Bangladesh Journal of Veterinary and Animal Sciences
Journal home page: www.bjvas.com
pISSN 2227-6416
eISSN 2709-2542

Research article

Screening of natural pigments from indigenous marine microalgae isolated from different coastal aquafarms of Bangladesh

Zahidul Islam[1], Helena Khatoon[2*], Mohammed Redwanur Rahman[2], Jakia Hasan[1]and Shanur Jahedul Hasan[1]

[1]Marine Fisheries and Technology Station, Bangladesh Fisheries Research Institute, Cox′s Bazar-4700, [2]Department of Aquaculture, Chattogram Veterinary and Animal Sciences University, Chattogram-4225.

A R T I C L E I N F O

Article history:

Received: 03/10/2021
Accepted: 23/02/2022

Keywords:

Indigenous microalgae, chlorophyll, carotenoids, phycobiliproteins, viability

[*]*Corresponding author:*
Cell: +8801716396008
E-mail: helena@cvasu.ac.bd

A B S T R A C T

Globalization demands something new and natural. Natural pigments from microalgae could be something like that. However, it is a long way from a Petri dish to market. However, it is going to happen. So, knowing the pigment concentration among different species is necessary because of its significant role in the global market. The present study is designed to estimate the Chlorophylls, carotenoids and phycobiliproteins (Allophycocyanin, Phycocyanin and Phycoerythrin) contents of four (4) different indigenous marine microalgae species i.e. *Chlorella* sp., *Chaetoceros* sp., *Nannochloropsis* sp. *and Tetraselmis* sp. The result showed significant differences in the concentration of pigments among the species. However, *Tetraselmis* sp. produced a significantly higher amount of chlorophyll a (2.68 µg/L ± 0.04), and Chlorophyll b (1.23±0.02 µg/L) where *Chaetoceros* sp. had higher chlorophyll c (0.29±0.01 µg/L) among all the species. In the case of carotenoids, *Chlorella* sp. produced the lowest carotenoids (0.56±0.02 µg/L), where the other three showed no significant difference. Allophycocyanin and phycoerythrin were reported significantly higher (0.0197±0.0006 µg/L) and (0.0029 ± 0.0002 µg/L) in *Nannochloropsis* sp. rather than other three species where no significant difference was found in the concentrations of phycocyanin among all the species. Whatever the amount is, these microalgae are important because of their novel features. The study's findings will help the producer select a definite species according to their target and needs.

To cite this paper: *Z. Islam, H. Khatoon, M. R. Rahman, J. Hasan and S. J. Hasan, 2021. Screening of natural pigments from indigenous marine microalgae isolated from different coastal aquafarms of Bangladesh. Bangladesh Journal of Veterinary and Animal Sciences, 9(2): 61-72.*

1. INTRODUCTION

Microalgae are unicellular microscopic organisms containing numerous bioactive compounds that can be commenced for various commercial uses. In particular, marine micro-algae can process some distinguished bio-chemical characteristics that are not available in higher plants (Ramussen et al., 2007). Moreover, microalgae' play a significant role in balancing the biological systems of nature and contribute to the production of various food, medicine, and cosmetics (Wijffels, 2008), producing various important natural pigments.

Pigments types and compositions of microalgae vary based on basis of the group and species types (Dring, 1982). Green algae possess mainly chlorophyll b, whereas red algae possess phycoerythrin and phycocyanin and brown algae

possess chlorophyll a, b and fucoxanthin. All the pigments constituents have specific functions in the physiological process of microalgae. For example, chlorophyll helps absorb and utilize light in photosynthesis, and carotenoids help protect the photosynthetic apparatus from photodamage. Broadly microalgae pigments can be classified into three different types, which are Chlorophylls (water-insoluble). Carotenoids (water-insoluble) and Phycobilins (water-soluble). Chlorophylls have green pigments. Carotenoids are yellow or orange pigments (Kalidas and Loveson 2005). Phycobiliproteins are allophycocyanin (bluish-green), phyco-cyanin (blue), phycoerythrin (purple) and phycoerythrocyanin (orange) (Sekar and Chandramohan, 2008). Various microalgae were reported as the potential for producing pigments (chlorophylls and carotenoids) (Hosikian et al., 2010). These are considered photosynthetic pigments (Britton, 1983). Chlorophyll is known as one of the most important bioactive compounds of microalgae. Chlorophyll a, b and c are photosynthetic pigments that consist of very different chemical structures and are present in the form of porphyrin macrocycle (Britton, 1983; Brown et al., 1991; Costache et al., 2012). Chlorophyll helps absorb and uses light energy to produce carbohydrates from carbon dioxide and water. This light-harvesting component plays a significant role in the survival of microalgae (Humphrey, 2004).

With the change of market demand and regulations, artificial colours are replaced by natural colours in the food industry (Spears, 1988). The colouring of products is very important because producers try to develop uniform products where consumers want attractive products (Timberlake and Henry, 1986). Chlorophyll is a very costly natural food colouring agent used to replace artificial colours (Spears, 1988). Commercialization of these kinds of natural colouring agents is necessary to cope with the world's rising demand. Derivatives of chlorophyll are also used as pharmaceutical products as it is reported to accelerate wound healing activities by more than 25% (Smith and Livingston, 1945). It is also found that chlorophyll can prevent bacterial growth (Carpenter, 1949). During ulcers treatment, chlorophyll derivatives help eliminate pain and improve the appearance of affected tissue (Cady and Morgan, 1948). Lanfer-Marquez et al. (2005) reported antioxidant properties of chlorophyll derivatives. Because of these novel properties, chlorophyll is considered an important pigment.

Carotenoids are one of the most valuable natural pigments abundant in various fruits, vegetables and algae. It functions as an accessory, light-harvesting pigments and protects the photo-synthetic apparatus against photo-damage (Ben-Amotz et al., 1987). More than six hundred types of carotenoids have been reported where astaxanthin, β-carotene, lutein, canthaxanthin etc. are the most common. Kauar et al. (2009) mentioned the polyene system effects on carotenoids and their distinctive molecular structure and light absorbing characteristics.

In today's world, naturally synthesized caro-tenoids demand increases day by day, where most commercially available carotenoids are chemically synthesized (Jin et al., 2003). Using of carotenoids is expanding in various food industries because of their different pro-vitamin activity. In addition, β-carotene shows anti-oxidant and anticancer properties (Becker, 2004). Without that, β-carotene is also used in animal feed as a retinol source, human food as a food colouring agent, and cosmetics as additives (Johnson and Schroeder, 1995; Edge et al., 1997). Besides, carotenoids are used as pigment sources for salmon and trout.

Phycobiliproteins are photosynthetic coloured proteins with different functions in the plant physiological system. It consists of 50% of cyanobacteria's total protein and great nitrogen reserve source (Kauar et al., 2009). The chromophore's presence in different microalgae phycobiliproteins is classified into four groups (Gantt, 1980; Rowan, 1989; Ducret et al., 1998). These are Phycocyanin (PC), Phycoerythrin (PE), Phycoerythrocyanin (PEC) and Allo-phycocyanin (APC).

Phycobiliprotein is also greatly importantas a natural pigment considering today's commercial need. Primarily its use was reported as natural colour but so many studies show these proteins have shown great potential in the pharma-ceutical sector. These have significant anti-inflammatory, antioxidant, hepatoprotective and free radical scavenging properties that can be

easily isolated and safely be used in cosmetics and food colouring (Henrikson, 1989; Romay et al., 2000). Phycocyanin, an important protein mainly used as food items like as colourant in chewing gums, candies, soft drinks, dairy products and cosmetics like lipstick and eyeliners (Santiago-Santos et al., 2004) and is also used as a dye in the pharmaceutical and cosmetic industry (Batista et al., 2006). Pure phycobiliproteins are also used as fluorescent labelling (Telfer, 2001).

The algae based economy spreads across 23 European countries and covers different environments and production methods (Rita et al., 2021). Bangladesh is a densely populated country and has a potential industrial sector. Introducing microalgae-based natural pigments in the food, pharmaceuticals, and cosmetics industry is timely demand to produce the best quality products. To correctly confirm the proper use of microalgae pigments, it is essential to determine the types and amount of pigments present in various microalgae. However, there is no data available in the context of indigenous microalgae of Bangladesh. Hence, this study was aimed to extract and choose the amount of chlorophylls, carotenoids and phycobiliproteins presence in four selected indigenous microalgae species *(Chlorella* sp., *Chaetoceros* sp., *Nannochloropsis* sp., *and Tetraselmis* sp.) isolated from different coastal aqua farms of Cox's Bazar coast.

2. MATERIALS AND METHODS

Collection of microalgae sample for isolation

Coastal aquafarms are known as a good source of marine microalgae. Therefore two different aquafarms of Cox's Bazar coast were selected as microalgae sampling stations. These are Irawan Softshell Crab Farm, Chowfaldandi, Cox's Bazar, Bangladesh (21°51'23.74" N, 92°01'25.28" E) and Beximco Shrimp Farm, Khurushkul, Cox's Bazar, Bangladesh (21°45'29.21" N, 91°97'15.73" E). Three-four liter water samples were collected using a Van Dorn water sampler (Wildlife Supply Company, Saginaw, Michigan) and filtered by using a 100 µm mesh-sized plankton net. Then the filtered sample was preserved at refrigerated conditions before transfer into the laboratory. Without that, physical and chemical parameters (temperature (°C), salinity (ppt), dissolved oxygen (DO), conductivity, and pH) of the sampling station were monitored by using a multi-parameter kit (YSI meter 556 MPS, US) to maintain a perfect laboratory condition.

Isolation of pure microalgae stock

Initially, the collected samples were normalized and cultivated using Conway media (Tompkin et al., 1995). Then pure cultures were isolated by performing serial dilutions and using micropipette on a micromanipulator with the help of a microscope. Pure species were identified on the basis of their morphological characteristics through observing under the light microscope (Al-Kandari et al., 2009)**.** Then the culture was maintained in a test tube using Conway media. Finally, the pure algal cultures were transferred in 250 mL Erlenmeyer conical flasks. The culture was maintained with a constant temperature range between 25±2^{o}C at 24 hours at 150 µE m^{-2} s^{-1} light intensity by using fluorescent light with continuous aeration with using a natural sterile air pump (Duong et al., 2012; Salama et al., 2013).

Culture of microalgae

Each species was cultured separately (3 cultures for each species) for pigments extraction and determination. Initially, the inoculation was the same in every culture, approximately 3%. The culture was maintained in Conway culture medium at 25± 2°C temperature, maintaining 24 hours of continuous light at 150 µ E m^{-2} s^{-1} intensity (Duong et al., 2012; Salama et al., 2013). Every species was harvested at their stationary phase for pigments analysis.

Determination of growth kinetics

Growth curve experiments were done based on cell density and biomass to determine their growth pattern and kinetics.

Daily growth kinetics was acquired by using a hemocytometer, and the following parameters were calculated for each strain, according to Guillard et al. (1973)

Growth rate (μ) = (LnN_1 - LnN_0) / (t-t_0)

N_0 and N_1 represent the number of cells at time t_0 and t, respectively.

Determination of natural pigments

Standard laboratory methods were followed to determine the natural pigments. For chlorophyll determination trichromatic approach was followed. Initially, 10 mL of each sample were filtered (47 mm Ø Whatman® GF/C glass microfiber filter papers) for extraction of microalgae. Then the filter papers were stored in freezing conditions using airtight plastic bags for three weeks. After three weeks, each filter paper was placed in a centrifuge tube with 2-3ml 90% aqueous solution (Mixing of 90 parts of Acetone with ten parts of $MgCO_3$ Solution) and macerated at 500 rpm for 1 minute. Then the volume was adjusted up to 10 mL using 90% aqueous acetone solution. Then the samples were steeped for 2 hours at 4^{o}C temperatures. Then the samples were centrifuged for 20 minutes at 500g, and the clear supernatant was transferred into new tubes. Finally, chlorophyll a, b, c was determined according to Aminot et al. (2001).

According to Shaish et al. (1992), carotenoid was determined. For phycobiliproteins determination cultures were harvested at their stationary phase through centrifuging. The cell pellets were washed 2-3 times with distilled water. The harvested biomass was dried in a hot air oven at 40 °C for 12 hours. Dried powder (40 mg) was then soaked in 10 ml phosphate buffer (pH 7.0; 0.1 M), vortexes and stored at 4°C for 24 h. Phycobiliproteins were extracted by centrifuging at 6000 rpm for 10 minutes. Finally, the supernatant was collected. Finally, the amount of phycocyanin (PC), phycoerythrin (PE) and allophycocyanin (APC) in the sample were calculated using equations and the extinction coefficients from Siegelman and Kycia (1978).

Statistical analysis

MS Excel was used to measure the mean and standard deviation of the mean of the data. IBM SPSS (v.26.0) was used for the post hoc test. A post hoc test was done when the ANOVA tested the significant difference in the concentrations of pigments among the microalgae species.

3. RESULTS

Isolation and identification of microalgae

All the collected samples were cultured in laboratory conditions following the standard method. Then the sample was processed to isolate single species. A total of 4 species were successfully isolated from the sampling water, where more than ten species were identified. Finally, the following four isolated species were mass cultured for natural pigments extraction and determination. Table 1 shows the list of isolated species and their source of collection.

Water quality parameters of conway media

The physical characteristics of media water are shown in Table 2. The data were recorded before and after autoclave. For physical properties, there was a slight change before and after autoclave, where pH (7.85) and dissolved oxygen (4.83 mg/L) slightly decreased to 7.72 and 4.53 mg/L, respectively. On the other hand, temperature (24.6 ºC) and salinity (29.3 ppt) slightly increase to 25.2 ºC and 30.1 ppt. Overall, there is no significant difference ($p > 0.05$) for all physical properties before and after autoclave.

Table 1. Name of the isolated species with their sources

Sl. No.	Species	Source
01	*Chlorella* sp.	Irawan Softshell Crab Farm, Chowfaldandi, Cox's Bazar, Bangladesh
02	*Chaetoceros* sp.	Beximco Shrimp Farm, Khurushkul, Cox's Bazar, Bangladesh
03	*Nannochloropsis* sp.	Beximco Shrimp Farm, Khurushkul, Cox's Bazar, Bangladesh
04	*Tetraselmis* sp.	Beximco Shrimp Farm, Khurushkul, Cox's Bazar, Bangladesh

*All those species were isolated through continuous serial dilution and microscopic observation

Table 2. Physical properties of media water before and after autoclave

Physical Properties	Before Autoclave	After Autoclave
pH	7.85 ± 0.12	7.72 ± 0.17
Temperature (ºC)	24.6 ± 0.63	25.2 ± 0.70
DO (ppm)	4.83 ± 0.68	4.53 ± 0.53
Salinity (ppt)	29.3 ± 0.38	30.1 ± 0.11

*Values are mean ± standard deviation (Where n=3)

Table 3. Growth kinetics of the Microalgae Cultured in Conway Media

Species	Growth Pattern (Days)				Growth rate (μ day^{-1})
	Lag Phase	Exponential Phase	Stationary phase	Death Phase	
Chlorella sp.	2	3 - 6	8	9	0.38[b]
Chaetoceros sp.	2	3 - 6	8	9	0.35[c]
Nannochloropsis sp.	2	3 - 7	10	11	0.25[d]
Tetraselmis sp.	2	3 - 6	7	8	0.60[a]

*Cell density and optical density were measured to determine the growth pattern

Growth kinetics

Growth pattern and growth kinetics data are shown in Table 3. All the species showed more or less similar growth patterns during their lag phase and exponential phase. However, in the stationary phase, *Tetraselmis* sp. reached its stationary at day eight, where *Chlorella* sp., *Chaetoceros* sp. reached at day nine and *Naannochloropsis* sp. at day 11. In the case of growth rate, significant differences were found among all the species.

Pigments concentrations

***Chlorella* sp.**

Figure 1 shows the concentrations of the pigments in *Chlorella* sp. *Chlorella* sp. produced highest amount of carotenoids (0.56±0.02 µg/L) among all the pigments. In case of chlorophyll; *Chlorella* sp. produced highest amount of chlorophyll a (0.48 ± 0.05 µg/L) than chlorophyll b (0.19±0.05 µg/L) and Chlorophyll c (0.06±0.01 µg/L). In case of phycobiliproteins; allophycocyanin (0.0103 ± 0.0005 µg/L) production was higher than phycoerythrin (0.0023±0.05 µg /L) and phycocyanin (0.0025±0.0005 µg /L).

***Chaetoceros* sp.**

Figure 2 shows the concentrations of the pigmentsin *Chaetoceros* sp. It has shown that *Chaetoceros* sp. produced the highest carotenoids (1.36±0.2 µg/L) among all the pigments. . In case of chlorophyll; *Chaetoceros* sp. produced highest amount of chlorophyll a (1.3±0.09 µg/L) than chlorophyll b (0.039±0.02 µg/L) and Chlorophyll c (0.29±0.01 µg/L). In case of phycobiliproteins; allophyco-cyanin (0.0100 ± 0.0006 µg/L) production was higher than phycoerythrin (0.0019±0.0002 µg/L) and phycocyanin (0.0017±0.0005 µg/L).

***Nannochloropsis* sp.**

Figure 3 shows the concentrations of the pigments in *Nannochloropsis* sp. *Nanno-chloropsis* sp. produced the highest carotenoids (1.68 ± 0.3 µg/L) among all the pigments. In case of chlorophyll; *Nanno-chloropsis* sp. produced highest amount of chlorophyll a (0.48±0.04 µg/L) than chlorophyll b (0.046±0.003 µg/L) and Chlorophyll c (0.01±0.0 µg/L). In case of phycobiliproteins; allophycocyanin (0.0197 ± 0.0006 µg/L) production was higher than phycoerythrin (0.0029±0.0002 µg/L) and phycocyanin (0.0027 ± 0.0006 µg/L).

***Tetraselmis* sp.**

Figure 3 shows the concentrations of the pigments in *Tetraselmis* sp. T*etraselmis* sp. produced the highest Chlorophyll a (2.68 ± 0.04 µg/L) among all the pigments. In addition *Tetraselmis* sp. produced 1.23 ± 0.02 µg/L of chlorophyll b, 0.10 ± 0.01 µg/L Chlorophyll c and 1.51 ± 0.05 µg/L of carotenoids. In case of phycobiliproteins; allophycocyanin (0.0113 ±

0.0004 µg/L) production was higher than phycoerythrin and (0.0018 ± 0.0001 µg/L) phycocyanin (0.0018±0.0004 µg/L).

Total phycobiliprotein

Total phycobiliprotein concentrations of each species are shown in Figure 5. No significant difference (p> 0.05) was found among *Chlorella* sp., *Chaetoceros* sp., and *Tetraselmis* sp. However, *Nannochloropsis* sp. produced the highest (0.0253±0.002 µg/L) of phycobiliproteins among the species.

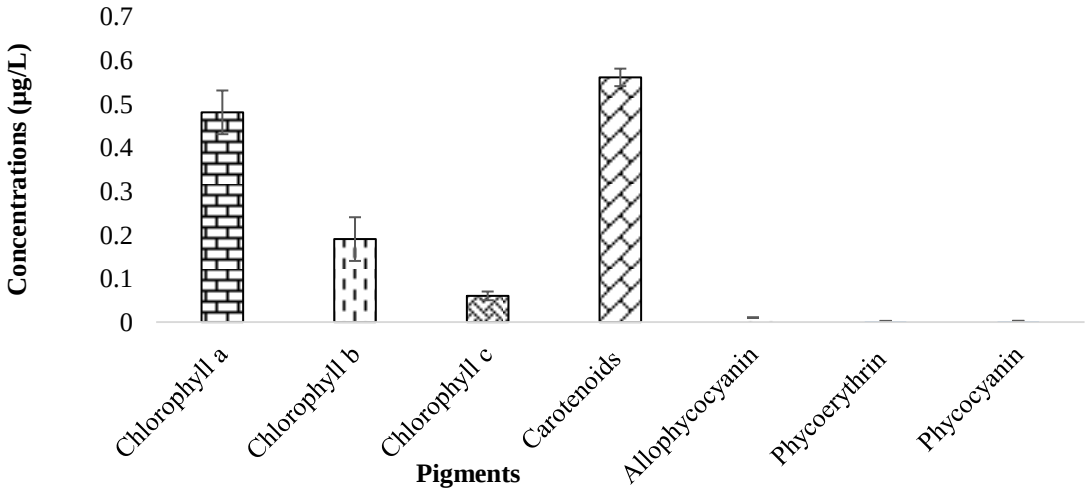

Figure 1. Pigments concentrations of *Chlorella* sp. in Conway culture media. Values are mean ± standard deviation (n=3)

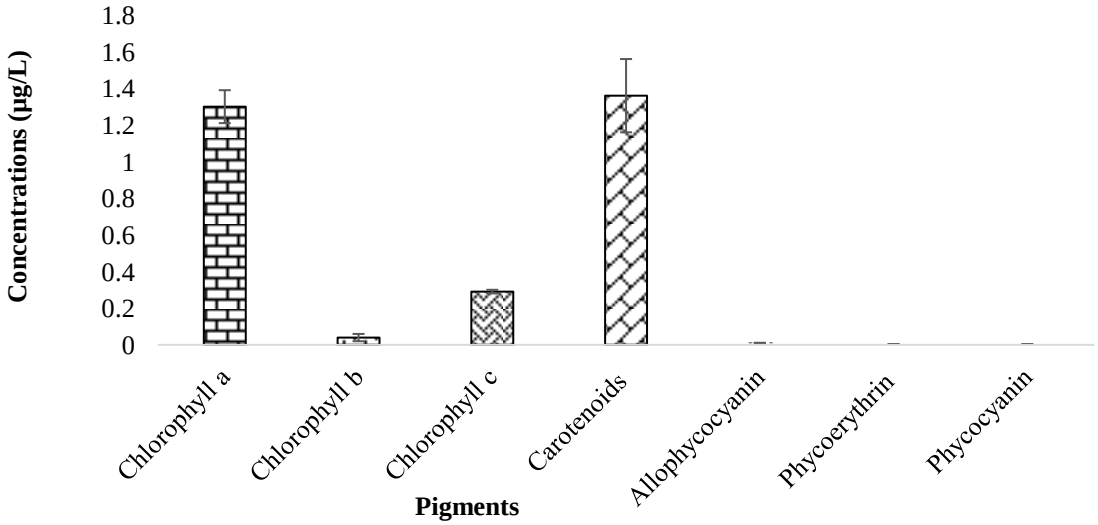

Figure 2. Pigments concentrations of *Chaetoceros* sp. in Conway culture media. Values are mean ± standard deviation (n=3)

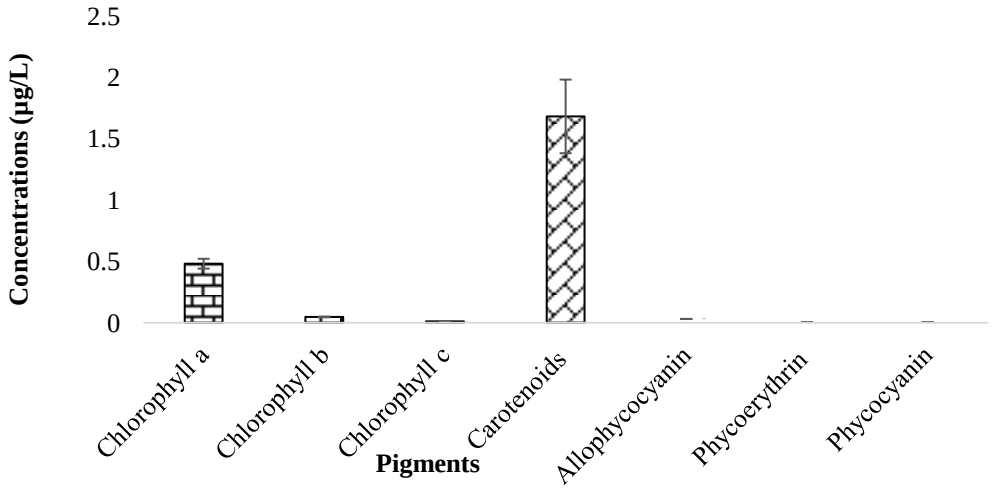

Figure 3. Pigments concentrations of *Nannochloropsis* sp. in Conway culture media. Values are mean ± standard deviation (n=3)

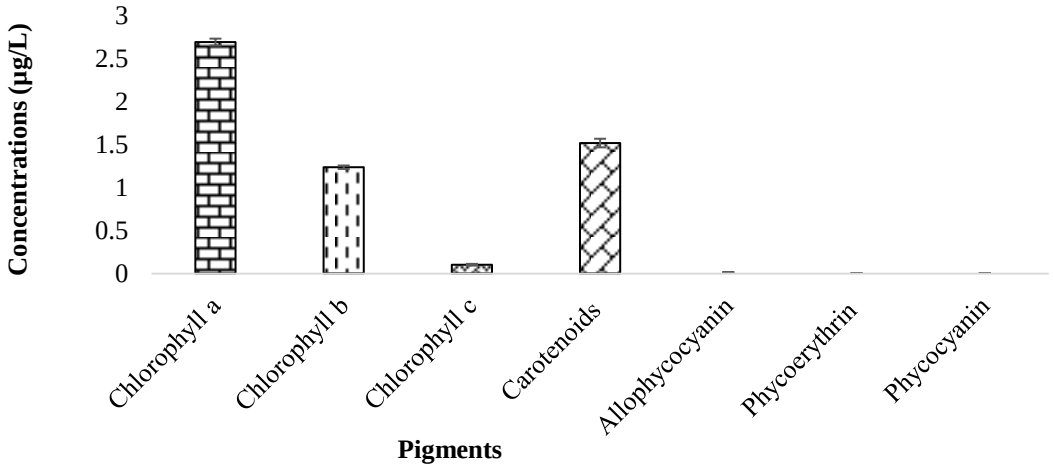

Figure 4. Pigments concentrations of *Tetraselmis* sp. in Conway culture media. Values are mean ± standard deviation (n=3)

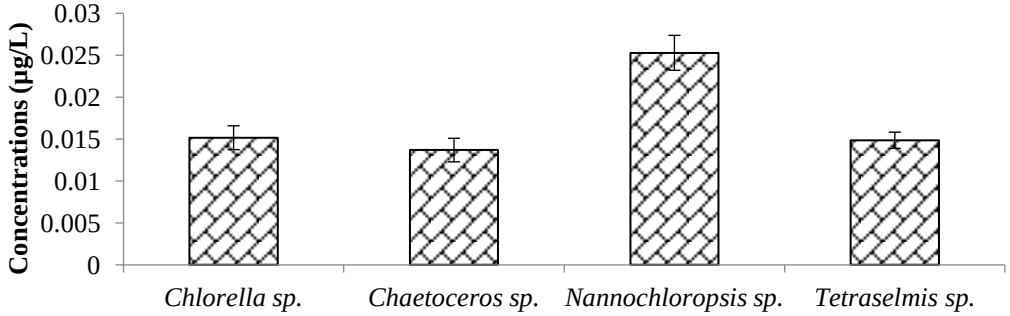

Figure 5. Total Phycobiliprotein production of microalgae in Conway culture media. Values are mean ± standard deviation (n=3)

4. DISCUSSION

All the isolated four (4) species (*Chlorella* sp., *Chaetoceros* sp., *Nannochloropsis* sp., and *Tetraselmis* sp.) are commercially important. However, the only use (as live feed) of these species is reported in different shrimp hatcheries of Bangladesh. Although the species are being used in so many ways (as food, feed, nutraceuticals, and pharmaceuticals) worldwide, no precise use has been recorded in aspects Bangladesh. Moreover, there is no precise data is available of commercially important indigenous microalgae species of Bangladesh. So, this study was designed to initialize a profile of available natural pigments of these commercially important indigenous species. In-situ culture of microalgae is mandatory to maintain pure culture and analysis. That is why maintaining a proper culture condition is necessary. According to Food and Agriculture Organization (FAO), the ranges of physical parameters for culturing microalgae are as follows; temperature (16-27 °C), salinity (12-40 ppt), and pH (7-9). All the physical parameters of the cultured media were within the range. Different types of microalgae can tolerate temperature fluctuation up to 15 ºC lesser than their optimum, where growth may be reduced, but a temperature of only a few degrees higher than optimum can cause cell death (Mata et al., 2010). However, according to Laven and Sorgeloss (1996), all the parameters were within the range during the experimental period.

The growth phase of microalgae consists of five growth phases: the lag phase, exponential phase, phase of declining relative growth, stationary phase, and death phase (Lavens and Sorgeloos, 1996). All the experimental microalgae species showed distinct growth phases (lag, growth, stationary, and death). Environmentally viable microalgae also need a definite period to physiologically adjust and adapt to a new environment (Barsanti and Gualtieri, 2006). Understanding the growth kinetics is essential to utilize the microalgae for the perfect use. All the cultures were harvested at their stationary phase for pigments analysis in this experiment.

Figure 1-4 shows the pigments concentrations in different microalgae. It has been observed that chlorophyll and carotenoids are the major constituents of pigments considering the phycobiliproteins. These are known as photosynthetic pigments, which are essential in photosynthesis (Britton, 1983).

Chlorella sp., *Chaetoceros* sp., *Nannochloropsis* sp., and *Tetraselmis* sp. all the species produced definite pigments, whereas *Chaetoceros* sp. and *Teraselmis* sp. produced higher than the other two species. Chlorophyll a and carotenoids are found the most produced pigments considering all others. Lavin (2000) reported chlorophyll a as the primary pigments, where the others functioned as accessory pigments.
Whatever the production is, concentrations of specific pigments vary among the species. In the case of chlorophylls, all the cultures were harvested at their stationary phase. Grung et al. (1992) show that chlorophyll-a concentration is the same in all algae groups, but different results were observed in the present study. *Tetraselmis* sp. showed a higher amount of Chlorophyll a and b than the other three species, whereas *Chaetoceros* sp. produced the highest amount of chlorophyll c than the other three. According to Danesi et al. (2011), higher chlorophyll concentration resulted in high cell concentration, but, considering chlorophyll c, the opposite pattern had observed. Chlorophyll a is higher considering each species chlorophyll concentrations since chlorophyll a is the main pigment where chlorophyll b and c are accessories pigments may or may not be related with chlorophyll a (Lavin, 2000). The photosynthetic rates of living organisms were significantly affected by the alteration of light intensity and the light regime, which consecutively influenced its growth (Pandey et al., 2010). Increased availability of light also may cause a decrease in the content of chlorophyll a and carotenoids (Alves de Oliveira et al., 2014). The variations in the amounts of culture nutrients also affect chlorophyll concentrations without other factors that can influence chlorophyll contents of microalgae, such as light, temperature, water quality, and cell extraction method. Finally, solvent for extraction is important because it directly affects chlorophyll concentrations and varies based on the solvent (Wellburn, 1994).

Carotenoids are usually determined from the dried biomass of microalgae. Biomass is

generally harvested when the growth condition is optimum. Velichkova (2014) described that biomass content directly affects carotenoids biosynthesis. In this study, *Nannochloropsis* sp. produced the highest carotenoids (1.68µg/mL ± 0.3) among four species. Melina et al. (2016) found that *Tetraselmis* sp. produced 2.6 µg/mL of carotenoids, almost double than our findings. Sirakov and Vekichkova (2014) found that *Nannochloropsis maculate* produced 0.836 µg/mL of carotenoids using a different culture medium. That indicates carotenoid production varies based on the culture medium. In addition, carotenoid concentration varies in various environmental parameters, chlorophyll concentrations, the solvent used for extraction, and species (Techetel and Ruppel, 1992; Rise et al., 1994; Sartory and Grobbbelaar, 1984). However, the extraction method and solvent used for extraction according to the species would ensure the proper result.

Many studies have been done on microalgae considering their multidisciplinary functions, especially for their organic biomass. Phycobiliproteins have unique light-absorbing properties. Parmar et al. (2011) reported that different dried biomasses of algae are ground and milled to produce commercial pigments and nutraceuticals. As mentioned earlier, phycobiliproteins are allophycocyanin (bluish-green), phycocyanin (blue), phycoerythrin (purple) and phycoerythrocyanin (orange) (Sekar and Chandramohan 2008). In this experiment, *Nannochloropsis* sp. showed maximum production of pigments in all cases, whereas other species showed different natures in different cases. The result showed that phycobiliproteins production varies among species. The most abundant pigment was allophycocyanin compared to phycocyanin and phycoerythrin. Phycobiliproteins concentration varies because levels of pigments tend to be reduced in high light exposure to prevent photo-oxidation damage caused by the production of free radicals. Lee (2008) stated that phycobiliprotein production varies in species in light regime due to chromatic adaptation. Chen et al. (2013) narrated that microalgae cell growth and pigment production are affected due to environmental change. It is reported that phycobiliproteins production was higher when the light availability was low (Alves de Oliveira, 2014). The phycoerythrin has been found to occur in small concentrations due to its high dependence on pH, regardless of the contribution of light (Cuellar-Bermudez et al., 2014). Phycocyanin production also tends to lower, probably due to artificial lighting (Reichert et al., 2006).

The study results have developed a complete quantitative profile of pigments of four indigenous microalgae of Bangladesh. With modernizations, natural pigments are replacing the synthetics product in the global market. As it is essential to select proper microalgae for target pigment production, the results of the study will help the producers select proper microalgae to accomplish their target pigments productions.

5. CONCLUSION

Finally, it can be said that the microalgae-based industry could be good business for our country, considering its pigments profile. Besides, microalgae also have some other importance in aspects of their food and feed value in the global platform. Whatever it is, skilled manpower and indigenous adaptation techniques are the pre-requisite to initialize this considerable industry in any new environment. For that reason, indigenous microalgae are best suited because of their environmental adaptability. The findings of this study will help to understand the growth behaviour and pigments profile of the selected four important commercial microalgae in aspects of Bangladesh. So far, Proper optimization of culture, expansion, extraction, and utilization is a timely demand to introduce this vast feasible industry in our country.

ACKNOWLEDGEMENT

This study was supported by the research grant funded by Bangladesh Fisheries Research Institute (BFRI), and UGC MS research grants. Authors are grateful to Chattogram Veterinary and Animal Sciences University for their laboratory support.

REFERENCES

Al-Kandari, M., Al-Yamani, F.Y. and Al-Rifaie, K. 2009. Marine phytoplankton atlas of Kuwait's Waters, numerous plates and figures. Kuwait: Kuwait Institute for Scientific, 1-350.

Alves de Oliveeira, C., Olieveira, W. C., Ribeiro, S. M. R., Stringheta, P. C. and Galvao do Nascimento, A. 2014. Effect of light intensity on the production of pigments in Nostoc

Aminot A. and Rey, F. 2001. Chlorophyll a: Determination by spectroscopic methods. ICES Techniqnes in Marine Environmental Sciences, 30: 17.

Barsanti, L. and Gualtieri, P. 2006. Algae: Anatomy, Biochemistry, and Biotechnology. Boca Raton, FL: CRC Press, Taylor and Francis Group, 10: 27-29.

Batista, A. P., Raymundo, A., Sousa, I. Empis, J. 2006. Rheological characterization of coloured oil in water food emulsions with lutein and phycocyanin added to the oil and aqueous phases. Food Hydrocolloids, 20: 44-52.

Becker, E.W. 2004. The nutritional value of microalgae for aquaculture. In: A. Richmond (ed). Microalgae for Aquaculture. Handbook of Microalgal Culture, Blackwell, Oxford, London, 380-391.

Ben-Amotz, A., Gressel, J. and Avron, M. 1987. Massive accumulation of phytoene induced by norflurazon in *Dunaliella bardawil* (chlorophyceae) prevents recovery from photoinhibition. Journal of Phycology, 23: 176-181.

Britton, G. 1983. The biochemistry of natural pigments. Cambridge University Press, 133 - 140.

Brown, S. B., Houghton, J. D. and Hendry, G. A. F. 1991. Chlorophyll breakdown. In Scheer H (Ed): Chlorophylls. Boca Raton, CRC Press, 465 - 489.

Cady, J.B. and Morgan, W.S. 1948. Treatment of chronic ulcers with chlorophyll: review of a series of fifty cases. The American Journal of Surgery, 75(4): 562 - 569.

Carpenter, E.B. 1949. Clinical experiences with chlorophyll preparations: with particular reference to chronic osteomyelitis and chronic ulcers. The American Journal of Surgery, 77(2): 167 - 171.

Chen, C. Y., Kao, P. C., Tsai, C. J., Lee. D. J. and Chang, J. S. 2013. Engineering stratergies for simultaneous enhancement of C-phycocyanin production and CO_2 fixation with Spirulina Platensis. Bioresource Technology, 145: 307-312.

Costache, M. A., Campeanu, G. and Neata, G. 2012. Studies concerning the extraction of chlorophyll and total carotenoids from vegetables. Romanian Biotechnology Letters, 17(5): 7702 - 7708.

Cuellar-Bermudez, S. P., Aguilar-Hernandez, I., Cardenas-Chavez, D. L., Ornelas-Soto, N., Romero-Ogawa, M. A. and Parra-Saldivar, R. 2014. Extraction and purification of high-value metabolites from microalgae: essential lipids, astaxanthin and phycobiliprotein (minireview). Microbial Biotechnology. John Wiley & Sons Ltd and Society for Applied Microbiology, 1 – 20.

Cysewski, G. R. and Todd, L. R. 2004. Industrial production of microalgal cell mass and secondary products-species of high potential Haematococcus. In: A. Richmond (ed). Handbook of Microalgal Culture. Biotechnology and Applied Phycology. Blckwell Science, Oxford, UK, 281 -288.

Danesi, E. D. G., Rangel-Yagui, C. O., Sato, S. and de Carvalho, J. C. M. 2011. Growth and content of Spirulina platensis biomass chlorophyll cultivated at different values of light intensity and temperature using different nitrogen sources. Brazillian Journal of Microbiology. 42: 362-373

Dring, M. J. 1982. The biology of marine plants. Edward Arnold. pp 1-8.

Ducret, A., Muller, S.A., Goldie, K. N., Hefti, A., Sidler, W. A., Zuber, H. and Engel, A. 1998. Reconstitution characterization and mass analysis of the pentacylindrical allophyco-cyanin core complex from the cyanobacterium Anabaena sp. PCC 7120. Journal of Molecular Biology, 278: 369-388.

Duong, V. T, Li, Y, Nowak, E. and Schenk, P. M. 2012. Microalgaeisolationand selection for prospective biodiesel production. Energies, 5: 1835–1849. doi:10. 3390/en5061835

Edge, R., Mc Garvy, D. J. and Truscott, T. G. 1997. The carotenoids as antioxidants-a review. Photochemistry Photobiology, 141: 189-200.

Gantt, E. 1980. Structure and function of phycobilisomes: Light harvesting pigment complexes in red and blue green algae. International Review of Cytology, 66: 45 - 80.

Grung, M. F., D'souza, M. L., Borowitzka, M. and Jensen, S. L. 1992. Algal carotenoids. 1. Secondary carotenoids 2. Haematococcus pluvialis aplanospores as a source of (3s, 3s')-astaxanthin esters. Applied Phycology, 4: 165-171.

Guerin, M., Huntley, M.E. and Olaizola, M. 2003. Haematococcus astaxanthin: applications for

human health and nutrition. Trends Biotechnology, 21: 210-216.

Guillard, R. R. L., Kilham, P. and Jackson, T. A. 1973. Kinetics of silicon-limited growth in the marine diatom *Thalassiosira pseudonana* Hasle and Heimdal (Cyclotella nana Hustedt). J. Phycol, 9: 233 - 237.

Henrikson, R. 1989. Earth and Food Spirulina. H Laguna Beach, CA: Donore Enterprises Inc.

Hosikian, A., Lim, S., Halim, R. and Danquah, M.K. 2010. Chlorophyll extraction from microalgae: A review on the process engineering aspects. International Journal of Chemical Engineering, 1-11.

Humphrey, A. M. 2004. Chlorophyll as a color and functional ingredient. Journal of Food Science, 69(5): 422 - 425.

Johnson, E.A. and Schroeder, W. A. 1995. Microbial carotenoids. Advance Biochemical Engineering Biotechnology, 53: 119-178.

Jin, E.S., Polle, J.E.W., Lee, H.K., Sang, M., Hyun. and Chang, A. 2003. Xanthophylls in microalgae from Biosynthesis to Biotechnological Mass Production and Applications. Microbiology and Biotechnology, 13:165-174.

Kalidas, C. and Loveson, E. 2005. Role of Microalgae pigments in Aquaculture. Aqua International, 34 -37.

Kaur, G., Khattar, J. I. S., Singh, D. P., Singh., Yadvinder. and Nadda, J. 2009. Microalgae: a Source of Natural Colours. Algal Biology and Biotechnology, 129-150.

Lanfer-Marquez, U. M., Barros, R. M. C. and Sinnecker, P. 2005. Antioxidant activity of chlorophylls and their derivatives. Food Research International, 38(8-9): 885–891.

Lavens, P. and Sorgeloos, P. 1996. Manual on the Production and Use of Live Food for Aquaculture. Rome: Food and Agriculture Organization of the United Nations.

Lavin, P. L. S. 2000. Nitrogenio organic intracellular: Extracao, papel dosiologico e acumilacao por microalgas maerinhas, em cultivos estanques," Dissertacao Universidate Federal Fluminense, Niteroi, Rio de Janeiro.

Lee, R. E. 2008. Phycology: Introduction,Basic characteristic of the algae. Cambridge University Press, 17

Mata, T. M., Martins, A. A. and Caetano, N. S. 2010. Microalgae for biodiesel production and other applications: A review, Renewable and Sustainable Energy Reviews. 14(1): 217-232. http//doi.org/10.1016/j.rser.2009.07.020

Melina, I., Sahay, S., Krishnika, A., Beena, B., Nair. and Jayalakshmy. 2016. Biochemical characterization of eight marine microalgae grown in batch cultures. Journal Algal Biomass, 7(3): 19-41.

Pandey, V. D., Pandey, A. and Sharma, V. 2013. Review article: Biotechnological applications of cyanobacteria phycobiliproteins. International Journal of Current Microbiology Applied Science, 2(9): 89 - 97.

Parmar, A., Singh, N. K., Pandey, A., Gnansounou, E. and Madamwar, D. 2011. Cyanobacteria and microalgae: a positive prospect for biofuels. Bioresources Technology. 102: 10163–10172.

Rasmussen, R. S., Morrissey, M. T. and Steve, L. T. 2007. Marine biotechnology for production of food ingredients, in Advances in Food and Nutrition Research. Academic Press, Boston, Mass, USA, 237 - 292.

Reichert, C. C., Reinehr, C. O. and Costa, J. A. V. 2006. Semicontinous cultivation of cyanobacterium Spirulina platensis in a closed photobioreactor. Brazilian Journal of Chemical Engineering, 23(1): 23-28.

Rita, A., Fatima, V. C., Javier, S. L., Costa, A. I., Annette, B., Silvia, F., Manuel, G. T., Fatemeh, G., Tanel, I, Martial, L., Micheal, M. M., Silvio, M., Cesar, P., Celine, R., Tryggvi, S. and Jorg, U. 2021. Current Status of the Algae Production Industry in Europe: An Emerging Sector of the Blue Bioeconomy. Frontiers in Marine Science, 7:1247. doi: 10.3389/fmars.2020.626389

Rise, M., Cohen, E., Vishkautsan, M. and Arad, S. 1994. Accumulation of Secondary Carotenoids in Chlorella zofingiensis. Jornal on Plant Physics. 144: 287-292.

Romay, C., Ledon, N. and Gonzalez, R. 2000. Effects of phycocyanin extract on prostaglandin E2 levels in mouse ear inflammation test. Arzneimittel for schung, 50: 1106-1109.

Rowan, K. S. 1989. Photosynthetic Pigments of Algae. Cambridge University, Press, New York.

Salama, E. S., Kim, H. C., Abou-Shanab, R. I., Ji, M. K., Oh, Y. K., Kim, S. H. and Jeon, B. H. 2013. Biomass, lipid content, and fatty acid composition of freshwater *Chlamydomonas mexicana* and *Scenedesmus obliquus* grown under salt stress. BioprocessBiosyst.Eng, 36:827 - 833.

Sartory, D. P. and Grobbelaar, J. U. 1984. Extraction of Chlorophyll a from Freshwater Phytoplankton for Spectrophotometric Analysis. Hydrobiologia, 114: 177 - 187.

Santiago-Santos, M., Ponce-Noyola, T., Olvera-Ramirez, R., Ortega-Lopez, J. and Ca~nizares-Villanueva, R. O. 2004. Extraction and purification of phycocyanin from Calothrix sp. Process on Biochemistry, 39: 2047–2052.

Sekar, S. and Chandramohan, M. 2008. Phycobiliproteins as a commodity: Trends in applied research, patents and commercialization. Journal of Applied Phycology, 20: 113-136.

Shaish, A., Ben-Amotz, A. and Avron, M. 1992. Biosynthesis of β-carotene in *Dunaliella*. Carotenoids Part A: Chemistry, Separation, Quantitation, and Antioxidation, 439 - 444.

Siegelman, H. and Kycia, J. 1978. Algal bili-proteins: Handbook of phycological method. Cambridge University Press, Cambridge, 71 - 79.

Sirakov, I. N. and Velichkova, K. N. 2014. Bioremediation of wastewater originate from aquaculture and biomass production from microalgae species *Nannochloropsis oculata* and *Tetraselmis chuii.* Bulgarian Journal of Agricultural Science, 20(1): 66-72.

Smith, L.W. and Livingston, A. E. 1945. Wound healing: an experimental study of water-soluble chlorophyll derivatives in conjunction with various antibacterial agents. The American Journal of Surgery, 67(1): 30 - 39.

Spears, K. 1988. Developments in food colorings: the natural alternatives. Trends in Biotechnology, 6(11): 283 - 288.

Techetel, B. W. and Ruppel, H. G. 1992. Lipid Bodies in *Eremosphaera viridis* De Bary (Chlorophyceae). Plant and Cell Physics, 31: 41-48.

Telfer, A. 2002. What is β-carotene doing in the photosystem II reaction centre? Philos. Trans. Royal Soc. Lond. Biol. Sci, 357: 1431-1439.

Timberlake, C. F. and Henry, B.S. 1986. Plant pigments as natural food colours. Endeavour, 10(1): 31-36.

Tompkins, J., De Ville, M. M., Day, J.G. and Turner, M. F. 1995. Culture Collection of Algae and Protozoa. Catalog of Strains. Ambleside, UK. pp 204-212.

Velichkova, K. 2014. Effect of different nitrogen sources on the growth of microalgae *Chlorella vulgaris* cultivation in aquaculture wastewater. Agricultural Science and Technology, 6(3): 337-340.

Wellburn, A. R. 1994. The Spectral Determination of Chlorophylls A and B, as well as Total Carotenoids, Using Various Solvents with Spectrophotometers of Different Resolution. Journal on Plant Physics, 144: 307-313.

Wijffels, R. H. 2008. Potential of sponges and microalgae for marine biotechnology. Trends in Biotechnology, 26: 26 - 31.