CHAPTER 1
INTRODUCTION

1.1 Background of the study:

Bangladesh's overall fishery production for 2017-2018 was 42.77 lakh metric tons, while inland open water (capture) accounts for 28.45% (12.17 lakh metric tons) and inland closed water (culture) accounts for 56.24% (24.05 lakh metric tons). On the other hand, the production of marine fisheries is 6.55 lakh MT and its contribution to total fish production is 15.31% with a growth rate of 2.71% (DoF, 2018). One of the main export products in Bangladesh is shrimp. Total production of shrimp and crawfish, including capture, increased from 1.60 lakh MT in 2002-03 to 2.54 lakh MT in 2017-18. Frozen seafood is the second largest export product in Bangladesh, after ready-made clothes.

Microalgae are extremely diverse organisms in global aquatic environments and they play a functional role in aquatic ecosystems as primary producers. About 50 microalgae species have been studied in detail with regard to their biochemistry and eco-physiology. In aquaculture systems, microalgae are considered very important in promoting live foods for raising fish and shrimp larvae. Many previous studies indicated that appropriate growth of aquatic organisms depends on the availability of essential nutrients and minerals. These essential nutrients like protein, lipid, carotene, vitamins, amino acids, polyunsaturated fatty acids and minerals come from microalgae (Habib et al., 2003).

The provision of live foods for aquaculture normally depends on availability of microalgae or zooplankton. Among microalgae, *Chlorella* is an important species used in aquaculture. This alga contains high protein, lipids and long chain polyunsaturated fatty acids (PUFA). *Chlorella*, together with rotifers, is usually used as feed for marine fish larvae. *Chlorella* have been produced in large quantities for food products, especially health foods, whereas during fish hatchery operation, small quantities of microalgae are required as live food for zooplankton and fish/shrimp larval rearing, and only in the production period. Production of microalgae biomass is relatively expensive due to high costs of the culture medium. Accordingly, alternative

low-cost media are currently being developed to produce microalgae with comparable nutritional values.

Waste water for aquaculture is usually high in waste nutrients such as nitrogen and phosphorus, total suspended solids, volatile suspended solids, biochemical demand for oxygen and chemical demand for oxygen (Mook et al., 2012).A build-up of solid waste inside the system should be avoided because when it decomposes, it can cause oxygen depletion and ammonia toxicity that contributes to eutrophication and nitrification of ecosystems receiving effluent.

Researchers have been researching the re-use of waste water from aquaculture to reduce the effect on the aquatic environment, and microalgae media are notable in practice. Wastewater provides all the essential nutrients required for the growth of algae. Due to its nutrient content and low cost, it is an appealing resource for algae production.In addition, because algae can absorb nitrogen and phosphorus, microalgae have a great potential for the removal of nitrogen (N) and phosphorus (P) in waste water.Waste water from municipal, agricultural and industrial activities is a source of nutrients for the cultivation of microalgae, which could significantly reduce the operation costs of algal production systems. Eutrophication of nearby water bodies could be caused by the mass volume of aquaculture waste water containing high concentrations of nitrogen and phosphorus produced during the year and when released untreated. On the other hand, many methods and technology are used to extract these nutrients, and this method of treatment is extremely expensive (Yuan et al., 2011) because the wastewater requires a lot of energy and maintenance purposes. The use of waste water will reduce the need for additional sources of nitrogen and phosphorus by around 55% (Yang et al., 2011).

The incorporation of wastewater from aquaculture activities as a nutrient source for the cultivation of microalgae could reduce the operating costs of the production systems for algae. The use of tropical marine microalgae therefore contributes positively to awareness of the effective cultivation of microalgae using waste water from the aquaculture industry. In order to optimize the growth and productivity of the microalgae, the parameters and ability to monitor those conditions can provide valuable information. Integrated aquaculture systems are regarded as a promising technology, but recent efforts have focused primarily on microalgae (Troell et al., 2003). Biological treatment is considered the most economically viable solution in

order to allow the reuse of water in these systems. These approaches are considered the most promising treatment technology with low investment and no secondary emissions. Therefore, current research work has investigated whether wastewater from shrimp hatchery is appropriate for microalgae cultivation with the goal of achieving substantial output of biomass and at the same time reducing the effect of wastewater on the ecosystem. As health foodstuffs as well as live food production for aquaculture, *Chlorella vulgaris* may contribute significantly.

1.2 Objectives of the Study

a. To evaluate the growth of *Chlorella vulgaris* cultured in waste water and Conway medium.
b. To determine the proximate composition of *Chlorella vulgaris* cultured in wastewater-and Conway medium.

CHAPTER 2

LITERATURE REVIEW

2.1 Aquaculture Wastewater

The increasing fish demands and driven by the huge profit from the export market led to a vast commercialization of the fish culture. However, along with the expansion of the aquaculture sector, concerns are evoked about the probable effects of aquaculture waste towards aquatic ecosystem. Aquaculture wastewater is defined as water discharged through sewers and drainage channels from aquaculture water systems once it has fulfilled its primary function. The main wastes are solid wastes, chemicals, and therapeutics. Solid wastes also known as particulate organic matter often consist of feaces or uneaten food. According to Liu et al. (2002), waste components also included the release of bacteria, pathogens and farmed species escapees. Wang et al. (2010) stated that the quality and quantity of waste from aquaculture depends mainly on culture system characteristics and the choice of species, but also on feed quality and management. There are a lot of efforts been done to treat or reduce the amount of wastewater discharge to environment and wastewater treatment is one of them. Main wastewater treatment types are divided into three stages, preliminary; removal of rags, rubbish, grit, oil and grease, primary; removal of floatable materials and secondary treatment; biological treatment to remove organic and suspended solids. The wastewater discharge to environment may still content high of ammonia, nitrogen and pathogen and have the potential to deteriorate aquatic ecosystem even the wastewater has been treated.

Nutrients (nitrogen, phosphorus) are usually concentrated in the wastewater discharged from intensive aquaculture, mainly from fish excrement and feed residue (Crab et al., 2007). To date, several biological and chemical methods have been successfully used to obtain a satisfactory quality of aquaculture effluent in the process of removing these nutrients, such as the common biological nitrification/denitrification process to remove nitrogen and the chemical precipitation process to remove phosphorus. While efficient, these methods are less environmentally friendly since they create chemical waste or sludge as a by-product,

which is generally considered to be environmental pollution. Aquaculture wastewater contains high concentration of nutrients needed by microalgae to use for growth.The total suspended substances of total phosphorus (P), total nitrogen (N), total carbon (C) were all high in aquaculture wastewater. Ammonia, nitrite, nitrate and many-dissolved organic nitrogen like urea, free amino acids and peptides in the wastewater can be the main nitrogen sources for microalgae. Microalgae are proven can adapt really well with the aquaculture wastewater as a medium. Furthermore, the microalgae grown in wastewater can accumulate valuable proximate and biochemical composition such as lipids and fatty acids that can be extracted from the dried biomass of algae and can be further used for other applications. Aslan and Kapdan (2006) used *Chaetoceros vulgaris* for nitrogen and phosphorus removal from wastewater with an average removal efficiency of 72% for nitrogen and 28% forphosphorus. Nutrient removal capacities of *Nannochloropsis* sp. have also been investigated. Microalgae were widely used to treat wastewater as it removes nitrogen, phosphorus, BOD and COD very efficiently (Wang *et al*., 2010). Ammonia nitrogen and phosphates in wastewater serve as a complete medium equivalent to chemical media from a kinetics standpoint (Li *et al*., 2011). It was also reported that the estimated cost of nutrients and CO_2in utilizing wastewater is not needed. So, the nutrients that are available in the aquaculture wastewater are beneficial for the microalgae growth. To sum up, aquaculture wastewater has the potential as alternative medium for the production of microalgae biomass as it has the necessary nutrient for microalgae growth.

Nutrients from aquaculture wastewater can be utilized by algae, which provide the co-benefit where the amount of wastewater discharge to ecosystem can be reduced and at the same time saving the amount of water needed and cost of preparation of medium for microalgae. In fact, various studies demonstrated the use of microalgae for production of valuable products combined with environmental applications (Bilanovic*et al*., 2009). Moreover, by removing nitrogen and carbon from water, microalgaecanhelplessentheeutrophicationeffectintheaquaticenvironment.

(Teresa *et al*., 2010). The biotechnology of growing microalgae in waste water is getting importance as biomass of these algae can be used as food and many other valuable products. Wastewater and CO_2 emission provide a means to reduce the expenses in microalgae cultivation.

Coupling of wastewater with microalgae cultivation provides an effective means of utilizing nitrogen and phosphorus with lipid accumulation. It is uneconomical to use artificial media for large-scale microalgae production, but using wastewater for algal growth will result in substantial cost reduction. Second, it assimilates large amount of organic carbon to produce its biomass which can further be processed for production. Growing algae in wastewater is the most feasible way to reduce the economic and environmental cost for production. Production of microalgae is an extensive method in terms of reclamation of wastewater containing high organic matter and nutrients (Ganapathi and Amin, 1972). Microalgae cultures offer an effective solution to tertiary and quaternary wastewater treatments due to the ability of microalgae to use inorganic nitrogen and phosphorous for their growth (Kumar *et al*., 2010). The main problem of large scale microalgae cultivation is large amounts of water containing nitrogen and phosphorus is needed. Large amount of wastewater can be used to replace commercial water.

It was reported that 30-40% (maximum 70%) of marinehatchery operating costs can be attributed to microalgae culture (Heasman*et al.,* 2001). Approximately around 5000 to 10000 tons of algal biomass is commercially produced worldwide (Bassam*et al*., 2013). However, the holding back nowadays is due to the high expense regarding construction material for cultivation system, proper agitation, CO_2 administration, and supply of large amount of water and nutrient resources as commercial media for microalgae. According to Chisti (2007), approximately 183 t of CO_2 is required to produce 100 t of algae biomass. If fresh water is used without recycling, in order to achieve 1 kg of lipid from microalgae biomass, would require about 3726 kg water, 0.33 kg of nitrogen and 0.71 kg of phosphate. To meet huge demands of energy in modern society, bio-energy production based on photosynthesis will require tremendous amounts of water for cultivation of microalgae. The global phosphate reserves are dwindling in amount and quality and it is assumed that phosphate rock production will peak in approximately 50-100 years and then decrease as the reserves are depleted. Therefore, the sustainable supply ofnutrients for microalgae production is a great importance in terms of economics, resource depletion, and environmentalprotection.

2.2 Microalgae

The main producer of the marine ecosystem is microalgae. Microalgae are small underwater plants which, without roots or leaves, have a diameter of between 1-50 μm. In addition, microalgae also undergo photosynthesis by using sunlight and converting carbon dioxide into biomass production. Microalgae, which are prokaryotic or eukaryotic photosynthetic microorganisms, can be categorized into two. The prokaryotic microorganism consists of cyanobacteria (Cyanophyceae), and green algae (Chlorophyta) or diatoms (Bacillariophyta) are eukaryotic microalgae (Li et al., 2008). Microalgae play an important role in the supply of energy and essential nutrients for the proper growth of aquatic species in open water (Habib et al., 2003). They are also major live foods for rotifers, cladocerans, zooplanktons and fish and shrimp larvae (Gallardo et al., 1995). In aquaculture, microalgae play a key role in developing aquaculture. In the commercial rearing of all growth phases of bivalve molluscs, larval stages of crustaceans and early growth phases of fishes, microalgae are commonly used as an important food source. This is because they rapidly replicate and can be harvested daily. They can also be used as food or as a food coloring addictive to essential nutrients. To be a useful aquaculture plant, microalgae must possess a number of main attributes. For example, they must have a sufficient intake size of 1 to 15 μm for filter feeders; 10 to 100 μm for grazers (Kawamura et al., 1998) and be readily digestable. They must have rapid growth rates, be adaptable to mass culture, and must also be stable to any temperature, light and nutrient variations in culture as may occur in hatchery systems.Microalgae are considered to be of high nutritional value.As pure biomass has strong consumer demand and value, pure products produced from microalgae have tremendous potential for commercialization. Biomass has been used as feed supplement, medication, nutraceutical, cosmeceutical, high health organism processing, animal color enhancement and potential biodiesel and biofuel production organisms (Chisti, 2007).Several unique characteristics, such as cell wall digestibility (Epifanio et al., 1981), cell size and biochemical composition, are thought to affect the nutritional value of microalgae (Fernandez-Reiriz et al., 1989).Tropical marine microalgae, *Chlorella vulgaris*, were chosen for the experiment. Because of their high nutritional content and the growth and development of larvae and juveniles, these marine microalgae have been widely used as live feed in the aquaculture industry.

2.2.1 *Chlorella vulgaris*

Domain :Eukaryota

Phylum : Chlorophyta

Class : Trebouxiophyceae

Order : Chlorellales

Family : Chlorellaceae

Genus : *Chlorella*

Species : *C. vulgaris*

The first algae with small globular cells to be isolated by Beijerinck in culture is *Chlorella* (green algae; Chlorophyta). One of the first microalgae to be considered for mass production and the first commercially produced microalgae was also *Chlorella*.The *Chlorella* genus is a single microalgae of 2.0-10.0 μm in diameter, spherical to ovoid, nonmotile, unicellular or colonial microalgae with a single cup of pyrenoid-shaped chloroplast (Bock et al., 2011).A thin wall of cellulose covers the cells. Chlorella was initially seen as a food source rich in protein, but it was also proposed as a biofuel source, it is commonly used as a healthy food and feed supplement, as well as in the pharmaceutical and cosmetics industry, Chlorella is the most cultivated eukaryotic algae. Proteins, carotenoids, certain immuno-stimulators, polysaccharides, vitamins, and minerals are included in it. For diesel replacements, *Chlorella* strains may be suitable. High quality biodiesel production from *Chlorella* sp. was obtained.

2.3 Growth Factors of Microalgae

Light, pH, temperature and nutrients are the key factors influencing the growth of microalgae (Tzovenis et al., 1997), but other factors such as salinity and aeration may also be important for a few species (Chu et al., 1996). Such variables also affect the physiological activities and biochemical composition of microalgae, which have been extensively studied (Brown et al., 1997).Microalgae can withstand a number of environmental conditions (Juneja et al., 2013). For algal growth, not only organic

carbon, substrate, vitamins, salts and nutrients are essential, but also balance with operational parameters; oxygen, carbon dioxide, pH, temperature, strength of light, removal of products and by-products.

2.3.1 Light

One of the most critical criteria for microalgae is light intensity, which is to assimilate inorganic carbon for conversion into organic matter. In general, microalgae rely on an adequate supply of carbon (40-50 percent carbon) and light to carry out photosynthesis processes for biomass growth (Moheimani, 2005). The criteria differ significantly with the depth of culture and the algal culture density. For instance, 1,000 Lux is suitable for Erlenmeyer flasks at higher depths and cell concentrations, the light intensity must be intensified to penetrate through the culture; 5,000- 10,000 Lux is required for larger volumes.The optimum light intensity for the growth of microalgae is in the range of 2,000- 5,000 Lux, according to Lavens and Sorgeloos (1996). Using fluorescence light for indoor culture can encourage better microalgae growth and cell division. Maximum light exposure, however, can become a limiting factor for the density of microalgae. The increase in cell density and specific growth rate is recorded that increase in light intensity up to a certain limit above which growth was inhibited.

2.3.2 Temperature

In both closed and open outdoor systems, temperature is the second most significant limiting factor for cultivating microalgae. For algae cultures, the optimum temperature is usually between 20-24 ° C, but with different compositions of the culture medium, species and strain cultivated, it can vary. Most species of microalgae are able to tolerate 16 to 27 °C (Lavens and Sorgeloos, 1996). In addition, above 27 °C will make the algae die. Many microalgae can easily withstand temperatures up to 15 ^{o}C lower than their optimum, but the complete loss of culture can result in exceeding the optimum temperature by only 2-4 ^{o}C (Teresa et al., 2010). Changes in light intensity can affect temperatures, which indirectly affect microalgae growth

(Huang et al., 2013). The effects of temperature on microalgae growth in the laboratory are well known, but the extent of the effects of temperature on annual outdoor biomass production is not yet adequately recognized.

2.3.3 pH

For most cultured algal species, the pH range is between 7 and 9, with the optimal range being between 8.2-8.7. In culturing microalgae, pH has a very vital function. Due to a pH that maintains failure, disturbances of several cellular activities may cause complete culture to collapse (Lavens and Sorgeloos, 1996).In microalgae cultures, pH is especially important because it interferes with the accessibility of CO_2, necessary for photosynthesis. In the case of high-density algal culture, the addition of carbon dioxide enables the correction of increased pH, which during algal growth can exceed limiting values of up to pH 9.

2.3.4 Nutrient Composition of Media

To assist the growth of microalgae, commercial media produce micronutrients and macronutrients. Some examples of macronutrients include nitrate, phosphate and silicate (Lavens and Sorgeloos, 1996). The most popular form of nitrogen in the culture medium is nitrate.Copper, zinc, cobalt, boron, iron and manganese are the most widely used trace metals, including (Probert and Klaas, 1999). Thiamin (B_1), cyanocobalamin (B_{12}) and occasionally biotin are also vitamins..However, on the other hand, depending on the time of culture growth, the concentration of dissolved nutrients appears to decrease significantly over time, reaching complete depletion.

2.3.5 Mixing and Aeration

Another essential growth parameter is mixing and aeration, as it homogenizes cell distribution, heat, metabolites and facilitates gas transfer. Furthermore, the rapid circulation of microalgae cells from the dark to the light zone of the reactor is desirable to promote a certain degree of turbulence, especially in large-scale production (Barbosa, 2003).High liquid velocities and turbulence levels, on the other

hand, can damage microalgae due to shear stress due to mechanical mixing or air bubble mixing (Eriksen, 2008). The optimal degree of turbulence depends on the pressure and should be investigated to prevent a reduction in productivity (Barbosa, 2003).

2.3.6 Salinity

Salinity can influence the growth and cell composition of microalgae, both in open and closed systems. Each marine microalgae has a different optimal salinity range that can increase due to high evaporation during hot weather conditions. Phytoplankton is typically affected by salinity changes in three ways: (1) osmotic stress (2) ion stress and (3) cellular ionic ratios changes due to membrane selective ion permeability (Moheimani, 2005).The growth of microalgae can be prevented by changes in salinity (Takagi et al., 2006). The simplest way to regulate salinity is to add fresh water or salt as needed.

2.4 Microalgae Growth Phases

Growth phase of microalgae consist of five growth phases which are, the lag phase, exponential phase, phase of declining relative growth, stationary phase and death phase (Lavens*et and sorgeloos*. 1996).

The initial phase is lag phase and during this phase the increase in the cell density is low because at this phase, the microalgae cell undergoes physiological adaptation of the cell metabolism involved in cell division and carbon fixation.

The second phase was the log phase or also known as the exponential phase. During this phase, the cell density increases as a function of time t logarithmic function:

$$Ct = C0.emt$$

with Ct and C0 = the cell concentrations at time t and 0, and m = specific growth rate. The specific growth rate is mainly dependent on algal species, light intensity & temperature.

The third phase is the phase of declining growth and at this time, cell division slows down and nutrients, light, pH, carbon dioxide or other physical and chemical factors begin to limit growth.

The fourth phase is the stationary phase and the cell density is constant for some times at this phase because the limiting factor and the growth rate is balance as this is the

phase with the highest microalgae density.

The last phase is the death phase. At this phase, the water quality deteriorates and nutrient depleted to the level incapable of sustaining growth and the cell density decreased rapidly and the culture collapsed.

2.5 Proximate composition

The word used in the feed sector is proximate composition, meaning the six components named moisture, crude protein, ether extract, crude fiber, crude ash and nitrogen-free extracts, which are expressed as the content (percent) of the feed. In order to understand the essence and properties of the subject feed, the calculated values of these six components in the feed are essential considerations.Species of microalgae can vary greatly in their nutritional value, and this may also change under different conditions of culture (Brown et al., 1997). Microalgae's nutritional composition relies on their environmental factors, growth rates or life cycle conditions (Richmond, 1986).The effects of light intensity, temperature, salinity and media nutrients on the growth and proximate composition of microalgae have been widely explored (Brown et al., 1997). Other 29 factors which can affect the nutritional value of microalgae are digestibility of cell wall structure and composition, shape and size, enzymes, nutrients and toxins.Overall, microalgae usually contain 30 to 40 percent protein, 10 to 20 percent lipid and 5 to 15 percent carbohydrate in the late-logarithmic growth process (Renaud et al., 1999).Brown et al. (1997) have a broader range of protein, lipid and carbohydrate levels that are 6-52%, 7-23% and 5-23% respectively.The proximate composition of microalgae, for example when nitrate is reduced, may dramatically change when cultivated through to the stationary process, carbohydrate levels may double at the expense of protein (Brown et al., 1993). In *Chlorella vulgaris*, protein, carbohydrates and lipids are analyzed for this experiment.

2.5.1 Protein

Protein composition of microalgae range from 6-52% as stated by Brown et al. (1997). High dietary protein provided best growth for juvenile mussels and Pacific oysters (Knuckey et al., 2002).There are a variety of variables affecting protein production in microalgae. The optimum temperature for microalgae protein development is within the 25 °C and 30 °C range stated that Renaud et al.,(1999). The

protein content in the microalgae would decrease from the optimum level at a higher temperature.

2.5.2 Lipid

Many species of microalgae can be induced to accumulate large lipid amounts (Sheehan et al., 1998), thereby leading to a high yield of oil. The average lipid content ranges between 1% and 70%, but some species can exceed 90% dry weight under certain conditions (Spolaore et al., 2006). Lipid content has been documented to range from 1%-85% for pure algae cultures, and the lipid shows varying lengths of the carbon chain, degrees of unsaturation, and polarity (Chisti, 2007).There are several factors, including light intensity (Yeesang and Cheirsilp, 2011), nitrogen (Illman et al., 2000) and phosphate, that influence lipid development in microalga. With the rise in light intensity, lipid output of microalgae would increase (Yeesang and Cheirsilp, 2011).The lipid output is reduced as the temperature gets higher. Opute (1974) reported that extremely high or low temperatures could decrease the output of lipid microalgae.

2.5.3 Carbohydrate

The composition of carbohydrates ranges from 5-23 percent, as shown by Brown et al (1997). Carbohydrate accumulation is mainly caused by the transfer of the protein metabolic pathway to the carbohydrate pathway (Markou et al., 2012). High-carbohydrate algal diets are recorded to produce the best growth for juvenile oysters (Enright et al., 1986).Several factors, such as nutrient restriction and other unfavorable environmental conditions, may contribute to the accumulation of carbohydrates in the microalgae, according to Markou et al. (2012). The iron that affects the photosynthesis system has influenced carbohydrate synthesis (Oijen et al., 2004).Changes in salinity in microalgae can change the carbohydrate content (Zhila et al., 2011).Limitation of nitrate results in a doubling of carbohydrate levels at the cost of protein (Brown et al., 1993). Previous studies have shown that carbohydrate production has been decreased due to the toxicity of copper (Markou et al., 2012).

CHAPTER 3

MATERIALS AND METHODS

3.1 Collection of Shrimp Hatchery Wastewater

Wastewater was gathered from Niribili Shrimp Hatchery, Cox's Bazar from the culture tank of shrimp (*Penaeus monodon*). Four sources of aquaculture waste water were selected for the screen .The results showed that the siphon wastewater had the highest increase in microalgae productivity due to the difference in the wastewater composition with collection time, thus being the key candidate as an aquaculture wastewater medium for microalgae. For screening process to cultivate the siphon, outlet, settling zone and retention point microalgae. Siphon operation is a practical cleaning process performed by farmers to renovate pond water by extracting the old one by draining it out along with shrimp faeces, exoskeleton and other waste materials at the bottom layer of the pond.Wastewater was collected per container in a batch container with a capacity of 20 L.

3.2 Media Preparation

3.2.1 Filtration and Preservation of Aquaculture Wastewater

Aquaculture wastewater was immediately filtered in the lab using vacuum filter pump in order to remove suspended solids and waste materials. Then, physical parameters(pH, salinity) were recorded in room temperature. The salinity recorded was 15 ppt. The filtered wastewater was stored in a cold room maintained at 20-21ºC.

3.2.2 Preparation of Conway Medium (Tompkins et al., 1995).

Three stock solutions (macronutrients, trace metal solutions and vitamins) were prepared by dilution of the chemical composition in water as shown in Table 1. The Conway medium was prepared by adding Solution A, Solution B and Solution C respectively 1 ml, 0.5 ml and 0.1 ml into 1 L of filtered and sterilized sea water at 28 ppt salinity.

Table 3.2.2: Chemical composition of Conway medium (Tompkins et al.,1995)

Constituents	Quantities
Solution A- Macronutrients	
Sodium nitrate ($NaNO_3$)	20g
Ferric chloride ($FeCl_3$)	1.3g
Boric acid (H_3BO_3)	33.4g
EDTA(b), di-sodium salt	45g
Manganous chloride (MnCl2, 4H2O)	0.36g
Sodium di-hydrogen orthophosphate (NaH2PO4, 2H2O)	20g
Distilled water	1000ml
Solution B- trace metal	
Zinc chloride (ZnCl2)	4.2g
Cobaltous chloride (CoCl2, 6 H2O)	4.0g
Ammonium molybdate ((NH4)6Mo7O24, 4H2O)	1.8g
Cupric sulphate (CuSO4, 5H2O)	4.0g
Distilled water	1000ml
Acidify with HCl to obtain a clear solution	
Solution-C Vitamins	
Vitamin B1	200mg
Vitamin B12	10mg
Distilled water	1000ml

3.3 Nutrient Analysis of Wastewater

The chemical analysis of aquaculture wastewater consists of total ammonium nitrogen (TAN), nitrite nitrogen (NO2-N) and soluble reactive phosphorous (SRP) were analyzed following Parsons et al. (1984) methods in laboratory and after sterilization process which is UV-exposure.

3.3.1 Total ammonium nitrogen (TAN)

Five mL samples of water were put in a test tube. 0.4 mL of phenol solution (20 g of analytical grade phenol dissolved in 200 mL of 95% v/v ethyl alcohol) and 0.4 mL of sodium nitroprusside were applied to the sample (1 g of sodium nitroprusside dissolved in 200 mL of MiliQH2O).To begin the reaction, 1 mL of oxidizing solution was then added. By mixing 100 mL of alkaline reagents (100 g of sodium citrate and 5 g of sodium hydroxide dissolved in 500 mL of MiliQH2O) and 25 mL of sodium hypochlorite solution, the oxidizing solution was prepared.The tubes were coated with parafilm and incubated at room temperature (20-27^{o}C) for 1 hour before the Shimadzu spectrophotometer was measured at 640 nm (Shimadzu UV-1601, Japan).

3.3.2 Nitrite (NO_2-N)

In a test tube containing 10 mL of water sample, sulfanilamide solution (0.2 mL) was applied. By dissolving 5 g of sulfanilamide in a mixture of 50 mL of concentrated hydrochloric acid, the sulfanilamide solution was prepared and diluted with MiliQH2O to 500 mL.Then, after 8 minutes, 1 mL of NED reagent (0.5 g of dissolved N-(1-napthyl)-ethylene diamine-dihydro-chloride in 500 mL of MiliQH2O) was applied to the tube and immediately mixed. Extinction was measured one hour later at 543 nm with a Shimadzu spectrophotometer (Shimadzu UV-1601, Japan).

3.3.3 Determination of Phosphate phosphorus (PO_4-P)

One mL of mixed reagent was added to the test tube containing 10 mL of water sample. Mixed reagent was prepared by mixing 100 mL of 0.02 M ammonium molybdate, 250 mL sulfuric acid, 100 mL of 0.31 M ascorbic acid and 100 mL of 0.002 M potassium antimonyl-tartrate. After 5 min and preferably within the first 2-3 hours, extinction was measured at 885 nm by using Shimadzu spectrophotometer (Shimadzu UV-1601, Japan).

3.4 Microalgae Sample Collection

Chlorella vulgaris microalgae selected in this study were collected from the Live Feed Research Corner Laboratory of Faculty of Fisheries, Chattogram Veterinary and Animal Sciences University. The pure seed, using Conway culture medium. The culture of *Chlorella vulgaris* has been preserved at 28 ppt salinity Sub-culturing of selected microalgae was performed every two weeks to maintain a pure and stable stock culture. With a 24 hour photoperiod, pure seed culture of all microalgae species was maintained at 30 ppt salinity in the Conway medium.Subsequently, the pure stocks were cultivated and maintained in indoor culture room condition at a temperature range of 23°C to 25°C with artificial light range 2000-3000 Lux with (light: dark = 24 h: 0 h) in the Live Feed Culture Laboratory with 30 ppt salinity. In order to preserve health and good stock, sub culturing is performed every two weeks.

3.5 Growth Curve Experiment

The growth curve experiment was performed to determine the pattern of the growth curve for microalgae.The experiment was conducted by preparing three replicates of sterilized Conway and waste water media with a volume of 200 mL in a 500 mL flask. $1x10^3$ cells/ml *Chlorella vulgaris* were inoculated and slowly shaken from the stock culture into the flasks containing each culture medium. In the monitored culture room at 23-25ºC with an artificial light range of 2000-3000 Lux (light : dark = 24 h: 0 h), the culture treatment was maintained on the rack. Throughout the experiment, aeration was continuously given. Cell density and optical density analysis were performed regularly. At the end of this experiment, the growth curve was plotted with cell density and optical density *Chlorella vulgaris* were established.

3.6 Experimental Design

For this experiment, five treatments consisting of 100% wastewater as control (T1); Conway media with 25% wastewater replacement (T2); Conway media with 50% wastewater replacement (T3); Conway media with 75% wastewater replacement (T4) and 100% wastewater (T5) were evaluated in triplicate using a complete randomized design. 1 × 103 cells/mL of Chlorella was inoculated from the stock culture into the flasks as initial cell density. The culture treatment was maintained on the rack in the controlled culture room at 23-25ºC with artificial light range 2000-3000 Lux with

(light:dark = 24 h: 0 h). Aeration was provided continuously throughout the experiment. Cell density and optical density were performed daily for growth studies.

3.7 Growth Parameter Analysis

3.7.1 Determination of Cell Density

Cell numbers was determined daily by placing an aliquot of well-mixed culture suspension on a rhodium-coated haemacytometer (Hawksley AC1000, UK). and with distilled water to make sure that it is free of dust, lint and grease. Small drop of properly mixed sample was transferred into the counting chamber. Meanwhile, the dropper was held at an angle until a small drop has arisen at the tip of it. The drop was released between the cover glass and the counting chamber base as a result of the capillary effect. The haemacytometer was checked to make sure there were no air bubbles and were allowed to settle 3-5 minutes for better counting. The cell density of the microalgae culture was calculated according to the following formula (Clesceri et al., 1989):

$$\text{Cell count (cells/mL) for 25 squares} = \frac{\text{Total number of cell counted}}{10\text{x } 4 \text{ x } 10\text{-}6}$$

Where,

10 = the squares of 2 chamber

4x10-6 = the volume of samples over the small squares area which is equivalent to $0.004mm^3$(0.2 x 0.2 x 0.1) expressed in cm^3 (mL).

3.7.2 Determination of Optical Density

The optical density of all cultures was determined daily using a UV-spectrophotometer (UV-VIS 1601, Shimadzu, Japan). The wavelength use for optical density were 540 nm (*Chlorella vulgaris*) (Lavens and Sorgeloos, 1996).

3.8 Proximate Composition Analysis

Microalgae were harvested during stationary phase to determine the nutritional profiles which are protein, lipid and carbohydrate.

3.8.1 Protein Analysis

According to Lowry et al., (1951) protein was analyzed. For every sample, 5-6 mg of freeze-dried microalgae was taken and made into 25 mL solution by mixing with distilled water. From the 25 mL of sample prepared, 0.5 mL was taken from each sample for protein analysis. Prior to that, Reactive 1 (1 % Potassium sodium tartarate) and Reactive 2 (2 g of Sodium carbonate in 100 mL of 0.1 N NaOH) were prepared. Mixed reagent was prepared by adding 1 mL of Reactive 1 to 50 mL of Reactive. Then, 0.5 mL of sample was added with 0.5 mL of 1 N Sodium hydroxide and it was kept in 100 °C water bath for 5 minutes. It was then cooled in a water bath and 2.5 mL of the prepared mixed reagent was added 10 minutes after cooling. The mixed solution was added with 0.5 mL of Folin reagent and was kept in dark places for 30 minutes. The reading of the mixed solution was taken with spectrophotometer (UV-1601, Shidmadzu) at the wavelength of 750 nm.

3.8.2 Lipid Analysis

Based on Marsh & Weinstein (1966) the lipid analysis was conducted by the sulphuric acid-charring method, following the carbonization method using tripalmitin as the standard after extracting lipids according to the method of Bligh & Dyer (1959). The samples were extracted from 4.5 mL of chloroform: methanol (1:2 concentration) and it was then centrifuged at 10 000 rpm for 10 minutes. After separating the supernatant from the biomass, 1.5 mL of chloroform and 1.5 mL of distilled water was added and the sample was centrifuged again to facilitate the separation of two phases. After centrifugation, the polar phase was removed with a pipette and evaporated under vacuum with a water-bath at 35 °C. The dry-residue was solubilized in 1 mL chloroform. Then, 3 aliquots of 200 µL each was taken from this solution and transferred into test tubes and the solvent was evaporated again. When completely dried, 2 mL of concentrated sulphuric acid was added. The carbonization process was carried out at 200 °C for 15 minutes, then the tubes were cooled and 3 mL of water was added into each tube. The optical density was measured at 375 nm.

3.8.3 Carbohydrate Analysis

Carbohydrate analysis was conducted based on the method of Dubois et al. (1956). For each sample, 5-6 mg was taken and made into 25 mL solution by mixing with

distilled water. Prior to analysis, 5 % phenol solution and concentrated sulphuric acid was prepared. Samples were analysed by adding 1 mL of 5 % phenolic solution and 5 mL of concentrated sulphuric acid. The optical density was measured at 488 nm in a spectrophotometer (Shimadzu UV-1601, Japan).

3.9 Statistical Analysis

Mean and standard deviation was calculated from the experimental data. Statistical Analysis was done in SPSS. Software by applying One Way ANOVA method. Here found no significant relationship between cell density and optical density but significant relationship was observed among protein, lipid and carbohydrate level in proximate composition analysis.

CHAPTER 4
RESULTS

4.1 Physicochemical Parameters of Aquaculture Wastewater

The physicochemical characteristics of collected aquaculture wastewater are shown in Table 4.1. The data were collected before and after UV treatment of wastewater. There was a small difference in physical properties before and after UV exposure, where pH (7.9), dissolved oxygen (5.26 mg/L) and temperature (24.2^0C) decreased marginally to 7.6, 5.1 mg/L and 22.7^0C respectively and the salinity (30.0 ppt) increases slightly to 31.2 ppt. Total Ammonium Nitrogen (TAN) was reduced from 4.720mg/L to 4.420mg/L, the concentration of Soluble Reactive Phosphorous (SRP) increased from 4.32 mg/L to 5.46 mg/L and the concentration of nitrite nitrogen (NO_2-N) was reduced from 2.821 mg/L to 2.212 mg/L after UV-light exposure.

Table 4.1: Physicochemical characteristics of the shrimp pond wastewater.

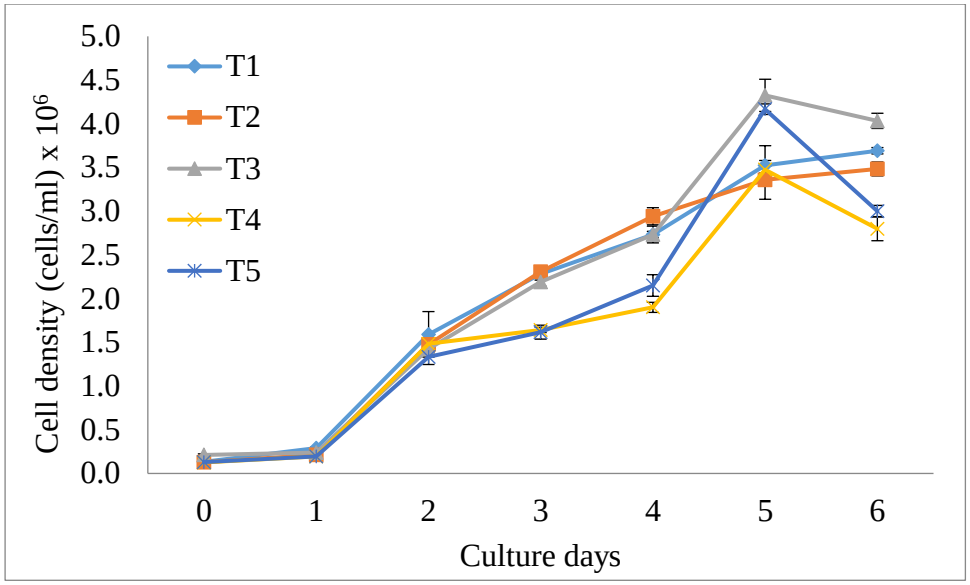
Properties	Before UV Treatment	After UV Treatment
Physical properties		
Ph	7.9	7.6
Temperature(^{o}c)	24.2.	22.7
Dissolved oxygen(mg/L)	5.26	5.1
Salinity(ppt)	30.0	31.2
Chemical properties(mg/L)		
Total Ammonium Nitrogen(TAN)	4.720	4.420
Nitrite nitrogen (NO2-N)	2.821	2.212
Soluble Reactive Phosphorus(SRP)	4.32	5.46

4.2 Growth Parameter Analysis

4.2.1 Growth of *Chlorella vulgaris* in Wastewater and Conway media

Figure 4.2.1(a) shows the cell density (cells ml^{-1}) of *C. vulgaris* cultivated in controlled environment in response to five different concentration of wastewater (T2 25 %ww, T3 50%ww, T4 75%ww, T5100%ww) and Conway media (T1). The present study revealed that *C. vulgaris* cultivated under different WW concentrations had reached their stationary phase almost at the same day. The initial cell density (cells ml^{-1}) remarkably changed after 7 days of cultivation in cultures with different ww concentrations. Maximum cell concentration was observed in different concentration at the Day 5. The figure shows that day 5 had highest cell density rather than other days. In day 5, maximum cell density found in T3 50% ww (4.325×10^6 cells ml^{-1}) and minimum cell density found in T2 25% ww (3.358×10^6 cell/mL). This results revealed that the *Chlorella vulgaris* cultured in 50% ww concentration in cultured media had significantly effected in growth performance.

The mean optical density readings of *C. vulgaris* cultured in different concentrations of wastewater media and Conway media are displayed in Figure 4.2.1 (b). In day 5, Maximum absorbance was found in different concentration of ww. Figure 4.2.1(b) shows that highest absorbance in T3 50% ww (0.40033 Abs) and lowest absorbance in T2 25% ww (0.26333 Abs). This result revealed that *Chlorella vulgaris.* Cultured in 50% ww concentration in culture media had significantly effected in growth performance.



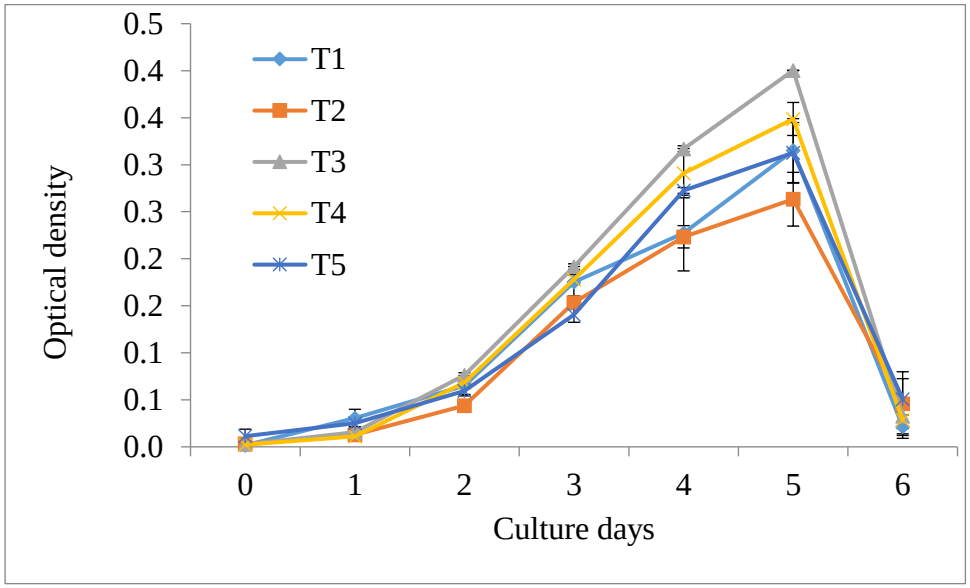


Figure 4.2.1: Cell density (a) and optical density (b) of *Chlorella vulgaris* cultured in Wastewater and Conway medium.

4.3 Proximate Composition

4.3.1 Protein content of *Chlorella vulgaris* cultured in Wastewater and Conway Media

Figure 4.3.1 shows the protein content (% dry weight) of the microalgae cultivated using Conway (T1) and waste water media are (T2 25% ww, T3 50% ww, T4 75% ww, 100% ww). The result showed that maximum protein content was found in Conway media and minimum protein content was found in T2 25% ww. There was significance difference (P > 0.05) in terms of protein content for *C. vulgaris* cultured in wastewater and Conway medium. However, protein content in *C.vulgaris* was significantly higher (P < 0.05) when cultured in Conway medium.

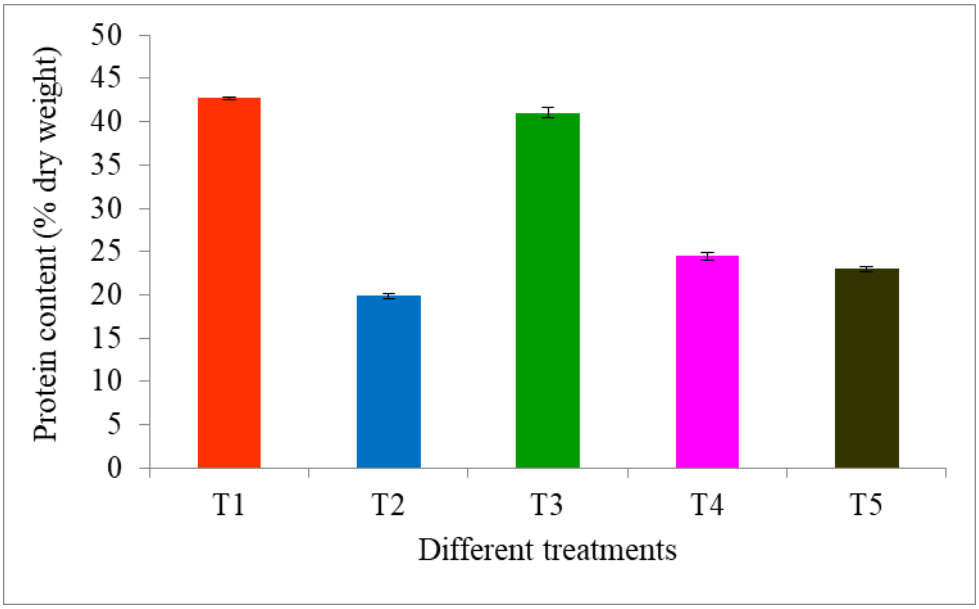


Figure 4.3.1: Protein content of *Chlorella vulgaris* in wastewater and Conway media.

4.3.2 Lipid content of *Chlorella vulgaris* cultured in Wastewater and Conway Media

The lipid content (% dry weight) in *C. vulgaris* cultured in Conway and different concentration of wastewater are shown in Figure 4.3.2. The result showed that highest lipid content was found in T4 (16% dry weight) and T5 (17% dry weight) and lowest lipid content was found in Conway medium. There were significance difference (p > 0.05) in terms of lipid content among different concentration of wastewater and Conway medium.

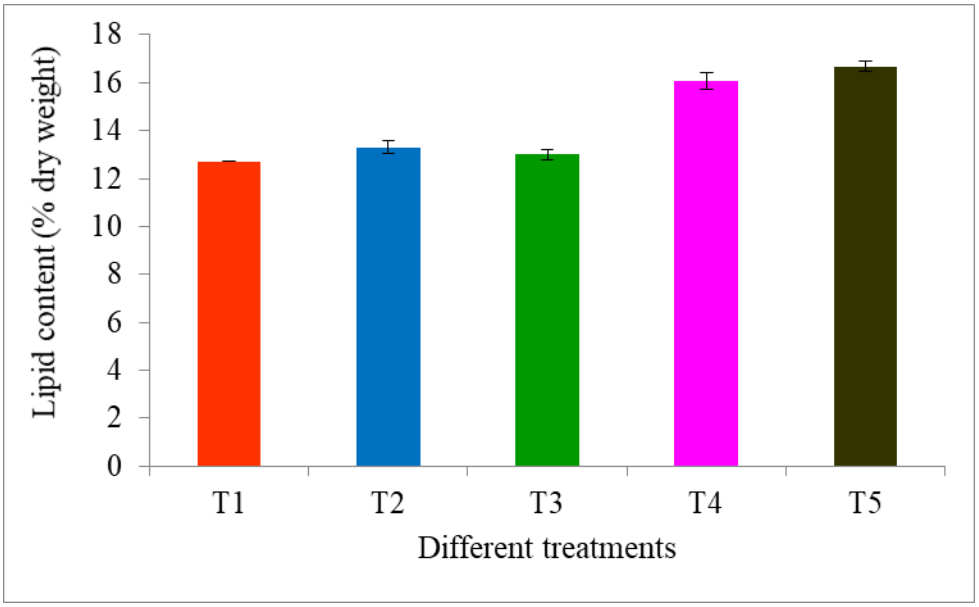


Figure 4.3.2: Lipid Content of *C. vulgaris* in wastewater and Conway media.

4.3.3 Carbohydrate content of *Chlorella vulgaris*cultured in Wastewater and Conway Media

The carbohydrate content (% dry weight) in *Chlorella vulgaries.* cultured in Conway medium and different concentrations of wastewater media are shown in Figure 4.3.3. The result shows that maximum carbohydrate content was in T3 50% ww and Conway medium (T1). Minimum carbohydrate content was found in T4 (75% ww) and T5 (100% ww). There was significance difference ($p > 0.05$) in carbohydrate content that cultured in different concentrations of ww.

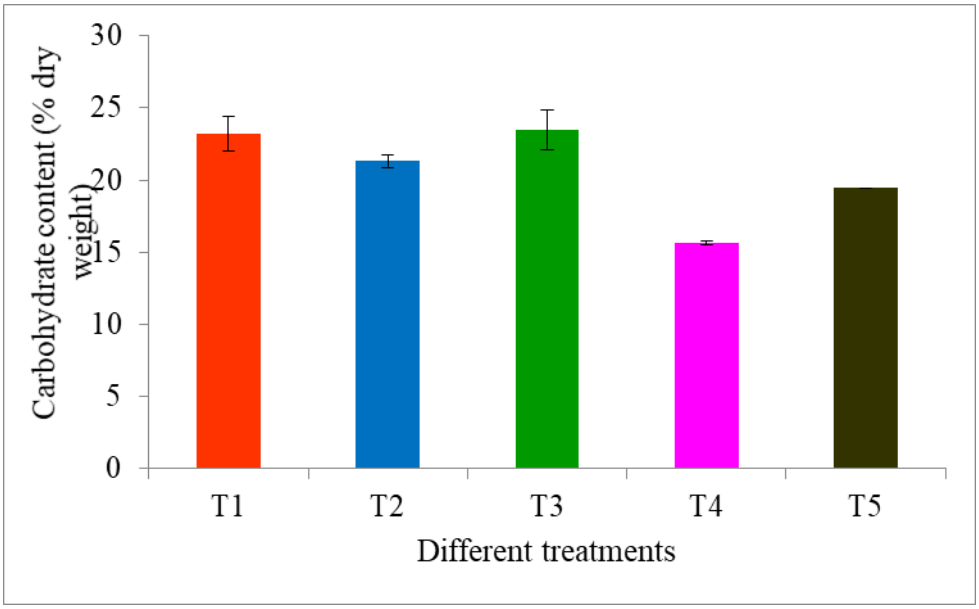


Figure 4.3.3: Carbohydrate content of *C. vulgaris* in wastewater and Conway media

CHAPTER 5

DISCUSSION

Nutrients availability of both the macro and micro plays a crucial role on the growth and biochemical composition of all microalgae (Xia et al., 2013). Adequate supply of nutrients mainly nitrogen, phosphorus is the principle to achieve higher growth rates in microalgal cells (Xia et al., 2013). The growth rate declines when the metabolic requirements and supplied nutrients are not balanced properly (Droop, 1975). There are also other important factors like temperature, light, salinity, pH etc. which might distress the growth and biochemical compositions of microalgae (Carvalho et al., 2009; Yeh and Chang, 2012). However in this study, different percentage of UV treated wastewater (25%ww, 50%ww and 75%ww) were applied as a source of nutrients along with commercial Conway media and compared with 100% commercial Conway media.

Physical water quality parameters need to maintain within the optimal range for the better growth of microalgae. FAO recommended the standard ranges of physical parameter for the production of microalgae which are temperature 16-27^{o}C, salinity 12-40 ppt and pH 7-9 (FAO, 1996). In this study, the pH (7.6), temperature (22.7^{o}C) and salinity (31.2 ppt) of wastewater after UV treatment which were used to replace commercial media found within the recommended FAO range. Both the pre and post treated physical parameters of aquaculture waste water within the optimum range (Table 4.1) to be used for microalgae culture.In general, the optimum temperature for phytoplankton growth is between 20 and 24^{0} C, although this may differ with the composition of the culture medium, the species and strain (FAO, 1996). Chisti (2008) suggested the ideal growth temperatures are usually between 20 and 30^{o}C for most marine microalgae.

However,many algal species with reduced growth rates, can withstand temperatures up to 15^{o}C lower than their optimum, but a temperature only a few degrees higher than optimal can cause cell death (Mata et al., 2010). In addition, culture treatment with an artificial light range of 2000-3000 Lux (light: dark = 24 h: 0 h) was maintained in the experiment.Phototrophs like the phytoplankton must obtain ample

light for their net growth to reach their light compensation point (Radmer et al., 1987). Laven and Sorgeloss (1996) also provide a simplified set of conditions for the cultivation of microalgae where all physical properties were within the range of this study (Table 4.1).

After exposure to UV light, all nutrients (TAN, NO_2-N and SRP) decreased in concentration. Theoretically, during the sterilization process, most nutrients are slightly lost. This was proved correct in this study, after exposure to UV-light, the nutrient concentration decreased significantly.

The data of this study shows aquaculture wastewater after UV treatment contains enough essential nutrients for microalgae growth where Total Ammonium Nitrogen (TAN) is 4.42 mg/L, Nitrite Nitrogen (NO_2-N) is 2.212 mg/L and Soluble Reactive Phosphorous (SRP) is 4.32 mg/L. Marine microalgae can utilise the inorganic nitrogen for their growth, metabolic activities and increase their cell density throughout the nitrogen-enriched condition. The wastewater medium provided nitrogen as nitrates, nitrites and ammonium salts and they were readily available in the inorganic form (Thompson et al., 1989). The number of nitrate-nitrogen (NO_3-N), nitrite-nitrogen (NO_2-N), ammonia-nitrogen (NH_3-N) and organically bonded nitrogen is Total Nitrogen (TN).Many of these nitrogen sources have been used for the processing of microalgae (Becker, 1994). Most ammonium in water is converted to toxic ammonia (NH_3) at high pH (>9), which can endanger marine species.In addition, for many types of microalgae, ammonium is preferable because it does not have to be reduced prior to amino acid synthesis and better biochemical enrichment in algal cells (Probert and Klaas, 1999).

Another main element necessary for normal growth of microalgae is phosphorus. The source of phosphorus was discovered naturally in waste water in the form of inorganic phosphate. Inorganic phosphate is a type of phosphate that is commonly added to the culture media and is favorable for the growth of microalgae (Probert and Klaas, 1999).For the better growth of microalgae, continuous supply of nitrogen and phosphorus play a major role. In the metabolism of microalgae cells, nitrogen is essential and the absorption of the nitrogen is directly related to photosynthesis. Nitrogen and phosphorus are the main nutrients for algal growth through the process of photosynthesis in presence of light. In the present experiment, total amount of

nitrogen and phosphorus in the collected wastewater samples were sufficient and therefore supported good growth of *C. vulgaris*. Moreover, the conway media contained all the balance nutrients recommended which also ensured proper growth of experimental algal strain.

The growth of *C. vulgaris* was determined through the measurement of cell density and optical density to verify the consistency of the result as each technique has its limitations. Based on Figure 4.2.1, there were no significant differences ($p > 0.05$) in terms of cell density and optical density in *C. vulgaris* when cultured either in Conway or wastewater media. *C. vulgaris* passed lag phase period by 2 days prior to starting both in Conway and wastewater media. According to Barsanti and Gualtieri (2006), microalgae need a certain time period to physiologically adjust and adapt to the new environment even though the cells were viable as they were not in the state to undergo division yet. Here in this study, similar dormant condition of cells for first two days was found and after that, they started quick division and the cell density and optical density rapidly picked onward days until the culture reached stationary condition.

Chan (2011) performed experiments to encourage the development of *Chlorella* sp. by cultivating microalgae in waste water from a fish farm and obtained a high growth rate.Similar research conducted by Chopin et al. (2012) also stated that the cultivation of some of the selected microalgae from the fish and shrimp farm in commercial medium and waste water showed a similar growth pattern. A study conducted by Becker,1994 using waste water from aquaculture shows good potential for growth and cell development.

Biochemical compositions are the means to evaluate the quality of cells in terms of nutrient accumulation and potential further utilization. Proteins, carbohydrates and lipids are the key components of algal cells (Becker, 1994). There are a variety of branch points in cells metabolism at which metabolic intermediates are differentiated between lipid synthesis and other products such as carbohydrates and proteins. In this study, *C. vulgaris* cultured in both Conway media and aquaculture wastewater had protein, lipid and carbohydrate content within the recommended range.

However, based on Figure 4.3.1, protein content (% dry basis) in *C. vulgaris* had significant difference ($p > 0.05$) when cultured in wastewater(50%) medium

(24.4555% dry weight) compared to Conway medium (42.6951% dry weight). But, 50%ww treatment had no significant difference in % protein content with the commercial Conway medium. Protein synthesis is directly affected by nitrogen consumption. Tropical Australian microalgae species found that tropical Australian microalgae can maintain 30% or more protein on dry weight basis.

On top of that, there was significant difference ($p > 0.05$) in terms of lipid content in *C. vulgaris* either in wastewater or Conway medium where 75% wastewater had the highest lipid content by percentage. Some of the algae species (*N. oculata* and *T. chuii*) are interesting and important microorganisms in the field of biotechnology because of their high lipid content, higher proteins and essential fatty acids (Ghezelbash et al., 2008). According to the results of this study (Figure 4.3.3), there was significant difference ($p > 0.05$) in terms of carbohydrate content in *C. vulgaris* cultured either in wastewater or Conway medium. The highest carbohydrate content was found in 50%ww followed by Conway medium and lowest in 75%ww. However, all the values of carbohydrate content in five different treatments were within the recommended range demonstrated in previous studies.

CHAPTER 6

CONCLUSION

The present study showed that wastewater from aquaculture could be used as an alternative medium for the cultivation of *C. vulgaris*under laboratory conditions. Our findings showed that aquaculture wastewater could promote *C. vulgaris* good algal growth to a similar extent as observed in the Conway medium.Nutrients are used in waste water which would otherwise have been discarded, thus lowering operating costs and protecting the world. Properly planned use of wastewater from aquaculture alleviates issues with water contamination and not only conserves important water supplies, but also takes advantage of the nutrients in the effluent.

CHAPTER 7

PROSPECTS AND RECOMMENDATION

Chlorella vulgaris is a very common microalgae in Bangladesh as well as over the world. It is fast growing microalgae rich with different kinds of nutrients. It contains all the essentials amino acids and about 14-19 minerals. *Chlorella vulgaris* are considered very important in promoting live foods for rising fish and shrimp larvae. *Chlorella vulgaris* have agreat potentials to produce food, fuels, fine chemicals and fertilizers on a commercial scale. To date,the value added production of *Chlorella vulgaris* still faces high production costs compared to other sources.These costs should be reduced by using aquaculture waste water. In addition, screening of microalgae based on the biochemical composition for efficient biomass production from waste water also plays a vital role in commercial applications.

Therefore, more detailed studies should be carried out, in particular sterilizing the process of wastewater aquaculture source for the mass production of microalgae. In order to evaluate the dynamics of aquaculture waste water, future work should also be carried out on more varied species of marine microalgae, especially one that has high application.

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APPENDICES

ANOVA

Protein

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	1375.207	4	343.802	89.731	.000
Within Groups	38.315	10	3.831		
Total	1413.521	14			

Homogeneous Subsets

Protein

			Subset for alpha = 0.05		
	T	N	1	2	3
TukeyHSD[a]	T2	3	19.8854		
	T5	3	22.9739		
	T4	3	24.4555		
	T3	3		40.7206	
	T1	3		42.6951	
	Sig.		.097	.733	
Duncan[a]	T2	3	19.8854		
	T5	3	22.9739	22.9739	
	T4	3		24.4555	
	T3	3			40.7206
	T1	3			42.6951
	Sig.		.082	.376	.245
Scheffe[a]	T2	3	19.8854		
	T5	3	22.9739		
	T4	3	24.4555		
	T3	3		40.7206	

	T1	3		42.6951	
	Sig.		.164	.817	

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

AppendixA : One way ANOVA of Protein Content of *Chlorella vulgaris* cultured in wastewater and Conway medium

ANOVA

Lipid

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	44.687	4	11.172	47.288	.000
Within Groups	2.362	10	.236		
Total	47.049	14			

Homogeneous Subsets

Lipid

			Subset for alpha = 0.05	
	T	N	1	2
TukeyHSD[a]	T3	3	12.6667	
	T1	3	12.7167	
	T2	3	13.3000	
	T4	3		16.0667
	T5	3		16.6667
	Sig.		.531	.578
Duncan[a]	T3	3	12.6667	
	T1	3	12.7167	
	T2	3	13.3000	
	T4	3		16.0667
	T5	3		16.6667
	Sig.		.158	.162

Scheffe[a]	T3	3	12.6667	
	T1	3	12.7167	
	T2	3	13.3000	
	T4	3		16.0667
	T5	3		16.6667
	Sig.		.648	.690

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

AppendixB : One way ANOVA of Lipid Content Of Chlorella vulgaris cultured in waste water and Conway media

ANOVA

CHO

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	124.903	4	31.226	11.877	.001
Within Groups	26.292	10	2.629		
Total	151.195	14			

Homogeneous Subsets

CHO

			Subset for alpha = 0.05		
	T	N	1	2	3
TukeyHSD[a]	T4	3	15.6307		
	T5	3	19.4512	19.4512	
	T2	3		21.3100	
	T1	3		23.1800	
	T3	3		23.5089	
	Sig.		.093	.071	
Duncan[a]	T4	3	15.6307		

	T5	3		19.4512	
	T2	3		21.3100	21.3100
	T1	3			23.1800
	T3	3			23.5089
	Sig.		1.000	.191	.144
Scheffe[a]	T4	3	15.6307		
	T5	3	19.4512	19.4512	
	T2	3		21.3100	
	T1	3		23.1800	
	T3	3		23.5089	
	Sig.		.158	.125	

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Appendix C: One Way ANOVA of Carbohydrate Content of *Chlorella vulgaris*cultured in wastewater and Conway media

Brief Biography of the Author

Sabiha Zaman Usha is the eldest daughter of Md Sharifu Zzaman and Mrs Shahnaj Zaman Tuku, was born and grown up in Chattogram. She has completed SSC from Bangladesh Nou Bahini School and College, Chattogram and HSC from Bangladesh Nou Bahini College,Chattogram. She has also achieved her BSc. degree in Fisheries from Chattogram Veterinary and Animal Sciences University. She is now a candidate of Master's degree of the same institute from the Department of Aquaculture. She has expertise on both field and laboratory works. She has done many farm works in Cox's Bazar district and microalgae laboratory research. Internship in Bangladesh's various fisheries related organizations and also in University Malaysia Terengganu, UMT is her advanced qualification besides academic study. She has a lot of experience on co-curricular activities. She has immense interest in research areas include, microalgae, fish breeding, microbiology, fish genetics, bio-floc technology, fish disease, ecology, and advanced aquaculture technologies. She is determined to make her a competent researcher and wants to reach in the apex of research world of fisheries in the world.