PRODUCTION TECHNOLOGY AND CHEMICAL COMPOSITION OF SPIRULINA (*Spirulina platensis*) IN BANGLADESH

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CERTIFICATE

This is to certify that the thesis entitled ***"STUDY ON PRODUCTION TECHNOLOGY AND CHEMICAL COMPOSITION OF SPIRULINA (Spirulina platensis)"*** *submitted to the Department of Horticulture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of* ***MASTER OF SCIENCE in HORTICULTURE****, embodies the result of a piece of bonafide research work carried out by* ***ASIF AHMED,*** *Registration No****. 13-05312*** *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, 2019
Dhaka, Bangladesh

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And let there be from you a nation inviting to good, enjoining what is right and forbidding what is wrong, and those will be the successful | ***Surah Ali 'Imran 3:104***

DEDICATED TO-

MY BELOVED PARENTS

To whom I owe every fiber of my being

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Author

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ABSTRACT

An experiment was conducted in the Rooftop of Horticulture of Sher-e-Bangla Agricultural University during the period of April to October, 2018 to develop a production technology and chemical analysis of spirulina. Eight large size (270/ L) food grade plastic container was used for the production of spirulina and made a sample of spirulina for chemical analysis. This experiment arranged in a Completely Randomized Design. Thirty days of production was carried out and find out a promising spirulina production technology. Climatic condition for spirulina production such as temperature light, photo inhibitor, oxygen concentrations, agitation, contamination, water quality, inoculums size were very important. A unique protocol for spirulina production was maintained. A series of activities like installation of the containers, chlorination of containers, de chlorination of containers, media preparation, strain inoculation in containers, intercultural operations, harvesting were done in this experiment. A spirulina sample was taken and made a chemical analysis of total ash, beta carotene, fat, moisture, protein, zinc and iron. By this study, beta carotene 99.24 (mg/100g), fat 0.14 (g/100g), total ash 11.24 (g/100g), moisture 14.53 (g/100g), protein 42.01 (g/100g), zinc 2.90 (mg/100g), iron 66.20 (mg/100g) were measured from supplied spirulina sample. Spirulina sample contains lower amount of fat 0.14 (g/100g), which was different from France 4.00 (g/100g), USA 5.00 (g/100g), Malaysia 6.00 (g/100g), Thailand 5-7 (g/100g). Standard quality spirulina production technology was obtained and produced world class spirulina in comparison to other standard type of spirulina produced in the world. This finding may be a source of valuable information for standard quality spirulina production system in Bangladesh.

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ABBREVIATIONS AND ACCORONYMS

AAS = Atomic Absorption Spectrophotometer

AEZ = Agro-ecological Zone

BARI = Bangladesh Agricultural Research Institute

Biol. = Biology

DAI = Days after Inoculation

et al. = And others

Hort. = Horticulture

i.e. = That is

j. = Journal

mm = Millimeter

CRD = Completely Randomized Design

Res. = Research

SAU = Sher-e-Bangla Agricultural University

Sci. = Science

Technol. = Technology

Viz. = Namely

BCSIR = Bangladesh Council of Scientific and Industrial Research

DI Water = Di-Ionize Water

PPM = Parts per Million

PPB = Parts per Billion

ABBREVIATIONS AND ACCORONYMS

DAE = Department of Agriculture Extension

NGO = Non-Government Organization

IFST = Institute of Food Science and Technology

UNDP = United Nations Development Program

OD = Optical Density

FAO = Food and Agriculture Organization

CHAPTER I

INTRODUCTION

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INTRODUCTION

Spirulina are multicellular and filamentous blue-green algae that has gained considerable popularity in the health food industry and increasingly as a protein and vitamin supplement to aquaculture diets. It grows in water, can be harvested and processed easily and has very high macro- and micro-nutrient contents. It has long been used as a dietary supplement by people living close to the alkaline lakes where it is naturally found – for instance those living adjacent to Lake Chad in the Kanem region have very low levels of malnutrition, despite living on a spartan millet-base diet. This traditional food, known as *dihé,* was rediscovered in Chad by a European scientific mission, and is now widely cultured throughout the world. In many countries of Africa, it is still used as human food as a major source of protein and is collected from natural water, dried and eaten. It has gained considerable popularity in the human health food industry and in many countries of Asia it is used as protein supplement and as health food.

During the sixtieth session of the United Nations General Assembly (Second Committee, Agenda item 52), a revised draft resolution on the "*Use of spirulina to combat hunger and malnutrition and help achieve sustainable development*" was submitted by Burundi, Cameroon, Dominican Republic, Nicaragua and Paraguay. As a follow up of this resolution, FAO was requested to prepare a draft position paper on spirulina so as to have a clearer understanding on its use and to convey FAO's position on this.

Spirulina is, like most cyanobacteria, an obligate photoautotroph, i.e. it cannot grow in the dark on media containing organic carbon compounds. It reduces carbon dioxide in the light and assimilates mainly nitrates. The main assimilation product of spirulina photosynthesis is glycogen. Spirulina shows an optimum growth between 35^0 C and 37 °C under laboratory conditions. Outdoors, it seems that an increase in temperature up to 39 °C for a few hours does not harm the blue-green algae, or its photosynthetic ability. Thermophilic or thermos tolerant strains of spirulina can be cultivated at temperatures between 35 °C and 40 °C. Such a property has the advantage of eliminating microbial mesophilic contaminants. The minimum temperature at which growth of spirulina takes place is around 15 °C during the day. At night, spirulina can tolerate relatively low temperatures. The resistance of spirulina to ultraviolet rays seems to be rather high (Richmond, 1986).

In the sixteenth century, when the Spanish invaders conquered Mexico, they discovered that the Aztecs living in the Valley of Mexico in the capital Tenochtitlan were collecting a “new food” from the lake (Sasson, 1997). Spanish chroniclers described fishermen with fine nets collecting this blue coloured “techuitlatl” from the lagoons and making a blue-green cake from it. Other legends say Aztec messenger runners took spirulina on their marathons. Techuitlatl was mentioned by naturalists until the end of the sixteenth century, but not after that, probably reflecting the loss of the lakes as they were drained for urban and agricultural development. The only remnant today, Lake Texcoco, still has a living algae spirulina population.

The current environmental conditions deteriorations, mental and physical stress, changes in the diet have been serious risk factors for the humans, increased the death rate and civilization diseases. These are the obvious reasons why new progressive trends are being extensively developed in modern medicine, pharmacology and biotechnology and more effective harmless medicaments are being sought for to treat and prevent various diseases. One of the trends in biotechnology is associated with blue green microalgae *spirulina platensis* which have been widely employed as food and feed additives in agriculture, food industry, pharmaceuticals, perfume making, medicine and science. *Spirulina platensis Spirulina sp.* has been used as food for centuries by different populations and only rediscovered in recent years. Once classified as the ―blue-green algae’’, it does not strictly speaking belong to the algae, even though for convenience it continues to be referred to in that way. It grows naturally in the alkaline waters of lakes in warm regions. Measuring about 0.1mm across, it generally takes the form of tiny green filaments coiled in spirals of varying tightness and number, depending on the strain. Its impressive protein content and its rapid growth in entirely mineral environments have attracted the attention of both researchers and industrialists alike.

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 algae 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. (Saranraj *et al*. 2013).

The dried cells of microorganisms such as bacteria, fungi, yeasts and algae that are grown in large scale culture systems as proteins, for human or animal consumption are collectively known as single cell protein. SCPs are characterized by; fast growth rate; high protein content compared to field crops; require less water and land and independent of climate; grow on wastewater; can be genetically modified for desirable characters such as amino acid composition and temperature tolerance. Among the various microorganisms used as sources of SCP, the blue green algae, Spirulina is considered as the best source. The composition of the biomass, including the high protein content, low content in nucleic acids, occurrence of high concentrations of vitamins and other growth factors and the presence of cell wall that is more easily digestible than that of other microbes indicate that Spirulina is a promising source of food or feed. (Jamal *et al* 2018).

Spirulina has been used as a complementary dietary ingredient of feed for fish, shrimp and poultry, and increasingly as a protein and vitamin supplement to aqua feeds. China is using this micro-alga as a partial substitute of imported forage to promote the growth, immunity and viability of shrimp. There has also been comprehensive research on the use of spirulina as aquaculture feed additives in Japan. (Jamal *et al* 2018).

Spirulina platensis is a microalga with appropriate composition to be used as a food supplement. It is commonly used by humans and animals as protein source. Several studies such as palatability, lack of toxicity and easy digestion, antioxidant actions, hypocholesterolemia, anticancer, immune stimulant, anti-inflammatory, antiviral, among others have been conducted to verify the possible benefits of spirulina and some properties have been verified (Rodriguez-Hernández *et al.,* 2001; Colla *et al.,* 2007).

Thus, the production of microalgae has received special attention recently, because, according to Rogatto *et al.* (2004), these microorganisms can be a good alternative source of protein in the diet. Among several microorganisms which have been studied, the blue-green alga spirulina is considered a promising microorganism due to its high protein content (65 to 70% DM) and great amount of vitamins and minerals (Kay, 1991).

Moreover, with the high reproduction rate of spirulina, it is estimated that some area available for their growth can produce 125 times more protein than the same area of corn (Furst, 1978).

Today, Spirulina is commercially cultivated in several countries, with a total annual production of a few hundred tones. A few other sites have ceased production, owing to high cost of production. Spirulina products in the form of pills and spray-dried powder are produced in Mexico, Taiwan, USA, Thailand, Japan and Israel for the health food market. Small amounts of Spirulina are extracted for the production of phycocyanin, known commercially as 'lima blue', used as a blue colorant for food and cosmetics. In addition, some Spirulina products are fortified with extracts of various herbs as well as some vitamins and minerals and sold as a relief for premenstrual syndrome. Another mode of production based on local inputs and simple techniques in rural, developing regions, yields human food as a supplement for protein-deficient diets. Indeed, the concept of mass cultivation of Spirulina is being pursued by people of two different social categories. One is the affluent industrialized society, where people (particularly vegetarians) are looking for natural foods and health-food additives for their diet. The other is represented by Third World societies in search of a rich source of protein that can be produced under local conditions using local resources, i.e. marginal land and saline water not suitable for conventional agriculture as well as animal and human wastes. In addition, the possibility of using Spirulina as a source of animal feed, particularly for fish, has attracted attention.

From the above discussion, the present study was undertaken with the followings objectives:

a. To develop spirulina production technology for Bangladesh.
b. To determine the chemical composition of the produced spirulina to compare with international one.

CHAPTER II

REVIEW OF LITERATURES

CHAPTER II

REVIEW OF LITERATURE

Spirulina is long held as a highly nutritious food for some decades. In 1974, World Health Organization described Spirulina as “an interesting food for multiple reasons, rich in iron and protein, and is able to be administered to children without any risk”, considering it “a very suitable food. Spirulina appears to have considerable potential for sustainable financial development, especially as a small scale crop for nutritional enhancement. Bangladesh is basically a poverty ridden, highly populous and malnourished country. The agriculture, despite making a huge leap forward, it just isn’t compatible enough to ensure total nutritional security in Bangladesh. Malnutrition plagues the majority of people, especially in children, adolescents and women. In addition, it causes individual tragedies such as maternal and child mortality, premature delivery, elevated risks of heart diseases and diabetes. Not only does it risk health, malnutrition also cuts a heavy figure in the national future as it results in lost productivity and reduced intellectual and learning capacity. In a country where the diet is dominated by cereal crops (about 70%) a diversified nutrient source is a must. Securing nutrition should be the first priority and this is where Spirulina comes in. Presence of some essential nutrients required by human beings makes Spirulina a modern day super food. Taking Spirulina as a food supplement will ensure nutrition security of Bangladesh. But the main problem is that to find out cont. effective production system in aspect of Bangladesh. In this situation the present work was conducted to find out the best production technology of spirulina. So here some important research works relevant to production of spirulina have been presented.

Paoletti, *et al.* (1975) studied the growth performances of *Spirulina platensis* and *S. maxima* under photo-limited conditions using Roux bottles of 1liter capacity incubated in a water bath at 30 °C. It was irradiated intermittently from one side with battery of fluorescent lamps (PAR intensity equal to 65.30 J/m^2/second) in light: dark cycle (12 hours:12 hours). The initial biomass concentration of spirulina was 350 mg (dry weight)/liter which attended to a maximum dry weight of 346 and 329 mg/liter in the cases of *S. platensis* and *S. maxima*, respectively. The culture solution was the standard bicarbonate-carbonate medium at pH 9–9.5 with bubbling air and 1 percent CO2.

Tomaselli *et al*. (1987) examined the growth of ten strains of *Spirulina platensis* and eight strains of *S. maxima*. They obtained three strains of *S. platensis* from different laboratories (strain Cl from Leonard, strain LB 1475/4 from the Cambridge Culture Collection and strain M135 from Teronobu's collection). The remaining 15 strains were isolated in their laboratory, mostly from water samples collected from Lake Texcoco (Mexico) and Lakes Monbolo and Rombou (Chad). The properties taken into consideration include: relative photosynthetic efficiency under photo-limited conditions, protein production, rate of dark respiration, behavior at temperatures above the optimum for growth, and tolerance of salinity.

Rijn and Shilo (1986) conducted an experiment on nitrogen limitation in natural populations of cyanobacteria (*Spirulina* spp. and *Oscillatoria* spp.) in Israeli fish ponds in summer. They found that carbohydrates synthesized at the lighted surface partially utilized for protein synthesis at the bottom of these ponds when cells labeled by ^{14}C under simulated pond conditions.

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

Bosma *et al.* (2007) conducted an experiment where volumetric productivities in the bubble column were predicted and compared with experimental volumetric productivities.

The light integration model over-estimated productivity, while the model in which we assumed no light integration under-estimated productivity. Light integration occurred partly (47%) during the period investigated. The average observed biomass yield on light was 0. 60g.mor1. The model of partly light integration predicted an average biomass yield on light of 0.57g.mol-l and predicted that productivity could have been increased by 19% if culture temperature would have been maintained at 24^0 C.

Reichert *et al.* (2001) studied the specific growth rate and productivity of two Spirulina platensis strains. Spirulina platensis strain used was found that low concentration (0.50g/L) and high renewal rates (50% w/v) resulted in high specific growth rate and productivity. These values are two to four times higher than those obtained in simple batch cultivation and indicate that the semi continuous cultivation of Spirulina platensis is viable.

Vree *et al*. (2015) conducted an experiment where it was observed in case of vertical photobioreactors, higher areal productivities and photosynthetic efficiencies, 19—24 g m-2 day I and 2.4—4.2 0/0, respectively, were found in comparison to the horizontal systems; 12—15 g m-2 Day-I and 1.5—1.8 0/0. The higher ground areal productivity in the vertical systems could be explained by light dilution in combination with a higher light capture. In the raceway pond low productivities were obtained, due to the long optical path in this system. Areal productivities in all systems increased with increasing photon flux densities up to a photon flux density of 30 molls m-2 day-1.

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

Reichert *et al.* (2006) studied that the cultivation of photosynthetic microorganisms such as the cyanobacterium Spirulina platensis has been studied by researchers in many countries because these organisms can produce products with industrial potential. We studied the specific growth rate (PX, Day-I) 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 semi continuous culture in two-liter Erlenmeyer flasks for 90 days (2160 h) at 300C 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 (PX = 0.111 Day-I) and high productivity (PX = 42.3 mg/L/day).

Saranraj *et al.* (2013) reported that, *Spirulina platensis* was cultivated in the conical flasks containing Zarrouk's medium alone [SPI] and Zarrouk's medium with three different concentrations (0.5 g/l [SP2 J, 1.0 g/l [SP3] and 1.5 g/l [SP4 1 of LFA supplementation. Among the four different supplementations used, SP4 which contained 441.5 g Lignite fly ash in one litre of Zarrouk's medium highly induced the growth and protein content of Spirulina platensis when compared to other supplementations. The optical density of Spirulina platensis was followed by SP_3

Richmond *et al.* (1990) carried out an experiment in open pond. This work represents an attempt to assess the relative contribution of the factors limiting productivity of *Spirulina platensis* in open raceways throughout the year. Temperature of the culture during daylight exerted the predominant effect on productivity and elevating the temperature resulted in a significant rise in productivity even in summer. Photo inhibition had a decisive role in summer in determining productivity of spirulina in open raceways in that growth almost ceased after mid-day. Contamination by other microorganisms, particularly S. minor and Chlorella sp. was estimated to reduce the net biomass yield by at least 15 to 20%, but measures to curtail the establishment of these species in the raceway have been devised. They found 20-25 g fresh weight per liter water.

Michele *et al.* (2007) conducted an experiment where they found under controlled conditions the maximum specific growth rate (g max) was 0.102 Day^{-I} the biomass doubling time (td) was 6.8 d, the maximum dry biomass concentration (X max,) was 1.94 g L Day-I and the maximum productivity (P max) was 0.059 g la-I Day-I, while the corresponding values in the greenhouse experiments were = 0.322 Day-I, d t = 2.2 d, x max - 1 73 g and P max - 0. 112 g L-1 Day^{-I}. Under controlled conditions the highest values for these parameters occurred when XO = 0.15 g la-I, while in the greenhouse XO= $0.4gI^{-l}$ produced the highest values.

Kumar *et al.* (2010) studied that growth analysis of cultures grown at different temperatures showed significant difference ($P < 0.05$) in growth pattern. Maximum biomass concentration (as dry weight) i.e. 0.73 g l-l was observed at temperature 35 ^{0}C and least i.e. 0.26 g l,-l was found at temperature 20 ^{0}C . During the growth of cultures, a wide range of temperature tolerance from 20 ^{0}C to 40 ^{0}C was observed. But at 45 ^{0}C, the growth was almost negligible (data not recorded). The maximum growth rate i.e. 0.091 doubling Day^{-I} was observed at 35 ^{0}C, but with further increase in temperature reduction in growth rate was observed. At 40 ^{0}C culture showed 0.041 doubling Day^{-I} which is almost half as compared to growth rate at 35 ^{0}C.

Po *et al*. (1978) studied that the production and nutritive value of *Spirulina platensis* on swine wastes. The alga contained 55 to 61 per cent crude protein. Three indoor culture ponds 0.65m^2 were designed and built under the light intensity of 500 foot candles. *Spirulina* yielded about 5 g m^2 d^{-1} A sample containing 2.038 of NH_3-N produced 16.25 mg of dry algae and contained 9.75 mg of protein. The protein showed a protein efficiency ratio of 2.25 and no toxic effects were noted.

Mitchell *et al*. (1974) demonstrated that *Spirulina maxima* significantly altered the storage and utilization of vitamins A and E. These were investigated by feeding diets containing 0, 2.7, 10.7, 18.7 and 26.7 per cent protein or by a mixture of them. Growth results indicated that rats did not utilize the diets containing *Spirulina maxima* as well as the case in control diet. The ingestion of *Spirulina maxima* caused a significant increase in dry matter and chloroform extractable crude fat in the faeces.

Krishna *et al*. (1981) studied the protein of *Scendes musacutus* and *Spirulina platensis*. These were fed to the rats upto the dosage of 800 mg kg^1 of body weight. The absence of gastrointestinal disorders such as diarrhea indicated that these algae were tolerated by animals even at 800 mg kg^{-1}. Application of these algae into albino rats did not elicit any skin allergy. Weight gains, PER and nitrogen balance studies with *Spirulina fusiformis* supplementation to poor rice diets showed significantly higher values of all parameters over casein supplementation.

Bergh *et al*. (1991) studied the bioavailability of vitamin B_{12} in rats using *Spirulina*. Two different seafood products nori and *Spirulina* were evaluated with synthetic cyanocobalamine as control, in 30 male weaning wistar rats, given a diet deficient in

vitamin B_{12} for six weeks, followed by a four week repletion period in which the rats were given supplements of equal doses of vitamin B_{12}. After repletion, cobalamine contents of serum and kidney were significantly lower and liver cobalamine content was higher, for both the nori and *Spirulina* fed rats than for the cyanocobalamine supplemented controls.

Kumar and Singh (1992) studied two strains of *Spirulina platensis* by enriching of cobalt and iodine, the repeated sub-culturing resulted in increased resistance to these trace metals. The cobalt tolerant strain which grew at 55mg $CoCl_2$ $6H_2O$ l^{-1} showed maximum uptake of 158.43 n mol Co ion mg^{-1}proteins which was 3.98 times higher than its parent. The iodine tolerant strain which grew at 7.0 g Kl l^{-1} showed maximum uptake of 0.65 m mol mg^{-1} of protein which was 1.25 times higher than its parent. Both the strains had the potential in relieving the deficiency of vitamin B_{12} and iodine in vegetarians.

Hayashi *et al*. (1994) reported the enhancement of antibody production in mice by dietary *Spirulina platensis*. Mice fed on a *Spirulina platensis* diet showed increased numbers of splenic antibody producing cells in the primary immune response to sheep red blood cells. *Spirulina* enhanced the immune response by stimulating macrophage functions, phagocytes and immunoglobin production.

Rushmikapoor and Mehta (1992) studied the lactation performance of dams fed *Spirulina platensis* supplemented diets. The composition of milk was estimated in terms of protein, fat and lactose of days 7, 14 and 21 of lactation. The rats were fed five different kinds of diets (Casein, *Spirulina*, wheat gluten, *Spirulina* + wheat gluten, *Spirulina*, devoid of vitamins and minerals) each providing 22 per cent proteins during the period of pregnancy and lactation. Casein and *Spirulina* containing dietary regiments were able to maintain fat levels even at later stage of lactation.

Manjit and Ahluwalia (1992) carried out the biochemical studies of *Spirulina* sugars and enzymes. Total water soluble sugars were more (7.31 per cent) than acid soluble sugars (6.19 per cent). The activities of α amylase (8.5 per cent), β amylase (5.33 per cent) and protease (1.07) were recorded. The activity of acid phosphatase was insignificant.

Sundararaman *et al*. (1994) studied the bioactive potential of marine Cyanobacteria in the animal based systems. Twelve different strains were administered to male albino wistar

rats. The strains of *Spirulina subsalsa*, Oscillatoriasalina, *Phormidium valderianum* were appeared as highly promising in their nutrition.

Manoj *et al*. (1992) reported the application of *Spirulina* for cancer chemo-prevention. Water extract of *Spirulina* was found to inhibit the lipid peroxidation to 76 per cent and the alcohol extract to 65 per cent. The chemical antioxidants like α tocopherol, Butylated hydroxyanisole and β carotene gave 35, 45 and 48 per cent respectively.

Nasima *et al*. (1996) reported that the Rice Husk Ash (RHA) and $NaHCO_3$ l^{-1} were used as a source of carbon in *Spirulina* culture. Addition of 2g of $NaHCO_3$ l^{-1} every other day supported better growth than 1 g RHA l^{-1} everyday. However, from an economic point of view RHA was preferred.

Hirajashi (2002) studied 12 adult males were administered an oral hot water extract of *Spirulina* and the number and activity of their natural killer (NK) cells was measured before and after treatment (NK cells destroy tumor cells by binding to them and delivering lethal chemicals that kill on contact). At the end of the study, there was a significant increase in the production and cancer killing ability to these NK cells. When their NK cells were exposed to a bacterial product after treatment, production of interleukin-12 (IL12), a measure of immune strength, was significantly increased in comparison to IL-12 production in NK cells without pre-exposure to *Spirulina*.

Gardillo *et al*. (1998) studied the effect of increased atmospheric CO2 on photosynthesis and growth of Spirulina. The increase of CO2 did not cause any change in maximum growth rate while it decreased maximum biomass yield as it affected the pigment content of the algae.

Vieira *et al*. (2003) reported that, the influence of nutrient for biomass production of *Spirulina platensis*, in open raceway ponds in addition of (carbon as sodium bicarbonate, nitrogen as urea, phosphate, sulfate, ferric iron, magnesium and potassium) on the growth rate of the cyanobacteria *Spirulina platensis.* In unsupplemented lagoon water production of *S. platensis* was 0.78 ± 0.01 g/l (dry weight basis) while the addition of 2.88 g/l of sodium bicarbonate resulted in 0.82 ± 0.01 g/l after 40 hours of culture.

Rafiqul *et al*. (2003) reported that, the maximum specific growth rate of 0.141 was found at 32 ^{0}C for *Spirulina platensis* and that of 0.144 was found at 37 ^{0}C for *Spirulina fusiformis*. Maximum biomass production of 2.4 g l-1 and chlorophyll *a* production of 16.6 mg l^{-1} were observed at 32 ^{0}C for *Spirulina platensis.* Maximum biomass production of 2.3 g l^{-1} and chlorophyll - *a* production of 14.2 mg l^{-1} were observed at 37 ^{0}C for *Spirulina fusiformis.*

Mohammad *et al*. (2012) studied that, *Spirulina platensis* is an important source of pharmaceuticals and nutraceuticals such as glinolenic acid (GLnA). GLnA yield of the culture medium (32 mg/L) were obtained at the highest light intensity of 5.0 klx.

Pandey *et al*. (2010) found that, the influence of pH for *Spirulina platensis* growth, protein and Chlorophyll a content were examined and 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.

Tasneem, F. (1990) reported that synchronous growth of Spirulina platensis was failed to grow both in liquid and solid media at its higher dilution. It was observed that minimum cell population is necessary to initiate and sustain Spirulina cultures. The culture filtrate had an absorbance of 0.96 on ninth day increased 50 per cent growth against control (0.63 absorbance).

Dubey (2006) reported that moderate light intensity in the cultivation of *Spirulina*, suggesting low light intensity at the beginning to avoid photolysis. He also noted that exposing *Spirulina* to high light intensity photolysis them.

Pandey *et al*. (2010) found that, the influence of light intensity for *Spirulina platensis* growth 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.

Mahadevaswamy and Venkatraman (1987) reported the presence of bacterial contaminants in outdoor cultivation of *Spirulina platensis*. The bacterial forms occurred in cultures were identified as aerobic spore formers. No the pathogenic forms affecting the products safety were identified.

Hofner *et al*. (1987) reported that the hexavalent chromium was most toxic and the zinc had little effect on the growth of *Chlorella fusce*. All elements tested arrest growth of *Spirulina maxima* at 10^{-4} M and smaller doses had no effect. Ahluwalia and Kochar studied the effect of mercuric chloride, cadmium chloride, nickel sulpahte and zinc chloride on the growth of *Spirulina platensis*. Among the metals, mercury had been most toxic and the algal growth was reduced even at 0.01 ppm. It was followed by cadmium (0.1 ppm). Higher doses resulted in fragmentation, lysis and death of the algae.

Phang *et al.* (2000) reported that, the waste water is usually discharged into the rivers, each factory producing about 10- 22 tons waste water per day which contains a very high carbon to nitrogen ratio (105:0.12), but it has been made more suitable for fermentation by anaerobic fermentation in an up-flow packed bed digestor. The digested effluent with an average C: N: P ratio of 24:0.14:1 supports growth of *Spirulina platensis*. The highest crude protein, carbohydrate and lipid content of the biomass were 68, 23 and 11%, respectively. The reduction in COD, ammoniacal-nitrogen and phosphate levels of the digested effluent reached levels of 98.0, 99.9 and 99.4%, respectively.

Nanda and Padhi (1992) studied the effect of sodium salt to 2,4-Dichlorophenoxyacetic acid (2,4-D), on the growth and pigmentation of *Spirulina platensis*. It was found to be very sensitive towards 2,4-D and the growth of algae inhibited at 5 mg ml^{-1}.

Ken *et al*. (1995) reported that the growth of *Spirulina platensis* was stimulated by adding 5Aminolevulinic acid (ALA, 500 mg l^{-1}). The photosynthetic activity was enhanced by rapid stimulation and accumulation of phycocyanin and chlorophyll II.

Wahal *et al.* (1974) reported that water-soluble sugars constituted the major carbohydrates of Spirulina. The low amount of starch was due to the high activity of α and β amylase in Spirulina. The in vitro digestibility of Spirulina had been reported using an amylase enzyme. A sufficient amount of protease activity indicated that the enzyme was mainly involved in protein turn over rather than in storage hydrolysis.

Balasubramanya and Sampath (1994) investigated the PER of Spirulina diet at 10 per cent protein level. Thirty-six albino rates of 21 days' old each were used as animal models for the experiment. Concentrated milk, casein and Spirulina diets were prepared at 10 per cent

protein level. The PER of Spirulina alga diet was found to be as high as (2.28) than that of casein (2.45) or milk (2.46).

Ishikawa *et al.* (1989) studied that, GLA has mainly respect to its therapeutic properties such as its ability to decrease blood cholesterol levels. Spirulina has a high amount of polyunsaturated fatty acids, 1.5-2.0 per cent of 5-6 per cent total lipid. Spirulina is rich in linolenic acid, stearidonic acid, eicosapentaenoic acid, docosahexaenoic acid and arachidonic acid.

From the review of literature, it is revealed that no works on spirulina production technology is measure in Bangladesh. So the present study was undertaken.

CHAPTER III

MATERIALS AND METHODS

CHAPTER III
MATERIALS AND MATHODS

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

3.1 Experimental site

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

3.2 Growth factors

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

3.3 Liquid media

The liquid used for the production of spirulina is a solution of mineral salts in water. This liquid has to supply the spirulina with all the chemical elements it needs. The pH of the culture media (i.e. its level of alkalinity) should be between 8.0 and l1.

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

3.4 Estimation of physio-chemical properties of cultural media

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

3.5 Research materials

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

3.6 Sources of materials

Spirulina strain was collected from "Energia Bangladesh Limited".

3.7 Design and layout of the experiment

Experiment was propelled in completely randomized design (CRD).

The experiment comprises total eight containers. Solution with spirulina strain was poured in each container, enclosing the container by cover.

3.8 Materials required for spirulina production

3.8.1 Materials: Materials which was required for spirulina cultivation as follows:

- ❖ Air pump
- ❖ Plastic tube
- ❖ Drilling machine
- ❖ Connector
- ❖ Harvesting net
- ❖ Crusher
- ❖ Container (270L)
- ❖ Syringe
- ❖ Polythene

3.8.2 Materials required for analysis of total ash of spirulina sample

- ❖ Porcelain crucible
- ❖ Furnace
- ❖ Hot plate
- ❖ Forceps
- ❖ Analytical balance
- ❖ Desiccator
- ❖ Stirring rod
- ❖ Wax pencil

3.8.3 Instruments required for analysis of protein of supplied spirulina sample

- ❖ Conical flask
- ❖ Burette
- ❖ Digestion tube
- ❖ Measuring cylinder
- ❖ Bowl
- ❖ Sieve
- ❖ Spatula
- ❖ Funnel

- ❖ Retort stand and clamp
- ❖ Dropper
- ❖ Analytical weighting balance
- ❖ Weighting boat

3.8.4 Materials required for analysis of fat from supplied spirulina sample

- ❖ Filter paper
- ❖ Thimble with steel thimble holder
- ❖ Mortar
- ❖ Oven glove
- ❖ Extraction beaker
- ❖ Spirulina sample
- ❖ Cotton
- ❖ Spatula
- ❖ Solvent (hexane)
- ❖ Whole beaker rack

3.8.5 Machines which were required for chemical analysis of spirulina

a. Atomic absorption spectrophotometer (Model: Shimadzu AA-700)

b. UV-spectrophotometer (Model: Evolution-201) (plate :02)

3.9 Laboratory

BTRT, IFST, BCSIR

2a Biotech Lab, Sher-e-Bangla Agricultural University

The aim of my research works is to develop a production technology in Bangladesh and to determine their chemical composition. In the past, an open pond system spirulina production system was developed by BCSIR. But they cannot successfully develop the production system in small scale in pots or container. In the roof top agriculture faculty building of Sher-e-Bangla Agricultural University was successfully developed a closed tank spirulina production system. After that, a spirulina sample was chemically analyzed from BCSIR. The results of the present study have been presented and discussed under the following headings.

3.10 Climatic condition for spirulina production

3.10.1 Temperature

Temperature in the range of 25^0C-35^0 C even if the outside temperature as 35^0 C was most ideally suited for getting maximum yield of spirulina. Below 20^0C, growth is practically nil, but spirulina does not die. Temperature above 35^0C leads to bleaching of cultures. Partial shading provided a culture of about 30^0 C even if the outside temperature was 38^0C.

Rafiqul *et al*. (2003) reported that, the maximum specific growth rate of 0.141 was found at 32^0 C for *Spirulina platensis* and that of 0.144 was found for *Spirulina fusiformis*. Maximum biomass production of 2.4 g /land chlorophyll a production of 16.6 mg/l were observed at 32^0 C for *Spirulina platensis*. Maximum biomass production of 2.3 g and chlorophyll - a production of 14.2 mg/l were observed at 37^0 C for Spirulina fusiformis. Colla *et al*. (2007) found that, temperature was the most important factor and that the greatest amount of gamma-linoleic acid (GLA) was obtained at 30^0 C, the fatty acid profile of the spirulina cultivated showing that (in order of abundance) palmitic, linoleic and linoleic acids were most prevalent.

3.10.2 Light

Spirulina required high light intensities during its growth phase. The optimum light intensity was between 20 and 30 K lux. Sundararaman *et al*. (1992) reported on the effect of different light quality on growth, protein and pigment synthesis of *Spirulina fusiformis.* When growing algae at a depth of 12–15 cm in open raceway ponds, self-shading governs the light availability to the single cell in the culture. Unless one uses a much diluted culture which allows penetration of light throughout the water column, a certain part of the culture will always fail to receive enough light to fulfill photosynthesis needs. Thus, almost by definition this kind of culture will be light limited. It was demonstrated in the early 1980s that augmenting cell concentration of the culture which increases self-shading resulted in a decrease of the growth rate. This kind of experiment was carried out during the summer, winter and spring, and findings indicated that the highest response of growth rate to cell concentration, i.e. self-shading, was observed in the summer. The initial interpretation was that in summer temperatures were high enough, so that main limitation for growth of spirulina outdoors was light. In winter and spring, however, when the temperature in the outdoor cultures was lower, the effect of self-shading was less pronounced (Vonshak, 1997).

3.10.3 Photo inhibition

Vonshak and Guy (1988) were the first to describe the phenomenon of photo-inhibition in outdoor-grown spirulina cultures. By following the in-situ photosynthetic activity of outdoor cultures grown at full solar radiation or under shaded conditions, they observed that shading could increase photosynthetic activity. It was also observed that shading

resulted in an increase in productivity. When the pond was shaded to reduce light intensity by 25 percent, the degree of inhibition was also significantly reduced. It is worth noting that once solar radiation lowers in the afternoon, recovery in photosystem-II activity occurs. Light availability in outdoor culture is highly dependent on cell concentration (Vonshak, 1997). After 15 years of study related to the role of light in productivity of outdoor algal cultures, spirulina, in particular, Israeli researchers reached a better understanding of the complicated light environment to which algal cells are exposed. It was demonstrated that due to extreme shifts in the level of light intensity, at least in spirulina cultures, photo inhibition may take place. The fact that photo inhibited spirulina cultures have a lower photosynthetic efficiency means that they require more light to reach the same level of activity as non-photo inhibited cells, thus, making photo-inhibited cultures actually light-limited. This finally leads to what may be seen as the paradox of light in outdoor spirulina cultures: during a significant part of the day, the outdoor cultures are photo inhibited and light limited at the same time (Vonshak, 1997). Vonshak *et al.* (1994) demonstrated that fluorescence measurements can be used as a fast reliable indication for photo inhibition in outdoor cultures of Spirulina platensis.

3.10.4 Oxygen concentrations

Assuming that the rate of photosynthesis can be used as an indication of the metabolic activity of outdoor algal cultures, the day-time changes in oxygen concentration in the pond are correlated with diurnal changes in light and temperature. In summer, the main limiting factor for growth of spirulina in outdoor culture is light; the daily peak in oxygen concentration is reached at the same time as light intensity is maximum. In winter, the main limiting factor is temperature because of a shift in the peak of oxygen which follows the peak in the pond temperature rather than light intensity (Vonshak, 1997).

3.11 Agitation

Agitation of algal cultures had the advantages of uniform distribution of $C0_2$ and prevention of thermal stratification. Many agitation devices had been reported which range from motor driven pumps, gravity flow, air light systems and manual agitation. Dubey (2006) found that, aeration, which could be achieved by rotators, and which provides agitation of growing cells to maintain the cells in suspension, has been described as very necessary in getting good quality and better yields of spirulina species.

3.12 Contamination

Contamination by different algal species may present a severe problem for microalgal cultures grown in outdoor open ponds. In most cases, the steps that proved effective in prevention of Chlorella contamination were maintaining a high bicarbonate concentration (e.g. 0.2 M), taking precautions to maintain the dissolved organic load in the culture medium as low as possible, and increasing winter temperature by greenhouse heating. Development of grazers in the culture, mainly the amoebae type, was prevented by the addition of ammonia (2mM) (Vonshak and Richmond, 1988). Experience indicates that contaminating organisms do not present a serious difficulty as long as good growth is maintained in a monoalgal culture. It is worth noting that no cyanophages attacking spirulina have been observed so far (Vonshak, 1997).

3.13 Water quality

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

3.14 Inoculums' size

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

3.15 Monitoring

Gitelson *et al.* (1995) of the Jacob Blaustein Institute for Desert Research, Ben-Gurion University of the Negev in Israel, have studied the optical properties of dense algal cultures outdoors and their application to the remote estimation of biomass and pigment concentration in *Spirulina platensis*. They have investigated the spectral properties of the reflectance and vertical attenuation coefficient of high-density productive algal ponds in the visible and near infrared region of the spectrum in as wide a range of biomass and pigment concentrations as possible. The objective of that research was to create the indices sensitive to pigment and biomass concentration which may serve as indicators for the physiological state of outdoor algal cultures. The Israeli researchers' findings may serve as a basis for remote real-time monitoring of phytoplankton quality in high-density productive algal ponds.

3.16 Production systems

In the United States of America, China, India, Thailand, Viet Nam and Taiwan Province of China, two types of open raceway ponds are used; the first, which is more capital-intensive, is lined by concrete (Thailand, India); the second is a shallow earthen tunnel lined with PVC or some other durable plastic. The cost and durability of the lining significantly influences the capital costs and thus the economic feasibility of this biotechnology. Any durable liner will add up to US$0.5 to the cost of production of each kg of algal biomass produced, demonstrating the need for cheaper lining such as low cost clay sealing. Such lining has to be tested for durability under turbulent flow and periodic cleaning of the pond (Vonshak, 1997).

3.17 Protocol for spirulina production

A series of steps have carried out for successful spirulina production as follows: (plate: 04, 05)

3.17.1 Installation of the containers

Eight container were placed horizontally on the rooftop of Sher-e-Bangla Agricultural University. Eight food grade silicon tube were coming out from a central plastic pipe which was directly connected with a motor (Model: GZLING 2RB 510 H26). The silicon tube was linked to container for supplying air. The motor connected with electric line performed two services at a time one was air circulation and another was agitation. The air circulation was continued at one-hour interval automatically by using timer. (plate: 03)

3.17.2 Chlorination of container

The Chlorination of container was carried out @ 0.02g/L H_2O with bubbling for one day

3.17.3 De chlorination of container

The de chlorination of container was done Ascorbic acid =0.04g/L with bubbling for one day.

3.17.4 Culture media

3.17.5 Media preparation

The culture medium was prepared by making four types of solution separately in four 1000 ml Erlenmeyer flasks for making 180 liters of culture medium. This volume was diluted with filtrated water-to obtain the initial intended concentration. The initial concentration of each solution is obtained using the dry biomass weight method. Then the culture medium

was prepared in an of 270L plastic container by pouring the solution sequentially for 180 liters of culture medium.

Table 1: List of the qualities of chemicals needed to prepare 180 L culture

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

(Habib *et al.* 2008).

3.17.6 Strain inoculation in container

A spirulina strain containing a high proportion of coiled filaments (less than 25 % straight filaments, if possible none at all), was choosen easy to harvest, and containing at least 1 % of gamma-linoleic acid (GLA) based on dry weight. Concentrated spirulina seed culture can be obtained either from the floating layer of an un agitated culture, or by re diluting a freshly filtered biomass (beware of lumps). A concentration of up to 0.03 g spirulina (dry) or 0.85g spirulina (fresh weight) per liter is permissible if storage and transportation last less than a week's time, and provided the seed culture be aerated at least two times a day. If aeration can be continuous, the concentration may be up to 10 g/l (weights of spirulina always refer to contained dry matter). The media was inoculated with 0.75g/l fresh weight basis. Manoj *et al.* (2011)

3.17.7 Intercultural operations

Apart from harvesting and feeding, a spirulina culture requires some attention in order to be kept in good condition. Agitation is a requisite. Continuous agitation however is not required. Agitation was carried out by a motor (Model: GZLING 2RB 510 H26). One third of full sun will saturate the photosynthetic capacity of spirulina, but shading is not required except to reduce the consumption of water (evaporation) or the temperature (< 38^0C) or

the pH (< 11.3). The temperature will practically never be too high, but the pH may soon become too high if insufficient carbon is supplied. The depth of culture must be kept between 10 and 20 cm. Accumulation of "white skins" and foam may float in the afternoon when the temperature of the culture goes above 35^0 C. These are not harmful as they will go back to the bottom again during the night, but their appearance is unpleasant and interferes light transmission during day time. They can be removed using a net. lf the concentration of spirulina is too low, the culture may be invaded by chlorella (a unicellular, edible alga). lt also interferes light transmission. So they can be removed using a net. Usual pathogenic bacteria do not survive the high p^H (> 9.7) of a spirulina culture in production, however a microbiological assay of the product should be made also at least once a week. Contaminations most generally occur during or after harvesting. The color of the culture should be deep green. If it turns yellowish, this may be due to either a lack of nitrogen or an excess of light (photolysis) or of ammonia (excess of urea). In the latter two cases recovery is generally possible within two weeks while resting the culture under shading. (Habib *et al.* 2008).

3.17.8 Harvesting

When the spirulina was in good condition, it was separated from the water ("harvesting") is an easy operation.

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

- The cool temperature makes the work easier,
- More sunshine hours will be available to dry the product, - The % proteins in the spirulina is highest in the morning.

There are basically two steps in harvesting:

- filtration to obtain a "biomass" containing about 10 % dry matter (1 liter = 100 g dry) and 50 % residual culture medium,
- removal of the residual culture medium to obtain the "fresh spirulina biomass", ready to be consumed or dried, containing about 20 % dry matter and practically no residual culture medium.

3.17.9 Harvesting index

Spirulina was harvested based on two methods.

a. Measuring optical density (01) by using spectrophotometer

When spectrophotometer indicates that OD become above 1 then spirulina biomass is ready to harvest

b. Using "Secchi disk" reading

The "Secchi disk" is a self-made instrument: a piece of white plastic fixed at the tip of a graduated rod. In case of "Secchi disk", after dipping it vertically into the spirulina culture up to 3cm if the white piece cannot see then it ready to harvest.

3.17.10 Washing and filtering

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

3.17.11 Pressing

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

3.17.12 Drying

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

Sun drying is the most popular among small producers, but requires a few precautions. Direct sun drying must be very quick, otherwise the chlorophyll will be destroyed and the dry product will appear bluish. Whatever the source of heat, the biomass to be dried must be thin enough to dry before it starts fermenting. A very simple technique was applied to

dry spirulina. Firstly, spirulina pest was poured in a syringe and pressed it. Finally, spirulina came out from the syringe very thin spiral rod shaped diameter of I to 2 mm. The total duration of the drying should not exceed a few hours, preferably 2 hours.

3.17.13 Crushing

crushing was accomplished by blender to fine powder. The dry chips or rods are usually converted to powder by grinding in order to increase their apparent density.

3.17.14 Storage

The best storage is in heat sealed, aluminized plastic bags. It can be stored in glassed made bottle

3.18 Data collection on different parameters

Observations of experimental data were extracted from each pot. (plate: 06) Data were recorded respectively on which parameters observation as follows:

Yield related

1. Fresh weight (g/L)
2. Dry weight (g/L)
3. Dry weight (kg/1000 L)

3.19.1 Optical density

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

3.19.2 Fresh weight (kg/180L)

After harvesting, the fresh biomass of spirulina was calculated by using electric balance.

3.19.3 Dry weight (g/180L)

After drying the fresh biomass lost its moisture than the dry weight was taken by using electric balance.

3.20 Parameter

3.20.1 Fresh weight (kg/180L)

I harvested fresh biomass from each 180L solution and got 3.488 kg fresh biomass of spirulina. Doucha and Livansky (2009) observed similar variation in his study on open pond algae production. Richmond *et al.* (1990) also found the similar kind of biomass production.

3.20.2 Dry weight (g/180L)

244 g dry weight was observed from each 180 L solution. Michele *et al.* (2007) also found similar type of dry weight in open and close tank system. Manoj *et al.* (2011) showed same type of dry weight in his study.

BCSIR tried to produce spirulina in open pond system but huge amount of contamination was occurred in that procedure. We developed a closed tank production system of spirulina that was really contamination free and economically favorable for both rural and urban area. By the help of this spirulina production system we produced quality spirulina. To determine spirulina quality, we conducted a chemical analysis in BCSIR lab under following points:

3.21 Chemical composition analysis of spirulina

3.22 Chemical analysis of Zn and Fe from the supplied spirulina sample

Sample was digested and then weight of the 5g spirulina sample. After that acid digestion was carried out. Sample was made in ash (furnace); next again acid digestion was done. Made the solution up to the mark with DI water. Then sample run by AAS and standard run, sample run. At the end of the procedure concentration result convert to final result ppm/ppb.

3.22.1 Sample preparation for Zn and Fe analysis

3.22.2 Stock solution corresponding to 1000mg/l

Weigh to the nearest + 0.0002gm, approximate 1.00 gm metal (minimum purity 99.5%) and dilute in a covered 250 ml glass beaker with 40 ml HNO_3. Then added 100ml of water. Boil to expelled nitrous fumes, cool, transfer to 1000ml volumetric flask and filled to the mark with water.

3.22.3 Standard solution corresponding to 10mg/l

Pipette 10.00 ml of stock solution into a 1000ml volumetric flask. Added 20ml of nitric acid, fill to the mark with water and mixed well.

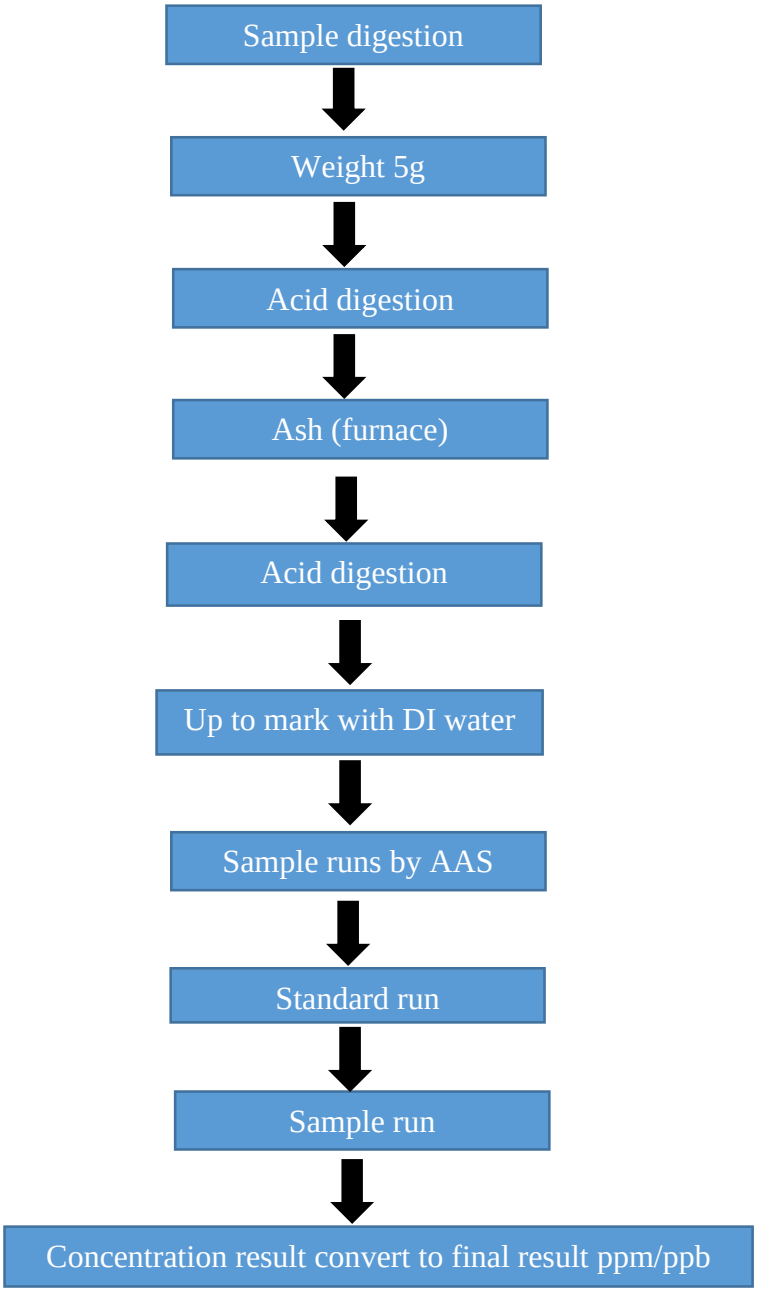


Figure 01: Sequential activities of the atomic absorption spectrophotometer

3.22.4 Standard solution corresponding to 0.4 mg/L

Pipette 20.00ml of standard solution into a 500 ml volumetric flask. Added 10ml of nitric acid, fill to the mark with water and mixed well. Prepared this solution on the day of use.

3.22.5 Standard solution corresponding to 0.02 mg/L

Pipette 5.00ml of standard solution into a 500ml volumetric flask. Added 10ml of nitric acid, filled to the mark with water and mixed well. Prepared this solution on the day of use.

Spoehr and Milner (1949) studied the chemical composition chlorella and find out similar type of result.

3.23 Chemical analysis of beta carotene by the help of UV-spectrophotometer

Added 2.5 ml of alkaline copper solution with 500µL of protein solution. Mixing properly and allowed to react 10 minutes. Added phenol reagent then rapidly mixing and wait for 60 minutes. At last measured the absorbance at 750 nm. (figure: 02)

3.23.1 Reagents

3.23.2 Alkaline copper solution: Mixed 50 mL of 2% Na2CO3 solution with 1 mL of 0.5% CuSO4 solution (can only be used for immediate analysis).

3.23.3 2% Na_2CO_3 solution: dissolved anhydrous sodium carbonate (2 g) and caustic soda (0.4 g) in 100 mL water

3.23.4 0.5% $CuSO_4$ solution: dissolved copper (II) sulfate pentahydrate (50 mg) and potassium sodium tartrate tetra hydrate (0.1 g) in 10mL of water.

3.23.5 Phenol reagent: Diluted commercial solution (2N phenol chemical reagent of Kanto chemical) to 1N.

3.24 Sample

Same concentrations are used both for the 10 mm rectangular cell and the micro cell of 10 mm.

Moorhead *et al*. (2005) studied same type of beta carotene chemical analysis and find out similar result.

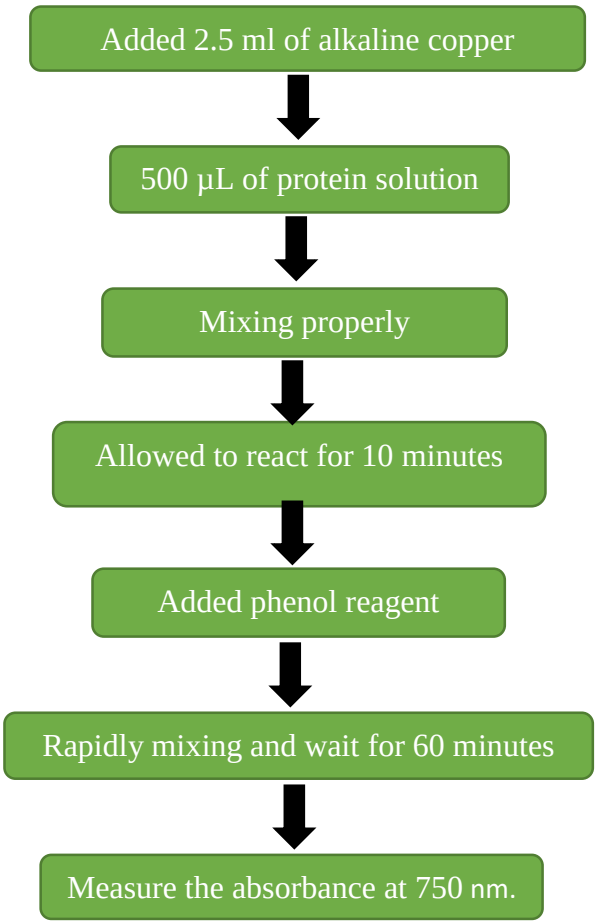


Figure 02: Sequential activities of UV-spectrophotometer methods

3.25 Analysis of total ash of spirulina sample

Two steps were involved in this procedure:

a. Crucible preparation

b. Total ash analysis

3.25.1 Crucible preparation

a) Mark a label on clean crucible(W_1)

b) Transfer the crucible into desiccator

c) Transfer the empty crucibles into muffle furnace

d) Adjust time 80 minutes and temp. 550^0 C

e) At the end of that, cool the furnace, and transfer crucible into desiccator for 30 minutes

f) Check analytical balance and calibrate

3.25.2 Total ash analysis

a) Weight crucible with sample 2g (W_2)

b) Crucible with sample on the hotplate for ash

c) Transfer sample into muffle furnace

d) Adjust Temp. 550^0 C and time 80 minutes

e) At the end of this process, cool furnace and transfer sample into desiccation for 30 minutes

f) Check analytical balance and calibrate (W_3)

Now Calculate total ash by following formula:

$$\text{Total ash content (g/100 g)} \Longrightarrow \frac{(W_3 - W_2)}{(W_2 - W_1)} \times 100$$

Where,

W1= Weight of crucible

W2= Weight of crucible + sample

W3= Weight of crucible + ash

Moorhead *et al.* (2005) studied same type of spirulina total ash chemical analysis and find out similar result.

3.26 Analysis of moisture of spirulina sample

3.26.1 Moisture analysis procedures

a) Weight of fresh spirulina sample

b) Pressed sample to remove some moisture

c) Kept in hot oven at 80^0 C for 48 hours

d) Weight of the dried sample

e) Measured moisture %

Moisture % determined by using following formula:

% of Moisture of supplied sample =

$$\frac{\text{Weight of fresh sample - Weight of oven dry sample}}{\text{Weight of fresh sample}} \times 100$$

Spoehr and Milner (1949) studied same type of spirulina moisture chemical analysis and find out similar type of result.

3.27 Analysis of protein of supplied spirulina sample

3.27.1 Sample preparation for protein analysis

a) 2 g of sample into digestion tube

b) Blank sample contain only conc. H_2SO_4 + Catalyst

3.27.2 Procedure for protein analysis

- In fume hood, 15 ml conc. H_2SO_4 was taken
- Catalyst Kjeltab cx into digestion tube
- Place the tube in the tube rack
- Putting the rack to the digestion block
- Set temperature and time
- Wait A few hours
- Turn on the water tap
- Place empty digestion tube
- Conical flask in distillation unit process cleaning program
- At the end of digestion process take the hot digestion tube from distillation unit
- Cool digestion tube with tap water

3.27.3 Carry out distillation process for blank and sample

- Prepare 50 ml boric acid, pour into conical flask
- Added 8 drops of indicators such as methyl red and bromocresol green
- Take out the blank digestion tube from the rack and placed it into the distillation unit
- After digestion, cool down the digestion tube with tap water

- Take the conical flask out and perform titration
- Clean the distillation unit

3.27.4 Run digestion sample

- Placed the conical flask (with 50 ml boric acid + indicators) and digestion tube with sample into the distillation unit
- Run digested sample
- After that performed titration

3.27.5 Run digested duplicate sample and then perform titration

- Carry out cleaning process after the distillation process ends

3.27.6 Titration

- Filled 0.1 M HCl into burette
- Record the volume of HCl before titration
- Perform titration
- Run HCl gradually from the burette into the conical flask and swix the conical flask along
- Continue the addition of HCl and swirling of flask until the end point is obtained
- When content changes from green to purple
- Record the volume of HCl (titrant)
- Perform calculating using formula

$$\% \text{ Nitrogen} = \frac{(\text{ml sample} - \text{ml blank}) \times N \times 14.007 \times 100}{\text{mg sample}}$$

Where,

N = Normality of titration to 4 places of decimal

Krishnakumari (1982) studied same type of spirulina protein chemical analysis and find out similar type of result.

3.28 Analysis of fat from supplied spirulina sample

3.28.1 Procedures of fat analysis of spirulina

- ✓ Preheat the sample to reduce moisture content by oven
- ✓ Cool the sample

3.28.2 Preheat extraction beaker

- ➢ Adjust the oven temperature to 105^0 C
- ➢ Preheat the extraction beakers in the oven for 1 hour
- ➢ Cool the extraction beakers in desiccator for 1 hour
- ➢ Weight the extraction beakers to obtain M_1 reading

3.28.3 Sample preparation

- ➢ Weight 2 g sample in a filter paper to obtain M_0 reading
- ➢ After that wrap it, and insert to the bottom of a thimble
- ➢ Next cover the sample with cotton wool

3.28.4 Solvent addition

- ➢ After that put beakers into the steel extraction beaker rack holder
- ➢ Bring the whole beakers rack with samples into fume hood
- ➢ Added the solvent which was hexane into extraction beaker

3.28.5 Extraction in soxtherm

- ➢ Insert the extraction beaker with solvent into soxtherm
- ➢ Started extraction
- ➢ After extraction take out extraction beakers from soxtherm and put into the steel extraction beaker rack holder
- ➢ Heat the extraction beakers in the oven at 105^0 C for another 1 hour if some of the residual solvents were not removed
- ➢ After that cool the extraction beakers in desiccator for 1 hour
- ➢ Weight the extraction beakers to obtain M_2 reading

3.28.6 Calculation of fat analysis

Fat content could be calculated by using the data obtained and the formula as below:

$$\text{Fat content} = \frac{(M_2 - M_1) \times 100}{M_o}$$

Where,

M_0 = Weight of the Sample

M_1 = Weight of the Extraction Beaker

M_2 = Weight of the Extraction Beaker with Oil

Krishnakumari (1982) studied same type of spirulina total ash chemical analysis and find out similar type of result.

Bleaching powder

$MgSO_4$

$FeSO_4.7H_2O$

Ascorbic acid

$NaHCO_3$

TSP

NaCl

K_2SO_4

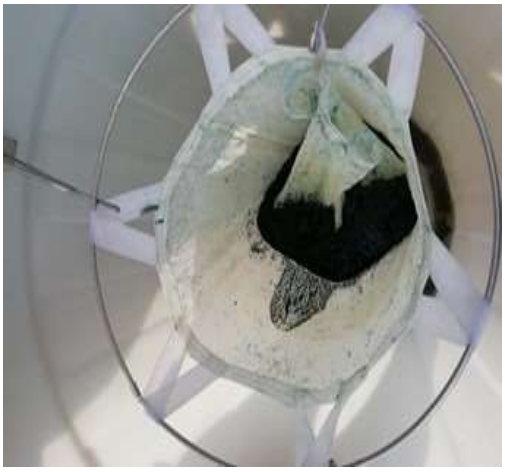
Na-EDTA

$NaNO_3$

Plate 01: Different necessary chemicals

Drilling machine

Connecting tube



Harvesting net

Electric motor

Container

Harvesting system

Plate 02: Different instruments for spirulina production

Atomic absorption spectrophotometer

UV-spectrophotometer

Optical density meter

P^H meter

Plate 03: Different kinds of machine for analysis purposes

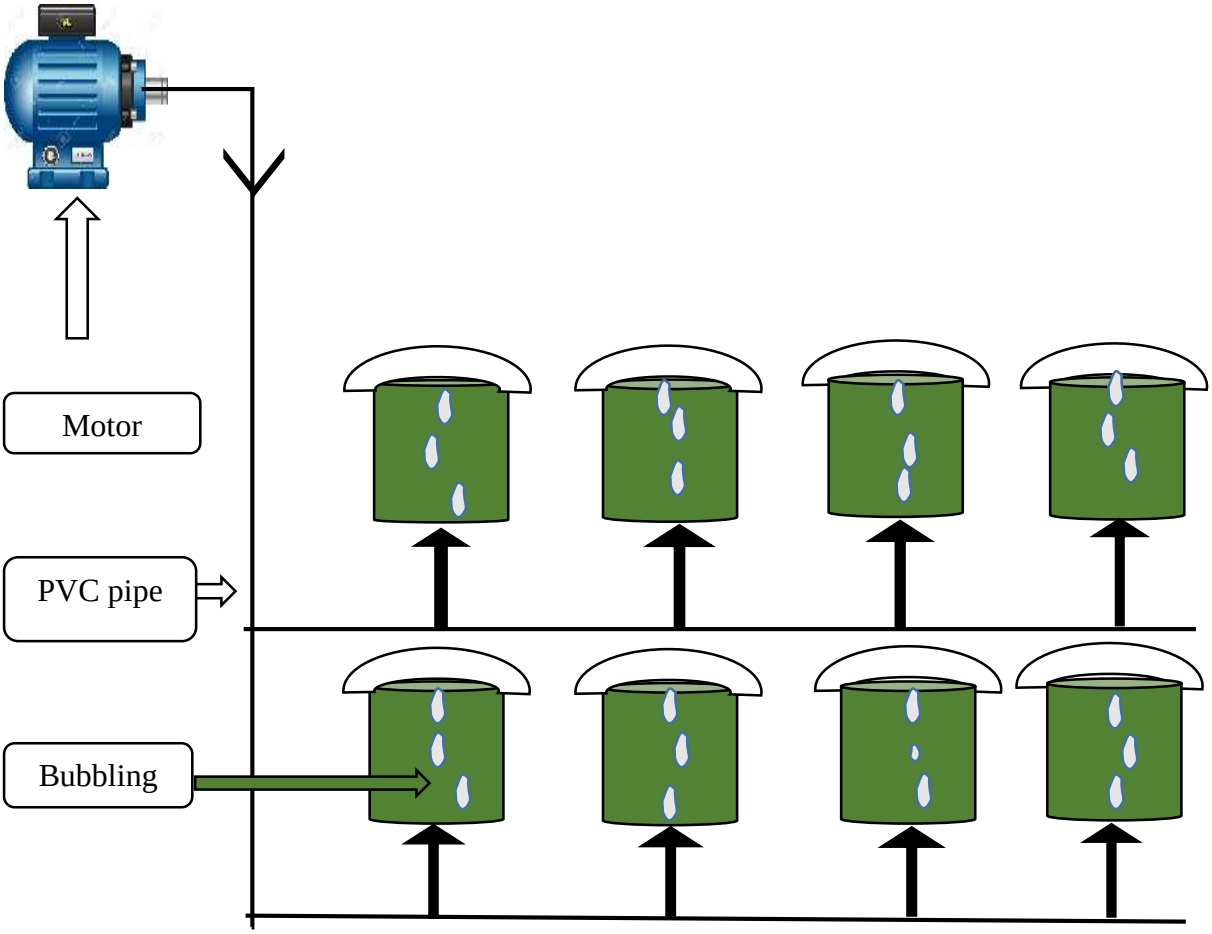


Plate 04: Schematic view of design and layout of the experiment

Installation

Inoculation

Bubbling and pumping system

Harvesting

Washing

Pressing

Plate 05: Sequential activities for spirulina production

Harvested spirulina

Drying process

Dry spirulina

Crushing

Spirulina powder

Spirulina storage

Plate 06: Sequential activities for Spirulina production

Fresh weight

Dry weight

UV-spectrophotometer measurement

OD measurement

P^H measurement

AAS measurement

Plate 07: Recording data

CHAPTER IV

RESULTS AND DISCUSSION

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Proximate composition of spirulina

Spirulina has high quality protein content (59–65 percent), which is more than other commonly used plant sources such as dry soybeans (35 percent), peanuts (25 percent) or grains (8–10 percent). A special value of spirulina is that it is readily digested due to the absence of cellulose in its cell walls (as it is the case for eukaryotic green microalgae such as Chlorella, Ankistrodesmus, Selenastrum, Scenedesmus): after 18 hours more than 85 percent of its protein is digested and assimilated (Sasson, 1997). The composition of commercial spirulina powder is 60 percent protein, 20 percent carbohydrate, 5 percent fats, 7 percent minerals, and 3–6 percent moisture, making it a low-fat, low calorie, cholesterol-free source of protein. (Table: 02)

Spoehr and Milner (1949) studied the chemical composition of spirulina and find out its component like protein 61.00 (g/100g), fat 6.00 (g/100g), total ash 9.00 (g/100g), moisture 6.00 (g/100g)

Moorhead *et al.* (2005) spirulina chemical analysis was performed and find out protein 67.00 (g/100g), fat 5.00 (g/100g), zinc 3.00 (mg/100g), iron 217 (mg/100g), beta carotene 225 (mg/100g), total ash 13.00 (g/100g).

Habib *et al*. (2008) studied the chemical composition of spirulina and find out protein 60.00 (g/10g), fat 7.00 (g/100g), total ash 11.00 (g/100g), moisture 9.00 (g/100g).

Table 02: Chemical analysis of fat, zinc, iron, beta carotene, total ash, moisture and protein from supplied spirulina sample

Sl. No.	Test parameter	Test values, SAU	FOI, France	USA	SAC, Thailand	IPGSR, Malaysia	BAU
1	Fat (g/100g)	0.14	4	5	6	6	7
2	Zinc (mg/100g)	2.9		3			
3	Iron (mg/100g)	66.2		217			
4	Beta carotene (mg/100g)	99.24		225			
5	Total ash (g/100g)	11.24	3	13	9	9	11
6	Moisture % (g/100g)	14.53			6	6	9
7	Protein (g/100g)	42.01	65	67	55-70	61	60

From the above discussion it can be mentioned that, in this study, spirulina sample contain lower amount of fat 0.14 (g/100g), which is different from France 4.00 (g/100g), USA 5.00 (g/100g), Malaysia 6.00 (g/100g), Thailand 5-7 (g/100g).

From the above discussion it can be notified that, produced spirulina contain zinc 2.90 (mg/100g), which was similar type like USA 3.00 (mg/100g) of spirulina produced in the world.

Produced spirulina contained 66.20 (mg/100g) of iron, which was different from USA 217.00 (mg/100g).

From the above discussion it can be mentioned that, produced spirulina contain beta carotene 99.24 (mg/100g) which was difference from USA 225.00 (mg/100g).

From the above discussion it can be notified that, produced spirulina contained total ash 11.24 (g/100g), which was different from USA 13.00 (g/100g), France 3.00 (g/100g), Thailand 3-6 (g/100g), Malaysia 9.00 (g/100g).

From the above discussion it can be mentioned that, in this study, spirulina sample contained moisture 14.53 (g/100g), which was different from Thailand 4-6 (g/100g), Malaysia 6.00 (g/100g).

Produced spirulina contained 42.01 (g/100g) of protein, which was different from USA 67.00 (g/100g), France 65.00 (g/100g), Thailand 55-70 (g/100g), Malaysia 61.00 (g/100g).

4.2 Chemical composition

Chemical composition of spirulina was analyzed in this experiment and find out some important component of spirulina like protein, fat, moisture, beta carotene, zinc and iron. By this study, beta carotene 99.24 (mg/100g), fat 0.14 (g/100g), total ash 11.24 (g/100g), moisture 14.53 (g/100g), protein 42.01 (g/100g), zinc 2.90 (mg/100g), iron 66.20 (mg/100g) were measured from supplied spirulina sample.

Fat content of standard spirulina is 4-7 g/100g. Fat content (0.14 g/100g) in our produced spirulina is very poor than standard spirulina. So our spirulina is standard type.

Zinc content of standard spirulina is 3 mg/100g. Zinc content (2.9 mg/100g) of produced spirulina is equivalent to standard spirulina. So produced spirulina is standard type.

Standard spirulina iron content is 217 mg/100g. Though Iron content (66.2 mg/100g) in produced spirulina is lower than standard spirulina but in comparison with available banana this amount was 220.67 parts higher. So our spirulina is Iron rich food.

Beta carotene of standard spirulina is (225 mg/100g). Though Beta carotene content (99.24 mg/100g) in produced spirulina was lower than standard spirulina but in comparison with available jackfruit this was 2.33 parts higher. So our spirulina is beta carotene rich food.

Total ash amount of standard spirulina is 3-13 g/100g. Total ash content (11.24 g/100g) of produced spirulina is equivalent to standard spirulina. So produced spirulina is standard type.

Protein amount of standard spirulina is (55-70 g/100g). Though Protein content (42.01 g/100g) of produced spirulina was quite lower than standard spirulina but 8.4 parts higher than available pulse crops. So our produced spirulina is rich in protein content.

CHAPTER V

SUMMARY AND CONCLUSION

CHAPTER V

SUMMARY AND CONCLUSION

5.1 Summary

Spirulina is a new consumer item in Bangladesh that is yet to get proper recognition. It is packed with nutritive and healthy benefits. Spirulina production is arguably the most cost effective method to tackle malnutrition and rural poverty. Government institutions like the Department of Agricultural Extension (DAE) should be made responsible for disseminating the Spirulina cultivation technology among the interested people who want to give Spirulina cultivation a go. Other governmental organizations and NGOs can also provide the required training and skill to cultivate Spirulina and make it a tool for sustainable growth.

So the main barriers of quality spirulina production are contamination, maintenance, growth rate, productivity and photosynthetic efficiency and the costly growth chamber. Bangladesh has a climate that is particularly favorable for production of spirulina which can enable us to exploit its high demand as dietary food supplement and boost a new processed food industry that has recently started its expansion.

For screening out suitable production technology of spirulina, an experiment was accomplished on the rooftop of agricultural faculty of Sher-e-Bangla Agricultural University during the period of April to October, 2018 to screen for finding out more economically convenient, nutritionally outstanding and easily available one for spirulina production in Bangladesh.

In spirulina production system, it could be maintained some important climatic condition like, temperature, light, photo inhibition, oxygen concentration etc. Spirulina production system was closed type system that totally contamination free. Water quality was very important factor in this production system. Appropriate inoculums size was used and continuous interval agitation was providing to stimulate multiplication of spirulina cells. To obtain quality spirulina continuous monitoring is required.

Protocol for spirulina production system was performed by following headings like installation of the containers, chlorination of containers, DE chlorination of containers,

culture media preparation, strain inoculation in containers, intercultural operation, harvesting, harvesting index, washing and filtering, pressing, drying, crushing, storage, data collection of different parameter like fresh weight, dry weight, optical density.

I harvested fresh biomass from each 180 L solution every 30 days' interval and got 3.488 Kg fresh biomass of spirulina. 244 g Dry weight was observed from each 180 L solution.

Protocol for spirulina chemical composition analysis was performed by following points like chemical analysis of Zn and Fe (sample preparation of different standard solution, sample digestion, weight 5g, acid digestion, ash furnace, acid digestion, up to mark with DI water, sample runs by AAS, standard run, sample run, concentration result convert to find result ppm/ppb), chemical analysis of beta carotene by the help of UV-spectrophotometer (reagent preparation, sample preparation, added 2.5 ml of alkaline copper solution, 500 µL of protein solution, mixing properly, allowed to react for 10 minutes, added phenol reagent, rapidly mixing and wait for 10 minutes, measured the absorbance at 750 nm), analysis of total ash of spirulina sample (crucible preparation, total ash analysis formula), analysis of moisture of spirulina sample (moisture analysis procedure, moisture analysis formula), analysis of protein of spirulina sample (procedure for protein analysis, carried out distillation process for blank and sample, run digestion sample, run digested duplicate sample and then performed titration), analysis of fat of spirulina sample (procedures of fat analysis, preheat extraction beaker, sample preparation, solvent addition, extraction in soxtherm, calculation of fat).

Chemical composition of spirulina was analyzed in this experiment and find out some important components of spirulina like protein, fat, moisture, beta carotene, zinc and iron. By this study, beta carotene 99.24 (mg/100g), fat 0.14 (g/100g), total ash 11.24 (g/100g), moisture 14.53 (g/100g), protein 42.01 (g/100g), zinc 2.90 (mg/100g), iron 66.20 (mg/100g) were measured from supplied spirulina sample.

By the help of this study, standard quality spirulina production technology was obtained and produced world class spirulina in comparison to other standard type of spirulina produced in the world.

5.2 Conclusion

It can be concluded from the findings of the study that the quality spirulina production system was developed under Bangladesh condition.1. According to the result, this production technology is the best spirulina production technology for Bangladesh condition. On the basis of discussion section, it can be cleared that, Spirulina production technology was the best production technology, first in our country. 2. Produced spirulina was the best quality spirulina according to the standard type spirulina produced in the world. Through the present study, it was evaluated based on the different performance as a base line for the researchers.

5.3 Recommendation

1. It is clear from the preceding discussion that the closed tank spirulina production system in roof top is a standard type of spirulina production system in Bangladesh and produced spirulina is as same as the world class quality spirulina.

2. The similar work can be conducted in other parts of the country to validate the result of the present results.

CHAPTER VI

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APPENDICES

APPENDICES

Appendix I. Monthly record of air temperature, relative humidity and rainfall of the experimental site during the period from April, 2018 to October, 2018

Months	*Air temperature (^{0}C)		*Relative humidity (%)	*Rainfall (mm) (total)
	Maximum	Minimum		
April, 2018	42.2	16.7	71	153.3
May, 2018	41.1	14.4	76	339.4
June, 2018	36.7	19.4	82	340.4
July, 2018	35	21.1	83	373.1
August, 2018	36.1	21.7	82	316.5
September, 2018	36.7	21.1	83	300.4
October, 2018	37.2	17.2	78	172.3

*Monthly average

*Source: Bangladesh meteorological department (Climatic and weather division) Agargaon, Dhaka-1207

Appendix II. Different information of the container which was used for spirulina production

Sl. No.	Items	Values
01	Container weight	5.00 kg
02	Container length	37.00 inches
03	Container breadth	32.00 inches
04	Container diameter	85.00 inches
05	Container thickness	1.27 cm