**A Seminar Paper**

**On**

# Use of Wastewater for Microalgae Culture and its Effect on Growth and Proximate Composition

Course Code: AQC 598

Term: Summer'2020

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**Use of Wastewater for Microalgae Culture and its Effect on Growth and Proximate Composition[1]**

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## ABSTRACT

Culture of microalgae by using wastewater provides many beneficial effects, among them the two mentionable are: removal of nutrient load from wastewater by microalgae and use of wastewater as cost effective culture medium for microalgal biomass compared to artificial growth medium. Microalgae which is considering as a potential source of nutrient like lipid, protein, carbohydrate, essential amino acid, fatty acid, antioxidant etc. This review summarizes the current approaches of microalgal biomass production using different types of wastewater specially in aquaculture, domestic, industrial, municipal wastewater. Again the effect of those wastewater on growth and proximate composition also reviewed. From the reviewed study it has found that, some strains of microalgae such as *Chlorella sorokiniana, C. vulgaris, C. minutissima, Tetraselmis chuii, Selenastrum sp.* are effective capability of growth in wastewater than the other strain of microalgae. In one finding biomass productivity of *C. vulgaris* (229 ± 7.3mg$L^{-1}$ $d^{-1}$) was found higher in wastewater along with the high nutrient removal efficiency (COD- 98.6%, TN- 91.68%, $NH_3$-N- 100%). Another finding showed the higher lipid and carbohydrate production using heterotrophic cultivation of C. *sorokiniana* in aquaculture wastewater are 39.1%, 36.1% (dry content weight), respectively. In one comparative study, the higher biomass concentration of *Chlorella minutissima* was found 4.69 g$L^{-1}$, while comparing with the other nutrient F/2 medium, was found 3.61 g$L^{-1}$. Another experiment found the highest cell density, when they cultured microalgae using wastewater + Conway medium (4.3 × 105 cells $mL^{-1}$) compared to those cultured using only wastewater (3.3 × 105 cells $mL^{-1}$) or Conway (2.9 × 105 cells $mL^{-1}$). The several findings also revealed the need of additional nutrient supplementation while cultivating the microalgal strain in different wastewater. The overall review concludes that the culture of microalgae in wastewater can be an effective method to minimize the production cost as well as nutrient removal from wastewater and production of valuable products from microalgae.

**Key Words:** Wastewater, Microalgae, Growth, Proximate composition, Culture medium.

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[1]A seminar paper for the course AQC 598, Summer, 2020

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# TABLE OF CONTENTS

# LIST OF TABLES

## LIST OF FIGURE

# CHAPTER I

# INTRODUCTION

Microalgae can referred as a microscopic algae, typically found in freshwater and marine water systems, living in both the water column and water sediment ( Thurman, 1997) . They are unicellular species which may exist individually, or in chains or groups with a size range from a few micrometers (µm) to a few hundred micrometers. Microalgae are the primary food source for a large number of aquatic organisms because of their high nutritional composition and play a key role in aquaculture development. Products made from microalgae have excellent potential for commercialization since pure microalgal biomass has high market demand and value. Microalgal biomass can be used as a live feed for fish larval rearing, premix for feed supplement, pharmaceutical, nutraceutical, cosmeceutical, production of high health organisms, and enhancement of animal color as it has higher pigment content. Microalgae, such as *Isochrysis sp*., *I. galbana*, *Nannochloropsis limnetica*, *N. maculate*, *Tetraselmis sp.* and *Skeletonema costatum*, are able to synthesize polyunsaturated fatty acids (PUFAs) [e.g. linoleic acid (LA, C18:2n-6), a-linolenic acid (ALA, C18:3n-3), stearidonic (SDA, C18:4n-3), arachidonic acid (ARA, C20:4n- 6), eicosapentaenoic acid (EPA, C20:5n-3), docosapentaenoic acid (DPA, C22:5n-3) and docosahexaenoic acid (DHA, C22:6n- 3) (Spolaore *et al*., 2006; Gouveia *et al* .,2008; Bellou *et al*., 2014; Nalder *et al*., 2015 ), molecules that play an essential role in reproduction, development and growth of fish, while their beneficial role in human health is well documented (Dunbar *et al*., 2014). In addition, some microalgae species synthesize protein of high nutritional value, and therefore are considered as an unconventional source of protein for animal consumption, while some other types of microalgae have the ability to store significant amounts of polysaccharides (Bellou *et al*., 2012). Microalgal species, such as *Dunaliella salina*, *Haematococcus lacustris*, *H. pluvialis* and *Porphyridium cruentum*, are able to synthesize pigments (i.e. beta-carotene, astaxanthin) and therefore they are of interest for pharmacy, cosmetology and food industry (Priyadarshani, 2012; Bellou *et al*., 2014 ; Richmond, 2014). Microalgae cultivation is generally realized in two kinds of systems: open raceways culture and photobioreactor culture. One of the challenges of this technology is related to the high maintenance capability and high cost of commercial culture medium as they required expensive synthetic nutrient in the medium. Therefore, it is ultimate necessary to find an alternative cost-effective culture medium.

Aquaculture is one of the fastest growing food industries now-a-days. According to FAO (2018), world fish production was 171 million tons in 2016, with aquaculture contributing 46.8% of the total. Aquaculture contributes to global fish production was 46.8% in 2016 while 25.7% in 2000 (FAO, 2016). This sector provides feed for 47% (51 million tons) of the global human fish consumption and its production is growing. This sector output is set to increase by 60–100% over the next 20–30 years to keep up with the increasing per capita fish consumption and the population (FAO, 2012). This growing industry generates wastewater rich in nutrients such as ammonia, nitrates, phosphates and organic load which usually termed as aquaculture wastewater that pollute the open water environment with excess nutrient discharged and fecal matter deposited into it (Gao *et al*., 2016; Lananan *et al*., 2014). Several studies demonstrated that microalgae have the potential for removing nitrogen and phosphorus from this wastewater. Another source of wastewater streams such as municipal (Lee *et al*.,2015) industrial (Dianursanti *et al*.,2014) and agricultural wastewater (Chen *et al*., 2015) as well as primary and secondary effluent, centrate and anaerobic digestion effluent (Ji *et al*., 2014) were exploited as suitable nutrient media for microalgae cultivation. These kinds of wastewater have their own characteristics and challenges such as nutrient variability and the presence of potential inhibitors that could affect the microalgal growth (Ji *et al*., 2014). In recent, many studies have developed strategies to overcome the challenges such as low nutrients, high turbidity, bacterial contamination and toxic materials associated with various wastewaters. These above kinds of wastewater sources utilized for algae cultivation also affect the scope of biomass for various applications. So, wastewater can be considered as a highly potential candidate due to its high nutrient content and large quantity of them may generated from the rapid growth of aquaculture sector. But the primary and secondary treatment processes is needed to obtain a clear, apparently clean effluent which is discharged into natural water bodies. Primary treatment is the elimination of the easily settled materials and secondary treatment is the oxidation of the organic material present in wastewater. This secondary effluent is filled with inorganic nitrogen (N) and phosphorus (P), and causes eutrophication and more long-term problems because of refractory organics and heavy metals that are discharged. However, each additional treatment step in a wastewater system greatly increases the total cost which can be possible to minimize by the microalgal cultivation into it.

The microalgae rely on many factors in the laboratory or in nature, such as temperature, light, salinity and nutritional factors that influence the growth, physiological activities and biochemical composition (Alsull and Omar, 2012). Microalgae are able to assimilate nitrogen from a diversify sources (Paasche and Kristiansen, 1982; Queguiner *et al*., 1986; Lund, 1987; Dortch, 1990; Cochlan and Harrison, 1991; Page *et al*., 1999). Ammonia, nitrite, nitrate and many-dissolved organic nitrogen's (urea, free amino acids and peptides) are considered as the main nitrogen sources for microalgae (Abe *et al*., 2002; Soletto *et al*., 2005; Converti *et al*., 2006). Microalgal cultures offer an elegant solution to wastewater treatments due to the ability of microalgae to use inorganic nitrogen and phosphorus for their growth

(Oswald, 1988b; 1988c; Richmond, 1986). In wastewater, nitrogen might be present in the form of ammonia ($NH_4^+$), organically bound nitrogen, or even nitrite ($NO_2^-$) and nitrate ($NO_3^-$), and phosphorus in the form of phosphates ($PO_4^{3-}$). These nutrients can be incorporated into algae cell biomass and subsequently removed from the wastewater. Using wastewater nutrients over synthetic nutrients improves sustainable microalgae production as nitrogen and phosphorus not only removed from wastewater but also re-used to produce extra biomass which finally helps to reduces excess nutrient discharge in the environment. Additionally, aquaculture wastewater contains relatively few pathogenic microorganisms and low amounts of heavy metals (Toze *et al.*, 2006; Abdel-Raouf *et al.*, 2012). Moreover, using the microalgal biomass produced by wastewater to develop animal feed or feed additives would be less of the biosafety concern on biosafety issue.

Microalgal cultivation using wastewater complies for the replacement of the large amounts of fresh water that is conventionally used for this purpose, thereby enabling the reuse of water resources; it also enables the development of the resulting microalgal biomass as aquaculture, livestock, and poultry feed (Guo *et al.*, 2013; Aleksandre *et al.*, 2015). However it can be stated that, the cultivation of algae in wastewater offers the combined advantages of treating the wastewater and simultaneously producing algal biomass, which can further be exploited for valuable products (Lam and Lee, 2012; Christenson and Sims, 2011) also wastewater is suitable for replacing fresh water in microalgal cultivation. The aim of this study leads to a reduction in the overall cost of microalgal production and also serves as an alternative way of wastewater treatment.

**Objectives of the Study**

On the basis of above fact the objectives of the reviewed paper will-

1. To highlight the potentiality of wastewater on growth and proximate composition of microalgae.
2. To highlight the potentiality of different microalgal species for treating wastewater.

# CHAPTER II

# MATERIALS AND METHODS

This seminar paper is completely a review paper so all of the information has been collected from the secondary sources. During the preparation of review paper key information were collected from various related books, journals, reports, publications etc. Findings related to seminar paper have been prepared from library facilities of Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU) and related internet web sites were used to accumulate information. Moreover, the valuable suggestions and information were provided by my major professor, course instructors, and other resource personnel. After collecting all the available information, this seminar manuscript is compiled and arranged systematically and chronologically to enrich this paper.

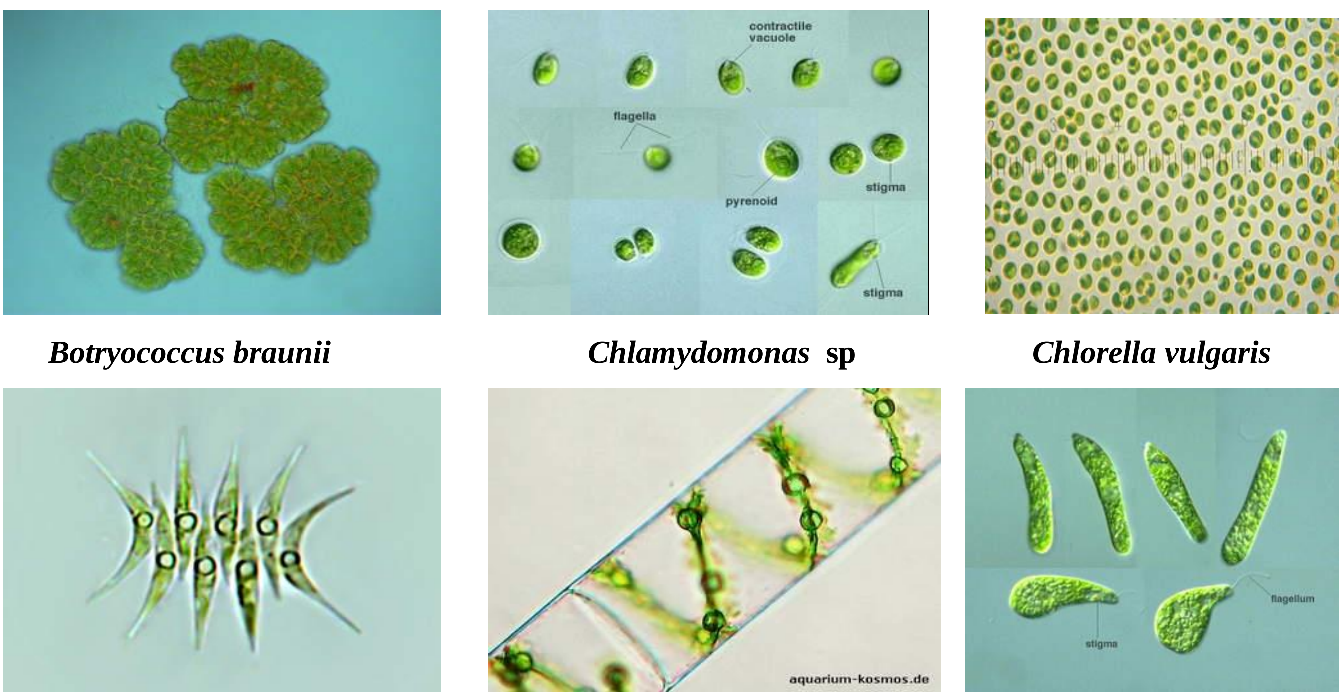
# CHAPTER III

# REVIEW OF FINDINGS

The detailed on the selected topic so far extracted and reviewed is discussed below under different sub-headings

## 3.1 Microalgae

Microalgae is a term use for a diverse group of unicellular organisms comprising eukaryotic protists, prokaryotic cyanobacteria, and blue-green algae (Day *et al*., 1999). Microalgae have a simple cellular structure and are a diverse group of photosynthetic eukaryotes ranging from unicellular to multicellular forms. The requirements of light, carbon dioxide, water, and nutrients (phosphorus and nitrogen as major nutrients) are essential for their growth. Microalgae can convert those necessities into energy in photosynthetic way and use that in cell development. The major chemical or nutritional constituents of microalgae are lipids, proteins, and carbohydrates with different compositions that are stored in the microalgae cell. The microalgae can widely be found in soils, ice, lakes, rivers, hot springs, and oceans, anywhere sunlight can reach. The growth rate of microalgae is 5–10 times faster than terrestrial food crops. According to recent statements (Raja *et al*., 2008), "microalgae are the untapped resource with more than 25,000 species of which only 15 are in use."




***Botryococcus braunii*** ***Chlamydomonas* sp** ***Chlorella vulgaris***

***Scenedesmus obliquus*** ***Spyrogyra* sp** ***Euglena gracilis***

**Figure 1: Microscopic view of some common microalgae.** (Source: Google and Wikipedia)

Lipid productivity of microalgae can have 15–300 times higher than in common oil crops and lipid accumulation of microalgae can be more than 50% under exhaustion of nutrients (Faried *et al*., 2017) and the minor constituents of microalgae are pigments such as phycobiliproteins, chlorophylls, and carotenoids, which can be used in industries such as pharmaceuticals, food, and cosmetics (Choo *et al*., 2017). Moreover, high nutritional profile is the main reason why microalgae can be used as an alternative feedstock or biodiesel.

### 3.2 Microalgae as a useful nutrient source

Microalgae can be considered as a rich source of nutrient such as protein, lipid, and carbohydrate useful for feedstock for fish as well as production for biofuel which already mentioned above. Microalgae lipids stored inside the cell can be divided to neutral lipids (acyl-glycerides and free fatty acids) and polar lipids (phospholipids and glycolipids). The neutral lipids functioned as energy supply while polar lipids act in forming cell membranes. (Table 1) shows the chemical compositions of various microalgae. Generally, lipids in microalgae are in the range of 20%–50% of total biomass (Tan, 2018). Hasnain *et al* (2018) stated that differences in species and culture conditions will produce different chemical constituents, particularly lipids, proteins, and carbohydrates of microalgae.

**Table 1: Chemical compositions of various microalgae (% dry matter)**

| Microalgae | Lipid (%) | Protein (%) | Carbohydrate (%) |
|---|---|---|---|
| *Botryococcus braunii* | 86 | 4 | 20 |
| *Chlamydomonas rheinhardii* | 21 | 48 | 17 |
| *Chlorella protothecoides* | 55 | 10–52 | 10–15 |
| *Chlorella vulgaris* | 14–56 | 51–58 | 12–17 |
| *Scenedesmus dimorphus* | 16–40 | 8–18 | 21–52 |
| *Scenedesmus obliquus* | 35–55 | 50–56 | 10–17 |
| *Spirogyra sp.* | 11–21 | 6–20 | 33–64 |
| *Chaetoceros muellerii* | 33 | 44–65 | 11–19 |
| *Porphyridium cruentum* | 9–14 | 28–39 | 40–57 |
| *Euglena gracilis* | 14–20 | 39–61 | 14–18 |
| *Prymnesium parvum* | 22–38 | 28–45 | 25–33 |

(Source: Tandon , 2017 and Shumbulo , 2018)

The lipid content of microalgae increased under the depletion of nutrients and the longer time of cultivation whereas protein decreased and the carbohydrate composition increased with cultivation time. However, it is microalgae species-dependent (Dong *et al*., 2016). Even though microalgae have a high lipid content, their cell walls are thick and rigid and covered by a rich complex of carbohydrates and glycoproteins (Zhou *et al*., 2017).So, the biomass conditions, particularly biomass concentration, dried/wet, and the growth stage of cultivation are some factors that need to be observed.

Microalgae can be a valuable source of carbon compounds, which can be utilized tremendously in biofuels, health supplements, pharmaceuticals, and cosmetics (Das *et al*., 2011). Microalgae can produce a wide range of bioproducts, which includes polysaccharides, lipids, proteins, pigments, vitamins, bioactive compounds, and antioxidants (Figure 2) (Brennan, 2010). It can be observed that the interest in microalgae as a renewable and sustainable feedstock for biofuels production has inspired a new focus in biorefinery.

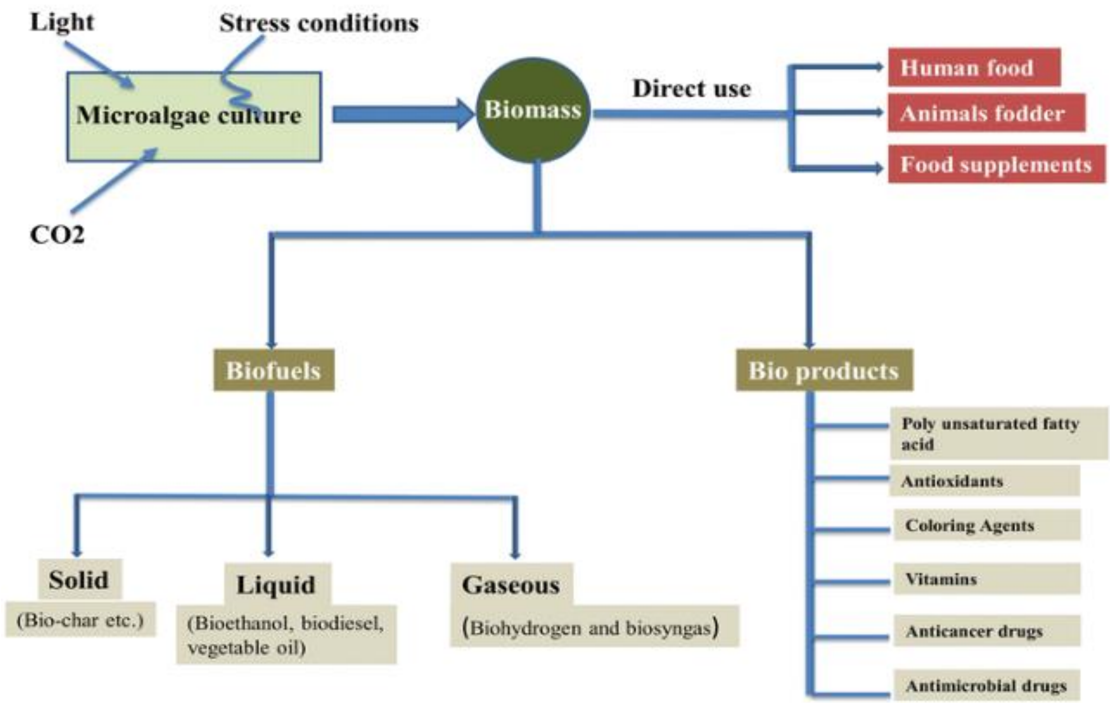



**Figure 2: Microalgae convert atmospheric $CO_2$ to carbohydrates, lipids, and other valuable bioproducts by using light.** (Source: Brennan, 2010)

Plaza *et al* (2009) stated that the industrial cultivation of microalgae to produce biofuels and bioproducts has increased dramatically over the last few decades. So, above circumstance indicates that algae are produced in quantity and sold directly as food and nutrient supplements, while their processed products or extracts are used in biopharmaceuticals and cosmetics (Luiten, 2003; Michael, 2013; Pulz, 2014).

### 3.3 Factors that affect Microalgae

Successful treatment of wastewater with microalgae requires good growth, and understanding of the factors that affect growth is therefore essential. The growth rate of algae and cyanobacteria is influenced by physical, chemical and biological factors (Table 2).

**Table 2: Factors that influence growth of microalgae during cultivation**

| | |
|---|---|
| **Abiotic factors, physical and chemical** | Light (quality, quantity) |
| | Temperature |
| | Nutrient concentration |
| | $O_2$, $CO_2$ |
| | pH |
| | Salinity |
| | Toxic chemicals |
| **Biotic factors** | Pathogens (bacteria, fungi, viruses) |
| | Predation by zooplankton |
| | Competition between species |
| **Operational factors** | Mixing |
| | Dilution rate |
| | Depth |
| | Addition of bicarbonate |
| | Harvesting frequency |

(Source: Becker, 1988)

According to the mentioned table above examples of physical factors are light and temperature. Chemical factors are constitutes of nutrients and carbon dioxide, where biological factors includes e.g. competition between species, grazing by animals and virus infections. Operational factors which also affect the factors mentioned above basically concerns bioreactor design, mixing and dilution rate.

### 3.4 Wastewater Characterization for Microalgal cultivation

Marcilhac *et al* (2014) describe the type of wastewater (WW) and its characteristics (e.g., pollutant concentrations, pHs, or color) determine the efficiency of algal-bacterial systems for wastewater treatment. More specifically:

- The initial carbon (C)/nitrogen (N)/phosphorus (P) ratio of the WW is effectively correlated with its biodegradability in absence of inhibitory or recalcitrant compounds, the optimum biodegradability ratio being 100:18:2 (g/g/g) (Posadas *et al*., 2014a). Most of the investigations carried out for the evaluation of WWT (domestic, industrial, agro-industrial and livestock effluents) by microalgae-based bioprocesses were conducted under carbon limitation (Park and Craggs, 2010) and also the addition of $CO_2$ from fuel gas or biogas represents a sustainable alternative to overcome C limitation in WWs with concomitant greenhouse gas mitigation and pH control of the algal-bacterial broth (Posadas *et al*., 2015; Serejo *et al*., 2015). Similarly, the concentration of C, N, and P in WW and their nature also influence the final algal-bacterial biomass composition (Posadas *et al*., 2015).

**Table 3: Concentrations of N and P as well as their molar ratios in different types of wastewaters**

| Wastewater sources | N (mg $L^{-1}$) | P (mg $L^{-1}$) | N:P ratio (molar) |
|---|---|---|---|
| Domestic | 20–85 | 5–20 | 11–13 |
| **Animal manure** | | | |
| Pigs | 800–2300 | 50–320 | 12–17 |
| Beef cattle | 63 | 14 | 10 |
| Dairy cattle | 185 | 30 | 4 |
| Poultry | 800 | 50 | 32 |
| **Industrial** | | | |
| Coke production | 757 | 0.5 | 3000 |
| Tannery | 273 | 21 | 29 |
| Paper mill | 11 | 0.6 | 41 |
| Textile | 90 | 18 | 11 |
| Winery | 110 | 52 | 5 |
| Anaerobic food waste | 1600–1900 | 300 | – |
| Olive mill | 530 | 182 | 2.9 |

(Source: Christenson and Sims, 2011; Cai *et al*. 2013)

- N-$NH4^+$ at concentrations higher than 100 mg N-$NH4^+$ $L^{-1}$ and pHs is greater than 8, inhibit photosynthetic activity in some microalgae species as a result of $NH_3$ toxicity (Posadas *et al*., 2014a). Based on the aqueous $NH_4^+$ =$NH_3$ equilibrium, microalgae inhibition increases at high pH value. Therefore effluents with high $NH4^+$ concentrations such as livestock wastewaters (≈600–3000 mgN- $NH4^+$ $L^{-1}$), centrates (≈400–800 mgN- $NH4^+$ $L^{-1}$) or anaerobically digested agro-industrial effluents (≈600–800 mgN- $NH4^+$ $L^{-1}$) need to be previously diluted or provided at low loading rates to avoid microalgae inhibition (Posadas *et al*., 2015; Gonzalez *et al*., 2008; Serejo *et al*., 2015).

- Heavy metals inhibit bacterial growth and photosynthesis and even generate morphological modifications in the microalgae cell walls at very low concentrations (Munoz and Guieysse, 2006). Cu, Cd, Cr, Hg, Pb, and Zn constitute the most common heavy metals found in WWs. As a matter of fact, Munoz *et al* (2006) reported *Chlorella sorokiniana* inhibition at Cu (II) concentrations of 2 mg $L^{-1}$, while Heng *et al* (2004) observed that Cd (II) and Pb (II) inhibited the growth of *Anabaena* sp by 50% at concentrations of 0.15 and 1.00 μg $L^{-1}$, respectively.
- Toxic organic pollutants such as salicylate, phenol, phenanthrene, and hydrocarbons also decrease the activity of microalgae and bacteria. For example, Borde *et al* (2003) found a complete inhibition of *Chlorella sorokiniana* growth at 10 mg phenanthrene $L^{-1}$, while a Pseudomonas strain used in symbiosis with this microalga was capable of biodegrading phenanthrene at 25 mg $L^{-1}$.
- The pH of the WW also influences WW biodegradability in microalgae-bacteria systems. Thus WWs with a pH outside of the optimal range for their treatment in Photobioreactors (7–9) (such as animal feed production or coffee WWs) are hardly biodegraded without any pH adjustment (Posadas *et al*., 2015).

### 3.5 Potentiality of wastewater as culture medium and its effect on growth and proximate composition of microalgae

Abhishek Guldhe *et al* (2016) done an experiment with *Chlorella* strain biomass production where *Chlorella sorokiniana* strain was used for the heterotrophic cultivation using aquaculture wastewater (AW) and BG11 nutrient medium .Microalgae were also grown in BG11medium and AW under phototrophic condition for comparative analysis. The nutrient supplementation of this experiments were carried out by adding 200, 400, 600and 1500 mg$L^{-1}$sodium nitrate in AW. Biomass productivity (mg$L^{-1}d^{-1}$) was calculated at late log phase gravimetrically (Singh *et al*., 2015b)and the nutrients removal efficiency was determined by analyzing nitrates ($NO_3^-$),nitrites ($NO_2^-$), TON, ammonia ($NH_4^+$) and phosphates ($PO_4^{3-}$) (Gupta *et al*., 2016). The removal efficiency in percentage was determined by using following equation (1)

$$\text{Percentage removal} = \frac{\text{Initial Concentration} - \text{Final Concentration}}{\text{Initial Concentration}} \times 100 \qquad (1)$$

Microalgal biomass collected from each experiment was analyzed for lipids, carbohydrates and proteins. The lipids obtained were measured gravimetrically and the percentage lipid content was determined based on lipid recovered from known weight of dry biomass. Lipids productivity was calculated according to equation (2) described by (Singh *et al*., 2015)

$$\text{Lipid Productivity} = \text{Biomass productivity} \times \frac{\text{Lipid Content}}{100} \qquad (2)$$

Where, biomass productivity is in $mgL^{-1}d^{-1}$, lipids content is in percentage per dry biomass weight. The quantitative analysis of protein was done by Lowry's method. Proteins productivity was determined in percentage per dry biomass weight using equation (3)

$$\text{Protein Productivity} = \text{Biomass productivity} \times \frac{\text{Protein Content}}{100} \qquad (3)$$

Total carbohydrates were quantified using the phenol-sulfuric acid method (Prajapati *et al*., 2013). Carbohydrate productivity was determined by the following equation (4)

$$\text{Carbohydrate productivity} = \text{Biomass productivity} \times \frac{\text{Carbohydrate Content}}{100} \qquad (4)$$

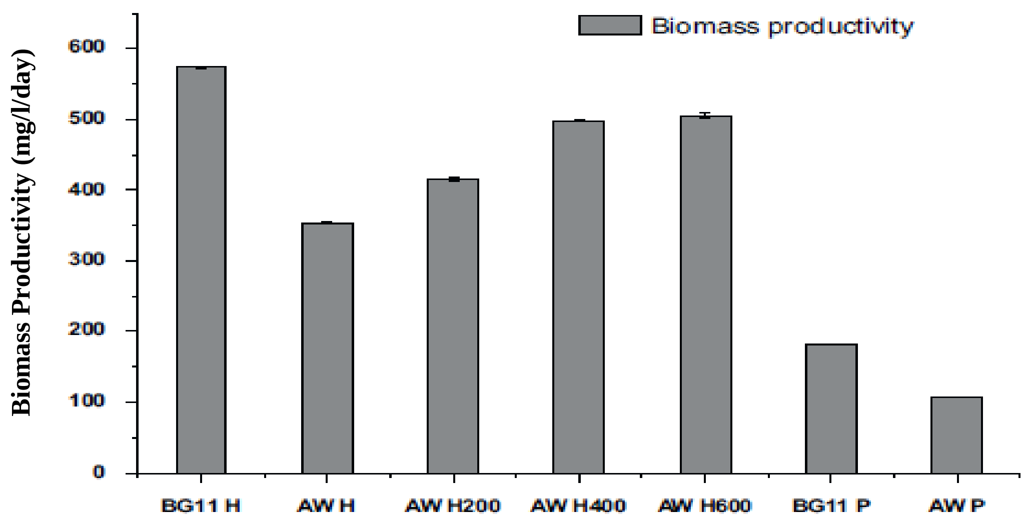
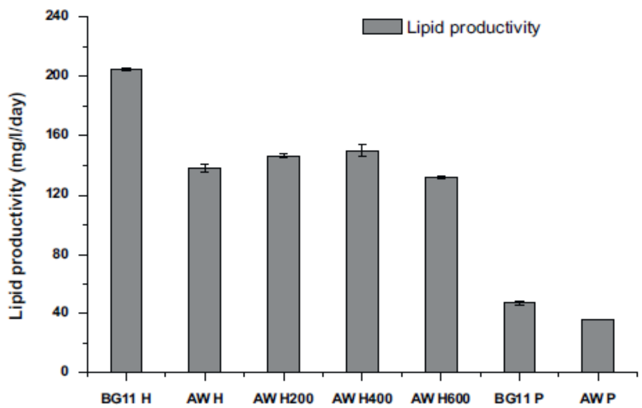


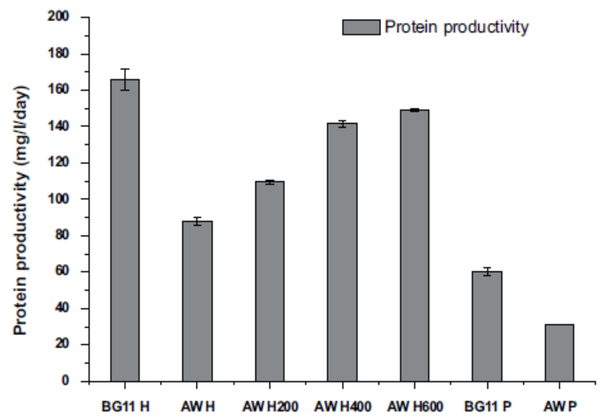

**Figure 3: Biomass productivities of *C. sorokiniana* in BG11 medium and aquaculture wastewater with different concentration of sodium nitrate supplementation.** (Source: Abhishek *et al*., 2016)

(Here, BG11 H- Blue green 11 media heterotrophic, AW H- Aquaculture wastewater heterotrophic, AW H 200- Aquaculture wastewater heterotrophic supplemented with 200 $mgL^{-1}$ sodium nitrate, AW H 400- Aquaculture wastewater heterotrophic supplemented with 400 $mgL^{-1}$ sodium nitrate,, AW H 600- Aquaculture wastewater heterotrophic supplemented with 600 $mgL^{-1}$ sodium nitrate, BG11 P- Blue green 11 media phototrophic, and AW P- Aquaculture wastewater phototrophic) **Error! Bookmark not defined.**

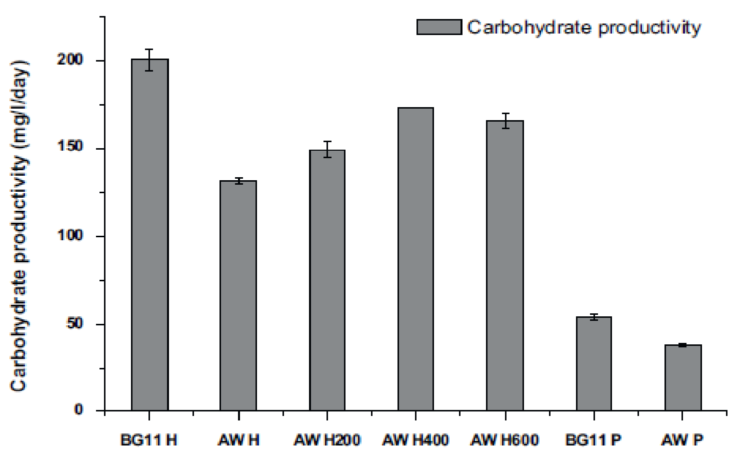
The heterotrophic cultivation of *C. sorokiniana* showed biomass yields of 2.47 $gL^{-1}$in AW and 4.02 $gL^{-1}$ in BG11 medium (Table 4).Higher biomass yield in synthetic medium (BG11) is because of the higher nutrient concentration compared to that in AW. Similarly, biomass productivity of 353.36 $mgL^{-1}d^{-1}$ in AW was found to be lower than the BG11 medium (573.79 $mgL^{-1}d^{-1}$). The lower biomass productivities indicates the need of nutrient supplement for microalgal cultivation in AW to achieve comparable biomass yields to that of the synthetic medium and also the biomass productivity in heterotrophic cultivation was 3 times higher than the phototrophic cultivation. This result clearly demonstrates that the heterotrophic mode of nutrition is the most suitable cultivation strategy for using aquaculture wastewater for microalgal biomass generation.




**(a)**




**(b)**




**(c)**

**Figure 4: Lipid (a), Protein (b) and Carbohydrate (c) productivities of *C. sorokiniana* in BG11 medium and aquaculture wastewater with different concentration of sodium nitrate supplementation.** (Source: Abhishek *et al*., 2016)

In the determination of lipid, carbohydrate and protein content and productivities the lipid content of *C. sorokiniana* cultivated using AW was 39.1% dry cell weight (DCW), while it was 35.75% DCW for synthetic medium. Similarly, carbohydrate content in *C. sorokiniana* grown using AW was 36.1% DCW, while it was 33.64% DCW for synthetic medium (Table 4). Protein content in the microalgal cells is directly related to the growth. Reduced growth is observed in microalgae grown on AW compared to synthetic medium. This has been reflected in the protein content also, the protein content for *C. sorokiniana* grown in AW was 24.57% DCW which was lower than the protein content 28.64% DCW of biomass generated using synthetic medium(Table 4).

**Table 4: Biomass, lipid, protein and carbohydrates yields in *C. sorokiniana* under different cultivation condition in synthetic media and aquaculture wastewater**

| Units | Biomass($gL^{-1}$) | Lipid content % | Protein content % | Carbohydrate content % |
|---|---|---|---|---|
| BG11 H | 4.02 | 35.75 | 28.64 | 33.64 |
| AW H | 2.47 | 39.1 | 24.57 | 36.1 |
| AW H200 | 2.9 | 35.3 | 26.38 | 35.97 |
| AW H400 | 3.49 | 30.15 | 28.42 | 34.71 |
| AW H600 | 3.54 | 26.1 | 29.46 | 32.79 |
| BG11 P | 2.54 | 26 | 29.46 | 29.74 |
| AW P | 1.51 | 33.45 | 29.46 | 35.43 |

(Source: Abhishek *et al*., 2016)

Generally, in microalgal cultivation a tradeoff is observed for biomass and lipid productivities with nitrogen stress. With increasing concentration of nitrogen in medium biomass productivities increases while lipid content decreases. In this experiment with sodium nitrate supplementation lipid productivity increased upto 400 $mgL^{-1}$ supplementation after that lower lipid productivity was observed with 600 $mgL^{-1}$. Similar trend was observed for carbohydrate productivity; highest carbohydrate productivity of was observed in *C. sorokiniana* grown in AW supplemented with 400 $mgL^{-1}$ sodium nitrate (Figure 4c). Protein productivity was found to be gradually increasing with the increasing concentration of sodium nitrate in AW. Highest protein productivity was observed with 600$mgL^{-1}$ sodium-nitrate in AW. The 400 $mgL^{-1}$ supplementation selected as optimum for biomass production resulted in protein productivity of 141.57 $mgL^{-1}d^{-1}$which is slightly lower than the highest (Figure 4b).

These results highlight the promising potential of cultivation of microalgae in AW for production of high quality biomass. Supplementation strategy has been found to be very effective to enhance the biomass productivity with adequate primary metabolites composition. The lipid and carbohydrates in microalgal biomass can be used for various biofuels synthesis such as biodiesel, bio-methane and bioethanol. Protein component of biomass makes it suitable for aquaculture or animal feed application.

Kabir *et al* (2018) was conducted an experiment with three isolated microalgae strains capable of growing in BG-11 medium *Chlorococcum humicola* and *Selenastrum sp.* from freshwater and *Chlorella vulgaris* was isolated from swine manure wastewater resource. Among these selected species, *C. vulgaris* grow more rapidly and there is a smooth increase in biomass yield and its exponential phase lasted for 4 days followed by stationary phase in next 3 days. While, in case of other two strains exponential phase remains for 6 days before entering into stationary phase.

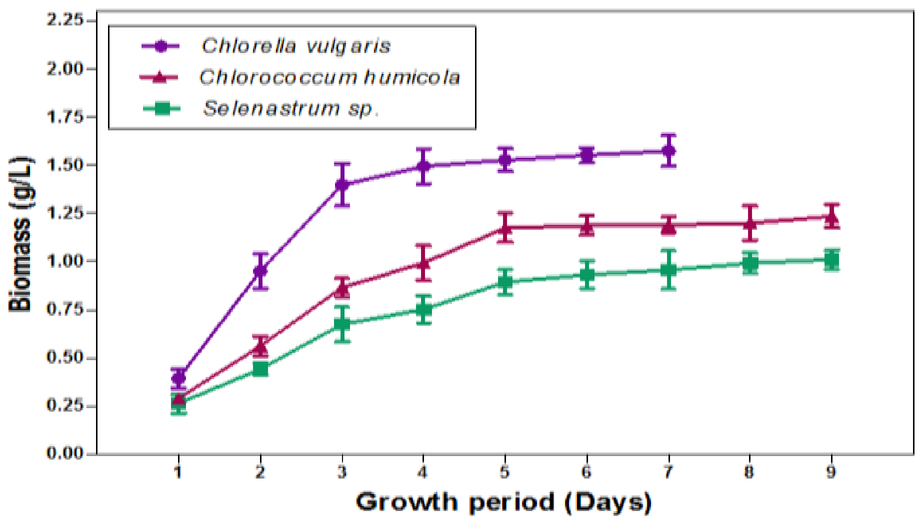
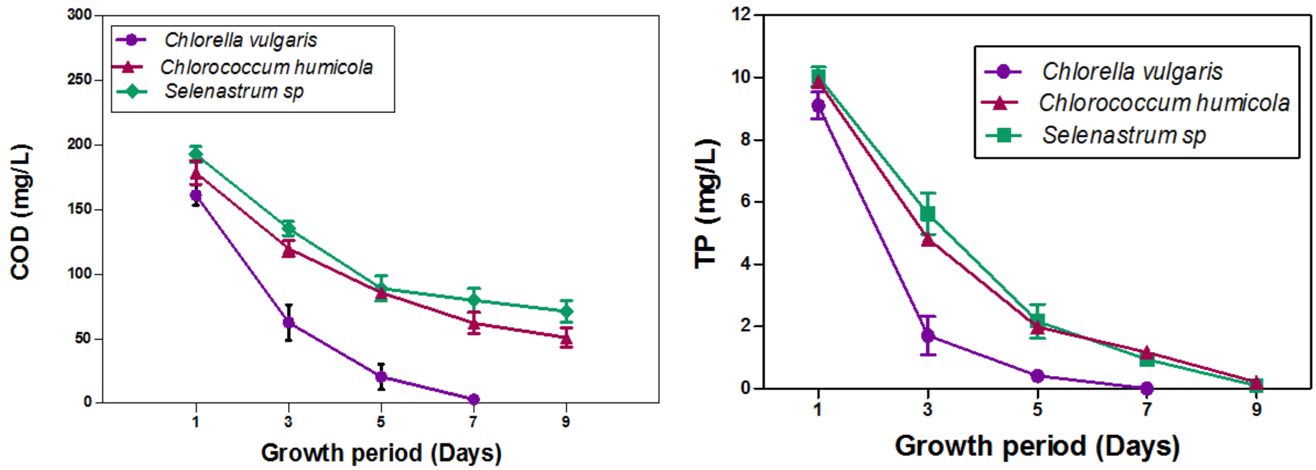



**Figure 5: Growth curve of microalgae in wastewater media.** (Source: Kabir *et al*., 2018)

Moreover, it was observed that biomass productivity of *C. vulgaris* (229 ± 7.3mgL$^{-1}$ d$^{-1}$) was higher than *C. humicola* (129 ± 3.94 mgL$^{-1}$ d$^{-1}$) followed by *Selenastrum sp*. (101.25 ± 8.41 mgL$^{-1}$ d$^{-1}$) although, these two strains have a growth period for 9 days. The highest growth rate and biomass productivity of *C. vulgaris* is because of its better acclimatization as it was selected among the top-performing microalgae strains in wastewater (Zhou *et al*., 2011) and may be due to its natural habitat in wastewater.

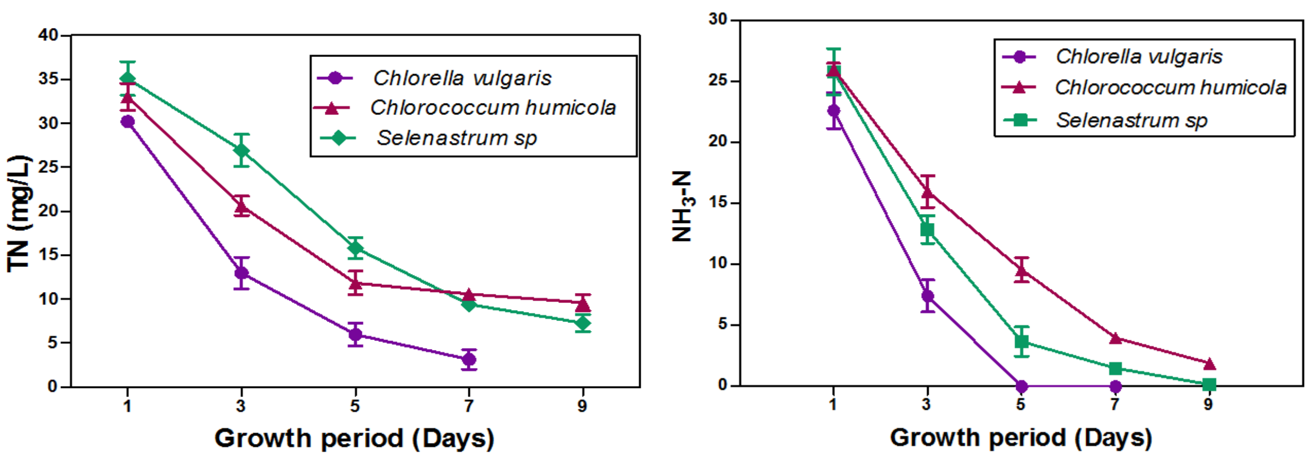
The removal efficiency (%) of COD (chemical oxygen demand), TP (total phosphorus), $NH_3$-N (ammonia) and TN (total nitrogen) by three microalgae species in sewage wastewater media (SWM) for batch cultivation mode was studied and illustrated in below Figures.




(a) (b)

**Figure 6: COD ($mgL^{-1}$) (a) and TP ($mgL^{-1}$) (b) removal rate of microalgae species in wastewater media.** (Source: Kabir *et al*., 2018)

The removal efficiency of COD was varied among different species and highest removal rate of 98.6% was achieved for *C. vulgaris* followed by *C. humicola* (77.37%) and *Selenastrum sp*. (68.35%). There was a rapid removal of carbon during first 4-5 days and COD removal rate was slow after that and this study was in line according to (Gutzeit *et al*., 2005). This might be because of less concentration of carbon and the remaining carbon source in a media may be slowly biodegradable material. Generally, carbon was considered as a limiting factor when algae were cultivated in sewage wastewater. Phosphorus removal rate was slow as compared to nitrogen and it may be due to the reason that nitrogen is a limiting factor in selected wastewater sample.




(a) (b)

**Figure 7: TN ($mgL^{-1}$) (a) and $NH_3$-N ($mgL^{-1}$) (b) removal rate of microalgae species in wastewater media.** (Source: Kabir *et al*., 2018)

The wastewater containing high ammonium concentration can be very effective to grow microalgae. In sewage wastewater the concentration of $NH_3$-N decreased rapidly and its removal efficiency was approximately 100% in two species except *C. humicola* (93%). T-N removal efficiency ranged from 91.68%, 80.61% and 74.37% in *C. vulgaris*, *Selenastrum sp*. and *C. humicola* respectively. The reason for fast removal of ammonia is its low concentration in wastewater. The removal of T-N was slightly lower as compared to ammonia representing that some organic nitrogen must be there, produced during microalgae cultivation and wastewater treatment process (Oswald *et al*., 1957).

Generally, microalgal biomass comprised of protein (6-52%), carbohydrate (5-23%) and lipids (7-23%) (Zhu, 2015). But this study showed that, the protein content of microalgae species grown on wastewater ranged from 13% to 15%. The reason for low concentration of protein in biomass may be rapid depletion of ammonia during cultivation. However, carbohydrate contents were high in *Selenastrum sp.* (23.96%) followed by *C. vulgaris* (18.23%) and *C. humicola* (8.67%). The highest lipid contents were observed in *C. vulgaris* (32.41%) as compared to other two species, this may be because of fast utilization of T-N as well as ammonia by this species as a result of nitrogen starvation.

**Table 5: Composition of microalgae grown in sewage wastewater growth media in batch mode under mixotrophic cultivation conditions**

| Species | Biomass Productivity ($mgL^{-1}$/d) | Biomass Concentration ($gL^{-1}$) | Lipid Content (%) | Carbohydrate content (%) | Protein content (%) |
|---|---|---|---|---|---|
| *Chlorella vulgaris* | 229 ± 7.3 | 1.574± 0.20 | 32.41 ± 2.4 | 18.23 ± 3.94 | 13.12 ±4.9 |
| *Selenastrum sp* | 129 ± 3.94 | 1.01± 0.15 | 21.62±1.9 | 23.96 ±0.9 | 15.67±3.6 |
| *Chlorococcum humicola* | 101.25 ± 8.4 | 1.235± 0.06 | 25.5 ± 3.1 | 8.67 ± 1.5 | 13.78 ± 2.1 |

**Note:** Data shown in mean ± standard error (Source: Kabir *et al*, 2018)

This experiment has demonstrated that it is feasible to grow microalgae in sewage wastewater under batch cultivation as *Chlorella vulgaris* shows the best growth in wastewater media and was capable of complete depletion of T-N, TP, NH3-N and COD as compared to *Selenastrum sp.* and *Chlorococcum humicola.* The biochemical composition of this study revealed that the carbohydrate and protein contents are present in normal range in all of the species and lipid contents were comparatively high in *C. vulgaris.* The limiting factor for growth of microalgae was $NH_3$-N and COD deficiency. So, more biomass productivity can be achieved by adding nitrogen and carbon in growth media which will ultimately increase the lipid production.

Khatoon et al (2017) conducted an experiment with a microalgal strain *Tetraselmis chuii* which was cultured in Conway culture medium (Tompkins *et al*., 199). *T.chuii* was grown in three different media, Conway, Conway + wastewater, and wastewater with triplicates. Growth parameters in terms of cell density and chlorophyll were measured. The specific growth rate (SGR) of microalgae was calculated using the following equation (5)

$$SGR = \frac{\ln(X_2/X_1)}{(t_2 - t_1)} \qquad (5)$$

Here $X_1$ is biomass concentration at the beginning of the selected time interval; $X_2$ is the biomass concentration at the end of the selected time interval; $t_2–t_1$ is the selected treatment period (in number of days) for the determination of biomass of microalgae species. Protein, carbohydrate and lipid were analyzed according to the methods of Lowry *et al* (1951), Dubois *et al* (1956), Marsh and Weinstein (1966), respectively.

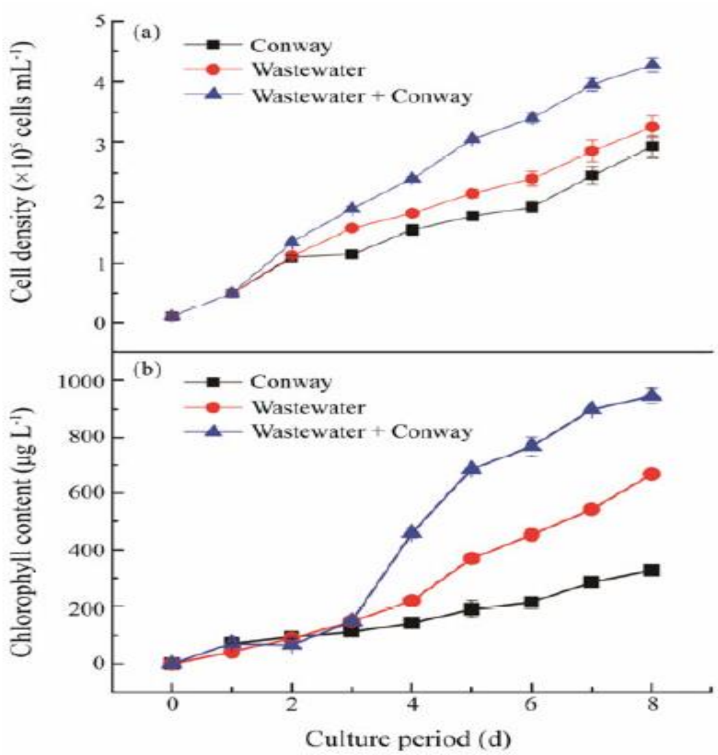



**Figure 8: Cell density (a) and Chlorophyll content (b) Verses culture period (day) for *Tetraselmis chuii* cultured in three different media.** (Source: Khatoon *et al*., 2018)

In this study, *T. chuii* grew well, even when they were cultured using aquaculture wastewater (Figure 8) The highest cell density was found when they were cultured using wastewater + Conway medium (4.3 ×

$10^5$ cells $mL^{-1}$) compared to those cultured using only wastewater ($3.3 \times 10^5$ cells $mL^{-1}$) or Conway ($2.9 \times 10^5$ cells $mL^{-1}$).

**Table 6: Specific growth rate of *Tetraselmis chuii* cultured in three different media**

| Medium | Initial density ($\times 10^5$ cells $mL^{-1}$) | Maximum cell density ($\times 10^5$ cells $mL^{-1}$) | Specific growth rate ($d^{-1}$) |
|---|---|---|---|
| Conway | 0.01 | 2.925 | 0.71 |
| Wastewater | 0.01 | 3.250 | 0.72 |
| Wastewater + Conway | 0.01 | 4.275 | 0.76 |

(Source: Khatoon *et al*., 2016)

The experiment showed that the use of aquaculture wastewater mixed with commercial medium for culturing microalgae is highly recommended to reduce the overall cost rather than relying fully on commercial medium. Other author found that microalgae can grow well in nutrient-rich wastewater such as fish or shrimp cultured wastewater (Khatoon *et al*., 2016; Chopin *et al*., 2012).

The proximate composition of *T. chuii* cultured in three different media is shown in below (Table 7). Significantly higher protein content (45.0 ± 0.2) % was observed in Conway + wastewater medium, followed by wastewater alone and Conway alone media at dry weight, respectively. In addition, significantly higher lipid and carbohydrate contents were also obtained in Conway + wastewater medium at (28.0 ± 0.7) % and (22.0 ± 0.1) % of dry weight, respectively.

**Table 7: Proximate composition (% dry weight) of *Tetraselmis chuii* cultured in three different media**

| Proximate analysis (% dry weight) | Conway | Wastewater | Conway + Wastewater |
|---|---|---|---|
| Protein | 26.3 ± 0.4 | 33.7 ± 1.1 | 45.0 ± 0.2 |
| Lipid | 16.5 ± 0.1 | 17.6 ± 0.3 | 28.0 ± 0.7 |
| Carbohydrate | 11.2 ± 0.2 | 12.1 ± 0.1 | 22.0 ± 0.1 |

**Note:** Data shown in mean ± standard error (Source: Khatoon *et al*., 2016)

According to Vasileva *et al* (2015), nitrogen is one of the crucial components for microalgae growth and protein biosynthesis. Several microalgae cultured in nutrient-rich condition can absorb the extra nitrogen from the culture medium and store it as proteins (Andersen, 2013). According to Mata *et al* (2009), *Tetraselmis* sp. is among the most common microalgae known to have a high concentration of lipid (20%–50% of dry weight). All treatments also showed much lower carbohydrate content compared to lipid. According to Ho *et al* (2012), the competition occurs between lipid and carbohydrate production under stress conditions because their synthetic pathways are closely related. Furthermore, biochemical composition of microalgae may vary among species depending on culture condition, environmental factors or different harvesting phases (Brown *et al*., 1997; Becker, 2004)

Malgorzata *et al* (2019) assessed the effect of nutrient in aquaculture wastewater on the growth of *Chlorella minutissima* and the biomass concentration increased to 4.69 g/L, while in the F/2 medium, it increased to 3.61 g/L. In the studies carried out by (Halfhide *et al*., 2014) a maximum mean biomass concentration for *Scenedesmus* (0.41 g/L) in the tilapia RAS wastewater was achieved after 36 h. The Optical density ($OD_{680}$) value in the F/2 medium gradually increased to 0.304 after eight days of incubation, and a slightly lower value was recorded after 10 days (0.292). Corresponding changes were observed during the cultivation of microalgae in wastewater, in which the highest optical density was observed after eight days, but this value was over 160% higher compared to F/2 medium.

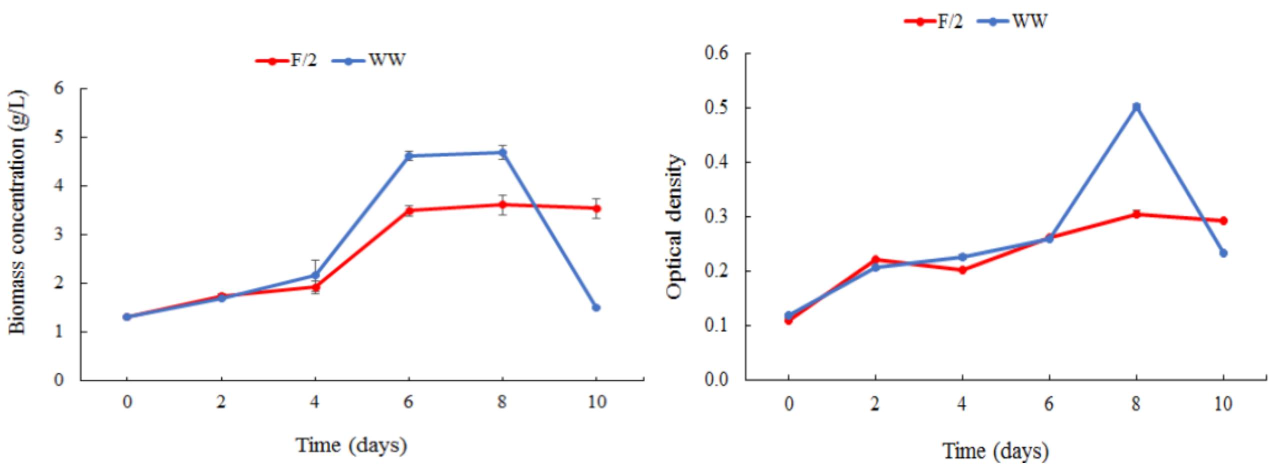



**Figure 9: Changes in biomass concentration and optical density during cultures of *Chlorella minutissima.*** (mean ± SD; F/2—synthetic and WW-wastewater). (Source: Malgorzata *et al*., 2019)

The productivity of *C. minutissima* biomass ascertained in the wastewater obtained from the aquaculture process was higher than that ascertained in other types of wastewater (e.g., municipal wastewater or wastewater resulting from animal (Liu *et al*., 2013). This difference may have been due to, among other factors such as varied content of nutrients in wastewater, cultivation conditions, and most importantly, the type of microalgae used in the purification process. It should be noted that the post culture sewage must be free from toxic components, including heavy metals, which are commonly found in industrial effluent, municipal waste stream, and mining wastewater (S.Alva, 2013), so that the obtained microalgal biomass can be used for the production of feed in aquaculture, among other applications.

# CHAPTER IV

# SUMMERY AND CONCLUSION

Microalgae have been utilized for several applications such as bioenergy generation, pigments, bioactive compounds, human nutrition, animal and aquaculture feed and synthesis of various compounds for pharmaceutical, nutraceutical and cosmetic industry and this biomass generation is required for these applications using wastewater have economic and environmental benefits. It has been primarily emphasized for bioenergy production nevertheless it is untapped resource for various bioactive and organic compounds.

From this review, different result showed that the wastewater could be used as an alternative source to cultivate microalgae. In this way, nutrients in the wastewater are utilized by algae otherwise, it would have been harmful to the environment and by growing algae in wastewater it reduces the medium costs and also saves the environment. The combining wastewater and nutrient medium showed the best one for culturing some algae such as *Chlorella, Tetraselmis* in terms of growth rate and biochemical composition. The biochemical composition revealed that the carbohydrate and protein contents are present in normal range in all of the species and lipid contents were comparatively high in *C. vulgaris*. Several studies also conclude that different species of *Chlorella* grow best in wastewater media and were capable of complete depletion of TN, TP, $NH_3$-N and COD. The limiting factor for growth of microalgae was $NH_3$-N and COD deficiency. So, more biomass productivity can be achieved by adding nitrogen and carbon in growth media which will ultimately increase the proximate composition.

The findings of other study have clearly highlighted the potentiality of aquaculture wastewater as a nutrient substrate for microalgal cultivation and heterotrophic mode of cultivation had shown better biomass and metabolites productivities than the phototrophic mode and also nutrient supplementation strategy improves the biomass growth as well as lipid, carbohydrate and protein productivities.

However, various challenges need to be overcome to make this approach economically viable and sustainable. Recent developments in microalgal biotechnology are certainly leading to overcoming some of the major challenges and can result in sustainable microalgal cultivation. Further investigations are required to ascertain the potential of wastewater grown microalgal biomass for different applications. Hence, more detailed study may improve or give a fruitful solution to utilize the wastewater for mass production of microalgae.

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