cultivation (Table 6), in 2nd cycle of cultivation T_3 (2.82) (Table 7) and in 3rd cycle of cultivation T_3 (2.75) (Table 8). In every cycle of cultivation T_2 treatment showed the highest benefit cost ratio although T_1 treatment gave the highest yield. No matter whether the highest yield is obtained with the T_1 treatment or the T_2 treatment, the T_2 treatment consistently had the best benefit-cost ratio. Similar result was observed by Madkaur *et al*., (2012). So,EcoSolv water + Modified media (T_2) more beneficial over EcoSolv water + Zarrouk's media (T_1).

CHAPTER V

SUMMARY AND CONCLUSION

Summary

Spirulina is micro filamentous blue green algae. It is used as high protein food source from Aztec civilization. Now-a-days Spirulina are cultivated all over the world in different culture media in different methods like open pond system, polythene tunnel and different close photobioreactor. Zarrouk's media used as a standard media in spirulina cultivation from 1966. In condition of Bangladesh some chemicals of zarrouk's media are highly expensive and sometimes not available in our local market. In this study we developed a cost effective media and evaluate the growth performance and economic analysis over standard zarrouk's media. To formulate a low cost medium for commercial spirulina production an experiment was conducted on the rooftop at the Academic Building, Sher-e-Bangla Agricultural University, Dhaka-1207 during November 2019 to March 2020. It was a single factor experiment having four treatments i.e. EcoSovl. water + Zarrouk's media (T_1), EcoSolv water + Modified media (T_2), Freshwater + Zarrouk's media (T_3), Freshwater + Modified media (T_4). These experiments carried up to 3rd cycles of cultivation and in 2nd and 3rd cycles of cultivation used 50% nutrients media with reuse the water. The experiment was outlined in Completely Randomized Design (CRD) with the four replications. Collected data were statistically analyzed for the evaluation of treatments to the selection of best culture media which is cost-effective. Summary of the experiment are described below:

The highest optical density (2.38) was observed in T_1 treatment followed by T_2 (2.09) and the lowest value (1.72) was observed in T_3 treatment. During 2nd cycle of cultivation the highest optical density (2.18) was observed in T_1 treatment followed by T_2 (1.83) and the lowest value (1.55) was observed in T_4 treatment. In 3rd cycle of cultivation the highest value of optical density (2.16) was observed in T_1 treatment followed by T_2 (1.95) and the lowest value (1.62) was observed in T_4 treatment.

The highest cell productivity (0.26 g/l/day) was observed in T_1 treatment followed by T_2 (0.23 g/l/day) and the lowest cell productivity (0.19 g/l/day) was observed in T_3 treatment in first cycle of cultivation. In 2nd cycle of cultivation the highest value of

cell productivity (0.24 g/l/day) was observed in T_1 treatment and the lowest value (0.17 g/l/day) was observed in T_4 treatment where T_2 and T_3 treatment give similar result (0.20 g/l/day). In 3rd cycle of cultivation highest value of cell productivity (0.24 g/l/day) was observed in T_1 treatment followed by T_2 (0.22 g/l/day) and the lowest value of productivity (0.18 g/l/day) was observed in T_4 treatment.

In first cycle of cultivation pH level was higher in T_1 and T_3 treatments than T_2 and T_4 treatments. Initial pH level of T_1 (9.73), T_2 (9.65), T_3 (9.69) and T_4 (9.63) and the highest pH value was observed in T_1 (10.37) at 6th day followed by T_3 (10.33) where T_2 (10.16) and T_4 (10.15). During 2nd cycle and 3rd cycle of cultivation initial pH of T_1 and T_3 was started above 10 where in 1st cycle of cultivation initial pH of T_1 and T_3 was above 9. Due to high initial pH 2nd cycle and 3rd cycle of cultivation was comparatively less productive than 1st cycle of cultivation.

In 1st cycle of cultivation the highest fresh weight (127.05 g) and dry weight (31.10 g) was observed in T_1 treatment followed by fresh weight (121.05 g) and dry weight (27.20 g) in T_2 treatment. The lowest fresh weight was observed in T_3 (102.82 g) and T_4 (105.45 g) which are statistically similar in result. The lowest dry weight was observed in T_3 (23.52 g) and T_4 (24.27 g). The highest Oscillatoria was observed in T_3 (12.65 g) and T_4 showed intermediate result (8.92 g), where T_1 and T_2 showed statistically similar in result. In 2nd cycle of cultivation the highest fresh weight was observed in T_1 (121.65 g) followed by T_2 (115.70 g) and lowest fresh weight was observed in T_4 (97.70 g) and T_3 showed intermediate result. In case of dry weight the highest result was found in T_1 (29.12 g) followed by T_2 (26.85 g) and the lowest result was found in T_4 (19.92 g) where T_3 showed intermediate result. The highest Oscillatoria was observed in T_4 (11.30 g) and the lowest result was observed in T_1 (8.85 g), where T_2 and T_3 was statistically similar in result. In 3rd cycle of cultivation the highest fresh weight was observed in T_1 (122.58 g) followed by T_2 (113.35 g) and the lowest fresh weight was observed in T_4 (96.55 g) and T_3 showed intermediate result. In case of dry weight the highest result was found in T_1 (28.60 g) followed by T_2 (24.65 g) and the lowest result was found in T_4 (20.50 g) where T_3 showed intermediate result. The highest Oscillatoria was observed in T_4 (13.02 g) and the lowest result was observed in T_1 (9.70 g), where T_2 and T_3 showed statistically similar in result.

In 1st cycle of cultivation the highest dry weight (2.22 g/l) was observed in T_1 treatment followed by T_2 (1.94 g/l) and the lowest dry weight was found in T_3 (1.68 g/l) treatment where T_4 (1.73g/l) showed intermediate result. During 2nd and 3rd cycles of cultivation the highest dry weight (2.08 g/l and 2.04 g/l) was observed in T_1 treatment followed by T_2 (1.91 g/l and 1.76 g/l) and the lowest dry weight (1.42 g/l and 1.46 g/l) was found in T_4 treatment, where T_3 (1.66 g/l and 1.62 g/l) showed intermediate in result. All 3 cycles T_1 showed the highest result.

The highest gross return was estimated (22200 Tk, 20800 Tk and 20400 Tk) in T_1 treatment followed by T_2 (19200 Tk, 19100 Tk and 17600 Tk) in 1st, 2nd and 3rd cycle of cultivation. Where the lowest gross return was calculated (16800 Tk) in T_3 treatment in 1st cycle of cultivation and the lowest gross return for 2nd and 3rd cycles of cultivation was calculated in T_4 treatments (14200 Tk and 14600 Tk).

The highest net return was estimated (11000 Tk) in T_2 treatment in 1st cycle of cultivation. In 2nd and 3rd cycles of cultivation the highest net return was calculated (14915 Tk and 14515 Tk) in T_1 treatment. Where the lowest net return was calculated T_3 (4435 Tk) in 1st cycle of cultivation, T_4 (10000 Tk) in 2nd cycle of cultivation, T_4 (10315 Tk) in 3rd cycle of cultivation.

The highest benefit cost ratio was observed in T_2 treatment (2.34, 4.54 and 4.19) in 1st, 2nd and 3rd cycles of cultivation and the lowest benefit cost ratio was noted in T_3 treatment (1.35, 2.82 and 2.75) in1st, 2nd and 3rd cycles of cultivation . In every cycle of cultivation T_2 treatment showed the highest benefit cost ratio although T_1 treatment gives highest yield.

Conclusion

From the above result, it can be concluded that EcoSolv water + Zarrouk's media (T_1) showed the highest yield in terms of productivity and dry weight (2.22 g/l, 2.08g/l and 2.04g/l) in 1st, 2nd and 3rd cycles of cultivation following (T_2) EcoSolv water + Modified media (1.94 g/l, 1.91 g/l and 1.76 g/l) where the lowest productivity was found in Freshwater + Zarrouk's media (T_4). Furthermore in case of benefit cost ratio EcoSolv water + Modified media (T_2) showed the highest BCR (2.34, 4.54 and 4.19) over T_1 treatments (1.91, 3.53 and 3.46) and the lowest BCR was observed in T_3

treatment in 1st, 2nd and 3rd cycles of cultivation. Looking upon the above circumstance, it can be enunciated that T_2 treatment was the most cost effective media for commercial Spirulina production.

Recommendations

Based on the findings of the present research, such two recommendations can be made:

1. Application of modified media would be the potential for minimizing the production cost.
2. EcoSolv water will be more suitable in compare to freshwater.
3. It could also be conducted in other locations to facilitate drawing valid conclusions.

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APPENDICES

Appendix I. Analysis of variance on optical density (OD) at different DAI in first cycle of cultivation

Source of variation	Degrees of freedom	Mean Square for optical density (OD)								
		1st	2nd	3rd	4th	5th	6th	7th	8th	9th
Factor A (Treatment)	3	0.003*	0.014*	0.10*	0.214*	0.250*	0.484*	0.513*	0.365*	0.437*
Error	9	0.00	0.00001	0.00	0.00006	0.0001	0.0001	0.002	0.0005	0.002

***: Significant at 0.05 level of probability**

Appendix II. Analysis of variance on optical density (OD) at different DAI in 2nd cycle of cultivation

Source of variation	Degrees of freedom	Mean Square for optical density (OD)								
		1st	2nd	3rd	4th	5th	6th	7th	8th	9th
Factor A (Treatment)	3	0.007*	0.011*	0.075*	0.076*	0.297*	0.322*	0.332*	0.273*	0.497*
Error	9	0.0001	0.00008	0.00004	0.0003	0.0001	0.0002	0.001	0.001	0.0003

***: Significant at 0.05 level of probability**

Appendix III. Analysis of variance on optical density (OD) at different DAI in 3rd cycle of cultivation

Source of variation	Degrees of freedom	Mean Square for optical density (OD)								
		1st	2nd	3rd	4th	5th	6th	7th	8th	9th
Factor A (Treatment)	3	0.0009*	0.008*	0.052*	0.079*	0.215*	0.185*	0.161*	0.234*	0.342*
Error	9	0.0	0.0001	0.0003	0.0003	0.0002	0.0009	0.0005	0.0003	0.0003

***: Significant at 0.05 level of probability**

Appendix IV. Analysis of variance on pH level at different DAI in 1st cycle of cultivation

Source of variation	Degrees of freedom	Mean Square for pH level								
		1st	2nd	3rd	4th	5th	6th	7th	8th	9th
Factor A (Treatment)	3	0.0074*	0.0063*	0.0116*	0.0145*	0.0382*	0.0523*	0.0058*	0.0117*	0.0328*
Error	9	0.0	0.00002	0.0005	0.0006	0.0008	0.0015	0.0004	0.0009	0.0005

***: Significant at 0.05 level of probability**

Appendix V. Analysis of variance on pH level at different DAI in 2nd cycle of cultivation

Source of variation	Degrees of freedom	Mean Square for pH level								
		1st	2nd	3rd	4th	5th	6th	7th	8th	9th
Factor A (Treatment)	3	0.0453*	0.0508*	0.0427*	0.0315*	0.0266*	0.0259*	0.0103*	0.0110*	0.0235*
Error	9	0.00001	0.0005	0.0005	0.00005	0.00007	0.0006	0.00008	0.00001	0.0013

***: Significant at 0.05 level of probability**

Appendix VI. Analysis of variance on pH level at different DAI in 3rd cycle of cultivation

Source of variation	Degrees of freedom	Mean Square for pH level								
		1st	2nd	3rd	4th	5th	6th	7th	8th	9th
Factor A (Treatment)	3	0.0211*	0.0243*	0.0326*	0.0198*	0.0221*	0.0102*	0.0283*	0.0216*	0.0415*
Error	9	0.00001	0.00004	0.00011	0.00002	0.0002	0.00006	0.0001	0.00018	0.00017

***: Significant at 0.05 level of probability**

Appendix VII. Analysis of variance on different biomass weight performance in 1st cycle of cultivation

Source of variation	Degrees of freedom	Mean Square for biomass weight			
		Fresh weight	Wash weight	Dry weight	Weight of Oscillatoria
Factor A (Treatment)	3	557.27*	197.01*	47.26*	32.36*
Error	9	6.28	4.47	4.16	0.322

***: Significant at 0.05 level of probability**

Appendix VIII. Analysis of variance on different biomass weight performance in 2nd cycle of cultivation

Source of variation	Degrees of freedom	Mean Square for biomass weight			
		Fresh weight	Wash weight	Dry weight	Weight of Oscillatoria
Factor A (Treatment)	3	497.17*	67.85*	65.13*	4.36*
Error	9	4.49	0.965	1.33	0.61

***: Significant at 0.05 level of probability**

Appendix IX. Analysis of variance on different biomass weight performance in 3rd cycle of cultivation

Source of variation	Degrees of freedom	Mean Square for biomass weight			
		Fresh weight	Wash weight	Dry weight	Weight of Oscillatoria
Factor A	3	562.79*	76.81*	47.01*	7.69*
Error	9	1.94	1.00	0.317	0.135

***: Significant at 0.05 level of probability**

Appendix X. Chemical price of Zarrouks media and Modified media used for Spirulina production			
Zarrouks Media	**Tk/kg**	**Modified Media**	**Tk/Kg**
$NaHCO_3$	80	$NaHCO_3$	80
$NaNO_3$	1200	$NaNO_3$	1200
NaCl	40	NaCl	40
K_2HPO_4	2700	$Ca_2(PO_4)_2$ (TSP)	20
K_2SO_4	1600	K_2SO_4	1600
$CaCl_2.H_2O$	1200	$CaCl_2.H_2O$	1200
$FeSO_4.7H_2O$	1000	$FeSO_4.7H_2O$	1000
$MgSO_4.7H_2O$	1100	$MgSO_4.7H_2O$	1100
EDTA	2700	Citric Acid	170

*Source Laby Shop.com

Appendix XI. Chemicals used to chlorination of freshwater	
Chemical	**Tk/Kg**
Bleaching powder	120
Ascorbic acid	39500

Appendix XII. Material cost of Spirulina production (1000 Liter)			
Input cost			
Cost Item	**Units**	**Units/Cost (TK)**	**Total cost (TK)**
1. Iron stand	4	6000	24000
2. Electric air pump	1	4200	4200
3. Food grade container (4 liter)	64	270	17280
4. Connecting T			350
5. Silicon pipe			600
6. PVC pipe	1		500
7. Harvesting net (500 mesh)	1		650
8. Labor	1		500
9. Electricity			300
10. Miscellaneous cost			200
Sub-total			48580

Appendix XIII. Non-material cost of Spirulina production (1000 Liter)			
Media cost	**Units (L)**	**Tk/ Liter**	**Total cost (Tk)**
1. Zarrouks media	1000	9.77	9770
2. Modified media	1000	6.40	6400
3. Mother culture	1000 g		1000
Sub-total			22570
Total Input cost (Material Cost + Non-material cost)			71150