IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)
e-ISSN: 2319-2402,p- ISSN: 2319-2399.Volume 12, Issue 3 Ver. II (March. 2018), PP 74-78
www.iosrjournals.org

Mass culture of phytoplankton & culture of *Spirulina platensis* using different nutrients

Tauhiduzzaman[1], Md. Asad Ali[1], Sheikh Sahiduzzaman[1]
[1](*Fisheries and Marine Resource Technology Discipline, Khulna University, Bangladesh)*
Corresponding Author: Tauhiduzzaman

Abstract: *An experiment was conducted to determine the effect of organic (poultry droppings, cow dung, urea, and mustered oil cake) and inorganic (TMLR) nutrients on the production of freshwater phytoplankton in transparent plastic bottles under the intensive condition. The abundance of phytoplankton varied from 0.3×10^5 to 7.24×10^5 individual per milliliter. Nine genus of phytoplankton were identified, but there were unidentified genus too. Among the organic and inorganic nutrient medium, in the TMLR there was found $7.24\pm0.56\times10^5$ individual per milliliter to be the most suitable for phytoplankton culture in term of total production. Nine genus of phytoplankton were identified as Peranema, Volvox, Spirogyra, Hormodium, Schizothrix, Nostoc, Peranema, Synedra, and Spirulina. In the TMLR treatment, Spirulina was $0.937\pm0.407\times10^5$ per milliliter that is more than other groups; it was greener than other treatments. Isolated Spirulina platensis was cultured in TMLR media with different doses: 1.4, 2.8, and 5.6 gm/L. In the 5.6 gm/L TMLR nutrient, the highest cell number was $9.26\pm0.42\times10^5$ per milliliter.*

Keywords: *Mass, Culture, Phytoplankton, Algae, Spirulina platensis, TMLR, nutrient*

I. Introduction

Plankton community consists of phytoplankton, primary producer and zooplankton which are dependent on phytoplankton for food [1]. Phytoplankton is considered as biological wealth of freshwater ponds, and it directly involved with other community of the water body from zooplankton to fishes. Phytoplankton represents the status of a water body and has sensitivity to change physiochemical parameters [2]. In hatchery, phytoplankton is used as food for raring the fish larvae. The success of hatchery fully depends on availability of live feed [3].

Larval rearing is at the same time one of the most lucrative and riskiest aquaculture systems. Some special strategies are needed to overcome risks during larval rearing. A larva cannot survive without immediate accessing of optimum live food, for example phytoplankton and zooplankton with other supplementary feeds. Supplemented artificial feed does not contain all essential components for better growth of larva. Therefore, plankton is required and fed with other feeds for healthy larvae [4].

Many trace minerals and vitamins are necessary for microalgae culture [5]. Organic fertilizers (for example, animal manures, mustered oil cake, soybean meal) are often used to cultivate planktons, but the fertilizers should have fine particle and lower composition of carbon and nitrogen because it allows fast decomposition [6]. Some naturally occurring nutrient media consist of soil extracts, vitamins, trace elements using for freshwater and saltwater phytoplankton effectively [7]. Some inorganic medium consisting of inorganic nitrates, phosphates, sulfate, other trace elements have been using aquaculture [8]. Size of phytoplankton culture varies from small scale to large scale. It may culture in 1L to thousands liter water [9].

Spirulina is microscopic blue-green algae which has no branch and enrich with vitamins, proteins, iron and essential fatty acids such gamma linolenic acid (GLA). *Spirulina* had been using as a food in Mexico for 1000 years during Aztec civilization. It is used as a good source of nutrients by the natives in the Lake Chad. Its safety for human has since been recorded through various studies by the United Nations Industrial Development Organi-zation (UNIDO). It can grow in high pH up to 11 where other most plankton cannot grow. At present, it is cultivated commercially and sold as a food through the planet because of its nutritional value. It is also recorded *Spirulina* helps enhancing the immune system, protecting intestinal lactobacillus, decreasing nephrotoxicity and radiation protection. Thus, *Spirulina* has been popular in hatchery [10]. The research was conducted to evaluate the culture medium for the successful propagation of phytoplankton especially *S. platensis***.**

II. Materials and Methods

Plankton collection: The experiment was conducted in Water Chemistry Laboratory of Fisheries and Marine Resource Technology (FMRT) Discipline of Khulna University from July to August. Water was collected from ponds of Fisheries and Marine Resource Technology (FMRT) Discipline at Khulna University campus longitudes 89°31'58.8"E and latitude 22°48'7.2"N.

Experimental set-up: Eight different organic and inorganic nutrient medium were used for freshwater phytoplankton growing. Organic nutrients medium were poultry droppings, cow dung, urea, mastered oil cake and TMLR medium [11] was only inorganic nutrient medium. Each treatment had three replicates.

Different medium:

Inorganic Nutrient		Amount (gm/L)
Treatment 8 (T_8)	TMLR	1.14
Organic Nutrient		
Treatment1 (T_1)	Poultry Droppings (Dry)	2.00
Treatment2 (T_2)	Cow Dung (Dry)	2.00
Treatment 3 (T_3)	Urea (Granular)	2.00
Treatment4 (T_4)	Mustered Oil Cake	2.00
Treatment5 (T_5)	(poultry droppings: cow dung: mastered oil cake=1:1:1)	2.00
Treatment6 (T_6)	(poultry droppings: cow dung: mastered oil cake=1:1:1)	2.50
Treatment7 (T_7)	(poultry droppings: cow dung: mastered oil cake=1:1:1)	3.00

TMLR medium was prepared diluting the following ingredients: $NaNO_3$ 1 gm, $NaH_2PO_4.H_2O$ 0.1 gm, Na_2SiO_3 0.01 gm, and $FeCl_3.6H_2O$ 0.03 gm are added to 1 liter water.

TMLR medium for Isolated *S. platensis*: Isolated *S. platensis* were cultured in 1.4 gm/L (t_1), 2.8 gm/L (t_2), 5.6 gm/L (t_3) TMLR media for 20 days.

Water collection and treatment: Pond water was treated and then kept in cylindrical and transparent plastic bottles for culturing. Light intensity plays an important role, but the requirements vary greatly with the culture depth and the density of the algal culture: at higher depths and cell concentrations the light intensity must be increased to penetrate through the culture. Light may be natural or supplied by fluorescent tubes. Overheating due to both natural and artificial illumination is avoided. Aerators were used for mixing the water and for better dissolved oxygen. Nutrients were measured by electric weight. One liter water was measured by one liter conical flask. pH meter, DO meter, Thermometer were used for analysis the water quality of culture system. Electronic microscope, Sedgwick-Rafter cell, Micro-pipette was used for qualitative and quantitative analysis of green water.

Quantitative and qualitative analysis of phytoplankton: The quantitative enumeration of the phytoplankton was carried out with the help of a Sedgwick-Rafter (SR) cell which is 50mm long, 20mm width and 1mm deep. Before filling the SR cell with sample, the cover glasses were diagonally placed across the cell and then samples were transferred with large bore pipette so that no air bubbles in the cell cover were formed. The SR cell was let stunned for at least 5 minutes to settle phytoplankton. Then plankton on the bottom of Sedgwick-Rafter cell was enumerated by compound microscope. The number of plankton in the S-R cell was derived from the following equation:

$$\text{No/ml} = \frac{C \times 1000\ mm3}{L \times D \times W \times S}$$

Where,

Where, C = Number of plankton; L = length of a strip in mm; D = depth of a strip in mm; W = Width in mm, S = number of strips counted [12].

. Phytoplankton cells were enumerated under a light microscope by using Sedwick-Rafter cell. Recognition of species is matter of experience. A series of pencil and ink drawing on postcards of the species of the observed were prepared to identify the organisms. Phytoplankton genera and species were identified using a variety of bibliographic references.

***S. platensis* Isolation:** *S. platensis* was isolated following the Micropipette Washing Technique [13]. A Pasteur's pipette was used to catch a *S. platensis*; this catching process continued for several times. Bold's Basal Medium was used for the propagation of the isolated planktons.

Statistical analysis: Statistical analysis was performed using Microsoft Excel and IBM SPSS Software Package Version 21.

III. Results

Temperature, pH, and Dissolved Oxygen: The temperature was 32.3±2.21°C, pH was7.4±0.5, and dissolved oxygen was 5.76±0.19 mg/L of the water of the culture system.

Phytoplankton Concentration: The maximum phytoplankton concentration was observed between 7th and 11th day of all treatment (Figure1). After 11 days, the concentration was decreasing day by day. The most abundant phytoplankton was found in the T_8, $7.24\pm0.56\times10^5$ on 11th day.

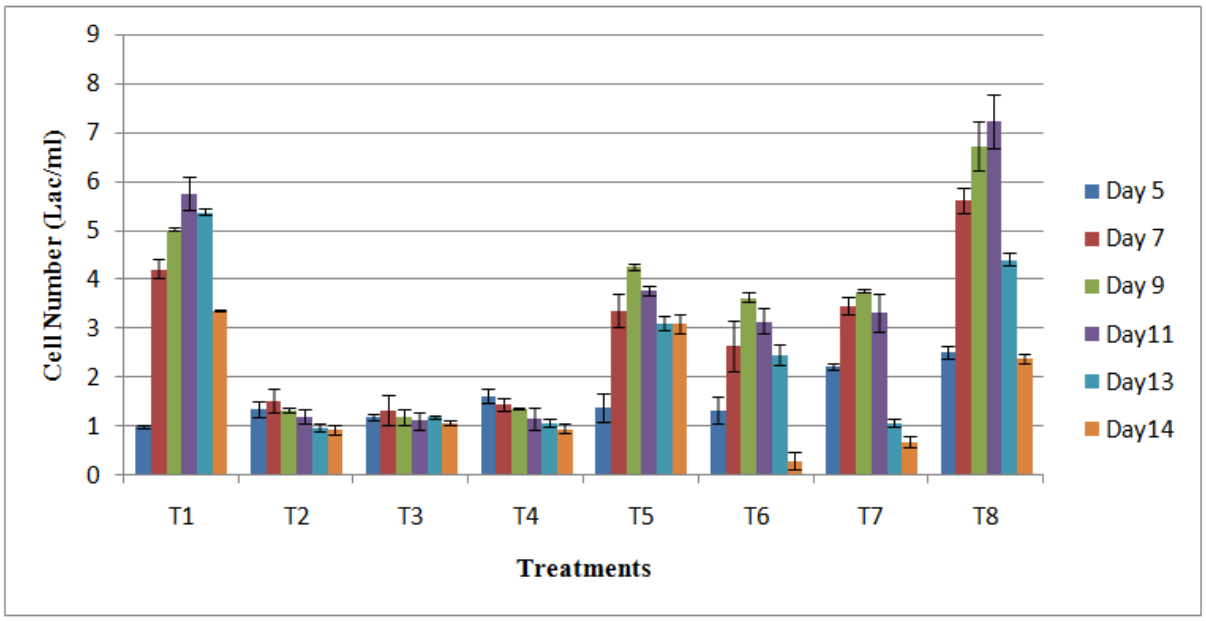

Figure1: Total phytoplankton abundance in mass culture

Spirulina was prominent in the TMLR medium (T_8), $1.41\pm0.063\times10^5$ individual per milliliter. It was on the 11^{th} day like other phytoplankton.

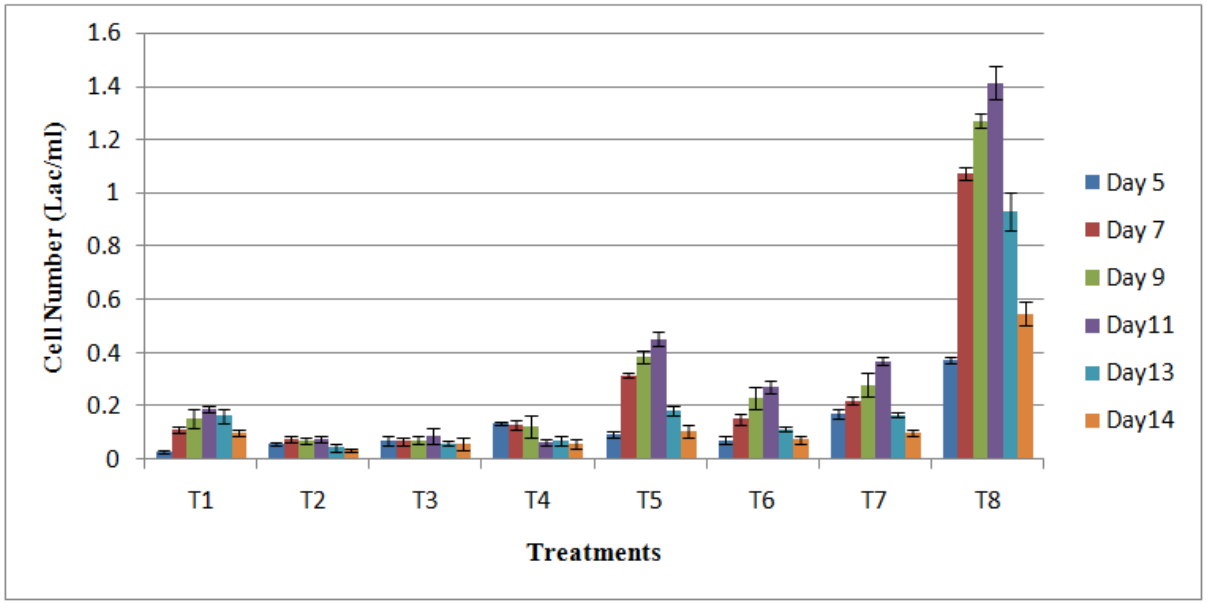

Figure 2: Abundance of *Spirulina* throughout the culture system

The number of *Spirogyra* varies throughout the culture system; it was 0.36 to 8.6 % of total cell number. The lowest number of Spirogyra ($0.009\pm0.0026\times10^5$ individual per milliliter) founded in the T_8 on the fifth day, and the highest number was in the T_5, $0.072\pm0.0063\times10^5$ individual per milliliter on the 11^{th} day.

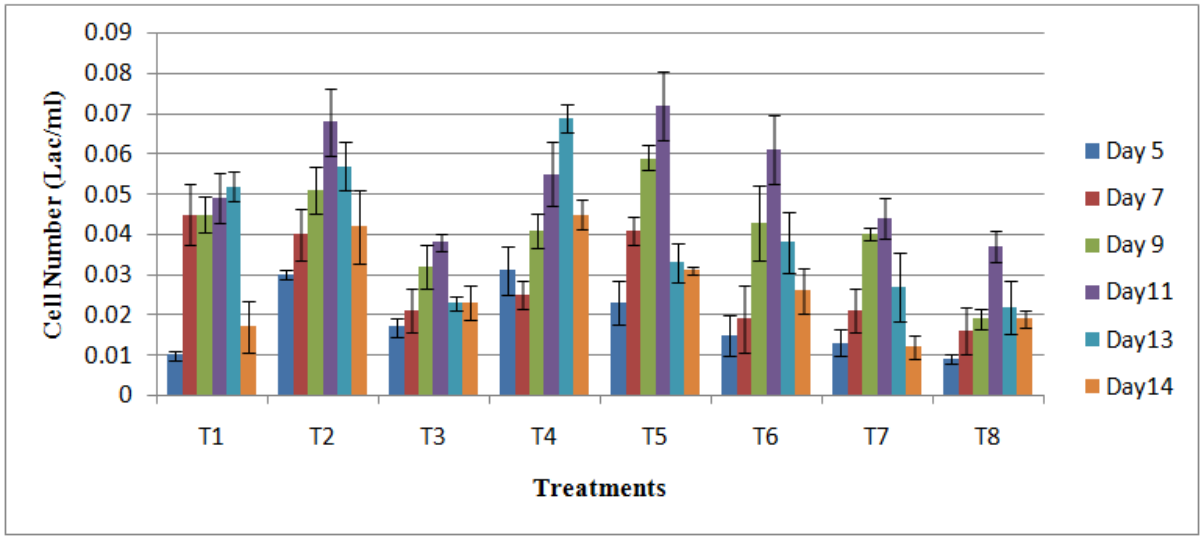

Figure 3: Concentration of *Spirogyra* throughout the mass culture system

Peranema sp., Volvox sp., Hormodium sp., Schizothrix sp., Nostoc sp., Peranema sp., Synedra sp., and some unidentified phytoplankton together were prominent (76.1 to 96.5%) in the mass culture system.

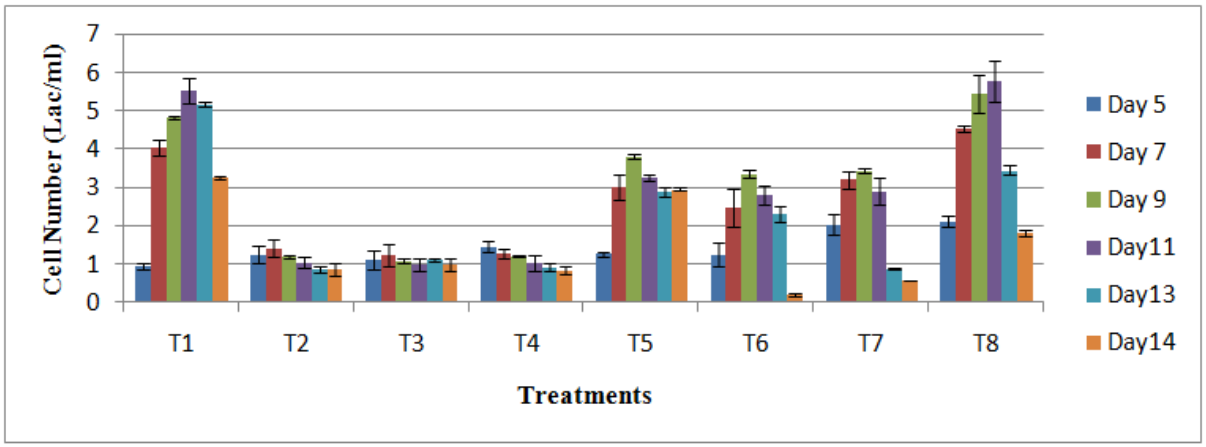

Figure 4: Total phytoplankton except *Spirogyra* and *Spirulina*

Abundance of *S. platensis* in isolated culture: *S. platensis* was the highest amount, 9.26±0.42 × 10^5 cells per milliliter water, at the maximum TMLR nutrient media (t_3). The phytoplankton culture system continued until 20th day although the treatment three (t_3) was not decreasing the cell number on 20th day.

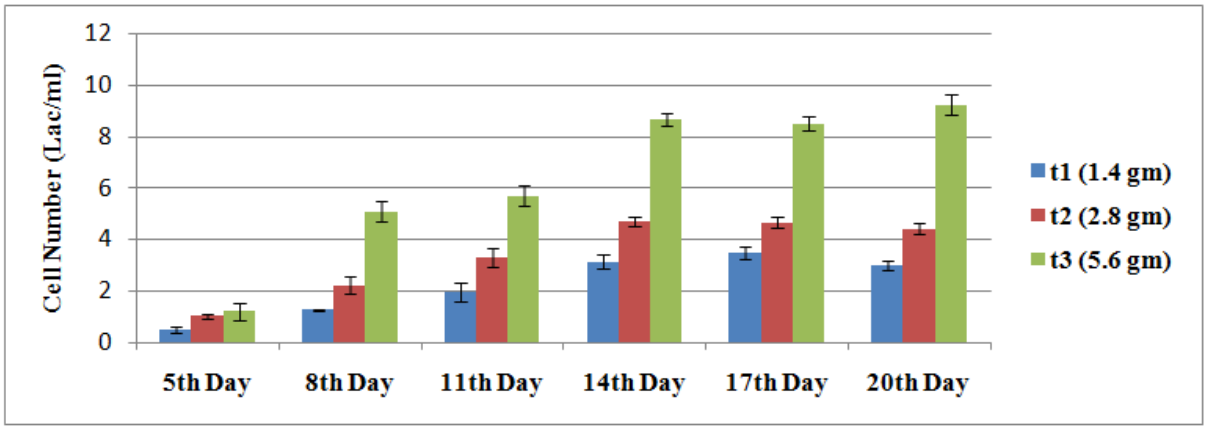

Figure 5: Abundance of *S. platensis* in the isolated culture system in different dosages

IV. Discussion

There were eight treatments and every treatment contains 1 liter of water. Every bottle was same in shape, transparency and so on. The treatments water exposed to 24 hours light.

During this study period (from 5th day to 14th day) the average abundance of phytoplankton were found in treatment T_1, T_2, T_3, T_4, T_5, T_6, T_7, T_8 respectively 10^5 -5.77×10^5, 1.35×10^5-1.52×10^5, 1.19×10^5-1.33×10^5, 1.37×10^5-1.62×10^5, 1.38×10^5-4.26×10^5, 1.34×10^5-3.64×10^5, 2.22×10^5-3.77×10^5, 2.51×10^5-7.24×10^5 individual per milliliter. These findings are similar to the findings of Hossain *et al*. (2007) [14]. They reported the abundance of phytoplankton in freshwater ponds varied from 60800 to 239400 cells per liter. Affan *et al*. (2005) also recorded the number of freshwater phytoplankton differed from 25.6 to 1590.6×10^3 per milliliter of quaculture ponds in Bangladesh [15]. In T8 treatment *Spirulina* was more amount than other groups (figure2), as a result; it looks greener than other treatments. As *Spirulina is* blue green algae, so it creates green color of water. As a result T_8 treatment looks greener than other treatments. T_1, T_2, T_3, T_4, T_5, T_6, T_7 have more diatom than T_8. Since pigments that diatoms use for photosynthesis yellow-brown because the sealgae use yellow-brown pigments to capture sunlight, unlike land plants, which primarily use green pigments. Diatoms use brown pigments so T_8 was less green. Diatoms grow on silica; poultry droppings, cow dung contain silica. As a result of abundance of Diatom, these treatments were less green. It was obvious that T_8 contains the highest number of mass phytoplankton cells as well as *Spirulina.* Thus, TMLR nutrient was selected for isolated *Spirulina*.

Nitrogen (N) and phosphorus (P) are crucial elements for the growth of phytoplankton. TMLR media contain both of them. Guillard (1975) media also contain them [16]. The density of *S. platensis* varied with dosages of nutrient media and days. If nutrient density increases the duration of the stationary phase of the culture system increases along with its cell density.

V. Conclusion

Live foods especially phytoplankton production is one of the most important operations of shrimp and fish hatcheries in Bangladesh. It is crucial that suitable foods be produced in sufficient amounts for the different larval stages. The result shows that the abundance and the types of phytoplankton vary with nutrients. Thus, the desired species of phytoplankton may culture using certain nutrient media.

References

[1]. Battish SK. Freshwater zooplanktons of India. Oxford and IBH publishing co. Ltd., New Delhi. 1992.
[2]. Reynolds CS. What factors influence the species composition of phytoplankton in lakes of different trophic status? Hydrobiol. 1998; 369/370: 11-26.
[3]. James PM, Fox JM. Hatchery techniques for Penaeid shrimp utilized by Texas A&M-NMFS Galveston Laboratory. Crustacean Aquaculture. 1983; 1: 129-154.
[4]. Lim LC. An overview of live feeds Production systems in Singapoor. 2003. In: Fulks W, Main KL (eds.) Rotifer and Microalgae culture systems. Proceedings of a U.S.Asia workshop. The Oceanic Institute, Honolulu, 187-201. 28-31 January 1991.
[5]. Ukeles R. American experience in the mass culture of microalgae for feeding larvae of the American oyster, *Crassostrea virginica*. 1976. In: Shelef G, Soeder CJ (eds.) Algae Biomass. Elsevier / North- Holland BiomediEal Press, New York, 287-306.
[6]. Geiger JG, Turner CJ. Zooplankton production and manipulation in striped bass rearing ponds. *Aquaculture*. 1990; 35: 331-351.
[7]. Creswell RL. Aquaculture Desk Reference. Van Nostrand Rheinhold, New York. 1993.
[8]. Hoff FH, Snell TW. Plankton culture manual, First edition, Florida Aqua Farms, Inc., Florida, USA. 1989.
[9]. Martin L, Arenal A, Fajardo J, Pimental E, Hidalgo L, Pacheco M, Garcia C, Santiesteban D. Complete and partial replacement of artemia nauplii by *Moina micura* during early post larval culture of white shrimp *Litopenaeus schmitti*. Aquaculture Nutrition. 2003; 12(2): 89-96.
[10]. Belay A, Ota Y, Miyakawa K, Shimamatsu H. Current knowledge on poten-tial health benefits of Spirulina. Journal of Applied Phycology. 1993; 5: 235-241.
[11]. Rajeswari MV, Balasubramanian T. Comparative study on growth of *Skeletonema costatum*: a microalga as live feed for aquaculture importance. International Journal of Research in Fisheries and Aquaculture . 2014; 4(3): 117-121.
[12]. APHA. Standard methods for the examination of water and waste water. American public health association, Washington. 1976.
[13]. Phang SM. Chu WL. University of Malaya Algae culture Collection, Cata-logue of Strains. Institute of Post Graduate Studies and Research, University of Malaya, Kuala Lumpur, Malaysia. 1999.
[14]. Hossain MY, Jasmine S, Ibrahim AHM, Ahmed ZF, Ohtomi J, Fulanda B, Begum M, Mamun A, El-Kady MAH, Wahab MA. A preliminary observation on water quality and plankton of an earthen fish pond in Bangladesh: Recommendations for future studies. Pakistan Journal of Biological Sciences. 2007; 10: 868-873.
[15]. Affan MA, Jewel MAS, Haque M, Khan S, Lee JB. Seasonal cycle of phytoplankton in aquaculture ponds. Algae. 2005; 20(1): 43-52.
[16]. Guillard RRL. Culture of phytoplankton for feed-ing marine invertebrates. 1975; pp. 29−60 in: Smith WL, Chanley HH (eds.). Culture of Marine Inverte-brate Animals. Plenum Press, New York, New York.

Tauhiduzzaman. "Mass culture of phytoplankton & culture of Spirulina platensis using different nutrients." IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT) 12.3 (2018): 74-78.