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WASTEWATER TREATMENT UTILIZING MICROALGAE FOR BIOREMEDIATION AND BIOMASS PRODUCTION

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Wastewater treatment utilizing microalgae for bioremediation and biomass production[1]

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ABSTRACT

Water shortages and environmental contamination have become unstoppable worldwide issues as a result of economic growth and population growth. This review summarizes the potentiality of microalgae in diverse wastewaters and critically evaluates their function in wastewater treatment in addition to exploring the potential of wastewaters for efficient microalgae biomass production. The trace elements nitrogen, phosphorus, and others that microalgae need for cell development are usually present in waste waters. As a result, microalgal bioremediation might be used in combination with present treatment methods as the primary biological approach to effectively treating wastewater. According to the reviewed study, several microalgae strains, including *Chlorella sorokiniana, C. vulgaris, C. minutissima, Tetraselmis chuii,* and *Selenastrum* sp., are more successful at growing in wastewater than other microalgae strains. With the *Scenedesmus* algae, the highest atrazine tolerance level was discovered. In one study, *Scenedesmus abundans* had a greater biomass productivity (3.55 mgL^{-1} d^{-1}) and a high nutrient removal efficiency (COD: 98.6%, TN: 91.68%, NH3: 100%) in wastewater. The state of microalgae bioremediation of wastewater along with biomass production and aims to serve as a guide for future studies in the area. The various results also showed that additional nutrient supplementation was necessary while growing the microalgal strain in various wastewaters. To enhance further, it is vital to understand the process by which microalgae eliminate nutrients and contaminants from wastewater.

Keywords: Wastewater, microalgae, bioremediation, biomass production

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Chapter I

INTRODUCTION

Environmental pollution and resource limitation are the primary problems that humanity will surely meet because as world population and economy continue to expand (Liu & Hong, 2021). Environmental pollution problems are becoming more and more problematic as the worldwide population, development, and civilization expand, as do their indirect impacts on aquatic ecosystems. The necessity for efficient and long-term management of urban wastewater represents only a few of the issues that face world today (Ukaogo *et al.*,2020). In terms of the sustainability both the current and future generations, the effects include an unsustainable development of wastes and wastewater, the dumping of wastewaters into freshwater resources, and global warming (Renuka *et al.*, 2015). In this scenario, the mixing of wastewater discharges in aquatic bodies is arising as one of the key problems to international stability due to the majority of the people in developing and underdeveloped countries depend on freshwater resources for their daily needs, either directly or indirectly (Renuka *et al.*, 2015). Thus, it is necessary to find low-cost, environmentally friendly technologies that are simple to use and can be accessed by the common people who have lower levels of education.

The methods used to remediate effluents or polluted water are generally classified as physical, chemical, and biological. Depending on the level and kind of pollution, they can be used either individually or together. Physical and chemical procedures are often more expensive. Moreover, the pH, conductivity, and total load of dissolved materials in the wastewater are all increased by most chemical procedures. The biological or bio-treatment of wastewater is a superior choice in this regard. The most used biological method for treating industrial and municipal wastewaters. The discharge of industrial and municipal wastewater creates major environmental issues to surrounding aquatic bodies (Yang *et al.*, 2008). Microalgae are an important element of the microbial variety of wastewaters, and they can help these wastewaters purify themselves (Sen *et al.*, 2013).

Microalgae (green expedient) has been reported as effective and powerful in the treatment of many types of residential, commercial, and industrial wastewaters because it needs less space. Reduced energy requirements, less minerals and organic residues, Efficacy in eliminating emerging pollutants (ECs) and heavy metals (HMs) is higher with aerobic treatment methods, while sludge formation is lower, when wastewater is used to grow microalgae, the amount of fresh water needed may be considerably reduced (by 90%), which also helps to balance the impact on the price of fertilizers (Gupta *et al.*, 2019). Hence, producing algae in wastewater has the dual benefits of treating wastewaters, reducing greenhouse gas emissions, and simultaneously creating algal biomass. The process of using microalgae to treat or bio-transform nutrients, contaminants, and CO_2 that are present in wastewaters while simultaneously producing biomass is known as bioremediation.

A possible approach shows up to be bioremediation, which involves using plants (including algae or lower plants) and associated microorganisms to remove or bio-transform contaminants from wastewater, such as fertilizers, heavy metals, etc. (Saeed *et al.*, 2022; Ali *et al.*, 2013). The use of microalgae for wastewater bioremediation was primarily limited to the effluents from treatment processes for cleaning and reducing elements like nitrogen and phosphorus that cause the environmental issue of eutrophication of waterbodies. Thus, it is essential to implement suitable treatment programs for the reduction and elimination of chemicals including ammonia (NH_4^+), nitrate (NO_3^-), and phosphate (PO_4^{3-}) (Lima *et al.*, 2020).

Algal biomass harvesting is the process of separating algae from their nutritional or growth media by utilizing methods including flocculation, centrifugation, floatation, filtration, or a combination of these methods. The cost, processing time, biomass species and quality, separation efficiency, and toxicity are some harvesting attributes or needs that affect the choice of an appropriate harvesting technique (Singh & Patidar, 2018). Biomass may be used for many purposes, including as a source of bioenergy resources (biogas and biofuels), food additives and protein supplements for animal and human feed, bio-ore for precious heavy metals, and medicines, cosmetics, and other useful compounds (Gupta *et al.*, 2019).

From this review paper, it would be possible to explore the wastewater treatment utilizing microalgae for bioremediation and biomass production.

OBJECTIVES

The study has undertaken to accomplish the following objectives:

- To understand the potentiality of wastewater treatment utilizing microalgae for bioremediation process.
- To explore the production of microalgae biomass from wastewaters treatment.

Chapter II

MATERIALS AND METHODS

All the information of this seminar paper has been collected from the secondary sources as it is just a review paper. During the preparation of this review paper, I went through various relevant books, journals, proceedings, reports, publications, internet etc. I got valuable suggestions and information from my major professor and my course instructors. After collecting all the available information, I myself complied the collected information and prepared this seminar 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

The word "algae" refers to the polygenetic group of single and multicellular organisms derived from many development pathways. They are unique and can range in structure from simple single cells to complex multicellular layers. Macroalgae are multicellular, non-microscopic, massive kinds of algae, whereas single-celled algae are known as microalgae (Vale *et al.*, 2022). Microalgae are adapted to an environment dominated by viscous forces. They are capable of performing photosynthesis which is important for life on earth. According to James *et al.,* (2019), microalgae generate approximately half of the atmospheric oxygen and use simultaneously the greenhouse gas carbon dioxide to grow autotrophically. Microalgae, together with bacteria forms the base of the food web and also provide energy for all the trophic levels above them.

Spirulina platensis *Spirogyra* sp. *Chlorella vulgaris*

S. maxima *Euglena* sp.

Figure 1. Some important microalgal species under microscope (Source: Rizwan *et al.*, 2018).

Thousands of microalgae exist on the earth but a few strains are used in the aquaculture. This is due to the difference in culture techniques. Over 50,000 species of microalgae, with a rich biodiversity exist all over the world. In case of Bangladesh, updated data is not available as we do not have a national microalgae collection and culture center.

3.2 Microalgal diversity in wastewater

The discharge of industrial and municipal wastewater creates major environmental issues to surrounding aquatic bodies (Yang *et al.*, 2008). In terms of nutrients, heavy metals, hydrocarbons, and other pollutants, wastewater is mostly numerous. Eutrophication is driven through the presence of nutrients, particularly nitrogen (N) and phosphorus (P), in the form of nitrate, nitrite, ammonia/ammonium, or phosphorus in wastewater (Liu *et al.*, 2010). Microalgae are a diverse group of organisms that include prokaryotic cyanobacteria and photoautotrophic eukaryotic microalgae. They are found in both freshwater and marine settings, and their thallus structure and habitats are quite diverse. A total of 152 species were identified by Yassin and Mahmoud, (2016) when they examined the phytoplankton diversity in sewage water combined with drain water. These taxa included Bacillariophyceae (60), Chlorophyceae (20), Cyanophyceae (20), Euglenophyceae (17), and Dinophyceae (9). Bacillariophyta constituted 39.4% of the total variety in the drain, making it the leading group.

3.3 Characteristics of wastewater

Waste waters are dumped into surface waters underneath the conditions of a permit that limits both the quantity and the level of contamination. Several contaminants include *Escherichia coli*, hazardous trace metals, and other organic and inorganic pollutants are found in both surface water and groundwater sources (Ahmad *et al.*, 2021). The major causes of water pollution are anthropogenic sources, including untreated industrial effluents, inadequate home waste disposal, and agricultural runoff (Choi *et al.*, 2018). Several parameters of wastewater are shown in Table 1.

Table 1. Wastewater Physico-Chemical Properties with Standard

Parameters	**Standard**		
	Bangladesh	**WHO**	**FAO**
Temperature (°C)	NA	NA	NA
DO (mg/l)	≥ 5.0	NA	NA
pH	6.0-8.5	6.5-8.5	6.0-8.5
EC (dS/m)	2.25	1.2	0-3
TDS (mg/L)	1000	2100	0-2000
CO_3^{2-}(mg/L)	NA	NA	0-3
HCO^{3-} (mg/L)	NA	58.4	0-610

Cl^{-} (mg/L)	600	600	0-1050
NO^{3-} (mg/L)	10	10	0-620
$PO_4{}^{2-}$ (mg/L)	6	NA	0-62
SO_4^{2-} (mg/L)	400	NA	0-960

(Source: Mridha, 2011)

There are many elements that contribute to the contamination of water bodies, including the agricultural industry, industrial production, mining, power generation, and others. This pollution will eventually have an impact on people in general (Hasan *et al.*, 2019).

3.4 Wastewater treatment using microalgae

Microalgae have several benefits, including the capacity to adapt to different climatic conditions and wastewater types, as well as the ability to remove specific toxins. Although microalgae are mainly photosynthetic by nature, some specific species may use various forms of organic matter in heterotrophic and mixotrophic modes, thus lowering the BOD/COD of the wastewaters (Ahmad *et al.*,2020). And the amount of nutrients in the wastewater was positively linked with the pace at which Chlorella removed nitrogen, phosphate, and chemical oxygen demand (COD) (Wang *et al.*, 2010). Figure 2 displays the effectiveness of microalgae in the removal of COD.

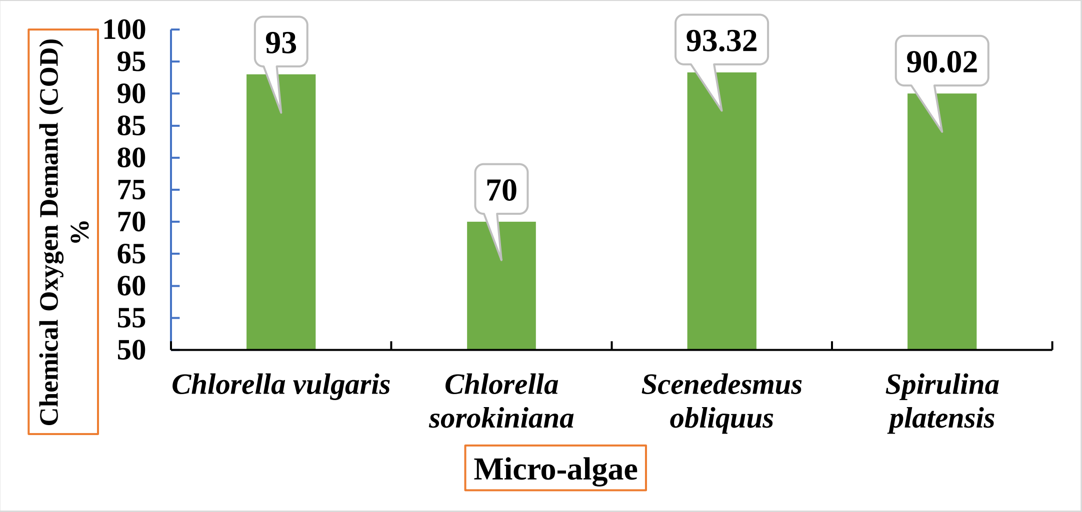

Figure 2. Effectiveness of microalgae in the removal of COD in wastewater (Source: Ahmad *et al.*, 2021).

3.4.1 Consumption of Nutrients

It is difficult to remediate wastewater that has a low nitrogen to organic carbon ratio (C/N). The addition of organics to such wastewater is frequently used to increase the effectiveness of bacterial nutrient removal as a source of energy. On the other hand, microalgae might increase their cell count while cleaning wastewater by using sunlight, dissolved inorganic co2 from the atmosphere, nitrogen, and other nutrients. Microalgal cellular nitrogen concentration may range from 3–10% depending on the strain type (Adamakis *et al.*, 2018). Microalgal/cyanobacterial strains have the potential to absorb a number of inorganic (such as ammonium, nitrate, nitrite, atmospheric nitrogen) and organic (such as urea, glycine, etc.) forms of nitrogen, but the effectiveness would again vary across strains and development conditions. Moreover, as a nitrogen source, microalgae might particularly absorb nitro and amino groups from various aromatic chemicals (such as aminonaphthalenes and nitrobenzonates), which would reduce the toxicity of the original contaminants (Choi *et al.*, 2018). Table 2 lists the removal rates of microalgal nutrients from various wastewaters.

Table 2. Microalgal nutrient removal efficiencies from different wastewater

Wastewater	**The Concentration of Contaminants in Wastewater (mg/L)**			**Microalgal Strain**	**Removal Efficiency (%)**			**References**
	TN	**TP**	**TOC**		**TN**	**TP**	**TOC**	
Municipal sewage water	116.1	212	-	*Chlorella* sp	94	89.1	-	Li *et al.* (2011)
	130	15	-	*Spirulina* sp.	79	93.3	-	Zhou *et al.* (2017)
	40.6	5.66	-	*Chlorella* sp.	82.4	50.9	-	Wang *et al.* (2010)
Agro-industry wastewater	1570	154	-	*Microalgal consortia*	49	70	-	Singh *et al.* (2010)
	44	88	495	*Scenedesmus obliquus*	34	65	42	Godos *et al.* (2010)
	44	88	495	*Algal consortia*	36	13	46	Godos *et al.* (2010)
Aquaculture wastewater	6.81	0.42	-	*Chlorella vulgaris*	86.1	82.7	-	Wang *et al.* (2010)
	41.3	4.96	-	*Tetraselmis suecica*	49.4	99	-	Michels *et al.* (2014)
	9.8	1.56	14	*algal-bacterial flocs*	58	89	71	Michels *et al.* (2014)
Aqueous phase wastewater from biomass to energy generation process	4223	504.7	13,917	*Tetraselmis* sp.	98.5	98	-	Michels *et al.* (2014)
	6900	1100	13,800	*Picochlorum* sp.	95.4	97.2	94.3	Das *et al.* (2020)
	9650	343	-	*Chlorella vulgaris*	59.9	94.6	-	Li *et al.* (2011)

*TN= Total Nitrogen; TP= Total Phosphorus; TOC= Total organic carbon (Source: Al-Jabri *et al.*, 2020)

3.4.2 Absorption of metals

Heavy metal levels in wastewater that's excessively high might prevent microalgal photosynthesis. Even so, microalgae may be used to remove metal from wastewaters by effectively concentrating the metal contaminants both inside and outside (Kumar *et al.,* 2015). Many metals, including Fe, Mn, Cu, Co, Zn, and Mo, are required by microalgal cells in trace levels for development. Yet, through different methodologies, microalgae are also able to capitalize on different heavy metals (such as Cd, Hg, Ni, Zn, Fe, Cu, Pb, Cr, etc.). Moreover, heavy metals may be transferred inside of cells through the cell membrane, lowering their quantities in wastewater (Kumar *et al.,* 2015). Table 3 lists the removal rates of several metals (such as Cd, Cr, Cu, Pb, Hg, Ni, and Zn) by certain microalgae.

Table 3. Metal reduction from wastewater by microalgae

Metals	Microalgae Strain	Removal Efficiency (%)	References
Cadmium	*Scenedesmus* sp.	73	Travieso *et al.* (1999)
	Chlorella sp.	33–41	Wong *et al.* (2000)
	Chlorella vulgaris	66	Travieso *et al.* (1999)
Chromium	*Chlorella vulgaris*	50.7–80.3	Sibli (2016)
	Scenedesmus sp.	92.89	Pradhan *et al.* (2019)
	Spirulina sp.	82.67	Rezaei *et al.* (2016)
Copper	*Spirulina maxima*	94.9	Rezaei *et al.* (2016)
	Chlorella vulgaris	96.3	Chan *et al.* (2014)
	Scenedesmus obliquus	72.4–91.7	Li *et al.* (2018)
Lead	*Chlorella vulgaris*	89.26	Malakootian *et al.* (2019)
	Chlorella sp.	66.3	Kumar & Goyal (2010)
	Psuedochlorococcum typicum	70	Shanab *et al.* (2012)
Mercury	*Chlorella vulgaris*	79–86	Fard & Mehrnia (2017)
	Chlorella vulgaris	34.21–93	Chan *et al.* (2014)
	Psuedochlorococcum typicum	97	Shanab *et al.* (2012)
Nickel	*Scenedesmus* sp.	97	Pradhan *et al.* (2019)
	Chlorella vulgaris	33–41	Wong *et al.* (2000)
	Chlorella miniate	60–73	Chong *et al.* (2000)
Zinc	*Chlorella* sp.	60–70	Chan *et al.* (2014)
	Synechocystis sp.	40	Chong *et al.* (2000)
	Scenedesmus sp.	98	Chong *et al.* (2000)

(Source: Al-Jabri *et al.,* 2020)

3.4.3 Organic Elimination

The microalgae can remove the organics from wastewater through three primary mechanisms: biodegradation, consumption, and biosorption. Although the removal of organic pollutants by microalgal sorption was only moderately effective (Wang *et al.,* 2019), microalgal cell walls may contain numerous polymer groups that might serve as potential sorption sites for organic pollutants. Despite the fact that the majority of microalgae are photosynthetic by nature, some of them have the ability to use different organics in either a mixotrophic or heterotrophic manner. This would allow the mixing of organic-rich streams (such as wastewater from food processing, glycerol from biodiesel plants, etc.) with other wastewater for combined treatment, improving the biomass and lipid yield (Choi *et al.*, 2018). In addition to bioaccumulation, microalgae can convert organic pollutants (such as phenolics, petroleum hydrocarbons, pesticides, polyaromatic hydrocarbons, and polychlorinated bisphenyls) into less toxic and non-toxic compounds or even completely mineralized products (such as CO_2), depending on the type of algae grown (monoculture or consortia) (Delrue *et al.*, 2016). Figure 3 depicts the CO_2 fixation rate of both pure and mixed microalgal strains as a function of culture time. Chlorella sp. had the greatest CO_2 fixation rate of all the mixed and pure strains utilized in the experiment. For mixed microalgae, Chlorella sp., the CO_2 fixation rates were 0.28, 0.957, 0.29, 0.14, 0.237, and 0.14 g/L-d (Park *et al.*, 2021).

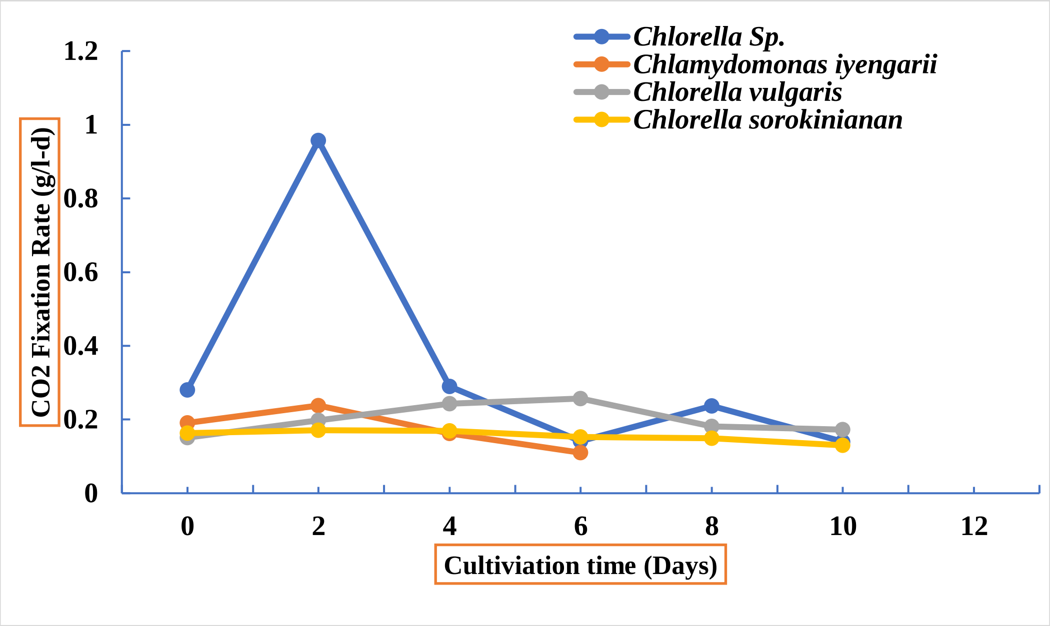

Figure 3. CO_2 fixation rate of pure and mixed microalgal strains with culture period using fitted biomass concentration by Gompertz model (Source: Park *et al.*, 2021).

3.4.4 Treatment of Emerging Contaminants (ECs)

Emerging contaminants (ECs) are mostly detected in landfill wastewater discharge and pharmaceutical and biotechnology wastewater. Pharmaceuticals, personal care products (PCPs), endocrine disrupting substances (EDCs), and pesticides are the most often seen ECs. Microalgae have the ability to eliminate ECs at concentrations between 9 and 24 g/L (Sakarika *et al.,* 2020). Following Table 4 exhibits the removal of ECs using various genera of microalgae.

Table 4. Efficiency of microalgae in removing Emerging contaminants (ECs)

Species of Microalgae	**Contaminant**	**Removal %**	**References**
Cymbella sp.	Naproxen	97.1	Ding *et al.* (2017)
Chlamydomonas sp.	17β-estradiol	93.9	Escapa *et al.* (2016)
Chlorella sp.	Ethinylestradiol	94	Cheng *et al.* (2018)
Chlorella vulgaris	Diazinon	94	Kurade *et al.* (2016)
Desmodesmus sp.	Triclosan-phenol	92.9	Wang *et al.* (2018)

(Source: Ahmad *et al*., 2021)

3.4.5 Passive contamination removal at high pH

In the waste stabilization pond, microalgae were shown to flocculate as early as 1970, particularly on warm, sunny days when the CO2 was depleted and the pH rose (Vandamme *et al.,* 2012). As a result, microalgae could also obliviously aid in the removal of the contaminant from wastewater. If there were no other source of CO2 outside air diffusion, the soluble carbonate would be consumed as the microalgae grew, raising the pH of the culture. A higher culture pH might cause certain contaminants to precipitate insolublely, making it easier to remove them from wastewater (Vandamme *et al.,* 2012), whereas ammonium could be transformed to ammonia and released into the atmosphere (Garcia *et al.,* 2000).

3.5 Production of microalgal biomass

One of the crucial steps in the microalgal bioremediation of wastewater is the removal of microalgal biomass from the treated wastewater. Hence, if the treated wastewater is to be used for other purposes, effective pre-harvesting of microalgae is also essential (Al-Jabri *et al.,* 2020). Although there are several methods for separating biomass from the majority of the culture, the choice of a harvesting method would mostly depend on the use to which the biomass would be put and the energy needed for each unit of biomass production. To create a biomass paste with a 20% solid content or greater, a two-phase harvesting technique is often used. A biomass slurry (usually 1-4%) is obtained by harvesting methods including sedimentation, flocculation, filtration, etc. in the first phase. This slurry can then be further concentrated (generally above 20%) using a centrifuge (Al-Jabri *et al.*, 2020). Table 5 lists the harvesting effectiveness of microalgae grown in various wastewaters. Table 6 also displays a comparison of several harvesting methods.

Table 5. Production efficiencies of microalgae grown in different wastewaters

Wastewater Type	**Type of Cultivation System**	**Strain**	**Harvesting Technique**	**Harvesting Efficiency (%)**	**References**
Urban wastewater	High-rate algal ponds	Mixed microalgae *	Coagulation flocculation	90	Gutiérrez *et al.* (2015)
Domestic wastewater	Photobioreactor	*Chlorella* sp.	Bioflocculation	98.9	Madkour *et al.* (2020)
Organic wastewater	-	Blue-green algae	Electrolytic flocculation	90	Cheng *et al.* (2020)
Pretreated swine wastewater	Photobioreactor	*Chlorella* sp.	Auto-flocculation	39.5	Sutherland *et al.* (2019)
Domestic wastewater	Photobioreactor	*Scenedesmus* sp.	Belt-filtration system	46–84	Sutherland *et al.* (2019)

* *Scenedesmus* sp., *Monoraphidium* sp., and *Amphora* sp. (Source: Al-Jabri *et al.*, 2020)

The primary constraint for large - scale microalgae cultivation appears to be producing. After using wastewater to develop microalgae, microalgae biomass recovery (microalgae harvesting) is an important factor of the production process of algal biomass. Foam flotation is an additional mechanical treatment option (Gutiérrez et al., 2015). Centrifugation is the most often used method for rapidly producing microalgae, and its yield may approach 98% (Martins et al., 2020).

Table 6. Energy requirement in microalgae biomass production

Harvesting Method	**Strain Name**	**IBC (mg/L)**	**HE (%)**	**CF**	**FBC (g/L)**	**Energy Needed (MJ/Kg)**	**References**
Self-cleaning Centrifugation	*Scenedesmus* sp.	1000	-	120	120	3.6	Molina *et al.* (2003)
	Mixed microalga	400–700	90	~100	40–70	9.45 $^{\varepsilon}$	
Electrocoagulation	*Chlorella vulgaris*	300–600	95	-	-	7.2	Vandamme *et al.* (2012)
Submerged Filtration	*Chlorella vulgaris*	410	98	14.7 $^{\varepsilon}$	6.07	2.3	Vandamme *et al.* (2012)
Vacuum Filtration (belt filter)	*Chlorella proboscideum*	1000		95	95	1.62	Molina *et al.* (2003)

Tangential flow filtration	*Tetraselmis suecica*	600	-	78	46.8	11.82	Martins *et al.* (2020)
Magnetic separation	*Botryococcus braunii*	1230	~92	-	-	13.17	Wang *et al.* (2018)
Dissolved air flotation	Mixed microalga	400–700	-	~100	40–70	5.87	Molina *et al.* (2003)
Ultrasound assisted harvesting	*Monodus subterraneus*	160–200	~83	~20	3.2–4	~200	Kim *et al.* (2014)
Pulse electrolysis	*Nannochloropsis oceanica*	~1000	96.4	-	-	1.8	Kim *et al.* (2014)

IBC = initial biomass concentration, CF = concentration factor, FBC = Final biomass concentration, HE = harvesting efficiency. $^{\varepsilon}$ was determined by the corelating centrifuge’s electricity consumption, harvesting efficiency, and initial biomass concentration. (Source: Al-Jabri *et al.*, 2020)

Biomass production of microalgae grown in wastewaters (Table 7). The production of value-added commodities using the harvested biomass is safe. The primary drawbacks are the high energy cost and the substantial deformation produced during the process, both of which may result in cellular damage (Martins et al., 2020).

Table 7. Biomass production of microalgae grown in wastewaters

Waste source	Microalgae	Biomass production ($g\ L^{-1}\ d^{-1}$)	References
Domestic wastewater	*Chlorella variabilis*	1.72	Tran *et al. (*2021)
	Scenedesmus abundans	3.55	SundarRajan *et al.* (2020)
	Chlorella sp.	0.73–1.38	El Asli *et al.* (2019)
Municipal wastewater	*Scenedesmus obliquus*	0.22	Ling *et al.* (2019)
	Scenedesmus sp.	1.81	Tripathi *et al.* (2019)
	Scenedesmus sp.	1.1	Arias *et al.* (2018)

(Source: Tran *et al.*, 2021)

Microbubbles are utilized in the flotation process to adsorb and draw microalgae cells to the liquid's surface for enrichment and harvesting. This method works well for harvesting low-density microalgae (Zhang et al., 2019). The process of separating solids from liquids using a porous membrane is known as filtration and includes dead-end, vacuum, pressure, and tangential flow filtering techniques (such as macro- filtration, ultra- filtration, micro- filtration, nano- filtration, and reverse osmosis) (Mantzorou & Ververidis. 2019).

Chapter IV

CONCLUSIONS

Microalgae-based bioremediation of wastewater represents a larger field for future study and development. The ability of microalgae to remove pollutants including chemical oxygen demands, Nitrogen, Phosphorus, heavy metals, and emerging contaminants is very promising. Using this method, it is useful to produce microalgae biomass as well as bioremediating wastewater (mainly through the removal of substances such as nitrogen and phosphorus). Also, because diverse wastewaters include significant levels of N and P, it is conceivable to use them as a source of nutrients to grow microalgae biomass, which may then be used to manufacture biofuels, animal feed and biofertilizer. Microalgae can help create a sustainable ecosystem by reducing the need for fresh water and land because they can be grown in photobioreactors and wastewater. Due to potential pollutants, microalgal biomass cultivated on wastewater is not yet totally safe. The utilization of microalgae presents a potentially beneficial alternative to traditional wastewater treatment, with the benefit of pursuing the objective of water treatment with lower operating and energy costs thereby gaining a resource such as microalgae biomass.

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Chapter I INTRODUCTION Environmental pollution and resource limitation are the primary problems that humanity will surely meet because as world population and economy continue to expand (Liu & Hong, 2021). Environmental pollution problems are becoming more and more problematic as the worldwide population, development, and civilization expand, as do their indirect impacts on aquatic ecosystems. The necessity for efficient and long-term management of urban wastewater represents only a few of the issues that face world today (Ukaogo et al.,2020).

In terms of the sustainability both the current and future generations, the effects include an unsustainable development of wastes and wastewater, the dumping of wastewaters into freshwater resources, and global warming (Renuka et al., 2015). In this scenario, the mixing of wastewater discharges in aquatic bodies is arising as one of the key problems to international stability due to the majority of the people in developing and underdeveloped countries depend on freshwater resources for their daily needs, either directly or indirectly (Renuka et al., 2015). Thus, it is necessary to find low-cost, environmentally friendly technologies that are simple to use and can be accessed by the common people who have lower levels of education.

The methods used to remediate effluents or polluted water are generally classified as physical, chemical, and biological. Depending on the level and kind of pollution, they can be used either individually or together. Physical and chemical procedures are often more expensive. Moreover, the pH, conductivity, and total load of dissolved materials in the wastewater are all increased by most chemical procedures. The biological or bio-treatment of wastewater is a superior choice in this regard. The most used biological method for treating industrial and municipal wastewaters.

The discharge of industrial and municipal wastewater creates major environmental issues to surrounding aquatic bodies (Yang et al., 2008). Microalgae are an important element of the microbial variety of wastewaters, and they can help these wastewaters purify themselves (Sen et al., 2013). Microalgae (green expedient) has been reported as effective and powerful in the treatment of many types of residential, commercial, and industrial wastewaters because it needs less space.

Reduced energy requirements, less minerals and organic residues, Efficacy in eliminating emerging pollutants (ECs) and heavy metals (HMs) is higher with aerobic treatment methods, while sludge formation is lower, when wastewater is used to grow microalgae, the amount of fresh water needed may be considerably reduced (by 90%), which also helps to balance the impact on the price of fertilizers (Gupta et al., 2019). Hence, producing algae in wastewater has the dual benefits of treating wastewaters, reducing greenhouse gas emissions, and simultaneously creating algal biomass.

The process of using microalgae to treat or bio-transform nutrients, contaminants, and CO2 that are present in wastewaters while simultaneously producing biomass is known as bioremediation. A possible approach shows up to be bioremediation, which involves using plants (including algae or lower plants) and associated microorganisms to remove or bio-transform contaminants from wastewater, such as fertilizers, heavy metals, etc. (Saeed et al., 2022; Ali et al., 2013).

The use of microalgae for wastewater bioremediation was primarily limited to the effluents from treatment processes for cleaning and reducing elements like nitrogen and phosphorus that cause the environmental issue of eutrophication of waterbodies. Thus, it is essential to implement suitable treatment programs for the reduction and elimination of chemicals including ammonia (NH4+), nitrate (NO3-), and phosphate (PO43-) (Lima et al., 2020). Algal biomass harvesting is the process of separating algae from their nutritional or growth media by utilizing methods including flocculation, centrifugation, floatation, filtration, or a combination of these methods.

The cost, processing time, biomass species and quality, separation efficiency, and toxicity are some harvesting attributes or needs that affect the choice of an appropriate harvesting technique (Singh & Patidar, 2018). Biomass may be used for many purposes, including as a source of bioenergy resources (biogas and biofuels), food additives and protein supplements for animal and human feed, bio-ore for precious heavy metals, and medicines, cosmetics, and other useful compounds (Gupta et al., 2019). From this review paper, it would be possible to explore the wastewater treatment utilizing microalgae for bioremediation and biomass production.

OBJECTIVES The study has undertaken to accomplish the following objectives: To understand the potentiality of wastewater treatment utilizing microalgae for bioremediation process. To explore the production of microalgae biomass from wastewaters treatment. Chapter II MATERIALS AND METHODS All the information of this seminar paper has been collected from the secondary sources as it is just a review paper. During the preparation of this review paper, I went through various relevant books, journals, proceedings, reports, publications, internet etc. I got valuable suggestions and information from my major professor and my course instructors.

After collecting all the available information, I myself complied the collected information and prepared this seminar 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 The word "algae" refers to the polygenetic group of single and multicellular organisms derived from many development pathways. They are unique and can range in structure from simple single cells to complex multicellular layers.

Macroalgae are multicellular, non-microscopic, massive kinds of algae, whereas single-celled algae are known as microalgae (Vale et al., 2022). Microalgae are adapted to an environment dominated by viscous forces. They are capable of performing photosynthesis which is important for life on earth. According to James et al., (2019), microalgae generate approximately half of the atmospheric oxygen and use simultaneously the greenhouse gas carbon dioxide to grow autotrophically. Microalgae, together with bacteria forms the base of the food web and also provide energy for all the trophic levels above them. / Figure 1. Some important microalgal species under microscope (Source: Rizwan et al., 2018).

Thousands of microalgae exist on the earth but a few strains are used in the aquaculture. This is due to the difference in culture techniques. Over 50,000 species of microalgae, with a rich biodiversity exist all over the world. In case of Bangladesh, updated data is not available as we do not have a national microalgae collection and culture center. 3.2 Microalgal diversity in wastewater The discharge of industrial and municipal wastewater creates major environmental issues to surrounding aquatic bodies (Yang et al., 2008).

In terms of nutrients, heavy metals, hydrocarbons, and other pollutants, wastewater is mostly numerous. Eutrophication is driven through the presence of nutrients, particularly nitrogen (N) and phosphorus (P), in the form of nitrate, nitrite, ammonia/ammonium, or phosphorus in wastewater (Liu et al., 2010). Microalgae are a diverse group of organisms that include prokaryotic cyanobacteria and photoautotrophic eukaryotic microalgae. They are found in both freshwater and marine settings, and their thallus structure and habitats are quite diverse.

A total of 152 species were identified by Yassin and Mahmoud, (2016) when they examined the phytoplankton diversity in sewage water combined with drain water. These taxa included Bacillariophyceae (60), Chlorophyceae (20), Cyanophyceae (20), Euglenophyceae (17), and Dinophyceae (9). Bacillariophyta constituted 39.4% of the total variety in the drain, making it the leading group. 3.3 Characteristics of wastewater Waste waters are dumped into surface waters underneath the conditions of a permit that limits both the quantity and the level of contamination.

Several contaminants include Escherichia coli, hazardous trace metals, and other organic and inorganic pollutants are found in both surface water and groundwater sources (Ahmad et al., 2021). The major causes of water pollution are anthropogenic sources, including untreated industrial effluents, inadequate home waste disposal, and agricultural runoff (Choi et al., 2018). Several parameters of wastewater are shown in Table 1. Table 1. Wastewater Physico-Chemical Properties with Standard Parameters _Standard _ _ _Bangladesh _WHO _FAO _ _Temperature (°C) _NA _NA _NA _ _DO (mg/l) _= 5.0 _NA _NA _ _pH _6.0-8.5 _6.5-8.5 _6.0-8.5 _ _EC (dS/m) _2.25 _1.2

_0-3 _ _TDS (mg/L) _1000 _2100 _0-2000 _ _CO32-(mg/L) _NA _NA _0-3 _ _HCO3- (mg/L) _NA _58.4 _0-610 _ _Cl- (mg/L) _600 _600 _0-1050 _ _NO3- (mg/L) _10 _10 _0-620 _ _PO4 2- (mg/L) _6 _NA _0-62 _ _SO42- (mg/L) _400 _NA _0-960 _ _(Source: Mridha, 2011) There are many elements that contribute to the contamination of water bodies, including the agricultural industry, industrial production, mining, power generation, and others. This pollution will eventually have an impact on people in general (Hasan et al., 2019). 3.4

Wastewater Treatment using Microalgae Microalgae have several benefits, including the capacity to adapt to different climatic conditions and wastewater types, as well as the ability to remove specific toxins. Although microalgae are mainly photosynthetic by nature, some specific species may use various forms of organic matter in heterotrophic and mixotrophic modes, thus lowering the BOD/COD of the wastewaters (Ahmad et al.,2020). And the amount of nutrients in the wastewater was positively linked with the pace at which Chlorella removed nitrogen, phosphate, and chemical oxygen demand (COD) (Wang et al., 2010).

Figure 2 displays the effectiveness of microalgae in the removal of COD. Figure 2. Effectiveness of microalgae in the removal of COD in wastewater (Source: Ahmad et al., 2021). 3.4.1 Consumption of Nutrients It is difficult to remediate wastewater that has a low nitrogen to organic carbon ratio (C/N). The addition of organics to such wastewater is frequently used to increase the effectiveness of bacterial nutrient removal as a source of energy.

On the other hand, microalgae might increase their cell count while cleaning wastewater by using sunlight, dissolved inorganic co2 from the atmosphere, nitrogen, and other nutrients. Microalgal cellular nitrogen concentration may range from 3–10% depending on the strain type (Adamakis et al., 2018). Microalgal/cyanobacterial strains have the potential to absorb a number of inorganic (such as ammonium, nitrate, nitrite, atmospheric nitrogen) and organic (such as urea, glycine, etc.)

forms of nitrogen, but the effectiveness would again vary across strains and development conditions. Moreover, as a nitrogen source, microalgae might particularly absorb nitro and amino groups from various aromatic chemicals (such as aminonaphthalenes and nitrobenzonates), which would reduce the toxicity of the original contaminants (Choi et al., 2018). Table 2 lists the removal rates of microalgal nutrients from various wastewaters. Table 2.

Microalgal nutrient removal efficiencies from different wastewater Wastewater _The Concentration of Contaminants in Wastewater (mg/L) _Strain _Removal Efficiency (%) _Ref. _ _ _TN _TP _TOC _ _TN _TP _TOC _ _ _Municipal sewage water _116.1 _212 _- _Chlorella sp _94 _89.1 _- _Li et al. (2011) _ _ _130 _15 _- _Spirulina sp. _79 _93.3 _- _Zhou et al. (2017) _ _ _40.6 _5.66 _- _Chlorella sp. _82.4 _50.9 _- _Wang et al. (2010) _ _Agro-industry wastewater _1570 _154 _- _Microalgal consortia _49 _70 _- _Singh et al. (2010) _ _ _44 _88 _495 _Scenedesmus obliquus _34 _65 _42 _Godos et al. (2010) _ _ _44 _88 _495 _Algal consortia _36 _13 _46 _Godos et al.

(2010) _ _Aquaculture wastewater _6.81 _0.42 _- _Chlorella vulgaris _86.1 _82.7 _- _Wang et al. (2010) _ _ _41.3 _4.96 _- _Tetraselmis suecica _49.4 _99 _- _Michels et al. (2014) _ _ _9.8 _1.56 _14 _algal-bacterial flocs _58 _89 _71 _Michels et al. (2014) _ _Aqueous phase wastewater from biomass to energy generation process _4223 _504.7 _13,917 _Tetraselmis sp. _98.5 _98 _- _Michels et al. (2014) _ _ _6900 _1100 _13,800 _Picochlorum sp. _95.4 _97.2 _94.3 _Das et al. (2020) _ _ _9650 _343 _- _Chlorella vulgaris _59.9 _94.6 _- _Li et al. (2011) _ _(Source: Al-Jabri et al., 2020) 3.4.2 Absorption of Metals Heavy metal levels in wastewater that's excessively high might prevent microalgal photosynthesis.

Even so, microalgae may be used to remove metal from wastewaters by effectively concentrating the metal contaminants both inside and outside (Kumar et al., 2015). Many metals, including Fe, Mn, Cu, Co, Zn, and Mo, are required by microalgal cells in trace levels for development. Yet, through different methodologies, microalgae are also able to capitalize on different heavy metals (such as Cd, Hg, Ni, Zn, Fe, Cu, Pb, Cr, etc.).

Moreover, heavy metals may be transferred inside of cells through the cell membrane, lowering their quantities in wastewater (Kumar et al., 2015). Table 3 lists the removal rates of several metals (such as Cd, Cr, Cu, Pb, Hg, Ni, and Zn) by certain microalgae. Table 3. Metal reduction from wastewater by microalgae Metals _Microalgae Strain _Removal Efficiency (%) _Ref. _ _ _ _ _ _ _Cadmium _Scenedesmus sp. _73 _Travieso et al. (1999) _ _ _Chlorella sp. _33–41 _Wong et al. (2000) _ _ _Chlorella vulgaris _66 _Travieso et al. (1999) _ _Chromium _Chlorella vulgaris _50.7–80.3 _Sibli (2016) _ _ _Scenedesmus sp. _92.89 _Pradhan et al. (2019) _ _ _Spirulina sp. _82.67 _Rezaei et al. (2016) _ _Copper _Spirulina maxima _94.9

_Rezaei et al. (2016) _ _ _Chlorella vulgaris _96.3 _Chan et al. (2014) _ _ _Scenedesmus obliquus _72.4–91.7 _Li et al. (2018) _ _Lead _Chlorella vulgaris _89.26 _Malakootian et al. (2019) _ _ _Chlorella sp. _66.3 _Kumar & Goyal (2010) _ _ _Psuedochlorococcum typicum _70 _Shanab et al. (2012) _ _Mercury _Chlorella vulgaris _79–86 _Fard & Mehrnia (2017) _ _ _Chlorella vulgaris _34.21–93 _Chan et al. (2014) _ _ _Psuedochlorococcum typicum _97 _Shanab et al. (2012) _ _Nickel _Scenedesmus sp. _97 _Pradhan et al. (2019) _ _ _Chlorella vulgaris _33–41 _Wong et al. (2000) _ _ _Chlorella miniate _60–73 _Chong et al. (2000) _ _Zinc _Chlorella sp. _60–70 _Chan et al. (2014) _ _ _Synechocystis sp. _40 _Chong et al.

(2000) _ _ _Scenedesmus sp. _98 _Chong et al. (2000) _ _(Source: Al-Jabri et al., 2020) 3.4.3 Organic Elimination The microalgae can remove the organics from wastewater through three primary mechanisms: biodegradation, consumption, and biosorption. Although the removal of organic pollutants by microalgal sorption was only moderately effective (Wang et al., 2019), microalgal cell walls may contain numerous polymer groups that might serve as potential sorption sites for organic pollutants. Despite the fact that the majority of microalgae are photosynthetic by nature, some of them have the ability to use different organics in either a mixotrophic or heterotrophic manner.

This would allow the mixing of organic-rich streams (such as wastewater from food processing, glycerol from biodiesel plants, etc.) with other wastewater for combined treatment, improving the biomass and lipid yield (Choi et al., 2018). In addition to bioaccumulation, microalgae can convert organic pollutants (such as phenolics, petroleum hydrocarbons, pesticides, polyaromatic hydrocarbons, and polychlorinated bisphenyls) into less toxic and non-toxic compounds or even completely mineralized products (such as CO2), depending on the type of algae grown (monoculture or consortia) (Delrue et al., 2016). Figure 3 depicts the CO2 fixation rate of both pure and mixed microalgal strains as a function of culture time. Chlorella sp.

had the greatest CO2 fixation rate of all the mixed and pure strains utilized in the experiment. For mixed microalgae, Chlorella sp., the CO2 fixation rates were 0.28, 0.957, 0.29, 0.14, 0.237, and 0.14 g/L-d (Park et al., 2021). Figure 3. CO2 fixation rate of pure and mixed microalgal strains with culture period using fitted biomass concentration by Gompertz model (Source: Park et al., 2021). 3.4.4 Treatment of Emerging Contaminants (ECs) Emerging contaminants (ECs) are mostly detected in landfill wastewater discharge and pharmaceutical and biotechnology wastewater.

Pharmaceuticals, personal care products (PCPs), endocrine disrupting substances (EDCs), and pesticides are the most often seen ECs. Microalgae have the ability to eliminate ECs at concentrations between 9 and 24 g/L (Sakarika et al., 2020). Following Table 4 exhibits the removal of ECs using various genera of microalgae. Table 4. Efficiency of microalgae in removing Emerging contaminants (ECs) Species of Microalgae _Contaminant _Removal % _References _ _Cymbella sp. _Naproxen _97.1 _Ding et al. (2017) _ _Chlamydomonas sp. _17ß-estradiol _93.9 _Escapa et al. (2016) _ _Chlorella sp. _Ethinylestradiol _94 _Cheng et al. (2018) _ _Chlorella vulgaris _Diazinon _94 _Kurade et al. (2016) _ _Desmodesmus sp. _Triclosan-phenol _92.9

_Wang et al. (2018) _ _(Source: Ahmad et al., 2021) 3.4.5 Passive Contamination Removal at High pH In the waste stabilization pond, microalgae were shown to flocculate as early as 1970, particularly on warm, sunny days when the CO2 was depleted and the pH rose (Vandamme et al., 2012). As a result, microalgae could also obliviously aid in the removal of the contaminant from wastewater. If there were no other source of CO2 outside air diffusion, the soluble carbonate would be consumed as the microalgae grew, raising the pH of the culture.

A higher culture pH might cause certain contaminants to precipitate insolublely, making it easier to remove them from wastewater (Vandamme et al., 2012), whereas ammonium could be transformed to ammonia and released into the atmosphere (Garcia et al., 2000). 3.5 Production of Microalgal Biomass One of the crucial steps in the microalgal bioremediation of wastewater is the removal of microalgal biomass from the treated wastewater. Hence, if the treated wastewater is to be used for other purposes, effective pre-harvesting of microalgae is also essential (Al-Jabri et al., 2020).

Although there are several methods for separating biomass from the majority of the culture, the choice of a harvesting method would mostly depend on the use to which the biomass would be put and the energy needed for each unit of biomass production. To create a biomass paste with a 20% solid content or greater, a two-phase harvesting technique is often used. A biomass slurry (usually 1-4%) is obtained by harvesting methods including sedimentation, flocculation, filtration, etc. in the first phase. This slurry can then be further concentrated (generally above 20%) using a centrifuge

(Al-Jabri et al., 2020). Table 5 lists the harvesting effectiveness of microalgae grown in various wastewaters.

Table 6 also displays a comparison of several harvesting methods. Table 5. Production efficiencies of microalgae grown in different wastewaters Wastewater Type _Type of Cultivation System _Strain _Harvesting Technique _Harvesting Efficiency (%) _References _ _Urban wastewater _High-rate algal ponds _Mixed microalgae * _Coagulation flocculation _90 _Gutiérrez et al. (2015) _ _Domestic wastewater _Photobioreactor _Chlorella sp. _Bioflocculation _98.9 _Madkour et al. (2020) _ _Organic wastewater _- _Blue-green algae _Electrolytic flocculation _90 _Cheng et al.

(2020) _ _Pretreated swine wastewater _Photobioreactor _Chlorella sp. _Auto-flocculation _39.5 _Sutherland et al. (2019) _ _Domestic wastewater _Photobioreactor _Scenedesmus _Belt-filtration system _46–84 _Sutherland et al. (2019) _ _* Scenedesmus sp., Monoraphidium sp., and Amphora sp. (Source: Al-Jabri et al., 2020) The primary constraint for large - scale microalgae cultivation appears to be producing. After using wastewater to develop microalgae, microalgae biomass recovery (microalgae harvesting) is an important factor of the production process of algal biomass. Foam flotation is an additional mechanical treatment option (Gutiérrez et al., 2015).

Centrifugation is the most often used method for rapidly producing microalgae, and its yield may approach 98% (Martins et al., 2020). Table 6. Energy requirement in microalgae biomass production Harvesting Method _Strain Name _IBC (mg/L) _HE
(%) _CF _FBC
(g/L) _Energy Needed
(MJ/Kg) _References _ _Self-cleaning Centrifugation _Scenedesmus sp. _1000 _- _120 _120 _3.6 _Molina et al. (2003) _ _ _Mixed microalga _400–700 _90 _~100 _40–70 _9.45 e _ _ _Electrocoagulation _Chlorella vulgaris _300–600 _95 _- _- _7.2 _Vandamme et al. (2012) _ _Submerged Filtration _Chlorella vulgaris _410 _98 _14.7 e _6.07 _2.3 _Vandamme et al.

(2012) _ _Vacuum Filtration (belt filter) _Chlorella proboscideum _1000 _ _95 _95 _1.62 _Molina et al. (2003) _ _Tangential flow filtration _Tetraselmis suecica _600 _- _78 _46.8 _11.82 _Martins et al. (2020) _ _Magnetic separation _Botryococcus braunii _1230 _~92 _- _- _13.17 _Wang et al. (2018) _ _Dissolved air flotation _Mixed microalga _400–700 _- _~100 _40–70 _5.87 _Molina et al. (2003) _ _Ultrasound assisted harvesting _Monodus subterraneus _160–200 _~83 _~20 _3.2–4 _~200 _Kim et al. (2014) _ _Pulse electrolysis _Nannochloropsis oceanica _~1000 _96.4 _- _- _1.8 _Kim et al.

(2014) _ _IBC = initial biomass concentration, CF = concentration factor, FBC = Final biomass concentration, HE = harvesting efficiency. e was determined by the corelating centrifuge's electricity consumption, harvesting efficiency, and initial biomass concentration. (Source: Al-Jabri et al., 2020) Biomass production of microalgae grown in wastewaters (Table 7). The production of value-added commodities using the harvested biomass is safe. The primary drawbacks are the high energy cost and the substantial deformation produced during the process, both of which may result in cellular damage (Martins et al., 2020).

Table 7. Biomass production of microalgae grown in wastewaters Waste source _Microalgae _Biomass production (g L-1 d-1) _References _ _Domestic wastewater _Chlorella variabilis _1.72 _Tran et al. (2021) _ _ _Scenedesmus abundans _3.55 _SundarRajan et al. (2020) _ _ _Chlorella sp. _0.73–1.38 _El Asli et al. (2019) _ _Municipal wastewater _Scenedesmus obliquus _0.22 _Ling et al. (2019) _ _ _Scenedesmus sp. _1.81 _Tripathi et al. (2019) _ _ _Scenedesmus sp. _1.1 _Arias et al. (2018) _ _(Source: Tran et al., 2021) Microbubbles are utilized in the flotation process to adsorb and draw microalgae cells to the liquid's surface for enrichment and harvesting.

This method works well for harvesting low-density microalgae (Zhang et al., 2019). The process of separating solids from liquids using a porous membrane is known as filtration and includes dead-end, vacuum, pressure, and tangential flow filtering techniques (such as macro- filtration, ultra- filtration, micro- filtration, nano- filtration, and reverse osmosis). Although membrane filtration has a high harvesting efficiency, the cost will surely rise as a result of membrane fouling and filter membrane replacement (Mantzorou & Ververidis. 2019).

Chapter IV CONCLUSIONS Microalgae-based bioremediation of wastewater represents a larger field for future study and development. The ability of microalgae to remove pollutants including chemical oxygen demands, Nitrogen, Phosphorus, heavy metals, and emerging contaminants is very promising. Using this method, it is useful to produce microalgae biomass as well as bioremediating wastewater (mainly through the removal of substances such as nitrogen and phosphorus).

Also, because diverse wastewaters include significant levels of N and P, it is conceivable to use them as a source of nutrients to grow microalgae biomass, which may then be used to manufacture biofuels, animal feed and biofertilizer. Microalgae can help create a sustainable ecosystem by reducing the need for fresh water and land because they can be grown in photobioreactors and wastewater. Due to potential pollutants, microalgal biomass cultivated on wastewater is not yet totally safe.

The utilization of microalgae presents a potentially beneficial alternative to traditional wastewater treatment, with the benefit of pursuing the objective of water treatment with lower operating and energy costs thereby gaining a resource such as microalgae

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