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قسم الأحياء

Algae-Based Bioremediation for Renewable Energy Production

A graduation research project submitted to the Department Biology in partial fulfillment of the requirements for the completion of the degree of Bachelor of science and Biology.

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This research project has been approved and accepted in part by fulfilling the requirement to obtain a bachelor's degree in biology.

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

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List of abbreviations

Abbreviation	Meaning
AD	Anaerobic digestion
AOM	Algal organic matters
ATP	Adenosine Triphosphate
BG-11	(Blue-green) Medium
BOD	Biological oxygen demand
COD	Chemical oxygen demand
DF	Dark Fermentation
DF	Dark fermentation
DOM	Dissolved organic materials
DP	Direct photolysis
DWW	Domestic wastewater
DWW	Dairy wastewater
ECs	Emerging contaminants
EOM	Extracellular organic matter
EPS	Extracellular polysaccharides
ETC	Electron transport chains
FAMEs	Fatty acid methyl esters
Fd	Ferredoxin
FTIR	Fourier-transform infrared spectroscopy
GHG	Greenhouse gas
H	Hydrogen
HHV	Higher heating value
HMs	Heavy metals
HRAPs	High-Rate Algal Ponds
HTL	Hydrothermal Liquefication

IOM	Intracellular organic matter
IP	Indirect photolysis
MFCs	Microbial fuel cells
MTs	Metallothionein's
NAD	Nicotinamide Adenine Dinucleotide
NR	Nitrate reductase
PAHs	Polycyclic Aromatic Hydrocarbons
PF	Photo-fermentation
PF	Photo-fermentation
POME	Palm mill oil effluents
PPCPs	Pharmaceutical and personal care products
PSI	Photosystem I
PSII	Photosystem II
ROS	Reactive oxidizing species
SC-CO_2	Supercritical carbon dioxide
TAGs	Triacylglycerols
TDN	Total dissolved nitrogen
TN	Total nitrogen
TOC	Total organic carbon
TP	Total phosphorus
VFAs	Volatile fatty acids
WHO	World Health Organization
WWTP	Wastewater treatment plants

List of symbols

Symbols	Meaning
°C	Temperature
°C/min	Heating rate
dry wt.	Dry weight
kg	Kilogram
M	Molarity
MJ/kg	Megajoules per kilogram
MPa	Megapascal pressure unit
S	Time
mW/m^2	Milliwatts per square meter
kJ/g	Kilojoule per gram
mg. g	Milligrams per gram
gL^{-1}	Gram per liter
gDW/L	Grams dry weight of the cell per liter
%v/v	Volume/Volume Percentage

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First, we would like to thank and praise Allah, who guided us to the right path and I gave us the strength and patience to face all obstacles in our lives and the ability to complete this project .

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Abstract

The potential of algae in environmental remediation and renewable energy production has been the focus of extensive research in recent years. Algae are known to efficiently remove various pollutants from wastewater, soil, and air while simultaneously producing biomass that can be converted into biofuels and other valuable products.

Our research provides an overview of the latest developments in the field of algae-based de-pollution and renewable energy. It covers the synthesis and characterization of different types of algae and their application in various de-pollution processes, including wastewater treatment, CO_2 capture, and heavy metal removal.

Furthermore, we provided a comprehensive overview of the latest technologies for converting algae biomass into renewable energy, including biodiesel, bioethanol, biogas, and hydrogen and the principles and mechanisms of these conversion technologies, along with their advantages and limitations.

Finally, we conclude our research with emphasizing the need for further research to fully realize the potential of algae in sustainable environmental remediation and energy production.

الخلاصة

كانت إمكانات الطحالب في المعالجة البيئية وإنتاج الطاقة المتجددة محور بحث مكثف في السنوات الأخيرة. من المعروف أن الطحالب تزيل بكفاءة الملوثات المختلفة من مياه الصرف الصحي والتربة والهواء بينما تنتج في نفس الوقت الكتلة الحيوية التي يمكن تحويلها إلى وقود حيوي ومنتجات أخرى ذات قيمة.

يقدم بحثنا لمحة عامة عن أحدث التطورات في مجال إزالة التلوث من الطحالب والطاقة المتجددة. ويغطي توليف وتوصيف أنواع مختلفة من الطحالب وتطبيقها في مختلف عمليات إزالة التلوث، بما في ذلك معالجة مياه الصرف الصحي، واحتجاز ثاني أكسيد الكربون، وإزالة المعادن الثقيلة.

علاوة على ذلك، قدمنا نظرة عامة شاملة على أحدث التقنيات لتحويل الكتلة الحيوية للطحالب إلى طاقة متجددة، بما في ذلك وقود الديزل الحيوي والإيثانول الحيوي والغاز الحيوي والهيدروجين ومبادئ وآليات تقنيات التحويل هذه، إلى جانب مزاياها وقيودها.

أخيرًا، نختتم بحثنا بالتأكيد على الحاجة إلى مزيد من البحث لتحقيق كامل إمكانات الطحالب في المعالجة البيئية المستدامة وإنتاج الطاقة.

Chapter (1)
Introduction

Introduction

The undesirable elements entering water can cause pollution and alter the water's quality (Alrumman *et al.,* 2016) also they have detrimental effects to the environment and human health (Briggs, 2003). Water is a crucial natural resource that we utilize for drinking and other human development requirements (Bibi *et al.,* 2016). Worldwide, access to clean drinking water is essential for maintaining human health. Water, an all-purpose solvent and may be a significant infection source. World Health Organization (WHO) statistics indicates that 80% of illnesses are water-borne. Numerous nations' drinking water does not adhere to WHO standards (Khan et.al., 2016). 3.1% of mortalities are attributable to unclean and subpar water (Pawari and Gawande, 2015).

Water pollution is mostly caused by the discharge of household and industrial effluent wastes, leaks from water tanks, marine dumping, radioactive waste, and atmospheric deposition. Industrial trash and heavy metals that have been improperly disposed of can build up in lakes and rivers, harming both people and wildlife. Industrial waste is the main source of the toxins that cause acute poisoning, immunological suppression, and reproductive problems. Infectious illnesses such as cholera and typhoid (Juneja and Chaudhary, 2013) and other illnesses including gastroenteritis, vomiting, diarrheic, skin issues, and renal problems are spreading through dirty water (Khan and Ghouri, 2011).

Water is very necessary in human life, there must be a solution to the problem of water pollution, also the fact that the world's population is expanding quickly and that this is increasing the rate at which wastewater is being produced further contributes to the rising energy requirement. Utilizing wastewater, in such a way, that the method employed treats the wastewater and creates some high value-added products that can be used again is the best way to fulfill both of these commitments (Ijaz*et al.,* 2016; Reungsang *et al.,* 2016).

Wastewater can be used to generate algae biomass-derived bioenergy through biomass conversion technologies. This technology is energy cheaper because it doesn't need to go through expensive processes. While a number of cutting-edge technologies are helping to address the problem of wastewater resource recovery, biological approaches show the greatest promise for effectively recovering vital resources from effluent (Bhatia *et al.,* 2020). Among myriad of biological approaches, microalage are seen as effective and ecofriendly candidates for wastewater treatment and biofuels production using microalgal biomass.

Additionally, there are growing worries about how to utilize waste and wastewater from diverse industrial effluents to create usable goods, particularly renewable energy (Akponah *et al.,* 2013). Where, shortage of fossil fuel resources in the near future necessitates the development of alternate energy sources. Therefore, the most effective methods involve in using wastewater to produce energy carriers such bioethanol, biogas, biodiesel which may be converted into electrical power (Kassongo and Togo, 2011).

This review discusses the efficiency of algae-based bioremediation technology and provides a comprehensive overview of the latest technologies for converting algae biomass into variable renewable energy sources including biodiesel, bioethanol, biogas, and hydrogen.

Aims of work

Our research aims to:

1. The importance of the mechanisms and processes involved in using algae to treat municipal, textile, agricultural, dairy and swine wastewater.
2. The potential of algae-based bioremediation and renewable energy production as a promising solution.
3. Understanding the applications of algae-based bioremediation, including inorganic and organic pollutant removal.
4. knowledge of mechanisms and processes involved in algae-based bioremediation, including bioadsorption, biosorption, bioaccumulation, biodegradation, and photodegradation.
5. The importance of using renewable algal biofuels and providing a competitive alternative to fossil fuels.
6. Understanding the mechanisms and processes involved in algae-based renewable energy production technologies including photosynthesis, anaerobic digestion, and hydrothermal liquefaction.
7. Description of algae-based integrated technology for wastewater treatment and bioenergy production.

Chapter (2)
Algae-based bioremediation

2.1 Techniques of Wastewater Treatment

Diverse contaminants that are present in the wastewater can be eliminated utilizing various methods. Based on the source and location, several treatment techniques are employed for wastewater treatment.

Biological oxygen demand (BOD) and suspended particles can both be decreased by 20–30% after first treatment (Hennebel *et al.,* 2015). The reduction of oil, grease, fats, sand, and coarse materials is a part of this process. Up to 85% of BOD and total suspended solids will be reduced by secondary treatment. This stage entails biological system-based breakdown of the dissolved sewage contents, as seen in Fig 2.1.

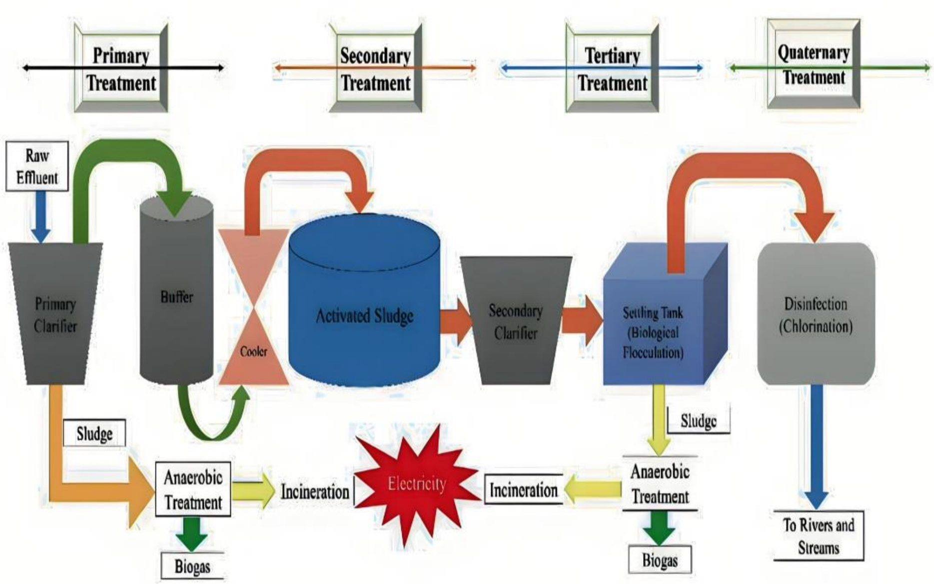


Fig. 2.1. Shows a schematic diagram of a waste treatment facility (Bhatia*et al.,* 2020).

The removal of biological materials from treated water that contains very little organic material and suspended sediments is the final stage of secondary treatment (Sharma *et al.,* 2012). Food in the form of organic materials is consumed by microbes in the wastewater and converted into carbon dioxide, water, and power. Up to 99% of sewage pollutants can be eliminated during tertiary treatment. Before releasing the water, some operators clean it by adding chlorine. Sometimes tertiary treatment is used to remove nitrogen and phosphorus. Advanced tools and technologies are used in tertiary treatment to further remove or discard impurities or specific pollutants (Leal *et al.,* 2010).

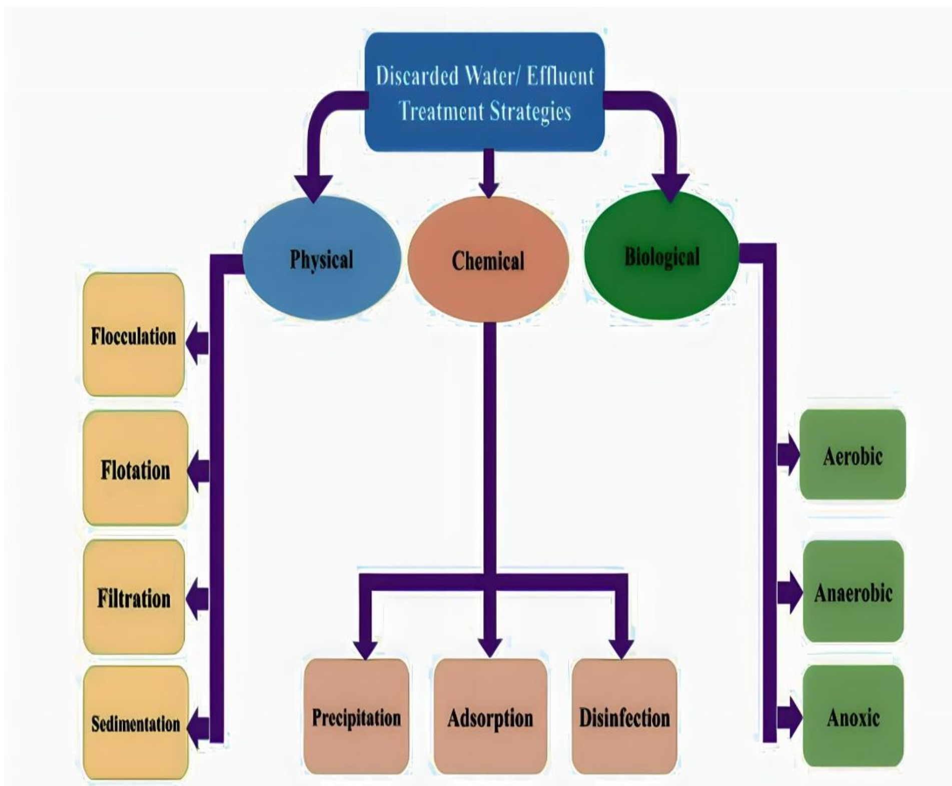


Fig. 2.2. Shows several wastewater treatment techniques (Bhatia *et al.,* 2020).

Fig (2.2) lustrates how wastewater or industrial effluent is physically treated using ultrafiltration, sedimentation, sand filtration, etc. depending on the type of substance present. In the chemical treatment chlorine is the most commonly used chemical as an efficient oxidizing agentand to eliminate the microorganisms that cause water deterioration. Ozone is yet another oxidizing disinfectant and is employed to clean sewage (Bhatnagar and Sillanpää, 2010).

In order to eliminate the hazardous pollutants, biological treatment methods utilize biological agents like plants and microbes or microalgae. Also using oxidation beds, aerated systems, and post precipitation, have been proposed for biological wastewater treatment. According to how microorganisms develop, it may be divided into several categories such aerobic, anaerobic, and anoxic systems as well as suspended growth and attached growth (Rathour *et al.,* 2020; Abioye *et al.,* 2014).

Previous reports indicated the high efficiency of microalgae in water bioremediation technology (Fig 2.3). The microalgae-based wastewater treatment and biomass recovery for production of high value-added products, biofuels and energy carriers have been previously discussed (Aishvarya *et al.,* 2015; Liu and Hong 2021).

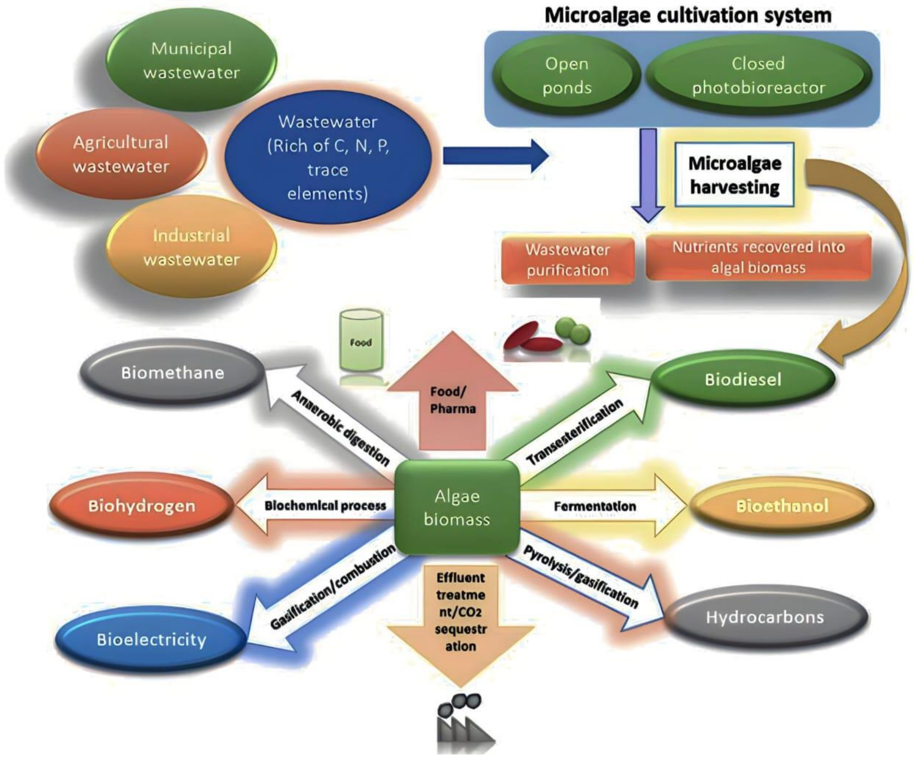


Fig. 2.3. Adapted microalgae's use in many areas for wastewater treatment combined with algae biomass-derived bioenergy production (Aishvarya *et al.*, 2015; Liu and Hong, 2021).

2.2 Wastewater treatment using microalgae

Commercial enterprises release a substantial amount of pollutants into the water stream. Most wastewater created is contaminated with a range of substances, including minerals, volatile organic compounds, oils and greases, heavy metals, and pesticides, depending on the kind of industry. In addition, industrial or sewage effluent is nutrient-rich, and the buildup of certain contaminants (nitrogen and phosphorus) may have a substantial impact on both freshwater and saltwater ecosystems. Before cleaning up some of these impurities, in addition to being fit for human consumption, the water may be safely released into the environment (Edokpayi *et al.,* 2017; Kalra *et al.,* 2021; Klimaszyk and Rzymski, 2017).

Microalgae use these nutrients from wastewater in order to carry out their cellular processes and generate useful biomass. Given the environmental issues brought on by a huge volume of wastewater containing poisonous and hazardous compounds, the cultivation of microalgae in wastewater can be beneficial and financially feasible. Numerous possible wastewater recycling systems have been researched in recent decades as a result of significant research efforts in the field of wastewater treatment (Edokpayi *et al.,* 2017; Kalra *et al.,* 2021; Klimaszyk and Rzymski, 2017). Water pollution results in a number of issues, including a lack of water for drinking and other necessities in homes and businesses. So, in order to increase water quality and decrease water shortage, wastewater treatment is required (Karimi-Maleh *et al.,* 2020).

Microalgae bioremediation is a potentially useful technique for treating industrial wastewater, according to recent studies. To ensure the production of valuable products, the growth of microalgae is essentially dependent on the presence of sufficient nutrients in the growth medium. For microalgae to thrive at their best, nutrients such as micronutrients, vitamins, trace minerals, and macronutrients like nitrogen and phosphorus are crucial (Ahmad *et al.,* 2016).

Wastewater contains the majority of the organic and inorganic nutrients that have been shown to be useful for microalgae production. Treatment with different microalgae strains is necessary for effective outcomes. Variable microalgae species such as *Chlorella* sp. (Ahmad *et al.,* 2018; Hariz *et al.,* 2019*)*, *Scenedesmus* sp., *Nannochloropsis* sp. (Emparan *et al.,* 2020), *Chlamydomonas* sp. (Ding *et al.,* 2016), and *Dunaliella salina* (Choi et al ., 2018; Takriff *et al.,* 2016) have been used to r wastewater treatment.

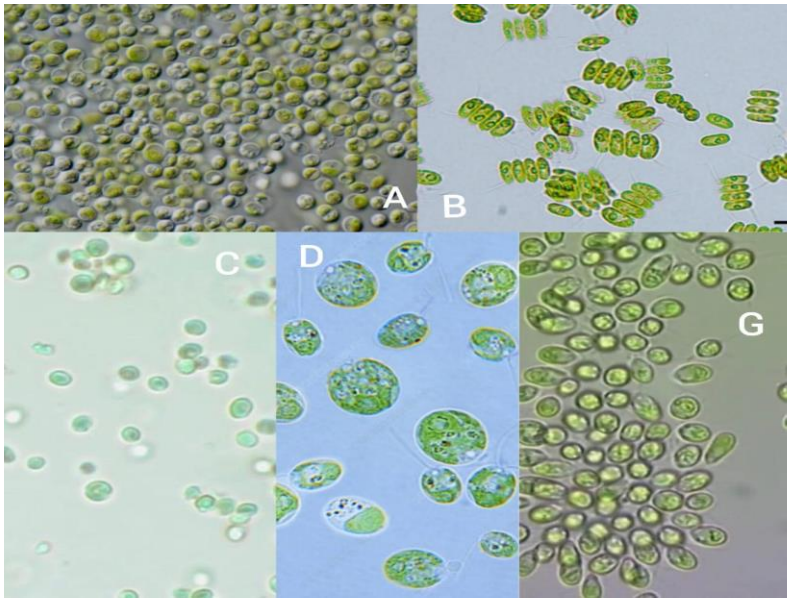


Fig. 2.4. Photomicrographs of microalgae in wastewater. A) *Chlorella* sp. B) *Scenedesmus* sp. C) *Nannochloropsis* sp. D) *Chlamydomonas* sp. G) *Dunaliella salina.*

2.2.1 Treatment of municipal wastewater

The majority of municipal wastewater is dumped by homes. They are typically dumped untreated into sewers, lakes, and rivers, which impedes community development. Total microbial load, pH, chemical oxygen demand (COD), biological oxygen demand (BOD), total nitrogen, phosphate, and potassium are among the physicochemical characteristics of municipal wastewater (Ibrahim *et al.,* 2020). Numerous studies in this field have shown that using municipal wastewater as a medium for growing microalgae may boost biomass output while also removing contaminants, including nutrients like nitrate and phosphate (Ye *et al.,* 2020; Qu *et al.,* 2020). Cultures of *S. obliquus* and *Desmodesmus* sp. were grown on a growth medium made from municipal wastewater that had had more than 75% of its nitrogen and phosphate removed.

The fatty acid composition revealed that when light intensity increased from 50, 150, and 300 E m^{-2} s^{-1}, oleic acid (18:1) and linoleic acid (18:3) contents increased and decreased, respectively. These findings imply that fatty acid synthesis and biomass productivity of microalgae are crucial for the manufacture of high-quality biodiesel. Additionally, a notable decrease in protein content was attained with a concurrent rise in fatty acid content (Nzayisenga *et al.,* 2020).

These findings imply that optimum light intensities are crucial for enhancing microalgal fatty acid synthesis and biomass productivity in order to produce high-quality biodiesel. Additionally, a notable decrease in protein content was attained, with a concurrent rise in fatty acid content (Nzayisenga *et al.,* 2020). The many kinds of municipal, agricultural, and industrial wastewater used for microalgae development and removal are displayed in (Table 1). Microalgae are fed by the nutrients in wastewater, which also help them grow and create biochemical components in their cells.

According to several research, the most nutrients are removed when microalgae are cultured in diluted effluent. An analogous process was during the bioremediation of untreated vineyard wastewater, *Chlorella vulgaris* was found. After 4 days of treatment, 20% (v/v) wastewater had the highest biomass content (2.63 gDW/L) and biomass productivity (0.66 gDW/L/day).

More than 92% and 50%, respectively, of the COD and polyphenol levels could be reduced with coculture. In order to lower production costs and use the resultant biomass as a source of biofuels, this study demonstrates that it is possible to use vineyard wastewater as a culture medium for microalgae growth (Spennati *et al.,* 2020).

Table (1): Microalgae nutrient removal, resource recovery, and lipid accumulation potential in wastewater.

Description	Microalgae	Removal (%)			Biomass	Lipids content	References
		COD	N	P			
Municipal wastewater	*Anabaena* sp.	98.6	100	96.5	215.7 mg/day	7.24	(Hena *et al.*, 2015)
Textile wastewater	*Chlorella zofingiensis*	_	93	90	32.4 g/L	21.6-25.4	(Zhou *et al.*, 2018)
	Scenedesmus obliquus	85.43	80.30	95.72	_	46.92	(Qu *et al.*, 2020)
	Scenedesmus sp. *HXY2*	96	96.6	94.5	_	15.56	(Ye *et al.*, 2020)
	Scenedesmus obliquus	_	78.5	95.2	0.529 g/L	21.9	(Eida *et al.*, 2018)
	Scenedesmus obliquus	99	99	100	2.68	39	(Pandey *et al.*, 2019b)

Textile wastewater	*Chlorella* sp. *WuG23*	75	75	_	58 mg/L/day	_	(Wirth *et al.*, 2018)
	Scenedesmus abundans	86.87	68.86	70.79	10.80 mg/L/day	_	(Brar *et al.*, 2019)
Dairy effluent	*Anabaena ambigua*	50	52.95	63.05	11.6 mg/L/day	_	(Brar *et al.*, 2019)
	Chlorella vulgaris	80.62	85.47	65.96	0.175 mg/L/day	_	(Choi, 2016)
Swine wastewater	*Arthrospira platensis*	98.4	98.8	100	0.52 g/L/day	158mg/L/ day	(Hena *et al.*, 2018)
	Scenedesmus sp. *ASK22*	90.5	100	91.24	1.22 cdw/L	30.7	(Pandey *et al.*, 2019a)
	Acutodesmus dimorphus	90	100	_	_	_	(Chokshi *et al.*, 2016)
	Tribonema sp.	56.6	89.9	72.7	_	42.4	(Chen *et al.*, 2020)
	Tribonema sp.	52.5	100	68-74	2.04g/L	26.5-55.4	(Huo *et al.*, 2020)

Molasses wastewater	*Monorphidium* sp. *FXY-10*	92.33	80	86	1.21g/L	92.33	(Dong *et al.*, 2019)
	Scenedesmus sp. *Z-4*	87.2	90.5	88.6	_	28.9	(Ma *et al.*, 2017)
Piggery wastewater	*C. vulgaris*	_	100	100	_	35-40	(Molinuevo-Salces *et al.*, 2016)
Aquaculture wastewater	Microalgal consortium of *Euglena gracilis* and *Selenastrum*	56-68	89	84-96	_	84.9mg/L	(Tossavainen *et al.*, 2019)
Domestic wastewater	*Scenedesmus* sp.	_	98.8	97.7	0.223 g/L/day	34.3 mg/L/ day	(Ren *et al.*, 2019)
	Scenedesmus sp.	69-96	94-98	73-82	0.196 g/L/day	65.2 mg/L/ day	(Nayak *et al.*, 2016)

2.2.2 Treatment of textile wastewater

Many contaminants, particularly dyes, are present in textile industry wastewater. If not effectively handled, these pollutants can have significant effects on aesthetics, eutrophication, decreased photosynthetic activity, and bioaccumulated toxins in aquatic environments. As a potential substitute for traditional wastewater treatment procedures, microalgal growth in textile dye effluent has been found. Through a bioconversion/biodegradation or biosorption process, dyes in wastewater are broken down while microalgae are growing. Also, compared to traditional treatment methods, microalgae have the added benefit of creating valuable biomass that may be turned into bioproducts, biofuels, and bioenergy during the bioremediation of textile wastewater (Premaratne *et al.,* 2021; Sharma *et al.,* 2021).

The removal of Reactive Red 120 (RR-120) textile dye from aqueous effluent was accomplished using microalgae and commercial activated carbon. In comparison to 94-98% of the activated carbon dye, *Spirulina platensis* was removed up to 94-99% at pH 2 and room temperature (Cardoso *et al .,* 2012). In a different investigation, textile wastewater was treated using mixed microalgal consortia (*Chlorella* and *Scenedesmus* sp.) in a fed-batch reactor. The fed-batch reactor was used for five cycles, each lasting 30–10 days, suggesting that the microalgae gradually adapted. The maximum total nitrogen removal (70%) and total phosphorus removal (95%) were accomplished. 68–72% of the textile effluent's color was eliminated.

According to this study, integrated microbial algal culture with textile effluent could be a good choice (Kumar *et al.,* 2018). Both free and immobilized algal cells can be used in the bioremediation of wastewater with microalgae.

Immobilized microalgae cells have been found to have various advantages over freely suspended ones for bioremediation. There are various advantages to employing immobilized microalgae cells for bioremediation as opposed to freely floating microalgae. The immobilization technique has the benefit of taking up less space and making it simpler to collect microalgae cells. After 6 days of culture, a study of the immobilized *Desmodesmus* sp. dye destination revealed that it eliminated methylene blue and malachite green by up to 98% (Al-Fawwaz and Abdullah, 2016). Found decolonization and nitrogen removal rates of 80% and 71% from textile wastewater at a pH of 12 and 150 microalgae beads, respectively.

Immobilized *Tetraselmis* sp. and *Chlorella* sp. Wu-G23 was also discovered for wastewater bioremediation (Adam *et al.,* 2015; Wu *et al.,* 2021). When employing wastewater-based microalgae growth as a potential way to save expenses associated with biomass production, wastewater characterization is essential since a high level of assurance is needed to achieve contaminant-free biomass (Jaramillo and Restrepo, 2017). It is crucial to comprehend the quantities of each nutrient when employing wastewater, especially the N: P: K ratio for microalgae development. The COD concentration also represents the wastewater's total organic load (dissolved and suspended particles). The amount of organic matter in the water discharged from industrial facilities is high (for example, total suspended solids, BOD, COD, minerals, oils, fats, heavy metals, and nutrients such as ammonia salts and phosphate) (Abdel-Raouf *et al.,* 2012; Kalra *et al.,* 2021; Su, 2021).

2.2.3 Treatment of agricultural wastewater

Processing products including palm oil, coffee beans, cassava, and sugarcane produces trash known as agricultural wastewater. The primary user of freshwater is industry, and livestock is increasingly a significant generator of effluent (Ummalyma *et al.,* 2021). Since byproducts and residues are inevitable in any productive industry, the agro-industrial and food processing sectors create huge volumes of wastewater. The release of wastewater into bodies of water without first treating it to remove nutrients and organic carbon causes eutrophication, which encourages the growth of unwanted organisms such aquatic macrophytes and cyanobacteria that produce toxins (Abdel-Raouf *et al.,* 2012).

The plant-based industry processes cassava (flour and starch), instant or lyophilized coffee, palm mill oil effluents (POME), and vinasse from sugar cane biorefineries. Because it generates a lot of biogas that can be utilized as a source of bioenergy and lowers running costs, anaerobic digestion is frequently employed as the first step in early wastewater treatment. This adds value to the process (Ahmad *et al.,* 2016).

One ton of crude palm oil needs 5 to 7.5 tons of water to produce, which produces a lot of wastewater in the form of POME (Ahmad *et al.,* 2016). Malaysia's palm oil industry generates a lot of POME and CO_2 (Ding *et al.,* 2020). POME is handled locally in two successive acidification, anaerobic, and aerobic digesting processes employing pond systems (Emparan *et al.,* 2020; Fernando *et al.,* 2021; Pascoal *et al.,* 2021). POME is processed utilizing inexpensive pond systems, with the possibility to use the biogas created by anaerobic digestion.

The total organic load (dissolved and suspended particles) of wastewater released into rivers is measured by COD, however wastewater still has a significant nutritional content even after aerobic digestion and COD. Within a research by Fernando *et al.,* (2021), POME is a different growing medium that microalgae may utilize to produce astaxanthin since it has high levels of N and P and little to no heavy metals.

In order to cultivate microalgae, the C: N: P ratio needs to be modified in accordance with the physicochemical makeup of the effluents from aerobically fermented POME (Khalid *et al.,* 2019). POME was used to cultivate the marine microalgae *Nannochloropsis oculata* and *T. suecica* as a substitute medium for lipid and biomass synthesis. On day 16, *N. oculata* and *T. suecica* both saw the highest specific growth rates (0.21 and 0.20 per day, respectively), as well as the highest lipid contents (39% and 27%) According to Shah *et al.,* 2016), algal culture in POME medium enhanced the removal of COD (95%), BOD (97%), TOC (75%), TN (91%), and oil and grease (95%).

Another research found that using *N. oculata* and *Chlorella* sp. resulted in the maximum elimination of COD (95–98%), BOD (90–98%), TOC (80–86%), and TN (80%) as compared to not using microalgae (Ahmad *et al.,* 2015). Freshwater is mostly consumed by agribusiness, and livestock is increasingly a significant generator of wastewater (Ummalyma *et al.,* 2021). Known as *Manipueira*, the wastewater created during the processing of cassava has a high COD because of the substantial organic loads present, but it also contains considerable amounts of nutrients that might be employed for the growing of microalgae in the bioremediation procedure (de Carvalho *et al.,* 2018).

That may be employed for the microalgae culture incorporated into the bioremediation procedure. 86.2–85.5% of COD and total solids were eliminated by using microalgae in a horizontal fixed-bed anaerobic reactor to treat effluent from cassava starch (Watthier *et al.,* 2019). In order to sequester CO_2 and create bioremediates, heterotrophic microalgae were grown on the wastewater left over after processing cassava. An alternate approach that employs the former as a nutrient source for the growth of microalgae biomass and the elimination of COD is the integration of cassava industrial operations with microalgae.

The following studies show that the combination of the cassava sector with the production of microalgae has progressed from a promise to reality: by use of *C. pyrenoidosa* (Yang *et al.,* 2008), *Phormidium* sp. (Francisco *et al.,* ., 2015), *Scenedesmus* sp. (Romaidi *et al.,* 2018), *Chlorella sorokiniana* and *Spirulina platensis* (Hadiyanto *et al.,* 2019), and using *Acutodesmus obliquus* in a pilot-scale open pond treatment (Selvan *et al.,* 2019). These investigations showed that the production of microalgae biomass significantly reduced COD and chemical components such nitrate, phosphate, sulfate, chloride, calcium, potassium, magnesium, sodium, phosphorus, and organic carbon.

2.2.4 Treatment of dairy wastewater

Ascochloris sp. may be grown with nutrients from wastewater from different processing streams in the dairy sector. By using this wastewater, a maximum COD removal rate of 95% was guaranteed, as was the quick formation of algal biomass. Maximum biomass production was attained in addition to lowering the variety of bacterial communities in wastewater (Kumar *et al.,* 2019; Zhu *et al.,* 2019).

After 8 days of growing in effluent from dairy farms without sterilizing, microalgae eliminated up to 89.7% COD. After 4 and 6 days of growth, total nitrogen from nitrite and ammonia was completely eliminated. According to reports, ammonia nitrogen is the preferred source of nitrogen for microalgae since it can be directly absorbed and takes very little energy for assimilation (Ding *et al.,* 2015). It has been estimated that during this integration process between microalgae production and the dairy sector, roughly 102 tons of liquid digestate from the dairy may be treated with simultaneous removal of N and P for every ton of *C. vulgaris* biomass generated.

Numerous types of nitrogen, such as nitrate, nitrite, nitric acid, ammonium, ammonia, molecular nitrogen, nitrous oxide, nitric oxide, and nitrogen dioxide, can be found in the environment (Barsanti and Gualtieri, 2022). In the context of producing biofuel, the symbiotic interaction between microalgae and bacteria can offer a number of competitive benefits over the development of pure axenic microalgae. The key technological restrictions related to the capital expense of sustaining pure microalgae growth are significantly reduced by the microalgae-bacteria combination.

Also contrasted with axenic growth in dairy wastewater (DWW) was the symbiotic development of *Tetraselmis indica* and *Pseudomonas aeruginosa* consortia. The consortium's greatest biomass output was 1454.88 mg/L, 38.80% more than the axenic growth of the pure microalgae culture in DWW. The consortium's greatest biomass output was 1454.88 mg/L, 38.80% more than the axenic growth of the pure microalgae culture in DWW. The consortium eliminated 79.83% of total dissolved nitrogen (TDN), 83.76% of (TDN), and 87.49% of COD.

Thus, the symbiotic microalgae-bacteria consortium promoted biomass growth and wastewater nutrient removal. Additionally, it could be helpful for biofuel technology (Talapatra *et al.,* 2021). After two reductions by glutamine synthetase and glutamate synthase, ammonic nitrogen in microalgae is utilized directly for the synthesis of amino acids (Crofcheck *et al.,* 2012). Ammonia, nitrate, and nitrite are transported through the plasma membrane into the cell via active transport, where they are absorbed by eukaryotic microalgae.

But nitrate reductase and nitrite reductase, two different enzymes, are required for two reductions before absorption is possible (Crofcheck *et al.,* 2012). The capacity of cyanobacteria to transform ambient nitrogen into ammonia, which may be utilized to generate amino acids and proteins, allows them to act as natural fixers of atmospheric nitrogen (Barsanti and Gualtieri, 2014; Cai *et al.,* 2013). The capacity of cyanobacteria to transform ambient nitrogen into ammonia, which may be utilized to generate amino acids and proteins, allows them to act as natural fixers of atmospheric nitrogen (Källqvist and Svenson, 2003; Lu *et al.,* 2018).

Microalgae in an autotrophic test environment adapted well to a medium containing 50 mg/L ammonium (NH_4^+), but they grew insignificantly in media containing 100 and 200mg/L NH_4^+, respectively, with maximal and specific growth rates equivalent to those of the control group. At 200 mg/L NH_4^+, the reduction happened on the second day, however at 100 mg/L NH_4^+, it didn't happen for six days before the biomass started to fall. With minimal microalgae growth recorded at 100mg/L NH_4^+, the data point to better tolerance under mixotrophic conditions; nevertheless, the same observation was reported for 200 mg/L NH_4^+ (Li *et al.,* 2019).

2.2.5 Treatment of swine wastewater

Carbon, phosphorus, and nitrogen are the three main chemical elements found in swine effluent. Numerous research has recommended using various microalgae species to clean swine wastewater. Through a process known as phosphorylation, microalgae could use phosphorus in the form of H_2PO_4 and HPO_4 to transform it into an organic molecule like ATP, which is a substantial source of energy.

Microalgae can take up phosphate from the pond system in the form of polyphosphates through a process known as passive absorption, which is influenced by environmental factors like temperature, light intensity, and phosphate content. To treat phosphorus-rich wastewater, however, the passive absorption method requires more research. Microalgae are both autotrophic and heterotrophic microscopic organisms with high photosynthetic efficiency, quick rates of growth, vast tolerance to harsh conditions, and respectable development under rigorous cultivation, making them efficient CO_2 capture organisms (Wang *et al.,* 2012).

Microalgae need essential nutrients in addition to water and other factors in order to produce biomass at a high pace. In swine manure, *Chlorella pyrenoidosa* is grown to lower the high organic content and increase the amount of lipids (Wang *et al.,* 2012). The pH of the growth media affects how phosphorus is partitioned because a high pH causes phosphorus to precipitate, making it available to the algal cells.

In one study, the pH effects were investigated in a combined system with *C. vulgaris* and *Bacillus licheniformis*. Compared to algae and bacteria alone, which alone removed 55 and 78% of the phosphorus, the combined system removed the most (92%). A pH of 7 was used for this study. However, in a different experiment, the ability to remove phosphorus caused the pH to decrease from 7 to 3, demonstrating that pH plays a significant role in the effectiveness of phosphorus removal (Liang *et al.,* 2013).

This treatment can be regarded as energy-positive if the biomass of the high algae pond treatment facility is combined with primary sludge to produce electricity or heat (Passos *et al.,* 2017). Conditions are necessary for high biomass production and effective pollution removal. Due to their capacity to flourish in these conditions, *Chlorella* sp. and *Scenedesmus* sp. are two of the most often used microalgae. Non-axenic piggery effluent contains pollutants (Mezzari *et al.,* 2017). Advised growing *Scenedesmus* spp. to get rid of *Salmonella* enterica serovar *Typhimurium* from swine digestate and stop animal husbandry-related Salmonellosis epidemics.

According to many researchers, microalgae initially adapt to wastewater conditions (Wang *et al.,* 2017). Neochloris aquatic was used in an N/P ratio of 1.5/1 to remove COD and NH_3-N to the greatest extent. Prior to removing nutrients from swine wastewater, it is necessary to assess the nutrient balance required for microalgae growth. Micronutrients, N, and P are abundant in anaerobically digested swine, which is necessary for the proliferation of microalgae (Ran *et al.,* 2021). *C. vulgaris* at a mixotrophic growth stage was used to propose an improved bioremediation technique for piggery effluent, increasing biomass output to an average of 2.56g/L (Delanka-Pedige *et al.,* 2021).

In terms of environmentally friendly wastewater technology, the membrane bioreactor came in second place, followed by the mixotrophic algal wastewater treatment system (Cheng *et al.,* 2020). Discovered that the ammonia and COD removal efficiency in treated swine wastewater were more significant than those in untreated swine wastewater when two auto-flocculation microalgae, *Tribonema* sp. and *Synechocystis* sp., were utilized. Improved biomass and protein production as well as the removal of COD, N, and P were achieved by using *C. sorokiniana* microalgae immobilized on the sponge as a solid carrier in swine wastewater and reusing the microalgae-loaded sponge for new cultivation (Chen, 2020).

2.2.6 Treatment of pharmaceutical wastewater

A substance with antibacterial, antifungal, or antiparasitic characteristics is an antibiotic. Infectious disorders in both people and animals are frequently prevented and treated with antibiotics. They are frequently applied to livestock to encourage growth (Kümmerer, 2009). The lactam antibiotic family known as cephalosporin is frequently used to treat and prevent bacterial infections by preventing the production of the peptidoglycan layer found on both Gram-positive and Gram-negative bacteria's cell walls (Magdaleno *et al.,* 2015). The effluents of cephalosporin producers contain a number of dangerous chemical substances, antibiotic residues, and inorganic salts that are harmful to the environment and biological life (Guo and Chen, 2015; Yang *et al.,* 2016). To treat wastewater including pharmaceutical and personal care products (PPCPs), microalgae-based technology has also been developed.

According to numerous studies, microalgae effectively remove PPCPs from wastewater, including antibiotics (Villar-Navarro *et al.,* 2018; Xiong *et al.,* 2017). One of the most crucial procedures for the elimination of certain antibiotics has been discovered as bioadsorption. It was discovered that each species of microalgae has a different ability for bioabsorption. In the first 10 minutes of treatment, 7-amino cephalosporanic acid was absorbed by *Chlorella* sp. (4.74 mg/g), *Chlamydomonas* sp. (3.09 mg/g), and *Mychonastes* sp. (2.95 mg/g) the initial 10 minutes of treatment (Guo *et al.,* 2016). The biomass of *S.quadricauda* that was isolated from its lipids had a 295 mg/g tetracycline adsorption capability (Daneshvar *et al.,* 2018).

Additionally, under ideal conditions*, Scenedesmus quadricauda* (295.34 mg/g) and *Tetraselmis suecica* (56.25 mg/g) reached their highest tetracycline adsorption capabilities (Daneshvar *et al.,* 2018). After 24 hours of treatment with the absorption mechanism, *Chlorella pyrenoidosa* demonstrated the maximum removal of cefradine (41.47%), which was 3.4 times greater than the clearance attained without the use of microalgae (12.37%) (Xiao *et al.,* 2021). Antibiotics adhere to internal proteins or other substances in active metabolic processes called bioaccumulation in living microalgal cells. Temperature, pH, time spent in contact with antibiotics, and antibiotic concentration are only a few of the environmental parameters that might affect bioaccumulation.

The best method for antibiotic eradication by microalgae is biodegradation (Xiong *et al.,* 2018). At a starting concentration of 159 mg/L, *Chlorella* sp. L38 biodegraded 72% of the florfenicol from the medium. This study reveals that *Chlorella* sp. L38 could be a suitable substitute alga for removing florfenicol from different water sources (Song *et al.,* 2019). In a different investigation, *Chlamydomonas* sp. Tai-03 was shown to have the maximum elimination of ciprofloxacin (100%) and sulfadiazine (54.53%) when it produced more than 1000 mg/L of carbohydrates per day. The removal of sulfadiazine was mostly accomplished by photolysis (33.60%), while the removal of ciprofloxacin was primarily accomplished by biodegradation (65.05%) (Xie *et al.,* 2020).

2.2.7 Removal of heavy metals

One of the most frequent elements of wastewater that causes toxicity in the aquatic environment and harms aquatic animals and plants is heavy metals. HMs infiltrate the food chain due to their resistance to breakdown and provide a health danger to higher plants and animals, including humans. Heavy metals can be absorbed and accumulated by some biological materials. In addition, using biomaterials for these objectives is a lot more ecologically beneficial than using traditional methods. Microalgae have been shown to be the most efficient biomaterial for heavy metal absorption and accumulation in previous research, which aimed to determine the most effective biomaterials in this respect (Hamouda *et al.,* 2016; Zeraatkar *et al.,* 2016).

In actuality, microalgae have been recognized as biosorbents with more potential than other sorbent kinds. Because of their high absorption capacities, low cost, and inherent abundance in the majority of ocean locations worldwide, microalgae have garnered great interest as biosorbents found in aquatic settings. Multifunctional macromolecules found in microalgae include lipids, proteins, and carbohydrates with a variety of negatively charged functional groups, including amino, hydroxyl, carboxyl, sulfhydryl, sulfate, phosphate, phenol, and others (Javanbakht *et al.,* 2014).

The outer layer of the cell wall is the first participant in the removal of heavy metals as a result of these negatively charged groups, which let ions from the surrounding environment to adhere (Leong and Chang, 2020; Singh *et al.,* 2021). For the biosorption of heavy metals, it is crucial to understand the structure, content, and properties of the cell wall (Podder and Majumder, 2017). The influence of physicochemical factors including pH, temperature, the presence of other metal ions, and the ratio of adsorbate to adsorbent must also be controlled because this non-metabolic process is extremely reliant on operating circumstances (Podder and Majumder, 2017).

Additionally, because this non-metabolic process is so reliant on operating circumstances, it is necessary to control the ratio of adsorbate to adsorbent as well as physicochemical factors like pH, temperature, and the presence of other metal ions (Zeraatkar *et al.,* 2016). Microalgae are resistant to heavy metals, such *Nitzschia-perminuta* and *Nitzschia-apalea*, may collect a lot of metals in their bodies, making them useful for remediating wastewater with high amounts of heavy metals (Chen *et al.,* 2013). To improve the heavy metal adsorption capacity of microalgae, several approaches have been suggested. When the capacity of the plant *Ceramium rubrum* to absorb copper from aqueous solutions was examined, it was discovered to have a bioabsorption capacity of 25.51 mg/g of total biomass.

Additionally, it was discovered that when treated with NaOH or CH_2OH, its efficacy rose to 42.92 mg/g and 30.03 mg/g, respectively (Imani *et al.,* 2011). *Phacus* sp. was also discovered to have greater thallium resistance in contaminated waters. The strain's pellicle-like body coating helps it handle thallium effectively (Płachno *et al.,* 2015). *Desmodesmus* sp. MAS1 and *Heterochlorella* sp. MAS3, acid-tolerant microalgae, may take up HMs like Fe and Mn. At an acidic pH of 3.5, the greatest removal of Fe (40-80%) and Mn (40-60%) was accomplished.

Cellular research indicates that in both strains, intracellular pathways predominate for the elimination of heavy metals (Abinandan *et al.,* 2019). All wastewaters include a sizable amount of heavy metals. Consequently, a thorough investigation of the absorption and accumulation of such metals is required, one that makes use of several microalgae strains under various stress circumstances.

2.3 Applications of algae-based bioremediation

Our world is currently confronting a number of economic and environmental concerns including the depletion of traditional energy sources, global warming, and water poisoning (Wasi *et al.,* 2013; Bano *et al.,* 2018). Different facets of environmental pollution have emerged in recent decades as a result of rising population, industrialization, and fast urbanization.

Untreated sewage (e.g. commercial, residential, and industrial effluent) can be dumped directly into aquatic environments including rivers, reservoirs, and the sea in locations where sewage treatment plant facilities are not well developed.

This is considered a rapid and affordable dumping technique. Water depletion is greatly affected by this. Additionally, the potential for considerable levels of dangerous pollutants to enter the human and animal food systems may have negative health effects (Hena *et al.,* 2021).

2.3.1 Application of algae in inorganic (heavy metals) pollutant removal

Metals with a relative high density compared to water are referred to as heavy metals. (Fergusson,1990). Heavy metals also include metalloids like arsenic, which can cause toxicity at low levels of exposure, on the basis that heaviness and toxicity are connected (Duffus, 2002). Environmental pollution by these metals has been a growing problem for ecological sustainability and worldwide public health in recent years.

Additionally, due to an exponential surge in theirusage in many industrial, agricultural, household, and technical applications, human exposure has increased significantly (Bradl, 2005). The environment is said to be exposed to heavy metals from geogenic, industrial, agricultural, pharmaceutical, household effluents, and atmospheric sources (He *et al.,* 2005). Mining, foundries, smelters, and other metal-based industrial processes are point sourcesof significant environmental pollution.

Heavy metal ions, which are typical abundant pollutants in industrial effluent including lead, copper, cadmium, zinc, and nickel, cause environmental degradation (O'Connell *et al.,* 2008; Tsekova *et al.,* 2010). The contamination of rivers, lakes, and oceans is also brought on by residualnutrients and heavy metal ions in home and agricultural wastewaters (de-Bashan and Bashan, 2010). Major health issues can result from thebiosorption and buildup of heavy metal ions in aquatic food systems in humans (Sridhara Chary *et al.,* 2008). Even in low quantities, heavy metal ions can be hazardous to people.

For instance, lead is extremelytoxic and can harm the neurological system, kidneys, and disrupt the metabolism of vitamin D, especially in youngsters

(ATDR, 2007). Nickel-based chemicals are acknowledged to cause cancer. (ATDR, 2005), and prolonged exposure to cadmium is linked to lung impairment, bonemineral loss, kidney damage, and increased risk of bone fractures. (ATDR, 2012).

The world's freshwater supplies and aquatic habitats canbe better protected by investigating novel ways to purify wastewater. Recovery of valuable metals from treated waters, such as gold and silver, and extraction of radionuclides like uranium from aqueous solutions, may minimize the cost of treatment (Wang and Chen, 2009).However, it is a significant financial barrier to treat wastewater that contains heavy metal ions. The principal physicochemical methods for removing heavy metal ions from wastewaters are chemical precipitation(Charerntanyarak, 1999), ion exchange (Dabrowski *et al.*, 2004), electrokinetic (Yuan and Weng, 2006), membrane processing (Qdais and Moussa, 2004), and adsorption (Lee *et al.*, 2012; Goharshadi and Moghaddam, 2015).

The development of physicochemical techniques has been hindered by a number of problems, including the high prices of chemicals at industrial scales and the ineffective removal of heavy metal ions. Additionally, the employment of alternative techniques is required by theconstriction regulations and limitations on effluent discharge into the environment. A more effective, less expensive, and environmentally friendly way to remove metal ions from wastewater is by biosorption of heavy metal ions using algae.

The sorption of radioactive and hazardous metal ions can, in fact, be accomplished using algae. (Pohl and Schimmack, 2006), moreover, to recover valuable metal ions such as gold and silver (Darnall *et al.*, 1986; Mata *et al.*, 2009a). However, to uselive algal systems at the desired level of treatment, it is essential to understand the maximal autotrophic output, which necessitates a thorough physiological characterization of the algal culture. The removalof heavy metal ions from wastewaters via biosorption, a method that mostly uses inert biomass and non-living algae, is thought to be novel. As the heavy metal ions frequently harm the living cells, there are few examples of employing live algae with a low sorption capacity (Lamaia *et al.,* 2005).

Additionally, there are significant changes in the sorption process according to the stage of algae development. More precisely, a numberof environmental conditions have an impact on live algae and have a direct bearing on their capacity for metal ion biosorption. Since intracellular uptake of heavy metal ions often occurs during the development phase of algae, which is when absorption occurs, live algae have more sophisticated absorption systems than non-living algae.

In contrast, dead algal cells take up metal ions by a process known as biosorption, which occurs on the surface of the cell membrane (Godlewska-Zyłkiewicz, 2001). Non-living algal biomass may be thought of as a collection of polymers such sugars, cellulose, pectins, glycoproteins, etc. with the ability to bind to heavy metal cations as adsorbents and provide the possibility of successful wastewater treatment (Arief *et al.,* 2008). A particular algal strain'sability for remediation is consequently determined by the hazardous amount of heavy metal ions present in different algae species, which can be extremely strain-specific. In other words, in addition to variationsbetween comparable species, a heavy metal ion may demonstrate a selective interaction with one particular strain of algae.

For example, *Desmodesmus pleiomorphus* cells were used to study the removal of cadmium ions, and it has been demonstrated that there was a 25% difference inthe strains' capacities for cadmium biosorption when using the 'L' and 'ACOI 561'. In terms of species differences (Romera *et al.*, 2007). discovered that the following macroalgal species had varying capacities for sorbing copper:

Ascophyllum nodosum > *Chondrus crispus* > *Asparagopsis armata* > *Spirogyra insignis* > *Fucus spiralis* are all followed by *Codium vermilara*. The greatest metal ion absorption occursat a low pH (3-5), and dried algal biomass demonstrates a larger metalion biosorption capacity compared to living algae.

These physicochemical parameters also influence the maximal capacity of metal ion removal for distinct micro and macro algae strains. The pH of the solution significantly affects the dissociation of the surface functionalgroups of dead algal biomass and the solution chemistry of heavy metalions (Pavasant *et al.*, 2006; Guo *et al.*, 2008). Surface functional groups on biomass cell walls and solution metal chemistry can both affect howpH affects metal absorption (Sheng *et al.*, 2004). Typically, after 120 minutes, the biosorption capability reaches an adequate level.

Microorganisms often accumulate heavy metal ions in two stages (Monteiro *et al.*, 2011a, 2012).The first stage involves rapid inactive biosorption on the cell surface and is absolutely unrelated to cellular metabolism. In the second stage, heavy metal ions actively sorb into the cytoplasm of algal cells. Intracellular ion uptake is the term for this phase, which is reliant on cell metabolism (Talebi *et al.*, 2013). In the biosorption and detoxification of heavy metal ions, intracellular ion uptake plays a significant role (Wilde *et al.*, 2006; Perales-Vela *et al.*, 2006).

The presence of various binding groups on the algal cell surface, such as hydroxyl, phosphoryl, carboxyl, sulphuryl,amine, imidazole, sulfate, phosphate, carbohydrate, etc., has been linked to the potential for heavy metal ion biosorption (Kaplan, 2013). Fourier transform infrared (FTIR) spectroscopy is used to examine the presence of active sites for heavy metal ion uptake in algal cells (Gupta and Rastogi, 2008). The amount of functional groups in the algae cells and the coordination number of the metal ion to be sorbed are two additional factors that affect an algae cell surface's ability to sorb a particular ion.

Also, the presence of binding sites for metal ions, the rates at which the metal ion forms complexes with the functional group, and thechemical state of these sites influence algal biosorption capacity. The deprotonation of carboxyl and phosphate groups on the algal cell surface results in the net charge of the cell surface being negative when binding groups are present (Mehta and Gaur, 2005).

The first barrier against the biosorption of heavy metal ions is the cell walls of algae. The majority of metal-binding sites are found in the polysaccharides and proteins which constitute the algal cell walls (Schiewer and Volesky, 2000). The differential ability of metal ions biosorption by the various algal strains is attributed to the variable cell wall components' quantity and distribution in distinct algal strains (Romera *et al.,* 2006). Compared to several algal strains, brown algae as a very promising option for biosorbentsof heavy metal ions and the biomass-metal ion affinity. Brown algae with alginate in their cell walls have astrong preference for the biosorption of lead ions (Romera *et al.,* 2007). In brown algae, alginate polymers are the main sorbents for heavy metalions, and the amount of binding sites on this polymer directly affects howwell it can absorb metal ions (Romera *et al.,* 2006; Davis *et al.,* 2003).

2.4.7.1 The main factors influencing heavy metal ion biosorption

Numerous variables, such as the concentration of metal ions and algal biomass, pH, temperature, and the presence of competing ions, may have an impact on the biosorption of heavy metal ions by algae. These factors will be reviewed along with any potential impacts they may have on the biosorption of metal ions in this section.

2.3.1.2 The influence of initial metal ion concentration

The initial concentration of metal ions in the solution phase has a significant impact on the ability of algal biomass to remove heavy metal ions. As the initial metal ion concentration rises, biosorption initially rises as well. A simultaneous rise in the concentration of metal ions and decreased metal sorption. (Singh *et al.*, 2010). The capacity of biosorption could be increased by using this phenomenon.

For example, Monteiro *et al.*, (2011) reported a 5-fold rise in initial concentrations of Pb and Cd ions to Cladophora fracta resulted in a relative decline in culture productivity, with total chlorophyll content loss, a reduction in the number of chloroplasts,and disintegrating cell walls being the main causes of cell death. It is important to note that once heavy metal ions have been bioabsorbed by algal cells, they are transported to the cell vacuole to show how living algal cells interact with lethal concentrations of heavy metal ions.

To avoid the inhibitory effects of anincreasing concentration of metal ions in the host cells, structural/binding proteins such metallothionein's (MTs) attach to adsorbed ions during this process. This mechanism enables the regular biochemical processes to be removed more effectively than at greater doses. For example, Mehta and Gaur, (2001a) reported that at concentrationsof 2.5 ppm, *Chlorella vulgaris* biomass can remove 69% and 80% of thecations Ni (II) and Cu (II), respectively.

Nevertheless, upon increasing Ni (II) and Cu (II) concentrations to 10 ppm;

the metalremoval rate was lowered to 37 and 42%, respectively. Thisdemonstrates unequivocally that the bio removal rates were lowered by almost 50% as the metal ion concentration rose from 2.5 to 10 ppm. Due to the toxicity of some heavy metal ions on living algal strains. Thus, adjusted concentrations of metal ion is required for the effective development of algae since metal ions absorption will be lowered by thedeath of algal cells (Shanab and Essa, 2007).

The effects of mercury, cadmium, and lead ion concentrations on *Scenedesmus quadricauda* development were examined. Results indicated that mercury ions had a deleterious impact on algal cells at any dose, lead and cadmium ions, at low concentrations (5-20 ppm), boosted algae development by increasing the amount of chlorophyll (Lamaia *et al.*, 2005).

However, the presence of too many hazardousheavy metal ions may cause denaturation of proteins, the replacement of vital components, or harm to the oxidative equilibrium of the living algae. The amount of oxidized proteins and lipids in the algae cells determines the severity of the stress on those cells. The ability of algaecells to withstand oxidative damage greatly influences how well they respond to heavy metal ions (Talebi*et al.*, 2013; Arunakumara and Zhang, 2008).

2.3.1.3 Living vs non-living algae

The process that contributes the biosorption of heavymetal ions in both living and non-living algae is primarily the ion exchange process, even if there are obvious distinctions between the accumulation of metal ions onto live algae cells and the biosorption of metal ions onto nonliving algal biomass (Monteiro *et al.*, 2012). While heavy metal bioremediation is often aided by the metabolic activities of live algae (Gadd, 1988), the biosorption of heavy metal ions from liquids using non-living algae has lately acquired favor.

Benefits of non-living biomass biosorption include the fact that non-living algae may absorb heavy metals more effectively than living algae, by a factor of several times (Mehta andGaur, 2005; Tsang *et al.*, 1999; Tam *et al.*, 2002). Additionally, one distinctive superiority of using dead biomass in heavy metal biosorption is the ability to recycle non-living algal biomass (Aksu and Donmez, 2001). Where, washing the biomass with deionized water and desorption agents such as HCl, NaOH, and $CaCl_2$ has been evaluated in many studies.

For instance, metal ions attached to the algal cell wall may be removed, while living algae are less resistant to physical and chemical recycling treatments than dead algae (Chen *et al.*, 2012). It is important to note that non-living algae may be treated simply by following physical and chemical protocols in order to increase adsorption capability (Arıca *et al.*, 2005). Using the non-living algae biomass, decreases the toxic effects induced by heavy metal exposure on the living system.

Additionally, there is noneed for extensive maintenance or the provision of additional growthnutrients (Arunakumara and Zhang, 2008; Franklin *et al.*, 2002).

However, a number of environmental conditions affect the biosorption of heavy metal ions by nonliving algae. Since most algaedevelop in neutral or slightly alkaline environments, pH variations have a higher influence on living than non-living algae (Kong *et al.*, 2010), and acidic conditions can influence how quickly algae grow, whereas basic medium may induce metal ions to precipitate (Skoog *et al.*, 2013; Fu and Wang, 2011).

Non-living algae are preferred for heavy metal removal in solutions with severe pH levels over living algae since, utilizing live algae complicates the control of the culturemedium's chemistry and increases the risk of undesired metal ion precipitation and bioremediation interference. The removal efficiencies of living and non-living algae could not be distinguished in any significant way; to be more specific, various living and non- living samples of *C. vulgaris* had the same ion removal efficiency for U^{4+} (Vogel *et al.,* 2010) and Ni^{2+} (Al-Rub *et al.,* 2004) biosorption.

In summary, compared to dead biomass, live cells with metabolic activity may exhibit increased metal ion absorption. They could alsoadsorb more diverse range of ions (Doshi *et al.,* 2007). Non-living cells, however, have quicker absorption kinetics. Successive adsorption-desorption cycles might successfully repurpose the deadbiomass resources (Areco *et al.,* 2012).

As a genuine candidate forlarge-scale bioremediation of industrial water, this method has been made possible by its low cost and simplicity of usage in non-living cells. Consequently, optimizing the interactions between algal strains, dead or living cells, and contaminants will result in the best removalefficiency.

2.3.1.4 Coupling wastewater treatment and biofuel production

The main constrain of wastewater applications is the high costof chemical-based treatments to remove extremely high concentrations of nutrients and harmful metal ions from wastewater (Gasperi *et al.,* 2008). The ability of algae to effectively remove heavymetal ions makes them incredibly reasonable tools for environmentally friendly and practically priced wastewater treatment (de-Bashan and Bashan, 2010; Pittman *et al.,* 2011).

Utilizing wastewaters for biomass production can drastically lower the capital, operating, and maintenance expenses for the production of microalgal biofuels (Park *et al.,* 2011). Systems that combine wastewater treatment with algae biomass production might reduce energy expenditures per unit by 20–25% and significantly reduce the cost of fertilizer and freshwater supplementation (Craggs *et al.,* 2011). Combining the production of microalgae biomass toward biofuels with wastewater bioremediation offers a solution to counteract eutrophication and industrial pollution while also producing sustainable energy (Lyon *et al.,* 2015).

A sustainable method to remove hazardous metals from wastewaters has been proposed forbioremoval of heavy metal ions utilizing microalgae (Mata *et al.*, 2009b). On the economic scale, there is a need to decrease the demand for chemical wastewater remediation, minimize the use of freshwater, and improve the compatibility of algal introduction in thewastewater treatment process (Lyon *et al.,* 2015; Lee, 2012; Zeng *et al.,* 2015; Kesaano and Sims, 2014).

Additionally, a variety of useful byproducts (including bioethanol and biodiesel), important nutrients, and bioactive substances may all be recovered from the generated biomass (Park *et al.*, 2011). Integrated algal-based treatment of wastewater and biofuel production may effectively eliminate potentially dangerous contaminants including remaining nutrients, toxic metal pollutants, and even transgenic algae from wastewaters while lowering inputs and costs of algal biomass production (de-Bashan and Bashan, 2010; Ruiz-Marin *et al.,* 2010).

When the removal of nutrients and heavy metal ions is necessary before wastewater is discharged, the coupled system is a helpful strategy. Additionally, the development of biofuels may lower the overall cost of capturing CO_2 from industrial sources or power plants. (Raeesossadati *et al.,* 2014). Maximizing autotrophic output is crucialfor realizing the suggested potentials of linked algal systems. High- rate algal ponds (HRAPs), which are extensively utilized on industrialscale across the world and play an efficient and cost-effective role in conventional wastewater treatment, might be used to apply it (Craggs *et al.*, 2012).

In compared to conventional wastewatertreatment technologies, HRAPs have cheaper startup and ongoing costs, require less sophisticated equipment to run, and provide all the advantages of linked systems to create biofuel (Craggs *et al.*, 2011, 2012).

2.3.2 Inorganic compounds

Typically, microalgae are autotrophic microorganisms, which means that they produce their own biochemical energy through photosynthesis using inorganic nutrients (such as N, P, and others) and inorganic carbon (CO_2) as their energy sources. (Huang *et al.*, 2010). The most often discussed method for absorbing and eliminating inorganiccontaminants in wastewater treatment is the autotrophic metabolism. Microalgae may eliminate inorganic N and P from primary effluent, secondary effluent, or centrate from sludge digestion, as shown in Fig. 2.5.

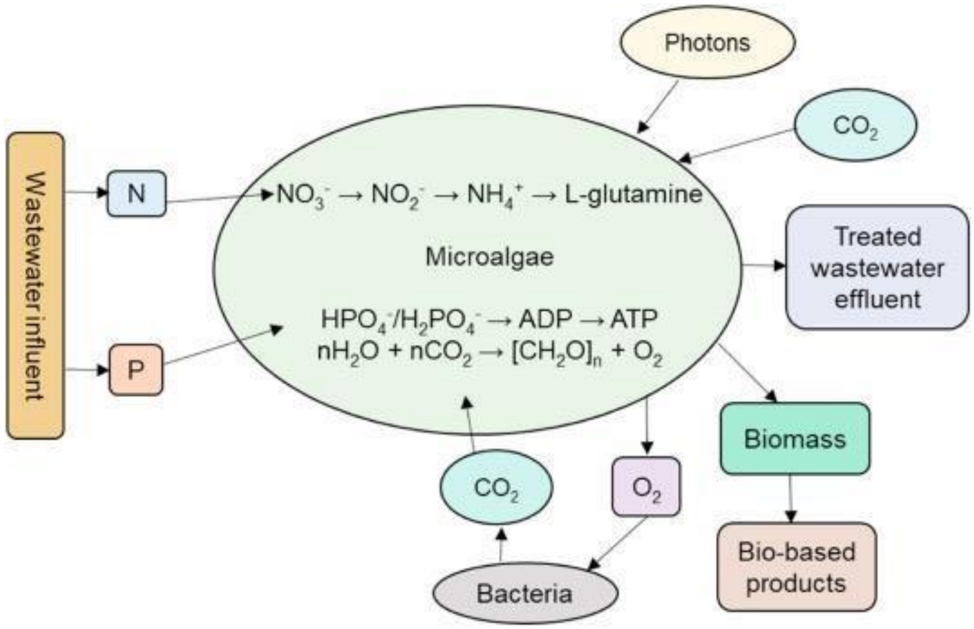


Fig.2.5. Uptake mechanism of nutrients and their intracellular interactions in microalgae cells. (Chai *et al.*, 2021).

The N/P ratio controls the N- and P-removal efficiency. The N/P ratio in microalgae can range from 8 to 45 gN/gP depending on the species. (Beuckels *et al.*, 2013: Christenson *et al.*, 2011). Some microalgae have the intriguing ability to ingest P in excess amounts (luxury absorption), which is stored inside the cells as polyphosphate granules and leads to increased P removal capabilities (Larsdotter, 2006; Solovchenko *et al.*, 2016). However, during wastewater treatment, P is typically the growth- limiting component (Wang *et al.*, 2010).

Algae mostly consume NH_4^+ and NO_3 in the case of N, with urea and nitrite (NO_2) coming in second and third (Larsdotter, 2006). Diverse wastewater types have been studied in various research for treatment by microalgae. It has been discovered that *Chlorella zofingiensis* can remove nutrients from effluent from pig farms. (Rawat *et al.,* 2011), whereas dairy wastewater was used to cultivate *C. pyrenoidosa* (Kothari *et al.,* 2012). Municipal wastewater has been observed to support the growth of *Chlorella* species and *C. vulgaris* (Li *et al.,* 2011; Ji *et al.,* 2013).

While total N was eliminated at a rate of around 80% of its initial concentration, total Pwas removed at a rate of between 80 and 90%. *Scenedesmus obliquus* and *S. acutus* cultivation in municipal wastewater resulted in 70-95% P and N removal. (Huang *et al.,* 2010; Ji *et al.,* 2013). Markou *et al.,* (2012) found 73% of initial COD removal by *Spirulina platensis*, whereas P and NO_3 were completely removed from olive-oil mill effluent . In order to cultivate *S. platensis* and *C. vulgaris*, poultry litter leachates were also investigated: N 99% of the original PO_4^{-3} was eliminated. (Markou *et al.*, 2016).

There have been reports of several organisms, including *Nanochloropsis* sp., *Chlamydomonas reinhardtii*, *Dunaliella tertiolecta*, and *Botryococcus braunii*, being able to absorb inorganic nutrients from wastewater. Environmental factors have a significant impact on how effectively nutrients are removed and biomass is produced during autotropic growth. The best pH range for algal development is between 7 and 9, and temperatures between 5 and 30 °C are recorded (Mohan *et al.*, 2015). But there are differences between species. Microalgae species should be able to adapt to seasonal temperature variations since they are crucial for wastewater treatment.

Study of Abdelaziz *et al.,* (2014) revealed that at temperature range from 10 °C and 22 °C, various strains could be grown in the secondary effluent of a treatment facilitywith removal efficiencies of 70% of the initial N and P. Light and temperature both affect algal growth as well as the removal of contaminants and pollutants; as light levels rise, so do growth rates,biomass productivities, and nutrient-removal efficiencies during autotropic metabolism (Gonçalves *et al.*, 2016). For instance, the NO_3^- removal effectiveness by *Chlorella kessleri* culture was better undercontinuous illumination than it was during diurnal cycles. (Lee andLee, 2001).

2.3.3 Organic compounds

Microalgae have the ability to metabolize organic forms of N andP that are present in wastewater, whereas bacteria play a significantrole in the mineralization of organic chemicals into inorganic forms (Healey,1973). Depending on the source of the effluent, these chemicals may be present in wastewater in high or low concentrations. Additionally, complex compounds like medications, personal care items, and endocrine disruptors can be linked with organic N and P. Even though their quantities are modest, the intricate structures of these compounds frequently prevent traditionalwastewater treatments from breaking them down. to be taken up bymicroalgae (Zhou *et al.,* 2014; Gadipelly *et al.*, 2014).

One of the primary sources of organic nitrogen is proteins, which can account for up to 60% of the organic nitrogen in effluent fromwastewater (Westgate and Park, 2010). According to the effluent source, urea is another organic source of N that can be as high as $20g/L^{-1}$. (Rittstieg *et al.*, 2001). According to earlier investigations, microalgae are effective bioremediator of these chemicals. For example, *Scenedesmus* sp. LX1 can remove up to 90% of the original urea content by absorption (Xin *et al.,* 2010). Protein removal has been reported by*Chlorella* sp. (McAuley,1986), *Tetraselmis striata (*Ricketts, 1990) and by algae consortia (Nilsson *et al.*, 1996) . When inorganic P is scarce, extracellular or cell-wall bound phosphatase enzymes can convert organic phosphates to orthophosphates. (Larsdotter,2006; Solovchenko *et al.*, 2016).

Agricultural effluents, which have organophosphorus pesticide concentrations of roughly 50 g L^{-1}, are the main source of organic Pin wastewater (Hultberg *et al.*, 2016). *Nostoc muscorum* showed efficient removal of organic P from insecticides (Ibrahim *et al.*, 2014), *Oscillatoria limnetica* (Salman and Potential, 2015) and *C. vulgaris* (Hultberg *et al.*, 2016). By changing their metabolism to mixotrophy or heterotrophy, several microalgal species may thrive on a variety of organic substances, including glucose, acetic acid, glycerol, urea, and xylose. (Markou*et al.*, 2012; Richmond *et al.*, 1986; Smith *et al.*, 2015).

Microalgal and bacterial mixotrophic metabolism can help in removing the organic pollutants from wastewater by biodegrading the contaminants or by using the organic molecules (Markou *et al.*, 2012). Higher cell densities than possible with autotrophic metabolism are made possible by mixotrophic metabolism's reduced dependence on light penetration in the culture (Smith *et al.*, 2015). Additionally, heterotrophic metabolism via dark fermentation enables anaerobic bacteria to mineralize N and P into NH_4^+ and orthophosphates for microalgae development as well as transform complicated carbon molecules into simple organic acids (Turon *et al.*, 2016).

Wastewater organic compounds may enhance biomass production. For example, Cabanelas *et al.*, (2013) found increased lipid and biomass synthesis by *Botryococcus terribilis* and *C. vulgaris* in municipal wastewater augmented with glycerol (50 mM). Chandra *et al.*, (2014) found 85% decrease in COD in municipal wastewater using a mixotrophic culture of diatoms, *Chlorella*, and

Scenedesmus (with glucose). A strategy to produce biomass with a certain composition, such as monounsaturated fatty acids for the generation of biodiesel, has alsobeen found to have impacts on the fatty acids profile as a reaction to glucose concentrations (Bhatnagar *et al.,* 2011; Smith *et al.,* 2015).

Although supplementing growth media with organic compounds might raise production costs, mixotrophic culture can be a viable way to achieve better biomass productivities. This indicates that only wastewaters containing organic substrates, such as sewage from homes, animal waste, waste from food processing, and polysaccharide hydrolysate (from starch or straw), are suitable for mixotrophy. (Zhang *et al.,* 2016).

2.3.3.1 Removal of Polycyclic Aromatic Hydrocarbons (PAHs) using algae-derived adsorbents

Natural sources of Polycyclic Aromatic Hydrocarbons (PAHs) include fossil fuels, although they can also result from the incomplete combustion of organic molecules (Yu *et al.,* 2011; Zhao *et al.,* 2015).

They have been found in the polluted water and soil. Studies on the biosorption of PAHs using various microalgal species have been previously investigated (Pathak *et al.,* 2018). Lei *et al.,* (2007) showedthe separation of PAHs from four microalgal biomass, including *Chlorella vulgaris*, *Scenedesmus platydiscus*, *Scenedesmus quadricauda*, and *Selenastrum capricornutum*, using fluoranthene (1.0 mg/L), pyrene (1.0 mg/L), and a combination of fluoranthene andpyrene (each at 0.5 mg/L).

According to their investigation, the extraction process is depending on the toxicant and algal type. *Selenastrum capricornutum* was reported as an efficient species in removing and transforming PAHs by 78% compared to*C. vulgaris* which had lower effect by 48%. With the exception of *S. platydiscus*, every species under study shown greater removal efficiency of fluoranthene than pyrene. Indicating thatthe presence of one PAH stimulates the removal of the other PAH, the removal rate of combined fluoranthene and pyrene was equivalent to or greater than the removal rate of either compound alone.

The non-living biomass of the brown seaweed *Sargassum hemiphyllum* was utilized by Chung *et al.,* (2007) to study the kinetics and equilibrium of the sorption of aqueous phenanthrene under various environmental conditions. Although there was no appreciable difference in the maximum sorption capacities, higher temperatures(15 to 35 °C) and shaking rates (50 to 250 rpm) resulted in increased sorption of phenanthrene.

Swackhamer and Skoglund (1993) observed that hydrophobic chemicals attach to the cellulose and lipid contents of microalgal cell walls, which have an impact on the biosorption process. A number of studies have been carried out to assess the contributions of different fractions of algal debris on adsorptive removal of organic pollutants in order to better understand the interactions between such pollutants and debris. Under light, dead algal cells may discharge materials like fattyacids and proteins from ruptured membranes into the medium (Kumar *et al.,* 2019; Widrig *et al.,* 1996). These findings imply that some cellular materials may be liberated from ruptured algal cell membranes and subsequently trigger interactions with contaminants.

In a recent study, Zhang *et al.,* (2019) examined the adsorption of three PAH substances (phenanthrene, benzopyrene, and naphthalene) on green algae and noted that the adsorption capabilityof the algae fell by as much as 25% when the lipid fractions were removed. This suggests that the lipid components are crucial to PAH sorption on algal biomass. However, the polysaccharide may have had less impact on the adsorption ofphenanthrene and benzopyrene.

Luo *et al.,* (2014) It was proposed that PAHs may be highly adsorbed by nonliving cells, which may be attributed to changes in the surface adsorptive properties of the microalgae cell after death and increased permeability of dead cell membrane in the absence of metabolic protection against the transport of pollutants into the cell. The adsorption is connected to the detritus of algae. After the polysaccharides portion was removed, the detritus began to develop more pores, which could be advantageousfor the adsorption of contaminants.

It has been demonstrated that dead *Selenastrum capricornutum* works effectively in eliminating phenanthrene, fluoranthene, and pyrene (Chan *et al.*, 2006). Algae-base adsorption, has been demonstrated as the fastest adsorptive method was used to remove the PAH pollution, followed by plant absorption. The main contributing factor could be physicochemical adsorption, which is independent on metabolism.

In related note, Avery *et al.,* (1998) foundthat the high potential of the binding sites allowed organic and inorganic contaminants to attach to the cell surface of algal biomass.They also concluded that cell walls might potentially offera large number of places for contaminants to bind, and extra binding sites would be more easily accessible in dead cells than in living ones.

Additionally, it has been noted that non-living cells are better able to adsorb PAHs than alive ones.

2.3.3.2 Nitrogen

Through sewage discharge from land where animal manure is kept or applied, organic nitrogen may enter to wastewater. In biological compounds including enzymes, peptides, proteins, chlorophyll, andenergy transfer molecules like ADP and ATP, organic nitrogen is theessential component. Nitrite (NO_2), nitrate (NO_3), nitric acid (HNO_3), ammonia (NH_3), ammonium (NH_4), and nitrogen gas (N_2)are among the inorganic sources from which organic nitrogen is formed.

Nitrogen is often found in wastewater in the forms of NH_4^+,NO_2 and NO_3. Eukaryotic microalgae are capable of converting inorganic nitrogen into organic forms through absorption. In a nutshell, the reduction of nitrate (NO_3) to nitrite (NO_2) and then to ammonium (NH_4^+), which is ultimately incorporated into amino acids (the organic form of nitrogen), is the transformation mechanism that occurs across the microalgae plasma membrane.

Nitrate reductase (NR), which is the reduced form of nicotinamide adenine dinucleotide (NADH), $C_{21}H_{27}N_7O_{14}P_2$, is found inside the microalgae, and is used as the first step in the absorption of nitrate. It transfers two electrons during the process that turns nitrate into nitrite. In the subsequent reduction of NO_2 to NH_4^+, ferredoxin (Fd) from microalgae and nitrite reductase, which is NADPH, $C_{21}H_{29}N_7O_{17}P_3$, formed from the photosynthetic process involving ADP, phosphate, and NADP, transfer six electrons.

All of the inorganic forms of nitrogen will be converted from this process to NH_4^+ in the microalgae's internal fluid. The intracellular fluid of microalgae contains glutamic acids (Glu), $C_5H_9NO_4$, which are neuroactive amino acids found in microalgae, and adenosine triphosphate (ATP), which is released from phosphorylation (the process of assimilation of phosphates into organic compounds) (Emparan *et al.,* 2019).

2.3.3.3 Phosphorus

Inorganic phosphorus, which is naturally present in lipids, nucleic acids, and proteins in wastewater, is crucial for the development and metabolism of microalgae. Inorganic phosphates are translocated across the plasma membrane of microalgae cells. Inorganic phosphorus, in the forms of monohydrogen and dihydrogen phosphate (HPO_4^{2-} and H_2PO_4), is incorporated into organic molecules during the metabolism of algae, in this case adenosine diphosphate (ADP) by phosphorylation.

Energy is needed for phosphorylation in order to create the desired result, ATP. The oxidation of respiratory substrates, the electron transport mechanism of the mitochondria present in eukaryotic microalgae, and light used during photosynthesis are all sources of energy. (Emparan et al., 2019). The light-dependent process for photosynthesis includes both photochemical and redox reaction stages. for ADP, phosphate (P), and NADP-based light-dependent phosphorylation is as follows: (Razzak et al., 2013):

$$2H_2O + 2NADP^+ + 3ADP + 3P + Photons_{(light)} \Rightarrow 2NADPH + 2H^+ + 3ATP + O_2$$

2.3.4 Dyes removal

Because of their large surface area and strong binding affinity, microalgae have been used to remove color and vinyl sulfone dye from textile effluent

(Andrade and Andrade, 2018; Chu and Phang, 2019). Microalgae cell walls engage in biosorption, electrostaticattraction, complexation, and bioconversion throughout the dye removal process. The surface of the algal biopolymer binds and collects dye ions, which then diffuse onto the solid phase of the biopolymer.

The functional groups included in extracellular polymers will aid in the biosorption of dye molecules onto the polymer surface(Kumar *et al.,* 2014). *Spirogyra*, amicroalga species, wasdemonstrated to be an effective biosorbent for the removal of reactive dyes. *Caulerpa lentillifera* and *Caulerpa scalpelliformis* biomass have the ability to biosorb away basic colors. Additionally, the removal of reactive dyes like Remazol Black B has frequently been accomplished using *C. vulgaris* as a biosorbent (Aksu and Tezer, 2005).

Microalgae break down the dyes into more basic chemicals by converting mono-azodye to aniline. *Chlorella vulgaris* has been reported to decrease 63-69% of (Supranol Red 3BW), a textile dye color in wastewater during a 10-day culture period. Researchers indicated the effectiveness of *Chlorella vulgaris* in dye color removing. In the medium lacking *C. vulgaris*, there was only a little (1.4%) color change of the Supranol Red 3BW textile effluent.

This shows that the active development of microalgae is what causedthe color loss. Additionally, the ability of five cyanobacteria strains (blue green microalgae)—*Anabaena flos aquae*, *Nostoc elepsosporum*, *N. linkia*, *A. variabilis*, and *C. vulgaris* to remove the red coloring caused by textile industry effluent was evaluated (Ghazal, 2018). The results of the experiment showed that every microalgae strain had a varying degrees of red dye elimination from the treated textile wastewater effluent. Following *N. elepsosporum*, which removed thedye completely, were *C. vulgaris* (96.16%), *A. variabilis* (88.71%), *N.linkia* (79.03%), and *A. flos aquae* (50.81%). The effectiveness of color removal by microalgae does not appear to be affected by the complexity of the textile effluent such as containing additional compounds, such as heavy metals.

2.3.5 Carbon dioxide fixation

In wastewater, carbon dioxide produced by microbial respiration can induce pH imbalance if present in large quantities. A few strains of microalgae are particularly good at reducing the pH of wastewaterbecause they can withstand high carbon dioxide concentrations. Microalgae have the ability to physiologically absorb carbon in the form of CO_2 from the environment and through the bacterial oxygenation of wastewater.

Carbon fixation often results in the final carbohydrate products $[CH_2O]_n$. Two channels may be distinguished in the total response; dark or light- independent reactions . Water serves as the electron donor in a light-dependent process like oxygenic photosynthesis, and oxygen is released following hydrolysis. In a number of subsequent steps, the carbon skeletons created by light-dependent and light-independent reactions are subsequently employed to create various organic molecules.

For instance, cellulose, a kind of carbohydrate, may be utilized as a precursor for the production of lipids and amino acids, which is why the process is known as CO_2 fixation. The bioremediation of wastewater is aided by this process because it also releases oxygen,which the bacteria subsequently use to lower the high organic loadsin the wastewater system through microbial metabolism (Satpati and Pal, 2020).

2.3.6 Biomass production

Through the use of biorefinery technologies, the biomass created by microalgae through wastewater treatment may be harvested and transformed into a range of fuel bioproducts and chemicals with added value (Chew *et al.,* 2017). For instance, biodiesel and biochar are produced by pyrolysis, biodiesel is produced through transesterification, bio-oil is produced through thermochemical conversion, and biomethane is produced through anaerobic digestion, among other processes (Klinthong *et al.,* 2015; Yu *et al.*, 2018).

As it acts as a passive biosorption process in the heavy metals absorption mechanism, biomass is essential for the removal of heavy metals from wastewater. Additionally, biochar is an excellent adsorbent for hazardous compounds found in wastewater treatment, including antimicrobials, surfactants, and dyes. Sludge-derived biochar improves soil qualities and lowers the carbon footprint of wastewater treatment facilities.

Slow pyrolysis, an anaerobic process, uses high temperatures (360-800 ^{o}C) and air pressure to break down biomass into charcoal residue, also known as biochar. Biochar produced from biomass by slow pyrolysis has a product distribution of 35 weight percent solid, 30 weight percent liquid, and 35 weight percent gas (Mohan *et al.,* 2014).

Table (2): Efficiency of microalgae in phytoremediation of various types of emerging contaminants (ECs) from wastewater.

Organism	ECs	Removal Mechanisms	Removal (%)	References
and *Chlorella vulgaris* other microorganisms	Tetracycline	Photodegradationand biosorption	69	(de Godos I *et al.*, 2012)
Chlamydomonas mexicana and *Scenedesmus obliquus*	Carbamazepine	Bioaccumulationand biodegradation	28–35	(Xiong J-Q *et al.*, 2016)
Microalgal consortium	Anti- inflammatory, and diuretics antibiotics	——	30–80	(Hom-Diaz *et al.*, 2017)
Chlorella pyrenoidosa	Triclosan	Photodegradation and biosorption	50–77.2	(Wang *et al.*, 2013)
Chlorella pyrenoidosa	Cefradine	Photodegradation	76	(Chen *et al.*, 2015)

Chlorella sorokiniana	Salicylic acid and paraceta- mol	——	70	(Escapa *et al.*, 2015)
Chlorella sorokiniana	Diclofenac, ibuprofen, par- acetamol and metoprolol	Photodegradation, biosorp- tion and photolysis	60–100	(de Wilt A *et al.*, 2016)
Lessonia nigrescens Bory and Macrocystis integrifolia Bory	Sulfamethoxazoleand sul- facetamide	Adsorption	—	(Navarro AE *et al.*, 2014)
Microalgal consortium	beta–blockers, antibiotic, antidepressant, relaxant muscle and others	--------	10–90	(Gentili and Fick ,2017)
Microalgal consortium	Pharmaceutical, personal care Products and endocrinedisrupting	Photodegradation and bio- transformation	>50	(Zhou G-J *et al.*, 2014)
Scenedesmus obliquus	Progesterone and	Photodegradationand	40–95	(Peng F-Q*et*

and*Chlorella pyrenoidosa*	norgestrel	biosorption		*al.*, 2014)
Desmodesmus subspicatus	17α–Ethinylestradiol	Bioaccumulation and bio-transformation	68	(Maes HM*et al.*, 2014)
Chlorellavulgaris	Estradiol	Biotransformation and bio-concentration	50	(Lai KM *et al.*, 2002)
Microalgal consortium	17α– Estrone, ethinylestra- diol,and 17ß–estradiol	Photodegradationand biosorption	83.9-95.4	(Shi W *et al.*, 2010)
Microalgal consortium	Ethinylestradiol	Biotransformation	-	(Della Greca M *et al.*, 2008)
Selenastrum capricornu-tumand Chlamydomonas reinhardtii	and ß–estradiol –ethynyle-stradiol 17	Photodegradation, biodegra- dationand adsorption	47–100	(Hom-Diaz *et al.*, 2015)

2.4 Mechanism of algae-based bioremediation

One of the most promising methods for removing of various contaminants is known as bioremediation (Mustafa *et al.,* 2021). Bioremediation is defined as the process of converting hazardous pollutants in soil and water into non-hazardous substances. However, some definitions limit the process to microbes only while others include plants (phytoremediation). In reality, biological remediation in nature involves both plants and microbes (Dwivedi, 2012). Different types of contaminants can be removed from microalgae through different methods Fig.2.6, including biosorption, bioaccumulation, biodegradation **(**Mustafa *et al.,* 2021), bio-adsorption, photodegradation and volatilization (Sutherland and Ralph, 2019)

Fig.2.6. Processes involved in algal bioremediation.

A variety of pollutants can be removed by microalgae, including those originating Fig 2.7 from domestic effluents, agricultural runoffs, textile, leather, pharmaceutical, and electroplating industries. Additionally, microalgae reduce carbon dioxide in their growth process and utilize micronutrients from effluents (Mustafa *et al.,* 2021).

The metabolism of microalgae changes significantly in response to even a little change in the concentration of certain contaminants, such as heavy metals. In order to identify these pollutants, microalgae can be utilized as a biological indicator. Algae have created a number of defenses against metal toxicity, though. These include metal sequestration by peptides such phyhelatins, decreased metal uptake, efflux, and extracellular detoxification (Cobbett and Goldsbrough, 2002; Tripathi *et al.,* 2006).

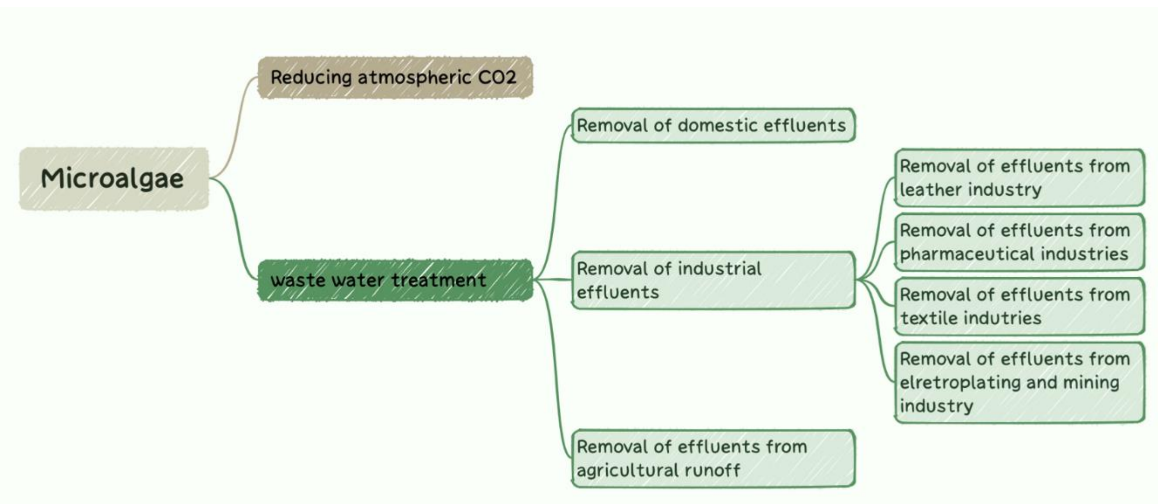


Fig.2.7. Flowchart showing potential use of microalgae in environmental remediation.

Algae have developed a number of enzymatic and nonenzymatic antioxidant defense mechanisms in addition to these tolerance mechanisms to combat the oxidative stress brought on by heavy metals. In response to heavy metal stress, nonenzymatic defense produces and accumulates intracellular antioxidants like glutathione, tocopherols, ascorbate, carotenoids, and proline.

Enzymatic defense involves increased expression of antioxidant enzymes like superoxide dismutase, catalase, and ascorbate peroxidase (Pinto *et al.,* 2003; Tripathi and Gaur, 2004). Varying species of microalgae have varying capacities for tolerating heavy metals, and these capacities are mostly influenced by the effectiveness of intracellular metal homeostasis processes and external factors like water's pH and ionic strength (Rangsayatron *et al.,* 2002).

As an example, *Phormidium* sp. could successfully hyperaccumulate cadmium, zinc, lead, nickel, and copper (Shanaab *et al.,* 2012; Rana *et al.,* 2013), while *Scenedesmus* sp. could tolerate the metals copper, nickel, cadmium, and zinc at lower concentrations (2-5 mgL^{-1}), and lead at relatively higher concentrations up to 30 mgL^{-1} (Shehata and Badr, 1980).

According to Rangsayatron et al. (2002), the blue-green alga *Spirulina platensis* demonstrated cadmium tolerance up to 100 mgL^{-1} and biomass elimination of approximately 98.04 mg Cdg1. According to Torres et al. (1997), *Phaeodactylum tricornutum*, a marine diatom, has a high cadmium tolerance of up to 22 mg L^{-1}.

Algae also have the capacity to eliminate emerging contaminants (ECs), which are essentially synthetic organic chemicals, some of the most frequently found ECs in aquatic habitats include pharmaceuticals, cosmetics, personal care items, insecticides, and flame retardants (Sutherland and Ralph, 2019).

Table (3): Removal efficiency of a range of emerging contaminants by microalgae.

Emerging contaminant	Microbial species (percentage removal efficiency	Pathways	Reference
17 α-Estradiol	*Scenedesmus dimorphus* (85)	Biodegradation	Zhang *et al.,* (2014)
17 β-Estadiol	*Chlamydomonas reinhardtii* (100), *Desmodesmus subspicatus* (95), *Nannochloris* sp. (60), *Selenastrum capricornutum* (88)	Bioadsorption, bio-uptake and biodegradation	Hom-Diaz *et al.,* (2015), Bai and Acharya (2019)
17α-Ethynylestradiol	*Chlamydomonas reinhardtii* (100), *Desmodesmus subspicatus* (68), *Nannochloris* sp. (60), *Selenastrum capricornutum* (60-95)	Bioadsorption, bio-uptake and biodegradation	Hom-Diaz *et al.,* (2015), Bai and charya (2019), Maes *et al.,* (2014),

7-amino cephalosporanie acid	*Chlorella* sp. (100), *Chlamydomonas* sp. (100), *Mychonastes* sp. (100)	Photodegradation and bioadsorption	Guo *et al.,* (2016)
Amitriptyline	*Chlorella sorokiniana* (68), *Chlorella vulgaris* (42), *Chlorella saccharophila* (92), *Coelastrella* sp. (60),	Bioadsorption	Gojkovic *et al.,* (2019)
Biperiden	*Chlorella sorokiniana* (35), *Chlorella vulgaris* (93), *Chlorella saccharophila* (89), *Coelastrum astroideum* (9), *Desmodesmus* sp. (41-71), *Scenedesmus* sp. (53), *Scenedesmus obliquus* (48)	Bioadsorption	Gojkovic *et al.,* (2019
Bupropion	*Chlorella sorokiniana* (60), *Chlorella vulgaris* (82), *Chlorella saccharophila* (88), *Coelastrella* sp. (89), *Coelastrum astroideum* (94), *Desmodesmus* sp. (86-90),	Bioadsorption	Gojkovic *et al.,* (2019

	Scenedesmus sp. (70), *Scenedesmus obliquus* (95)		
Caffeine	Mixed consortia (99), (26-81)	Biodegradation	Matamoro et al. 2016, Gojkovic *et al.,* (2019)
Carbamazepine	*Chlamydomonas mexicana* (35), *Chlorella sorokiniana* (10-30), *Desmodesmus* sp. (71), *Nannochloris* sp. (20), Mixed consortia (4-15), (20), *Scenedesmus obliquus* (35)	Bioadsorption and Biodegradation	Xiong *et al.,* (2017a), de Wilt *et al.,* (2016), Gojkovic *et al.,* (2019), Zhou *et al.,* (2014) , Bai and Acharya (2016), Gentili and Fick (2017), Matamoro et al. 2016
Ciprofloxacin	*Chlamydomonas mexicana* (13-56), *Dictyosphaerium* sp. (11), *Nannochloris* sp. (100), Mixed consortia (20- 30), (74-79)	Photodegradation, biodegradation	Xiong *et al.,* (2017), Gentili and Fick (2017), Bai and Acharya (2016), Hom-Diaz *et al.,* 2017.
Climbazole	Mixed consortia (30-70), *Scenedesmus obliquus* (88)	Biodegradation	Zhou *et al.,* (2014), Pan *et al.,* (2018)

Clomipramine	*Chlorella sorokiniana* (96), *Chlorella vulgaris* (100), *Chlorella saccharophila* (100), *Coelastrella* sp. (34), *Desmodesmus* sp. (29-42), *Scenedesmus* sp. (73), *Scenedesmus obliquus* (78)	Bioadsorption	Gojkovic *et al.,* (2019)
Codeine	*Chlorella sorokiniana* (50), *Chlorella vulgaris* (57), *Chlorella saccharophila* (42), *Coelastrella* sp. (46), *Coelastrum astroideum* (72), *Desmodesmus* sp.(37-80), *Scenedesmus* sp. (33), *Scenedesmus obliquus* (59)	Biodegradation and photodegradation	Gojkovic *et al.,* (2019)
Diclofenac	*Chlorella sorokiniana* (30),(40-60), *Chlorella vulgaris* (21), Mixed consortia (55), (92)	Photodegradation, biodegradation	Escapa *et al.,* (2015), de Wilt *et al.,* (2016), Villar-Navarro *et al.,* (2018), Matamoro et al. 2016
Estriol	*Scenedesmus dimorphus* (85)	Biodegradation	Zhang *et al.,* (2014)

Estrone	Mixed consortia (85), *Scenedesmus dimorphus* (85)	Biodegradation	Zhou *et al.,* (2014) , Zhang *et al.,* (2014)
Flecainide	*Chlorella sorokiniana* (71), *Chlorella vulgaris* (100), *Chlorella saccharophila* (100), *Coelastrella* sp. (52), *Coelastrum astroideum* (66), *Desmodesmus* sp. (72-96), *Scenedesmus* sp. (40), *Scenedesmus obliquus* (93)	Photodegradation	Gojkovic *et al.,* (2019)
Diphenhydramine	*Chlorella sorokiniana* (73), *Chlorella vulgaris* (98), *Chlorella saccharophila* (93), *Coelastrella* sp. (87), *Coelastrum astroideum* (87), *Desmodesmus* sp. (88-92), *Scenedesmus* sp. (86), *Scenedesmus obliquus* (85)	Biodegradation	Gojkovic *et al.,* (2019)
Fluconazol	*Desmodesmus* sp. (33)	Bioadsorption	Gojkovic *et al.,* (2019
Fluoxastrobin	*Synechococcus* sp.	Bioadsorption	Stravs *et al.,* (2017)

Hydroxyzine	*Chlorella sorokiniana* (76), *Chlorella vulgaris* (93), *Chlorella saccharophila* (93), *Coelastrella* sp. (80), *Coelastrum astroideum* (96), *Desmodesmus* sp. (87-100), *Scenedesmus* sp. (73), *Scenedesmus obliquus* (95)	Biodegradation	Gojkovic *et al.,* (2019)
Ibuprofen	*Chlorella sorokiniana* (100), *Nannochloris* sp. (40), *Navicula* sp. (60), Mixed consortia (98), (99)	Bio-uptake and biodegradation	de Wilt *et al.,* (2016), Bai and Acharya (2016), Ding *et al.,* (2017), Hom-Diaz *et al.,* (2017) , Matamoro *et al.*, 2016
Metoprolol	*Chlorella sorokiniana* (100), *Chlamydomonas reinhardtii*, *Dictyosphaerium* sp. (99)	Biodegradation	de Wilt *et al.,* (2016), Stravs *et al.,* (2017), Gentili and Fick (2017)
Naproxen	Mixed consortia (10-70), (89)	Biodegradation	Hom-Diaz *et al.,* 2017, Matamoro *et al.,* 2016

2.4.1 Bioadsorption

This process occurs passively (No energy required) and rapidly (Vidyashankar and Ravishankar, 2016). Since bioadsorption is a non-metabolic process, EC binding to the microalgal surface happens on both living and dead microalgal cell surfaces because the majority of the contaminant receptors in cells are still functional even after the cell has died (Choi and Lee, 2015). The bioadsorption of compounds by microalgae occurs either by adsorption onto cell wall components or by adsorption onto organic substances (e.g., extracellular polysaccharides (EPS) (Kaplan, 2013; Saavedra *et al.,* 2018). The negatively charged functional groups included in the EPS, such as sulfydryl (SH), phosphate (PO^3), 4-carboxyl (COO), and hydroxyl (OH), bind randomly to metal cations found in the aquatic environment (Pereira *et al.,* 2011).

Multiple binding groups, including OH, SH, COO, PO_3, NO_3, RNH_2, RS, and RO, facilitate the metal ion adsorption, the cell surface, the cytoplasm, and particularly the vacuoles all contain these binding groups (Zeraatkar *et al.,* 2016). The surface of the algal cell undergoes several chemical reactions during bioadsorption, including adsorption, ion exchange, surface complexation, chelation, and micro-precipitation (Dönmez *et al.,* 1999; Schmitt *et al.,* 2001).

The chemical composition of an ECs affects its capacity to adhere to microalgal cell surfaces. Through electrostatic interactions, hydrophobic, cationic ECs are actively drawn to the surface of the microalgal cell while hydrophilic ECs are repelled (Xiong *et al.,* 2017).

Compared to living microalgae, the nonliving microalgae have several advantages, such as: none of the contaminants are toxic to them; the bioadsorbed ECs can be removed by the use of a suitable desorbing agent, desorbed bioadsorbed ECs and microalgal biomass can be reused; operational costs, such as growth media, are substantially reduced since no microalgal culture maintenance is necessary (Mane *et al.,* 2011, Dixit and Singh, 2014). A potential strategy for improving ECs bio-adsorption by the microalgae is to modify the cell surface through pre-treatment techniques that are either physical (such as grinding, thermal drying, steaming, and lyophilization) or chemical (such as acid and alkaline conditions) (Sutherland and Ralph, 2019). For the medication tramadol, Ali et al. (2018) showed that chemically modified microalgal biomass (0.1 N NaOH) had a 70% greater bioadsorption rate than unmodified microalgal biomass.

Optimizing temperature, algal growth conditions, and exposure time are further methods for improving the bioadsorption of ECs by microalgae. Changes in temperature will affect the rate of ECs adsorption onto the surface of the microalgal cell because bioadsorption is a thermodynamic process. Whether the process is endothermic or exothermic will determine how temperature impacts the adsorption rate. Increased temperature has the opposite effect on exothermic processes than it does on endothermic processes, slowing down the rate of bioadsorption in the case of exothermic sorption activities (Sutherland and Ralph, 2019).

2.4.2 Bio-uptake (biosorption)

The contaminants enter the cell through the cell wall during bio-uptake, where it binds to intracellular proteins and other substances. Contrary to adsorption, bio-uptake of pollutants into the cell happens over the course of hours to days and only in living microalgal cells. Three primary ways of EC absorption by microalgal cells exist: (1) passive diffusion, (2) passive facilitation diffusion, and (3) energy-dependent/active uptake across the cell membrane (Sutherland and Ralph, 2019)

Since ECs passively diffuse through the membrane from a high (external) concentration to a low (internal) concentration, the cell is not required to expend any energy. Low molecular weight ECs that are non-polar and lipid soluble may theoretically diffuse past the cell membrane since the membrane is hydrophobic; however, polar molecules, molecules with high molecular weight, and ions cannot pass through passively. Two examples of ECs bio-uptake through passive cell membrane diffusion are the accumulation of the antibiotics triclosan and triclocarban by the filamentous green alga *Cladophora* sp. and the anti-epileptic drug carbamazepine by the green alga *Raphidocelis subcapitata* (Coogan *et al.,* 2007; Vernouillet *et al.,* 2010).

Passive-facilitated diffusion is the process where ECs diffuse across the cell membrane with the help of transporter proteins, whose role is to mediate the influx of polar molecules into the cell. The final mechanism is active transport of the ECs across the cell membrane, which requires the use of energy by the cell. Often in active transport, the compound moves against a concentration gradient, although this is not always the case. Regardless of the mechanism, bio-uptake affected by the physico-chemical environment, including temperature and pH, the metabolic state, or health, of the cell, and the presence of any metabolic inhibitors (Wilde and Benemann, 1993).

According to various research (e.g., Gattullo *et al.,* 2012; Maes *et al.,* 2014; Bai and Acharya, 2016), several lipophilic medicines are removed by microalgae mostly by absorption.

One difficulty with bio-uptake is that the accumulation of ECs inside the microalgal cell may lead to an excess of reactive oxygen species, which could cause oxidative damage to biomolecules, cellular malfunction, and ultimately cell death (Zhang *et al.,* 2011). The lifecycle stage may also influence the amount of ECs that is taken up by the cell. Lee et al. (2019) observed that the highest bio-uptake rates of the radionuclide caesium by the green alga *Haematococcus pluvialis* occurred when the cells were in the red cyst stage, while the lowest uptake rates occurred in the flagellate stage. Changes in the number of cellular potassium transporters at the different lifecycle stages is

thought to explain the measured differences in the caesium uptake rates by this species (Lee *et al.,* 2019).

The capacity of living algae cells to treat industrial wastewater depends on their development rate, which directly affects the concentration of their biomass and, in turn, their overall capacity for metal ion biosorption (Volesky, 2007). Several physical/chemical processes that alter the characteristics of the algal cell surface to create more binding sites can increase the amount of heavy metal ion uptake by algal biomass. Physical modifications to algal biomass, such as heating/boiling, freezing, crushing, and drying, typically result in an increased amount of metal ion biosorption (Zeraatkar *et al.,* 2016).

$CaCl_2$, formaldehyde, glutaraldehyde, NaOH, and HCl are the most frequently used algal pretreatments. Calcium binding to alginate as a result of $CaCl_2$ pretreatment is crucial for ion exchange (Rincón *et al.,* 2005; Bishnoi *et al.,* 2007). In particular, hydroxyl groups and amino groups are crosslinked more strongly by glutaraldehyde and formaldehyde (Ebrahimi *et al.,* 2009; Dabbagh *et al.,* 2008) While HCl denatures proteins (Srinivasa Rao *et al.,* 2005) or replaces light metal ions with a proton (Romera *et al.,* 2006), NaOH increases the electrostatic interactions of metal ion cations and creates the ideal circumstances for ion-exchange (Romera *et al.,* 2006).

2.4.3 Immobilized algae

Supporting materials for biomass immobilization can be natural biopolymers (like agar and alginate) or synthetic substances (like silica gel and polyacrylamide) (Bayramoglu and Yakup Arica, 2009; Bayramoğlu *et al.,* 2006; Mehta and Gaur, 2001). According to studies (Eroglu *et al.,* 2015), immobilized algae have a higher biosorption capacity than free algal cells and prevents biomass loss during the biosorption cycle (see Table 4).

The immobilization of biomass increases the capacity for photosynthetic activity (Bailliez *et al.,* 1986) and lessens the toxicity of particular chemicals (Bozeman *et al.,* 1989). Additionally, Al-Rub et al. (2004) found that it enables the repeated employment of algal cells during subsequent sorption/desorption cycles of metal ions bio removal from aqueous solutions.

Table (4): Comparison of biosorption capacity of metal ions using immobilized algal biomass vs. living algae.

Algae species	Immobilization system	Initial metal ion conc. (mg L^{-1})	Metal ion	Max. sorption (mg. g^{-1}) Living algae	Max. sorption (mg. g^{-1}) Immobilized algae	Reference
Chlamydomonas reinhardtii	Ca - alginate	500	Cd(II)	28.9	79.7	(Bayramoğlu *et al.,* 2006)
Chlorella sorokiniana	Loofa sponge	300	Cr(III)	58.8	69.26	(Akhtar *et al.*, 2008)
Scenedesmus quadricauda	Ca - alginate	600	Cu(II)	35.9	75.6	(Bayramoglu and Yakup Arica, 2009)
C. reinhardtii	Ca - alginate	500	Cu(II)	35.9	106.6	(Bayramoğlu *et al.,* 2006)
C. sorokiniana	Loofa spong	200	Ni(II)	48.08	60.38	(Akhtar *et al.*, 2004)

C. vulgaris	Blank alginate	100	Ni(II)	15.6	28.6	(Al-Rub *et al.*, 2004)
S. quadricauda	Ca - alginate	600	Ni(II)	9.7	30.4	(Bayramoglu and Yakup Arica, 2009)
C. reinhardtii	Ca - alginate	500	Pb(II)	230.5	308.7	(Bayramoğlu *et al.*, 2006)
S. quadricauda	Ca - alginate	-	Zn(II)	20.2	55.2	(Bayramoglu and Yakup Arica, 2009)

2.4.4 Bioaccumulation

In living microalgal cells, the active process (need energy) of bioaccumulation allows for the active uptake of contaminants, where they bind to internal proteins or other substances (Xiong *et al.,* 2018). In bioaccumulation, metal ions are accumulated inside the microalgal cells. When these ions are accumulated inside the algal cell organelles (vacuoles or thylakoids) (Zohoorian *et al.,* 2020). Not all contaminants that have been bioadsorbed onto the surface of microalgae can penetrate the cell and bioaccumulate (Wu *et al.,* 2012).

These processes take a long time to complete, starting with the uptake of metal ions and ending with the transport of those ions inside of the cell or any individual cell organelle. Ion-selective transport proteins found on the cell membrane aid in the transfer of metal ions (Arunakumara and Zhang, 2008)

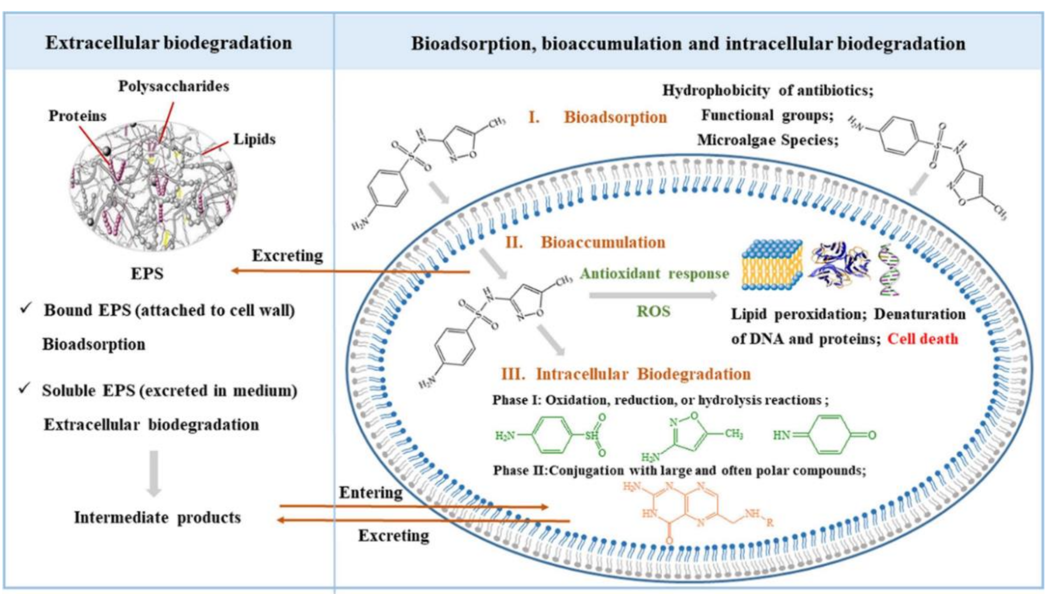


Fig. 2.8. Mechanisms involved in the removal of antibiotics by microalgae (Xiong *et al.,* 2021).

Antibiotics got into microalgal cells by passive diffusion during the elimination of trimethoprim, sulfamethoxazole, florfenicol, and carbamazepine, which led to bioaccumulation (Bai and Acharya, 2017, Song *et al.,* 2019, Xiong *et al.,* 2016).

Physicochemical conditions, including temperature, pH, contact time, and contaminant concentration, affect bioaccumulation (Xiong *et al.,* 2021). For example bioaccumulation of antibiotics in planktonic food webs was shown to be significantly influenced by temperature (Tang *et al.,* 2020). Furthermore, carbamazepine bioaccumulation in *Chlamydomonas mexicana* and *Scenedesmus obliquus* increased with increasing carbamazepine concentrations and cultivation times (Xiong *et al.,* 2016).

Moreover, the accumulation of antibiotics in microalgal cells might result in an overproduction of reactive oxygen species (ROS), including free radicals, hydroxyl radicals, perhydroxyl radicals, and alkoxy radicals, as well as nonradical forms (hydrogen peroxide (H_2O_2), and singlet oxygen (O_2) (Kurade *et al.,* 2016, Xiong *et al.,* 2018).

In general, ROS keep cells functioning at a baseline level, and they also serve as signal transducers (Zandalinas and Mittler, 2018). Due to their strong oxidative properties, ROS increased in microalgae after exposure to antibiotics, causing DNA and proteins denaturation, and causing cellular damage, mutagenesis and cell death (Kumar *et al.,* 2016). Antibiotics in microalgae and associated risks, it is imperative to control the concentrations of antibiotics (Xiong *et al.,* 2021).

2.4.5 Biodegradation

One of the most promising strategies for pollutant cleanup is the biodegradation or biotransformation of ECs by microalgae. Contrary to bio adsorption and bio uptake, which just serve as biological filters to concentrate and remove EC from the surrounding aqueous solution, biodegradation entails the breakdown of complex chemicals into simpler breakdown components by catalytic metabolic degradation (Sutherland and Ralph, 2019).

The complex process of biodegradation involves the breakdown of organic substances, either by biotransformation, which results in the production of several metabolic intermediates (Achermann *et al.,* 2018, Xiong *et al.,* 2019, Xiong *et al.,* 2020). or by pure or mixed microbial cultures through complete mineralization to CO_2 and H_2O (Alvarino *et al.,* 2016, Bouju *et al.,* 2012).

There are two main ways that microalgal biodegradation can take place: either through metabolic degradation, in which the ECs acts as the microalga's carbon source or electron donor/acceptor, or through co-metabolism, in which the EC is broken down by enzymes that are catalyzing the breakdown of other substrates already present (Tiwari *et al.,* 2017).

Some microalgal species can use mixotrophic growth strategies for metabolic degradation, where organic carbon and dissolved inorganic carbon are both taken up at the same time. This enables them to co-exist autotrophic and heterotrophic metabolisms. The microalgal biodegradation process can take place either intracellularly or extracellularly, or it can combine both methods, with the breakdown products first degrading extracellularly before being further degraded intracellularly (Tiwari *et al.,* 2017).

Extracellular degradation depends on the expulsion of enzymes into the EPS to serve as an external digestive system, whereas intracellular degradation depends on the bio-uptake of the ECs by the cell. In order to boost the bioavailability of the ECs for subsequent uptake by the cell, the EPS can also function as a surfactant and emulsifier (Xiong *et al.,* 2018). Phase I and Phase II enzyme families are both involved in the complex enzymatic process of microalgal biodegradation of ECs. Cytochrome enzymes, such as monooxygenase, dioxygenase, hydroxylase, carboxylase, and decarboxylase enzymes, are involved in phase I of biodegradation (Thies *et al.,* 1996; Pflugmacher and Sandermann, 1998).

These enzymes' primary function during biodegradation is to increase a contaminant's hydrophilicity by adding or removing hydroxyl groups via hydrolysis, oxidation, or reduction processes (Xiong *et al.,* 2018). In Phase II, enzymes such glutathione-S-transferases catalyze the conjugation of glutathione with a variety of substances having electrophilic centers, resulting in the opening of the epoxide ring to prevent oxidative damage to the cell (Xiong *et al.,* 2018).

Numerous enzymes have been identified as playing a part in the cellular defense, deactivation, and/or destruction of a variety of chemical compounds that cause cellular stress in microalgae (Wang *et al.,* 2019). These include catalase, glutamyl-tRNA reductase, mono(di)oxygenase, pyrophosphatase, carboxylase/decarboxylase, dehydratase, alkaline and acid phosphatase, transferase, and hydrolases (Elbaz *et al.,* 2010; Xiong *et al.,* 2018; Wang *et al.,* 2019). When the cells were treated to human and veterinary antibiotics, several of these enzymes, including superoxide dismutase and catalase, showed elevated activity in a variety of microalgal species (Aderemi *et al.,* 2018; Wang *et al.,* 2019).

The specific function of the several enzymes in the Phase I and Phase II enzyme families is not fully understood, and microalgal biodegradation of ECs is thought to be a highly complex process (Xiong *et al.,* 2018). The extracellular glycoprotein laccase is one enzyme that is believed to contribute to the biodegradation of pollutants by microalgae, multi-copper oxidases known as laccase glycoproteins catalyze the one-electron oxidation of a variety of substrates, including mono- and poly-phenols and aromatic amines, to radicals, which may then be subjected to cross-linking or depolymerization processes (Claus, 2004, Otto and Schlosser, 2014).

Have been found to be essential for the biodegradation of both phenol and synthetic dyes by microalgae (Kılıç *et al.,* 2011, Otto and Schlosser, 2014). In cultures of the cyanobacterium *Phormidium valderianum* during the biodegradation of phenol, increased secretion and activity of laccase and polyphenol oxidase have been observed (Shashirekha *et al.,* 1997), while laccase-mediated biodegradation of industrial dyes has been observed in the green alga *Gonium* sp. (Kılıç *et al.,* 2011). However, it has also been demonstrated that laccase plays no part in the microalgal biodegradation of other substances, such as ρ-chlorophenol, even if the responsible enzymes have not yet been identified (Forootanfar *et al.,* 2013).

With several investigations in Table 4 it is clear that biodegradation is mainly responsible for the removal of many ECs (Sutherland and Ralph, 2019). For example, microalgal biodegradation of the hormone progesterone and norgestrel has been successfully demonstrated in the two freshwater microalgae, *Scenedesmus obliquus* and *Chlorella pyrenoidosa* (Peng *et al.,* 2014), while Hom-Diaz et al. (2015) reported biodegradation of the hormones 17 β-estradiol and 17ά-ethinyles- tradiol by the microalgae *Selenastrum capricornutum* and

Chlamydomonas reinhardtii. In the microalgal transformation of progesterone and norgestrel, Peng et al. (2014) identified the primary processes as reduction (hydrogenation), hydroxylation, oxidation (dehydrogenation), and side-chain breakdown (Xiong et al. 2017).

Since most antibiotics are difficult to biodegrade because of their pharmacological stability (Martin-Laurent *et al.,* 2019) or resistance (Khetan and Collins, 2007; Xu *et al.,* 2011).

2.4.6 Photodegradation

One of the primary transformation mechanisms for antibiotic residue in natural water settings, particularly in surface layers, is photodegradation (Doll *et al.,* 2003; Dabic *et al.,* 2019). most antibiotics are extremely sensitive to light (Bonvin *et al.,* 2013; Baena-Nogueras *et al.,* 2017; Tian *et al.,* 2018). Depending on the pollutant structure, photodegradation can take place by direct photolysis or indirect photo-oxidation. When light is absorbed by a target molecule and used as energy to break the chemical bonds inside it, this process is known as direct photolysis. The importance of this mechanism depends on the light absorption spectrum of the pollutant under consideration, which can be affected by pH, temperature, and interaction with other molecules including ions and organics (Jiang *et al.,* 2010; Beliakova *et al.,* 2003).

Direct photodegradation is the process by which antibiotics are directly triggered to break down or degrade when they absorb light (Zepp and Cline, 1977; Wammer *et al.,* 2013; Tian *et al.,* 2018). In contrast, indirect photodegradation relies on oxidant species produced by photosensitizers under light such as hydroxyl radicals (•OH), singlet oxygen ($^1O_2$), superoxide (O_2^-), hydrogen peroxide (H_2O_2), and peroxyl radicals (OOR•) (Li and Hu, 2016; Tian *et al.,* 2018). Indirect photolysis has a reaction rate that is substantially faster than direct photolysis and is mostly responsible for the elimination of organic compounds (Xu *et al.,* 2011; Tian *et al.,* 2018). In one example, the norfloxacin degradation rate in a solution of *C. vulgaris* exposed to light is three times higher than that in a solution devoid of algae (Zhang *et al.,* 2012).

The indirect photodegradation of antibiotic residue by algae depends on the active oxidizing agents produced by photosensitizers (Wei *et al.,* 2021). Many components found in algal solutions, including extracellular organic matter (EOMs), enzymes, and chlorophyll, have the ability to operate as photosensitizers (Zhang *et al.,* 2012; Tian *et al.,* 2018). Extracellular organic matter (EOM) and intracellular organic matter (IOM) are the two major categories for algal organic matters (AOMs) (Li *et al.,* 2012; Lee *et al.,* 2018).

In photodegradation, EOMs frequently take the lead by generating active species when exposed to light (Tenorio *et al.,* 2017; Tian *et al.,* 2018; Tian *et al.,* 2019). This may be due to the proteins, carbs, and humic-like material that make up EOMs. (Henderson *et al.,* 2008; Li *et al.,* 2012; Qu *et al.,* 2012). The dissolved components of EOMs have a large role in photodegradation. For instance, the photolysis process benefits from the photochemical reactivity of humic and fulvic dissolved organic materials (DOMs) (Zuo *et al.,* 1996; Franke and Franke, 1999).

The word "DOM" refers to a variety of organic substances, including molecules like hemicellulose, hydrophilic organic acids, and fulvic and humic acids.

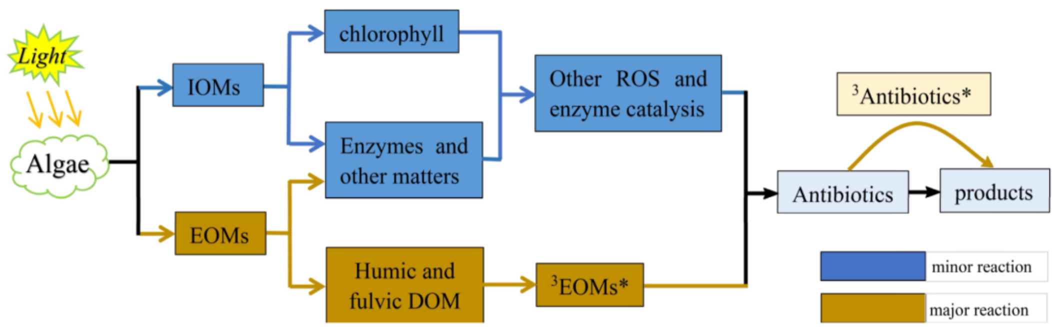


Fig. 2.9. The possible schematic for mechanism of the degradation of antibiotics in alga-containing water with irradiation (Wei *et al.,* 2021).

DOM is released by microalgae into the surrounding culture medium, and this substance may contribute to photodegradation enhancement through a variety of mechanisms, such as catabolic processes, redox cycling, the generation of hydroxyl radicals, or the inhibition of photo-oxidation through competitive reaction with radicals (Van Trump *et al.,* 2006, Norvill *et al.,* 2016).

According to Zhang et al. (2012), norfloxacin can be photodegraded by using the DOMs that are generated by irradiated algae as a photosensitizer . DOMs from algal EOMs are likely the primary catalyst for induced antibiotic photodegradation because Tranvik and Kocalj (1998) reported that they have a strong capacity to absorb photons. It is therefore logical to conclude that this is the case. Algal EOMs produce reactive oxidizing species (ROS) which start the photodegradation of antibiotics (Wei *et al.,* 2021).

The type of algae employed may have an impact on how effectively antibiotics are photolyzed by algae. Ge and Deng (2015) investigated the photodegradation of two fluoroquinolone drugs (enrofloxacin and ciprofloxacin hydrochloride) by two algae species (*Platymonas subcordiformis* and *Isochrysis* *galbana*). The photodegradation of the two medicines was aided by both algae. The average photolysis rate was improved by *P. subcordiformis* to 76.1%, compared to 68.7% with *I. galbana* treatment (Ge and Deng, 2015).

The physico-chemical characteristics of the pollutant, the intensity and wavelength of the light exposure, as well as the physico-chemical characteristics of the water body, all affect how quickly ECs photodegrade. In microalgal treatment systems, a higher concentration of dissolved organic molecules (DOM) may also promote photodegradation (Wei *et al.,* 2021).

The elimination of the pharmaceutical drug ibuprofen in a microalgal bioreactor may have been accomplished through indirect photodegradation in the presence of microalgal dissolved organic materials (de Wilt *et al.,* 2016).

2.4.7 Volatilization

The release of volatile organic compounds into the atmosphere from the liquid phase is known as the volatilization of ECs. the procedure is influenced by the physico-chemical characteristics of the ECs of interest (such as Henry's law constant) as well as the treatment system's operational parameters (such as aeration or agitation rates, temperature, and atmospheric pressure) (Tran *et al.,* 2018).

Microalgal-based purification techniques High sunshine and temperatures (in comparison to traditional wastewater treatment systems), along with high aeration rates provided by mixing devices (such as paddlewheels, bubble lift columns, and stirrers), may help increase the removal of volatile ECs (Wei *et al.,* 2021). For hydrophobic, volatile chemicals, such as musk scents, Matamoros et al. (2015) discovered that volatilization occurred during the summertime operations where both sunshine and temperatures were higher (Matamoros *et al.,* 2015).

Chapter (3)

Algae-based renewable energy production

3.1 Algae-based renewable energy

The environmental problems brought on by traditional fossil fuels and the rising need for energy can both be addressed by algae-based renewable energy. Algae are straightforward aquatic plants that can flourish in a variety of aquatic environments, including freshwater, saltwater, and wastewater. This makes them a more secure, unfocused, and effective type of plant. Algae use a variety of mechanisms to fix harmful gases like carbon dioxide and produce biomass out of sunshine, nitrogen, and phosphorus. Algae have been investigated for a variety of uses, such as food, bioactive compounds, and biofuels (Sulieman *et al.,* 2020; Gawali and Jadhav, 2022; Du, 2020).

Three fossil fuels—oil, coal, and natural gas—provide more than 80% of the energy we use today. The burning of fossil fuels is responsible for about 98% of carbon emissions. Using less fossil fuels would result in a significant decrease in the emissions of carbon dioxide and other pollutants. This can be done by either using less energy overall or by switching to renewable fuels in place of fossil fuels. So, the use of alternative energy sources is expected to increase in the future (Demirbas and Demirbas, 2011).

Algae-based biofuels are thought to be a more environmentally friendly substitute for fossil fuels than petroleum-derived fuel. When compared to fossil fuels, algae-based biofuels can lower greenhouse gas emissions by 30 to 50%. Algae are a viable source of biofuels since they can repair greenhouse gases and have a high capacity for producing lipids (Gawali and Jadhav, 2022; Nandan, 2020). Consumers are growing increasingly interested in algal biomass and its applications in a variety of algae-based products. Algae of some species are nutritious and suitable for human consumption. Moreover, they are a component in livestock feed and can be transformed into organic fertilizers. A new spectrum of goods is available through the extraction of biologically active substances from algae that can be employed in the food, pharmaceutical, cosmetic, and agricultural industries. Due to their distinct composition, interest in algae is constantly growing. They are well to be a rich source of biologically active substances, including lipids, proteins, carbs, minerals, polyunsaturated fatty acids, and antioxidants (Michalak and Chojnacka, 2018).

3.2 Algae as sustainable energy source

The need for a better quality of life results in regular use of non-renewable energy sources. The usage of conventional fuel results in the release of greenhouse gases, particulate matter, and CO_2 into the environment (Verma and Mishra, 2020). Algae can serve as a source of renewable energy since it can be directly transformed into fuels like biodiesel, bioethanol, and biomethanol. Due to algae's greater output non-edible oil extraction and rapid growth without having to compete for land with food production, there is increased interest in the manufacture of biodiesel from algae.

Renewable energy sources are becoming more essential to the achievement of sustainable energy and environmental protection due to the drawbacks of fossil fuels (Najafi *et al.,* 2011). The conventional biofuels that are now produced from agricultural products like corn, sugarcane, oilseed plants, and some animal fats are also being replaced by these algae oils. They don't require a lot of farming, unlike oilseed crops, so they help preserve agricultural society lands available for food crops (Verma and Mishra, 2020).

3.3 Promising Fuels from Algae

Crude algae oil is a promising resource for renewable energy. It can be used directly as fuel or as a raw material for the production of other fuels such as diesel, biodiesel, jet fuel, and gasoline. Additionally, anaerobic digestion of biomass yields biogas, while algal carbohydrate-derived sugars can be fermented to create bioethanol. Another potential application is the production of biohydrogen. Algae is a renewable resource that can be grown quickly and sustainably, making it an attractive alternative to fossil fuels. Many scientists and companies are working to develop cost-effective ways to produce algal biofuels, which have the potential to reduce our reliance on non-renewable energy sources and mitigate the impacts of climate change. (Choo *et al.,* 2020; Demirbas and Demirbas, 2011).

3.3.1 Biodiesel

The mono-alkyl esters of vegetable or animal fats are what are referred to as biodiesel. Trans esterifying the source oil or fat to make biodiesel results in a product with a viscosity that is similar to that of petroleum diesel. Transesterification refers to the chemical process that transforms oil into the fatty ester that corresponds to it (biodiesel). Biodiesel is a type of biofuel that is typically made from methyl esters that are produced through the transesterification of organic oils from plants or animals. The transesterification reaction for biodiesel is fairly straightforward as shown in Fig (1) (Demirbas and Demirbas, 2011).

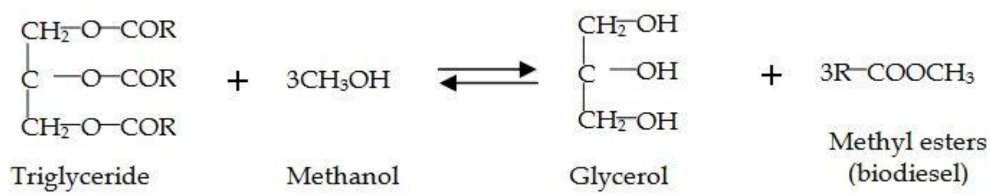


Fig 3.1. Reaction of biodiesel (Romero et al., 2011).

There are numerous well-known industrial techniques that can be used to produce biodiesel from microalgae, with base catalyzed transesterification with alcohol being the most popular. A triglyceride-containing fat or oil reacts reversibly with an alcohol to produce fatty acid alkyl ester and glycerol (Bajhaiya *et al.,* 2010). A method for producing algal biodiesel is shown in Fig (2). Numerous variables need to be taken into account and optimized at each stage, such as energy and material inputs (such as nutrients and energy for mixing during development) and the proper handling of waste products, such as used medium and leftover biomass (Scott *et al.,* 2010).

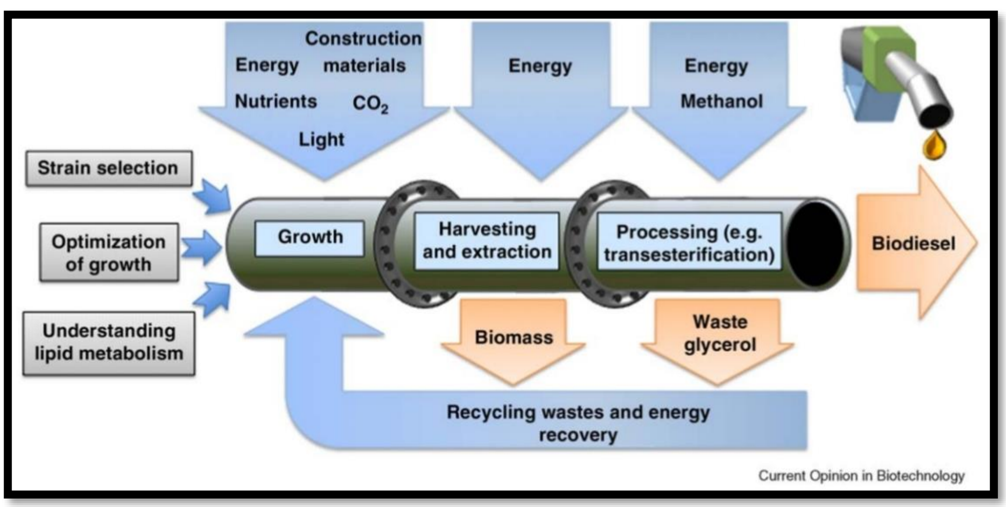


Fig 3.2. illustrating the key steps in the production of algae-based biodiesel (Scott *et al.,* 2010).

3.1.1.1 The advantages of algae biodiesel

Algae-based biofuels have several environmental benefits. Firstly, they can minimize the negative impact on the environment and land use. Secondly, they can reduce and significantly control pollution in the environment (Smith *et al.,* 2010). Thirdly, due to their ability to thrive all year long, algal oil productivity is higher than that of conventional oil seed crops. Finally, they degrade spontaneously and quickly through biodegradation, making them somewhat harmless for the environment.

It is not necessary to use herbicides or insecticides when growing algae. Algae is a renewable resource that can be grown quickly and sustainably, making it an attractive alternative to fossil fuels. Many scientists and companies are working to develop cost-effective ways to produce algal biofuels, which have the potential to reduce our reliance on non-renewable energy sources and mitigate the impacts of climate change (Datta *et al.,* 2019).

3.3.2 Biogas

When compared to kerosene, coal, diesel, and biomass solid fuels, biogas produced through anaerobic digestion emits less nitrous oxide, particulate matter, and GHG (Wiley *et al.,* 2011). Methane (CH_4) and carbon dioxide (CO_2) are the two main components of biogas, although it also typically contains hydrogen sulfide (H_2S) and other sulfur compounds (Ramarai and Dussadee, 2015). Methane from 55 to 75% and CO_2 from 25 to 45% make up the majority of biogas. Particularly in rural locations, biomethane from the biogas can be used as a fuel for heating, electricity generating, and transportation (Oncel, 2013).

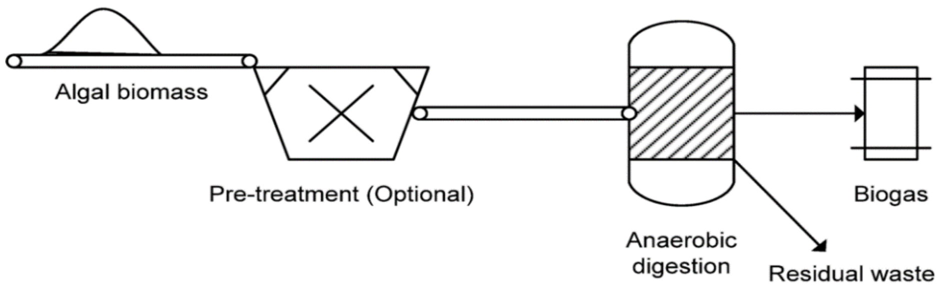


Fig 3.3. Simplified biogas generation from algal biomas (Aitken and Antizar-Ladislao, 2012).

3.3.3 Biohydrogen

A potential sustainable energy source and viable replacement for fossil fuels is the creation of biohydrogen. When the sustainability of the global population began to be negatively impacted by the limited availability of fossil fuels and the pollution they generated, interest in the large-scale generation of biohydrogen began to grow. Because biological processes primarily operate at room temperature and pressure, they require less energy than chemical or electrochemical systems do to make hydrogen.

Biophotolysis and photosynthesis-hydrogen generation using a variety of microbial species are the main areas of attention in the current development of algal hydrogen production Photosynthetic microorganisms like cyanobacteria and green microalgae need sunlight to produce hydrogen (Show *et al.,* 2019; Shaishav *et al.,* 2013). As a potential clean, renewable, and environmentally friendly energy source, bio-hydrogen shows promise.

With little energy use, bio-hydrogen can operate at room temperature and pressure. The four main classes of biohydrogen production techniques can be roughly characterized as indirect biophotolysis, direct biophotolysis, dark fermentation, photo fermentation, and a combination of these processes (Saifuddin and Priatharsini, 2016; Chandrasekhar *et al.,* 2015).

Based on its reliance on light, the creation of biohydrogen from algae follows particular different methods. While dark fermentation takes place in the absence of light, biophotolysis and photofermentation both use light to produce hydrogen (Pathy *et al.,* 2022). Significant obstacles to present biological hydrogen generation systems include High cost Low H_2 productivity Expensive photobioreactors for biophotolysis, High-cost Expensive bioreactors Needs high intensity light for photofermentation, and Low chemical oxygen demand (COD) removal Incomplete substrate conversion Difficult fermentive substrate utilization for dark fermentation (Kumar Gupta *et al.,* 2013).

3.4 Production of biofuel using algae

The term "biofuel" refers to any hydrocarbon fuel produced quickly from organic matter (living or extinct organisms). There will be a 40% increase in demand for fossil fuels between 2010 and 2040. Hence, in order to meet our energy needs, the necessity for alternate energy sources has been researched. Solar, wind, and biomass-based biofuels are examples of renewable energy sources (Vaishnav, 2021). One of the most important sources of sustainable biofuels for the coming of renewable energy is algae.

Algae are an adaptable feedstock that can metabolize a variety of disposal streams, including urban sewage and carbon dioxide from industrial exhaust gases, to create a wide range of goods with variable composition and purposes. These materials include lipids, which can be converted into biodiesel, carbs, which can be converted into ethanol, and proteins, which can be consumed by both humans and animals (Menetrez, 2012).

Algae, in contrast to other biomass sources, has a high biomass production for each unit of light and area. has a high starch or oil content, doesn't need fresh water or land for farming, and meets the criteria for nutrients, it can be satisfied by either seawater or wastewater. Many chemical compounds, including lipids and carbohydrates, are produced by algae. It is possible to obtain gasoline using these biomolecules recognized as biofuel. Aquatic life forms known as algae can be either unicellular or multicellular.

Although the concept of manufacturing microalgal biofuel is not new, it is currently receiving serious consideration in light of the rising cost of petroleum. Concern over global warming and its connection to the consumption of fossil fuels is another factor driving serious attention (Rajkumar *et al.,* 2014).

The adaptability of these little organisms is actually their genuine defining characteristic Fig (4). Algae are capable of producing a wide range of biofuels and are able to survive and adapt to a wide range of environmental conditions (Jones and Mayfield, 2012).

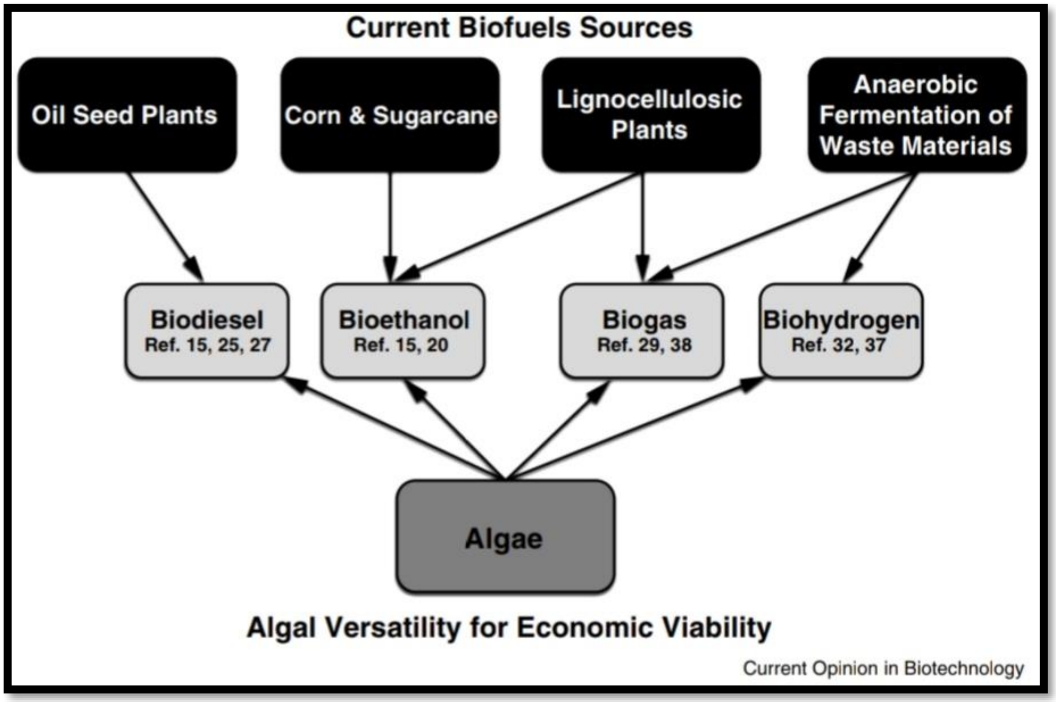


Fig 3.4. shows current plant resources biofuels compared to algae (Jones and Mayfield, 2012).

These biofuels can now be made from a variety of plant resources, although many of them compete with food production. Algae are one type of photosynthetic organism that may provide a wide range of biofuels applications that will surely support other, less flexible systems. References displayed demonstrate their adaptability (Jones and Mayfield, 2012).

3.4.1 Generation of Biofuels

Biofuels can be classified into three generations. The first generation of biofuels uses cultivated plants such as sugarcane, sugar beet, maize, palm, soybean, and sweet sorghum as feedstocks. Plant sugars or starches are fermented with yeast to produce bioethanol, and plant oils are extracted to produce biodiesel.

However, these processes have negative impacts on the food and water sectors and the environment. The second generation of biofuels uses non-food plants such as Jatropha, grass, switchgrass, silver grass, and non-edible parts of current crops. The third generation of biofuels uses algal biofuel, which emerged as a promising alternative to reduce excessive usage of hazardous pesticides, water use, and land use. Algae can be grown quickly and sustainably, and algal biofuels have the potential to reduce our reliance on non-renewable energy sources and mitigate the impacts of climate change (Vaishnav, 2021).

3.4.2 Strain selection for biofuel production

Because of the enormous diversity of the group, which has evolved over billions of years, finding the perfect algae strain for biofuel production is made easy. The potential genetic variety of algae is orders of magnitude greater than that of land plants or animals, yet this richness has just lately been studied. This potential is evident in the variety of algae species being investigated for fuel production, including green algae, diatoms, and cyanobacteria.

Algal strains must be able to withstand changes in salinity, temperature, light, and pathogen load in agricultural environments. To ensure their widespread use, strains that are designed for production in different environments and during different seasons will be required.

Industrial bioreactor habitats are more stable than open ponds because they can be managed. To ensure efficient operations, it will be crucial to find algal strains that grow rapidly to high cell densities and generate large amounts of lipids while consuming the least amount of energy. Any culture system will experience some degree of contamination, and since strict sterilization will almost definitely be prohibitively expensive, strains will need to be sufficiently resistant to infections in order to produce large yields (Georgianna and Mayfield, 2012). They are capable of producing triacylglycerols (TAGs). TAGs are thought of as a viable feedstock for making biodiesel. They have the capacity to synthesize enormous quantities of lipids, ranging from 20 to 50 percent of dry weight. Several species of microalgae produce lipids to varying degrees (Bharathiraja et al., 2015).

Table (5): Oil content of microalgae (Chisti, 2007).

Microalgae oil content	**(% dry wt.)**
Botryococcus braunii	23-75
Chlorella sp.	28-32
Crypthecodinium cohnii	20
Cylindrotheca sp.	16-37
Dunaliella primolecta	23
Isochrysis sp	25-33
Monallanthus salina	>20
Nannochloris sp.	20-35
Nannochloropsis sp.	31-68
Neochloris oleoabundans	35-54
Nitzschia sp.	45-47
Phaeodactylum tricornutum	20-30
Schizochytrium sp	50-77
Tetraselmis suecica	15-23

Both the algal growth rate and the oil content of the biomass affect oil productivity, which is the mass of oil generated per unit volume of microalgal broth each day. For the production of biodiesel, high oil productivity microalgae are preferred. Many lipids, hydrocarbons, and other complex oils are produced by microalgae depending on the species (Chisti, 2007). The sector for algae biofuels is no longer viable economically due to a variety of technical obstacles.

The most essential prerequisite for efficient algal biofuel production is fast-growing strains with high oil yield. Also, it's necessary to create and optimize oil extraction techniques and conversion technologies.

Now a days analyzing the possibility of microalgae as a third-generation biofuel feedstock. The various procedures for producing biofuel from microalgae are depicted in Fig (5) (Pragya *et al.,* 2013).

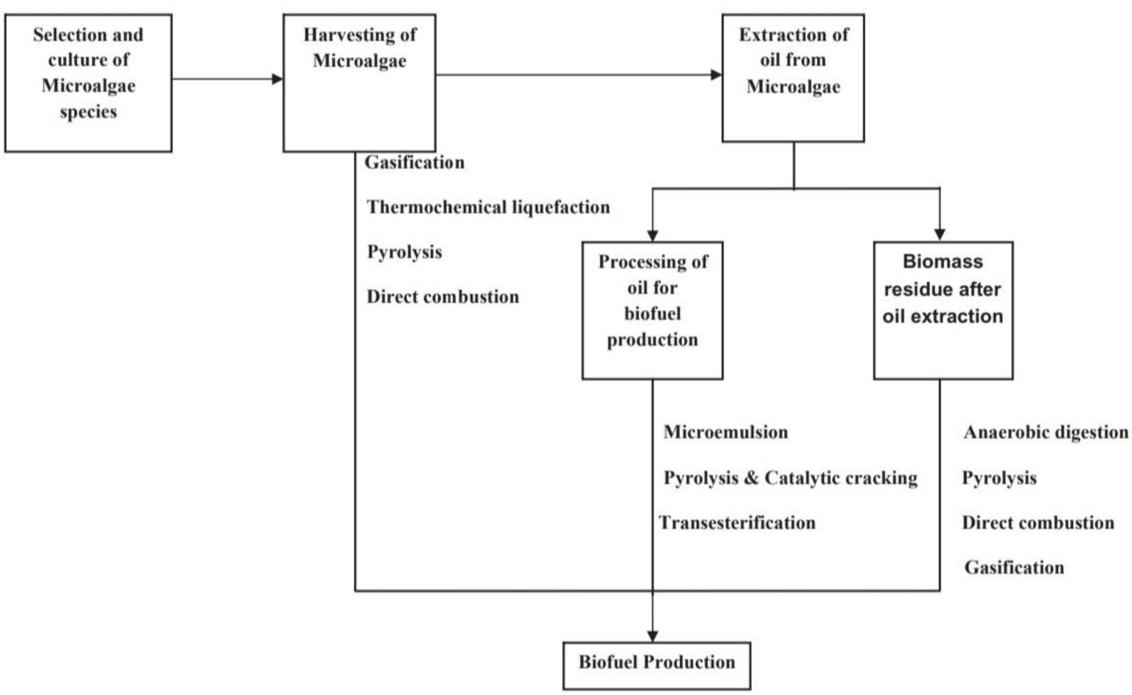


Fig 3.5. The process of turning algae into energy involves several processes (Pragya *et al.,* 2013).

3.4.3 Methods for harvesting microalgae

Water removal from the algal culture is necessary for further processing of microalgal biomass into biofuels. 20% to 30% of the entire production costs are attributable to harvesting alone. Therefore, an effective harvesting technique is crucial for the mass production of biodiesel (Rawat *et al.,* 2011; Amaro *et al.,* 2011).

The method of harvesting microalgae can typically be broken down into two steps. Bulk harvesting, the initial stage of that process, separates microalgal biomass from the bulk culture. The algal slurry is further concentrated in the second step, thickening. The energy cost of thickening exceeds that of bulk harvesting (Chen *et al.,* 2011).

3.4.3.1 Harvesting techniques

3.4.3.1.1 Centrifugation

Centripetal acceleration is used in the harvesting process to divide the algal culture into areas of higher and lower density. The algae and water are then separated by draining the extra medium (Harun *et al.,* 2010).

3.4.3.1.2 Gravity sedimentation:

Using this technique, suspended particles form concentrated slurry, which is then separated from the fluid above, by gravity. It is frequently used to separate microalgae from water and is very energy efficient (Rawat *et al.,* 2011).

3.4.3.1.3 Filtration

In this process, filters that hold back algae while allowing water to pass through them are used to cultivate algae. The procedure is repeated until there is a thick algal paste in the filters (Harun *et al.,* 2010).

3.4.3.1.4 Flocculation

The process of flocculation involves the association of solute particles in a solution to create floc aggregates, which aid in settling.

To balance out this negative charge on the surface of algae, chemicals known as flocculants are used. Flocculants shift the negative charge and enable microalgae cell aggregation. Flocculation improves harvesting effectiveness when paired with filtering or sedimentation (Rawat *et al.,* 2011; Harun *et al.,* 2010).

3.4.3.1.5 Electrolytic process

Microalgae travel toward the anode during this process, when their surface charge is neutralized and the cells subsequently group together to form aggregates. This method is quite effective and removes between 80% and 95% of the algae cells (Chen *et al.,* 2011).

3.4.3.1.6 Flotation

Air or gas bubbles connect to solid particles during the gravity separation process known as flotation, which subsequently lifts the solid particles to the liquid's surface. Microalgae can be harvested more successfully and advantageously by flotation than by sedimentation (Rawat *et al.,* 2011).

3.4.3.1.7 Electrophoresis techniques

Without the need for any chemical additions. Charged algae are induced by an electric field to leave the solution. The microalgal flocs are attracted to and carried to the surface by hydrogen produced by the electrolysis of water (Chen *et al.,* 2011).

3.4.4 Extraction of lipid/oil for biodiesel production

Oil extraction follows harvesting. After being extracted, the lipid is transformed into biodiesel. Lipid extraction can be carried out via solvent extractions, physical procedures, chemical methods, or a mix of the two. The extraction process should be quick, scalable, efficient, and gentle on the lipids being extracted (Maceiras *et al.,* 2011; Rawat *et al.,* 2011).

3.4.4.1 Oil and lipid extraction techniques

3.4.4.1.1 Method of solvent extraction

Using this technique, solvents are used to extract the oil from the algae. Lipids can interact in a variety of ways, and these interactions must be overcome for effective extraction. The two most used techniques for extracting lipids from algal biomass are Soxhlet extraction and Bligh and Dyer's method. Hexane is used in the Soxhlet method and a mixture of chloroform and methanol is used in the

Bligh and Dyer's procedure to extract lipids (Rawat *et al.,* 2011; Kim *et al.,* 2012).

3.4.4.1.2 Extraction of supercritical carbon dioxide (SC-CO_2)

It is one of the innovative green technology approaches that holds out the possibility of replacing the conventional organic solvent lipid extraction techniques. A typical extraction device comprises of an oven module-installed feed pump for compressing and delivering liquid CO_2 to the extraction vessel as well as a heated micro-metering valve to depressurize incoming SC-CO_2 (Pragya et al., 2013).

Table (6): efficiency of various oil extraction techniques (Pragya *et al.,* 2013).

Algae species	Technique	% of oil extracted
***Nannochloropsis* sp.**	SC-CO_2	25
Spirulina platensis	SC-CO_2	77.9
***Chlorococcum* sp.**	SC-CO_2	81.7
***Chlorococcum* sp.**	Soxhlet	45
Chlorella vulgaris	Bligh and Dyer's method	10.6

3.4.4.2 Factors affecting extraction

The selection and effectiveness of harvesting methods for microalgae are influenced by various inherent characteristics of the cells. These features include density, surface charge, size, shape, hydrophobicity, salinity of the surrounding medium, adhesion and cohesion properties, as well as settling or floating velocities, all of which are significant factors when it comes to separation (Griffiths *et al.,* 2011).

3.4.5 Biofuel production from microalgal biomass

The biomass of microalgae can be changed by biochemical and thermochemical processes to produce biofuel. Fig (6) depicts the process by which microalgae biomass is converted into biofuel and its byproducts (Choo *et al.,* 2020).

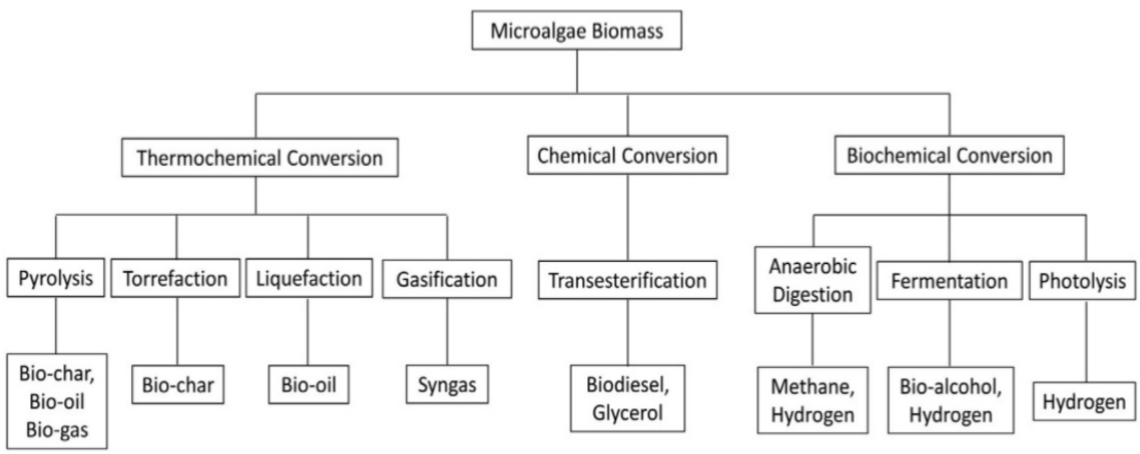


Fig 3.6. the process by which microalgae biomass is converted into biofuel and its byproducts (Choo *et al.,* 2020).

3.4.5.1 Conversion methods for algae-based biofuels

3.4.5.1.1 Transesterification

Transesterification yields biodiesel in the form of fatty acid methyl esters (FAMEs). Triacylglycerol or triglyceride is transformed into FAME (biodiesel), a non-toxic, low-molecular-weight, less viscous fuel that can be used right away in engines. A quick procedure that combines lipid extraction and transesterification in one step is direct transesterification, also known as in situ transesterification. By changing the reaction equilibrium toward greater FAME yields and improving extraction effectiveness, this single-step technique outperforms the conventional two-step method in terms of FAME yield. Chemical solvent serves as both the reactant for the transesterification reaction and a solvent for removing lipid from microalgae cells. Through in situ transesterification, using n-hexane and chloroform as solvents, methanol as the reactant, and sulfuric acid as the catalyst, respectively, the yield of more than 98% FAME was achieved.

By employing hexane as a cosolvent and 0.5 M sulfuric acid at 90C for 2 hours, *Chlorella pyrenoidosa*, which has a lipid content of 56.2%, produced 95% biodiesel (Suganya *et al.,* 2016; Kiran *et al.,* 2014; Rawat *et al.,* 2013).

3.4.5.1.2 Fermentation

A metabolic process known as fermentation uses microorganisms to turn organic substrates like sucrose, bagasse, cellulose, or starch into ethanol.

Depending on the need for oxygen during the process, a process can be either (1) aerobic or (2) anaerobic (Lee and Lee, 2016). Microalgae with a reasonably high concentration of carbohydrates can be fermented to make bioethanol.

Although most microalgae species have very few carbohydrates, cultivating conditions like nutrient levels and light source can be changed to alter the algae's biomass composition. The microalgae species *C. vulgaris* is perfect for producing bioethanol since it has a high starch content (around 37%) and can convert ethanol by up to 65% (Shuba and Kifle, 2018).

Microalgae biomass or starch must first undergo pretreatment to be milled into sugars before being combined with *Saccharomyces cerevisiae* yeast and water in a fermenter to undergo fermentation. After fermentation, the diluted alcohol that results is pumped to a holding tank for distillation. The distillation process can generate concentrated ethanol with a 95% purity level, which is appropriate as an additive or a replacement for transportation fuel (Choo *et al.,* 2020).

3.4.5.1.3 Pyrolysis

Without oxygen or air, pyrolysis is a thermal process that converts dry biomass into solid fuel (biochar), liquid fuel (biooil), and gaseous product at a temperature of 400 to 600 C and atmosphere pressure. The medium-low calorific value of the biofuel that is produced through the pyrolysis process is the goal. The pyrolysis process can be categorized into many modes as indicated in Table 7, depending on the operating state (temperature, heating rate, and duration) (Choo *et al.,* 2020).

Table (7): Various Pyrolysis Modes (Choo *et al.,* 2020).

Pyrolysis mode	Temperature (°C)	Heating rate (°C/min)	Residence time
Slow	400	0.1-1.0	>10 s
Fast	580-1000	10-200	0.5-10 s
Flash	700-1000	>1000	<0.5 s

Biochar, which can be utilized for power generation, carbon sequestration, and fuel utilization, is produced via slow heating rates and prolonged heating times. Greater liquid and gas yield (around 70%–80%) is achieved with fast pyrolysis compared to that in slow pyrolysis (15%–65%), as a result of the faster heating rate and shorter duration in fast pyrolysis. Microalgae biomass's quick pyrolysis is simpler and more effective than that of other lignocellulosic biomass because it lacks phenolic chemicals (Choo *et al.,* 2020).

3.4.5.1.4 Torrefaction

The thermochemical conversion process known as torrefaction uses biomass to create coal fuel (biochar). Torrefaction produces liquids and gases as byproducts, but solid coal fuel is the primary end product. Biochar is a carbon-rich material made from biomass that is created thermally from organic feedstock under an oxygen-free environment (Sukiran *et al.,* 2017).

3.4.5.1.5 Liquefaction

The liquefaction process, which yields biooil, is carried out at low temperature typically between 250 and 350 °C and high H_2 pressure typically between 5 and 20 MPa. This method is ideal for moisture feedstock like microalgae since it doesn't require drying because it has a very high-water content (>80%) (Choo *et al.,* 2020).

3.4.5.1.6 Biophotolysis

Because it is renewable, emits no CO_2 when burned, has a higher heating value (HHV) per unit mass (141.65 MJ/kg), and can be converted into electricity by fuel cells, hydrogen gas is a very versatile and effective fuel energy carrier. At the moment, hydrogen is created by the reforming of fossil fuels, which produces

9 kg of CO_2 for every kilogram of H2 produced as well as minute amounts of nitrogen dioxide and sulfur dioxide.

When these air pollutants enter the atmosphere without being properly treated, acid rain will form as a result. A technique for producing hydrogen gas from microorganisms is known as biohydrogen production. In biological electrolysis, which is a component of photosynthesis, water molecules are split into proton (H^+) and oxygen (O_2).

In the usual photosynthesis route, hydrogen reacts with carbon dioxide to produce glucose and oxygen. However, when anaerobic conditions exist or a particular type of microalgae is present, hydrogen is produced during photosynthesis rather than carbohydrates. Many microalgae species from the *Chlamydomonas, Chlorella, Scenedesmus, Tetraspora,* etc. genus can produce hydrogen using the hydrogenase enzyme.

Biohydrogen can be produced using three techniques. The first technique is direct biophotolysis, which involves the splitting of water's protons and electrons at photosystem II (PSII) to produce hydrogen. The second technique is indirect biophotolysis, in which the breakdown of internal carbon molecules produces electrons and protons. The third technique is a fermentation process in which H and H2 are the products of a reaction between electrons produced by the oxidation of cellular endogenous substrate. These methods require intensive research to make biological H2 production economically viable without requiring large energy inputs. The energy sources for biohydrogen production include solar energy, organics, and electricity (Choo *et aal.,* 2020).

3.5 The advantages of algal biofuel

Algae biofuel is a promising alternative to fossil fuels due to its environmental benefits. Algae biofuel cuts greenhouse gas emissions by 30 to 50% when compared to fossil fuels (Gawali and Jadhav, 2022). Microalgae are easy to cultivate in difficult conditions and have low production costs, making them a viable source of bioenergy. A lot of microalgae are photosynthetic, which means they use CO2 and sunlight to build biomass, helping to cut down on greenhouse gas emissions (Alazaiza *et al.,* 2023). Algae biofuel avoids competing for resources like land, fertilizer, and water with food production. Algae can also be cultivated using wastewater as a source of water and nutrients, which can also be used to recover nutrients from the wastewater (Alala, 2022). Algae can synthesize lipids more effectively than plants using sunlight. Algae biofuel is a sustainable, carbon-free kind of transportation that can help meet the world's growing energy demand (Durakovic and Memon, 2016).

3.6 The disadvantages of algal biofuel

Algal biofuel has a lot of benefits, but there are some drawbacks as well. The high cost of manufacture, which restricts its economic feasibility, is one of the main drawbacks (Nandan, 2020; Hu *et al.,* 2021). A significant quantity of land, water, and fertilizers are also needed for the large-scale growth of algae for the generation of biofuels, which may have an adverse effect on the environment (Sameera *et al.,* 2011). Another drawback is that other industries, including agriculture and aquaculture, compete for resources, which can result in disputes over the use of land and water (Gawali and Jadhav, 2022). At last, finding the best algal species for biofuel production is still difficult because there have only been a few thousand algae species evaluated as potential sources of biofuel, and none of them were perfect (Sameera *et al.,* 2011).

3.7 What are the challenges facing the large - scale industrialization of algae biofuel?

The excessively high cost of algal biofuel, which restricts its commercial viability, is one of the difficulties facing its large-scale manufacturing. To tackle this problem, effective ways to enhance the production of algae biofuels on a wide scale and their use in real world applications are required (Hu *et al.,* 2021; Saad *et al.,* 2019).

Other difficulties include finding the optimal algal species for the generation of biofuels as well as increasing the productivity and economic viability of algae by increasing their carbon efficiency and photosynthetic efficiency (Saad *et al.,* 2019; Laurens *et al.,* 2017). Additionally, in order to satisfy the high biomass requirements for the manufacture of biofuels, it is crucial to create methods that are practical and allow for the economical, effective, and high-density growing of algae (Nandan, 2020).

3.8 Algae-based renewable energy production via photosynthesis

A promising substrate for the generation of bioenergy is microalgal biomass. Microalgae are an appropriate biomaterial to make biodiesel, bioethanol, biohydrogen, methane, and bioelectricity. Fig. 3.7 shows the many options for producing biofuel from microalgae.

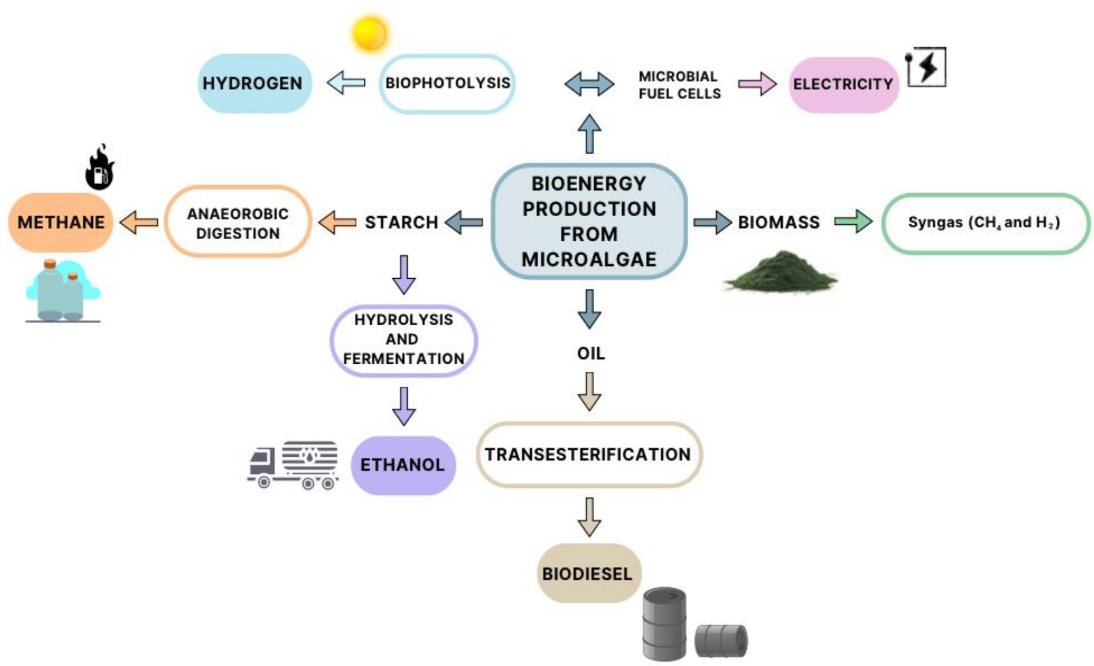


Fig. 3.7. Scheme of bioenergy production from microalgae.

3.8.1 Biohydrogen

The primary substance for producing energy is fossil fuel, which is used to supply energy needs. However, it is a finite resource, and usage rates are correlated with greenhouse gas (GHG) emission rates (Patel *et al.,* 2014). As a result, in recent years, there has been an increase in study interest in finding clean, non-toxic, and sustainable alternatives to fossil fuels as a source of energy. Energy carrier hydrogen (H_2) has a high calorific value of 122 kJ/g and a 2.75-fold higher heating efficacy than hydrocarbon fuels (Patel *et al.,* 2014; Tommasi *et al.,* 2008).

In addition, the only byproduct of the burning of H_2 is water (H_2O). It has been used in recent years to generate energy using fuel cells or internal combustion engines. Although H_2 is not a naturally occurring substance on Earth, it is suitable as a clean and renewable fuel. It can be created through steam reforming, which uses syngas, natural gas, coal, and waste biomass to generate 50% of the total H_2 produced (Oey *et al.,* 2016). However, because they require a high temperature (970-1100 K) and produce large amounts of CO_2, these processes are not regarded as environmentally friendly on a commercial basis (Medisetty *et al.,* 2020; Kothari *et al.,* 2008).

Additionally, gasification (of coal and biofuels), electrolysis of water, partial oxidation, pyrolysis, and biological processes all contribute to the production of H_2 (Sharma and Arya, 2017). Photosynthetic microorganisms, including microalgae, generate biologically renewable hydrogen through fermentation and bio photolysis (microbial photolysis). As a result, compared to thermochemical processes, it is a cost-effective or environmentally friendly procedure.

3.8.1.1 Bio photolysis pathways of H_2 production by microalgae

There is evidence that certain species such as *Chlamydomonas reinhardtii, Chlorella fusca, and Scenedesmus obliquus* can generate H_2 through either direct photolysis (DP) or indirect photolysis (IP) mechanisms (Choi *et al.,* 2011; Mithuna *et al.,* 2013; Oncel *et al.,* 2014). The capacity of H_2 production through bio photolysis relies on the propensity of microorganisms, environmental factors, enzymes, metabolic pathways, and biorefinery process (Show *et al.,* 2018).

Microalgae make biomolecules by following oxygenic pathways. In this, salts are necessary for its metabolic activity, and H_2O serves as a growth medium, CO_2 as a carbon supply, and light as an energy source. Light is essential in the photolytic process of H_2 generation in microalgae. Chlorophyll (a) or (b) and other light-harvesting complexes primarily absorb light and assist in fixing carbon, while particular enzymes carry out the procedure (Oh *et al.,* 2011; Show *et al.,* 2018). Table 9 provides a summary of the various biological techniques and their advantages and disadvantages.

Table (9): Advantages and disadvantages of different microalgal H_2 production methods, their brief biochemical mechanisms **(**Goswami *et al.,* 2021).

Biological Mechanism	**Biochemical reaction**	**Duration**	**Yield in Range**	**Advantages**	**Shortcomings**
Direct photolysis DP-H_2	H_2O + light→ H_2	5–10 days (depends on microalgal species)	1.2–73.5 mL L^{-1}	Utilizes H_2O and sunlight Not required energy or ATP	Low yield Sensitivity to O_2
Indirect photolysis IP-H_2	CO_2 + H_2O + light → Glucose+ low O2 → H_2	148–168 h	6.625–243 mL L^{-1}	Overcome O_2 sensitivity Microorganism cultured in simple medium (sulphur deprived media) Inexpensive technique	Production capability is low Only perform by few microalgae and cyanobacteria

Photo-fermentation PF-H_2	Biomass→ biomolecules→ light + PNS bacteria→ H_2	96–120 h	125.0 mL g^{-1}VS	The most efficient process compares to other modes High substrates transformation efficacy compares to the DF Utilizes waste products for cultivation of microorganisms	The requirement of the light source Required large area and specialized anaerobic bioreactor PF-H_2 yield is low
Dark Fermentation DF- H_2	Biomass→ biomolecules→ DF bacteria→ H_2	48–72 h	16.2–135 mL H_2 g^{-1} VS	It does not depend on the O_2 Produces a wide range of organic acid as by-products Not require light source for accomplished of process	It required pre-treatment process, Substrates conversion efficiency is lower compare than PF, Produces CO_2, CO and H_2S

3.8.1.2 Direct photolysis H_2 (DP-H_2) production

DP is a naturally occurring mechanism that is also known as light-dependent pathways and is initiated by microalgae. Equations 1a and 1b (Eq. 1a and 1b) describe the photochemical oxidation reaction as taking place in two stages (Show *et al.,* 2018). In the first step the photosystem II (PSII) is activated by sunlight. H_2O is then split into proton (H^+), electron (e^-), and oxygen (O_2) with the aid of the enzyme H_2O -plastoquinone oxidoreductase, which is found in the thylakoid. Finally, the electron is moved across the photosystem I (PSI) and ferredoxin (Fd) from PSII. In the second step, moving e^- to Fd to [Fe-Fe]-hydrogenase causes to create the molecule DP-H_2. Using light energy as a power source, e moves.

Additionally, ATP synthase uses the proton gradient created by H^+ (which is created by the oxidation of H_2O) to make ATP (Show *et al.,* 2018). This mechanism is very helpful because it can be controlled by solar energy and does not require extra nutrients (carbon sources) or ATP for the stability of the process. It is an enzyme-dependent process that is efficient in anaerobic conditions or low oxygen levels because [Fe-Fe]-hydrogenase is oxygen-sensitive (Show *et al.,* 2018).

$$2H_2O + 4H^+ + \xrightarrow{\text{Sunlight}} 4_{e^-} + O_2 \text{ ………………..…..…….. Eq. (1a)}$$

$$4H^+ + 4_{e-} + Fe_{red} \xrightarrow{\text{Hydrogenase}} 2H_2 + Fe_{ox} \text{ ……………… Eq. (1b)}$$

Here**, Eq. 1a**: Photosynthesis process; **Eq. 1b:** H_2 production biochemical reaction.

3.8.1.3 Indirect photolysis (IP-H_2) production

The IP-H_2 system, which is a two-step procedure as shown in Figure 1b, overcomes the DP limitations through a strategic strategy. In the first step, the microalgae fix CO_2 and then transform it into metabolites and internal carbon storage materials. It aids the microalgae in lowering in-situ O_2 concentrations that stop the electron transport chains (ETC) and create a prerequisite for the following stage. The second stage involves the degradation of biomolecules (the glycolytic and citric acid processes), which produces the electron. For the synthesis of IP-H_2, the electrons are transformed to plastoquinone and subsequently passed to the [Fe-Fe]-hydrogenase enzyme (Jiménez-Llanos *et al.,* 2020).

According to reports, the oxygenic photosynthesis process in PSII is inhibited by the sulphur-deficient medium, which lowers the level of oxygen (Anwar *et al.,* 2019). The absence of sulfur stops PSII activity, lowers photosynthetic activity, and shifts to respiration mode. The total anaerobic conditions are caused by the low in-situ O_2, which lowers the activity of mitochondrial respiration. The anaerobic environment encourages in-situ IP-H_2 generation (Show *et al.,* 2018). In Eqs.2a and 2b, the metabolic process that produces IP-H_2 is described (Jiménez-Llanos *et al.,* 2020).

$$12H_2O + 6CO_2 + \xrightarrow{\textbf{Light energy}} C_6H_{12}O_6 + 6O_2 + 6H_2O\text{…..………Eq.(2a)}$$

$$C_6H_{12}O_6 + 12H_2O + Fe_{red} \xrightarrow{\textbf{Hydrogenase}} 12H_2 + 6CO_2 + 6H_2O\text{…...…Eq.(2b)}$$

Here, **Eq. 2a:** Metabolism of glucose formation; **Eq. 2b:** IP-H_2 production in anaerobic condition.

The main barriers to the commercialization of microalgae based H_2 platforms are (a) low rates of productivity, (b) high costs of operation, (c) subpar process architecture, and (d) a lack of information about how to increase strain capacity. However, the combination of various methods can enable the production of renewable H_2 at a reasonable cost. Figure 3.8 presents examples of the various integration methods.

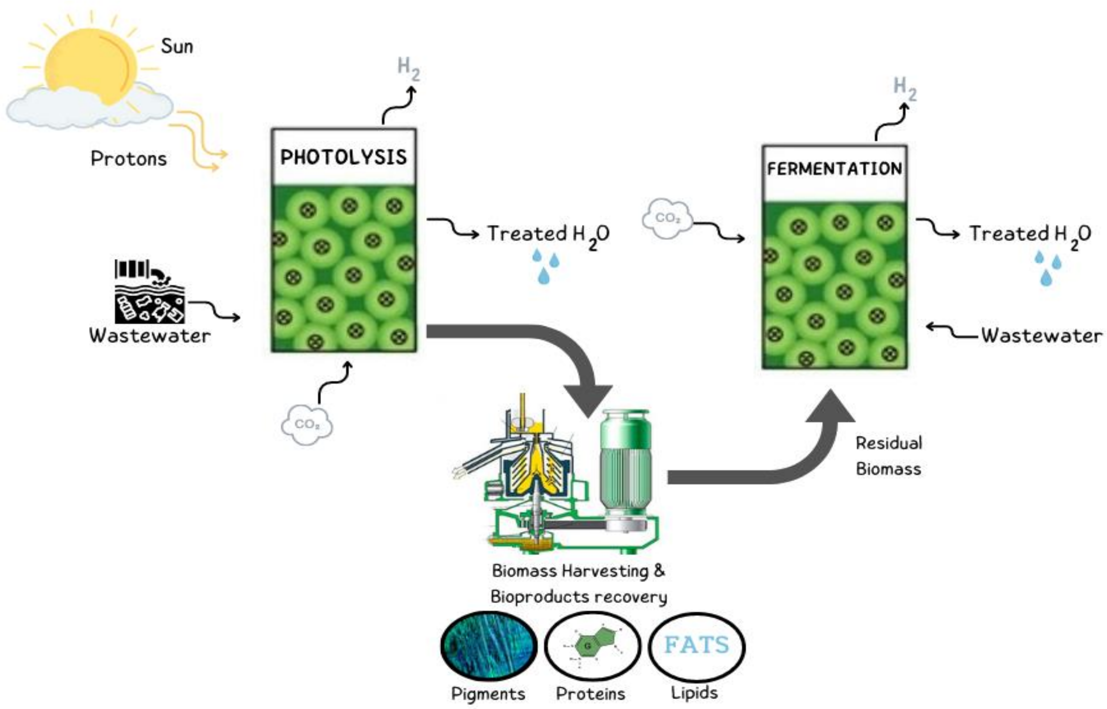


Fig. 3.8. Advance integrated approach for microalgal H_2 production.

3.8.2 Bioethanol

Bioethanol is another renewable biofuel that can be divided into three different types: first generation bioethanol, which is made from sugar and starch crops; second generation bioethanol, which is mostly made from lignocellulosic biomass; and third generation bioethanol, which is based on microalgae.

Microalgae are a different, less expensive source of substrate for the manufacture of bioethanol because they can store significant volumes of biomass made of carbohydrates (John *et al.,* 2011).

Many microalgae species, including *Spirulina, Chlamydomonas,* and *Chlorella*, have significant concentrations of glycogen starch (greater than 50% dry weight), which can be enzymatically changed into glucose and then into bioethanol (John *et al.,* 2011; Demirbas, 2011). However, various pre-treatment methods have also been employed, such as those utilizing acids, alkalis, or supercritical CO_2 (Lam and Lee, 2012).

Glucose is transformed into bioethanol and carbon dioxide in the following reaction:

$$C_6H_{12}O_6 \Rightarrow 2CH_3CH_2OH + 2CO_2 \quad \text{……………..……..……………..…Eq. (3)}$$

Numerous studies have supported the efficiency of microalgae grown on municipal, industrial, and swine wastewater to bioethanol production with concurrent wastewater treatment. According to study by Onay (2018), bioethanol was created from *Nannochloropsis gaditana* in diverse municipal wastewaters. With f/2 media as a control, microalgae were grown at 24 ± 2. The bioethanol yield of *Nannochloropsis gaditana* ranges from 70.3 ± 2.4 mg. g biomass^{-1} to 94.3 ± 5.5 mg. g biomass^{-1} in different municipal wastewaters (0, 30, 60, and 100%), with %30 wastewater having the greatest bioethanol yield (94.3 ± 5.5 mg. g biomass^{-1}).

In the study of Reyimu and Özçimen (2017), different ratios of seawater and municipal wastewater were used as a growth medium for cultivating *Nannochloropsis oculata* and *Tetraselmis suecica* under the same growth conditions. Additionally, the dry weight and carbohydrate content of several microalgal species were examined to assess the bioethanol productivity of these two microalgal strains, which were grown in the best wastewater medium. The findings show that *T. suecica* is well suited for ethanol production using municipal wastewater as a culture medium.

It was also investigated whether microalgae might accumulate carbs and extract nutrients from swine effluent. In 10% unsterilized swine wastewater, *Chlorella sorokiniana* AK-1 and *Chlorella vulgaris* ESP-31 were cultivated, and they produced maximum levels of 42.5% and 189 mg L^{-1} d^{-1} carbohydrates, respectively. The maximal carbohydrate productivity and total nitrogen removal efficiency of *C. vulgaris* ESP-31 were increased to 266 mg $L^{-1}d^{-1}$ and 54.2%, respectively, at 25% wastewater and 25% BG-11 concentration.

The yield and concentration of the bioethanol produced by the ethanol fermentation of the biomass were 84.2% and 4.2 gL^{-1}, respectively. Overall, unsterilized swine wastewater was shown to be a cost-effective nutrient source for microalgal growing, which further boosts the economic viability and environmental compatibility of bioethanol production with concurrent swine wastewater treatment (Acebu *et al.,* 2022).

Hirano *et al.,* (1997) studied the ethanol generation from more than 250 strains of microalgae as early as 1997. *Chlamydomonas reinhardtii* (UTEX2247), among them, was found to have the highest starch concentration (45%), followed by *Chlorella vulgaris* (IAM C-534) (37%). More recently, microalgae have been used to simultaneously make biodiesel and bioethanol since lipids are removed before the fermentation step. Lipids were isolated from *Chlorococum* sp. using supercritical CO_2 at 60 °C. After that, *Saccharomyces bayanus* ferments the sugars to produce bioethanol. In this situation, supercritical CO_2 functions as a dual agent extractor, removing both lipids and carbohydrates from the cell wall in a single process. This approach to large-scale production appears to be promising (Harun *et al.,* 2010).

3.8.3 Biodiesel

Recently, the production of biodiesel has received a lot of attention due to the global energy crisis. Microalgae are a good substitute for third generation biodiesel feedstocks since they have a better photosynthetic efficiency than conventional crops (Ahmad *et al.,* , 2011). Compared to diesel fuels made from petroleum, biodiesel made from microalgal lipid is more environmentally benign and sustainable. Microalgae have several benefits as feedstocks for biodiesel, including the ability to amass significant amounts of triacylglycerols, grow quickly, fix atmospheric CO_2, adapt to a wide range of conditions, including harsh ones, and use nutrients from wastewater (Hu *et al.,* , 2008).

According to studies by Li *et al.,* (2008) and Pittman *et al.,* (2011), integrating the production of microalgal biodiesel with wastewater treatment has the potential to reduce freshwater usage, lower the cost of adding nutrients for microalgal growing, and eliminate nitrogen and phosphorus from effluents. Microalgal oil content and composition vary depending on various microalgae and growth conditions, including temperature, nutrient availability, and light intensity (Converti *et al.,* , 2009; Solovchenco *et al.,* , 2008; Li *et al.,* , 2009, 2010). Under stress conditions including nitrogen shortage, phosphate restriction, and high Fe^{3+} concentration, the cellular lipid content of different types of microalgae considerably increased. (Liu *et al.,* , 2008; Khozin-Goldberg and

Cohen, 2006; Illman *et al.,* , 2000). The oil content of several microalgae, including *Scenedesmus* sp, *Chlorella* sp, and *Neochloris oleoabundans*, can reach 20% to 50% of total cell dry weight (Gouveia and Oliveira, 2009), demonstrating the great potential for biodiesel generation.

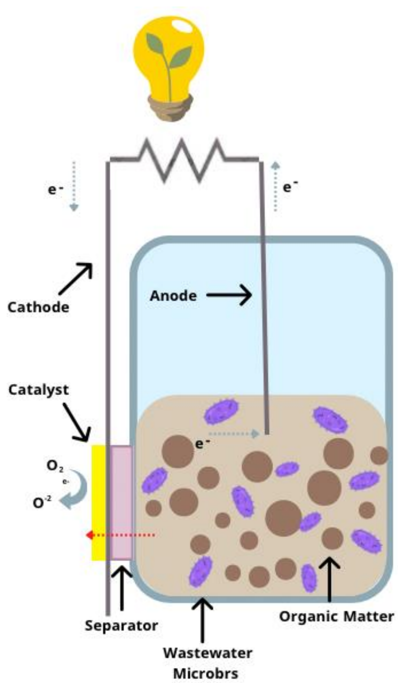
In the study of Chinnasamy *et al.,* (2010), It was discovered that growing a native consortium of microalgae in wastewater can convert 63.9% of the lipid harvested from those algae into biodiesel. Industrial plant wastewater has a complicated chemical makeup. Although industrial wastewater lacks carbon, it does include nitrogen and phosphorus, both of which are essential for the formation of algae. The purpose of this study was to find out whether it was possible to remove nitrogen and phosphorus from industrial wastewater and to identify a promising strain for using microalgae to produce renewable energy.

Microalgae have also been investigated for their capacity to remove nutrients from domestic wastewater (DWW) while generating biomass rich in lipids for the generation of biodiesel. Eight microalgae were grown in the (DWW) to assess their ability to remove nutrients and produce biomass. *Chlamydomonas reinhardtii* displayed the best performance, reducing the total phosphorus (TP) of DWW from 2 mg L^{-1} to 0.02 mg L^{-1} with a treatment efficiency of 99.15%. Treatment effectiveness increased to 99.07% for total nitrogen (TN). *Chlamydomonas reinhardtii* and *Chlorella pyrenoidosa* exhibited a decrease from 18.35 to 0.17 mg L^{-1}. *Chlorella sorokiniana* had the greatest lipid content, which was measured at 36.93%.

3.8.4 Bioelectricity

3.8.4.1 Microalgae used in MFCs

Microbial fuel cells (MFCs) are technologies that can cleanse wastewater while also producing energy. They use microorganisms to convert wastewater's organic matter's chemical energy into electricity. The cathode receives the electrons and protons generated at the anode as a result of microbial metabolism. At the cathode, platinum is typically used to catalyze the reduction of ambient oxygen, which then combines with protons to produce water (Hernández-Fernández *et al.,* 2015; Logan, 2009; Logan *et al.,* 2006). A typical air-cathode single-chamber MFC's schematic is shown in Fig. 3.9 It also exhibits the reactions that generally occur at both electrodes when the anodic microorganisms employ acetate as a substrate (Du *et al.,* 2007).



Anodic reaction

$$CH_3COO^- + 2H_2O \Rightarrow 2CO_2 + 7H^+ + 8e^- \quad \text{Eq. (4a)}$$

Cathodic reaction

$$O_2 + 4e^- + 4H^+ \Rightarrow 2H_2O \quad \text{Eq. (4b)}$$

Fig. 3.9. Scheme of an air-cathode single-chamber microbial fuel cell.

Using microalgae is one of the most innovative developments in MFCs. Microalgae can be utilized as a substrate at the anode to remove nutrients or to trap CO_2 produced in the cathode (Walter *et al.,* 2015; Salar-García *et al.,* 2016; Gajda *et al.,* 2015; Ieropoulos *et al.,* 2010).

Numerous studies over the past ten years have focused on the integrating of microalgae and MFCs.

In 2009, Powell *et al.,* (2009) created an MFC with *Saccharomyces cerevisiae* in the anodic chamber and *Chlorella vulgaris* in the cathode. The algae culture served as an effective electron acceptor and grew by utilizing the CO_2 produced in the cathode. With this setup, 2.7 mW/m^2 cathode could be produced.

However, a distinction between microalgae-MFCs and photo microbial fuel cells must be made. Photo microbial fuel cells can only function with light, but microalgae-MFC can function in both light and darkness (Xu *et al* 2015; Cui *et al.,* 2014). Microalgae MFC setups can be divided into single chamber, double chamber, or photosynthetic sediment types (see Fig. 3.10).

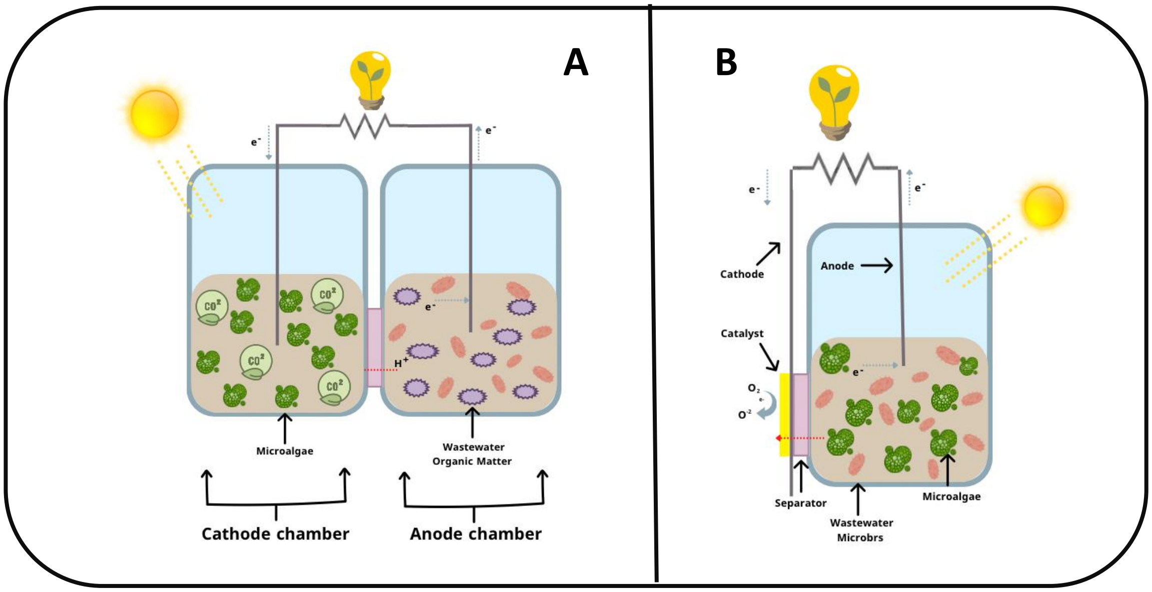


Fig.3.10. Scheme of two microalgae-MFC configurations: **A)** microalgae in the cathodic chamber to capture the CO_2 produced and **B)** microalgae as carbon source for microorganisms in the anodic chamber.

Several studies have created effective microbial fuel cells (MFCs) for combined bioelectricity generation and wastewater treatment.

Oscillatoria sp. and *Scenedesmus* sp. were two different kinds of microalgae that were utilized in the MFC as a biocathode. The experimental findings demonstrated that *Oscillatoria* sp. and *Scenedesmus* sp., respectively, had peak power densities of 32.5 ± 0.5 and 28.5 ± 0.3 mW/m^2. The findings of this study suggested that the development of energy-efficient wastewater treatment systems and the prospect of bioelectricity generation utilizing algae as a biocatalyst for economic feasibility (Naina Mohamed *et al.,* 2023)

According to Ribeiro *et al.,* (2022) Bioelectricity production and synthetic wastewater bioremediation were evaluated in a bench-scale microalgae-microbial fuel cell (MMFC) system. The studies were carried out in triplicate for 7 days with 3 different combinations: MMFC1 (*Escherichia coli* + *Desmodesmus subspicatus*), MMFC2 (*Pseudomonas aeruginosa* + *D. subspicatus*), and MMFC3 (E. coli + *Pseudokirchneriella subcapitata*). On the seventh day of operation, the MMFC 1 produced 560 mV (p 0.05) more bioelectricity than the MMFC 2 and MMFC 3. With a considerable reduction in total phosphorus (TP) and total organic carbon (TOC) after 7 days, the microalgae demonstrated a high bioremediation effectiveness. In conclusion, the MMFC 1 with *D. subspicatus* in the cathodic chamber and *E. coli* in the anodic chamber was the most successful in producing bioelectricity.

3.9 Algae-based renewable energy production via Anaerobic Digestion

Biomethane is one of the most promising alternative fuels that ability to facilitate the transition from the current fossil fuel-dependent energy to a sustainable energy future using anaerobic digestion (AD) process. Methane combustion produces less CO_2 than other hydrocarbon fuels while producing more heat per unit mass than other complex hydrocarbons (molecular mass to heat of combustion ratio: 16.0 g/mole to 891 kJ/mole) (Shuba and Kifle, 2018).

Microalgae can be a more appealing feedstock among these sources because of their five to ten times faster growth rate, high biomass yield, suitability for cultivation in nonarable lands and nutrient-rich wastewaters, and potential ability to use CO_2 and thereby reduce atmospheric CO_2 concentration (Stephens *et al.,* 2013; Kröger and Müller-Langer, 2012; Ward *et al.,* 2014). The end products of AD include a solid organic residue high in nitrogen and biogas mostly made of CH_4 (55–75%) and CO_2 (25–45%) (Li *et al.,* 2011; Harun *et al.,* 2010).

The AD's long-established method of producing biogas has many advantages, including minimal sludge generation, low prices, and minimal energy use (Adarme *et al.,* 2017).

There are two different types of AD that can be used to produce methane from microalgae or other biomass: liquid AD and solid-state AD. Although the fundamentals of both approaches are identical, they differ in terms of the physical characteristics of the system, particularly the moisture content of the substrate load (Li *et al.,* 2011). There are numerous steps that can be taken to produce methane from the AD of microalgae comprising cultivation, harvesting, pre-treatment, and then AD of the pre-treated microalgae (Fig. 3.11).

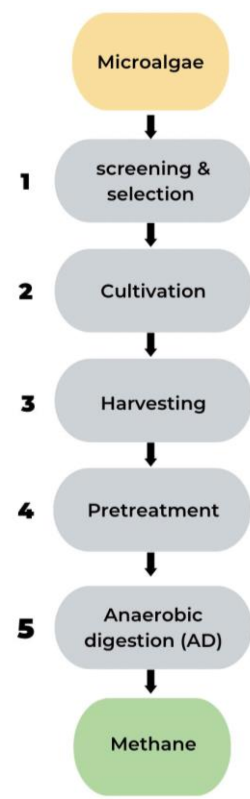


Fig. 3.11. Overview of methane production from microalgae through anaerobic digestion.

3.9.1 Cultivation of Microalgae

Most microalgae strains are strictly phototrophic, meaning that light is a necessary condition for the growth of microalgal cells. However, certain heterotrophic strains are light independent and can develop using organic substrates as a carbon source. Though autotrophic farming is frequently practiced, heterotrophic cultivation is acknowledged for having comparatively higher biomass productivity (Tijani *et al.,* 2015).

Numerous techniques, including batch, fed-batch, repeated fed-batch, semicontinuous, and continuous systems, can be used for microalgae cultivation (Tan *et al.,* 2018). In general, there are two main systems used to microalgal cultivation: closed photobioreactor systems and open raceway ponds. From a techno-economic standpoint, the former is more convenient than the latter.

However, the potential for undesirable microbial contamination in the raceway pond cultivation system is a bottleneck (Balat, 2011). For the phototrophic culture of a single microalgae species, photobioreactors offer controlled conditions. As a result, the cells can carry out the photobiological activities required for cell proliferation (Mata *et al.,* 2010). It also supplies the appropriate light intensity.

Table (10): Compares two microalgae cultivation methods: open raceway ponds, and photobioreactors. (Chisti, 2007) with the permission from Elsevier, copyright 2007.

Parameters	Photobioreactor facility	Raceway ponds
Biomass productivity (kg/year)	100,000	100,000
Volumetric productivity (kg/m^3/d)	1.535	0.117
Areal productivity (kg/m^2/d)	0.048	0.035
Biomass concentration (kg/m^3)	4.00	0.14
Dilution rate (/d)	0.384	0.250
Area needed (m^2)	5681	7828
CO_2 consumption (kg/year)	183,333	183,333
System geometry	132 parallel tubes/unit; 80-m-long tubes; 0.06 m tube diameter	978 m^2/pond; 12 m wide, 82 m long, 0.30 m deep
Number of units	6	8

3.9.2 Harvesting and Processing of Microalgae

Concentrating and separating mature biomass from the growth medium are the main steps in the harvesting of microalgae. Microalgae cultures contain extremely dilute dry matter, with dry matter concentrations ranging from 3–4 g/L in closed systems to 0.5–0.75 g/L in open pond systems (Fasaei *et al.,* 2018). The diluted biomass solution is subsequently concentrated to include between 10 and 25 % dry solids or more (Barros *et al.,* 2015).

Three strategies can be used to concentrate the microalgae cultures from the cultivation medium: (i) one-step harvesting and dewatering; (ii) one-step harvesting with a separate dewatering step; and (iii) one-step harvesting with two steps of subsequent dewatering (Fasaei *et al.,* 2018).

However, the second strategy, in which microalgal biomass is thickened in the first stage to a slurry typically containing 1-5% of biomass for aiding the separation of biomass from culture media, achieves the most typical and universal harvesting technique. The dewatering of the thickened biomass, which results in microalgal sludge with an average concentration of 20% biomass, is the key component of the secondary stage (Deconinck *et al.,* 2018).

Microalgal cultures can be concentrated by physical, chemical, or biological methods. The most promising physical techniques for destroying microalgal cells, for instance, include electrolysis and ultrasonic noises (Hincapié Gómez and Marchese, 2015; Bosma *et al.,* 2003). In the chemical concentration of microalgae, cultures are coagulated using various chemical compounds, including inorganic and organic ones, or the neutralization of the microalgal negative charge utilizing nanoparticles for enhanced coagulation (Deconinck *et al.,* 2018; Lee *et al.,* 2015).

The biological concentration of microalgae is typically dependent on the bio-flocculation that can be accomplished by introducing natural flocculation (Alam *et al.,* 2016). The concentrated microalgae biomasses are then dried for improved shelf life and handling convenience in the following bioprocessing phases.

3.9.3 Pretreatment of Microalgae

The microalgal biomass must undergo an additional pretreatment phase to make it more digestible before being subjected to the digesting step. As of now, numerous pretreatment techniques have been investigated for lignocellulosic biomass under the major categories of physical (mechanical and thermal), chemical, physicochemical, and biological approaches (Zabed *et al.,* 2017). These techniques are also used for microalgae pretreatment under the same circumstances (Cardeña *et al.,* 2017; Córdova *et al.,* 2018; Jankowska *et al.,* 2017).

Physical pretreatment techniques work by increasing the surface area and pore volumes of the microalgae biomass. This type of pretreatment typically entails mechanically shrinking the biomass, which is energy-intensive and ultimately unsustainable for commercial facilities (Zabed *et al.,* 2016c). Furthermore, size reduction alone is insufficient for effective conversion; other strategies must be used. Acids like H_2SO_4 and alkalis (like NaOH) are frequently utilized in the chemical pretreatment procedure (Zabed *et al.,* 2017c). However, in addition to needing acid or alkali, chemical pretreatment also needs high temperatures.

Although thermochemical pretreatment has been shown to be effective for increasing methane yield from microbial biomass (Penaud *et al.,* 1999), some techno-economic issues like reactor corrosion, the need to remove or neutralize chemicals, and energy consumption may make it difficult to implement on a large scale.

Another pretreatment strategy is biological pretreatment, which is seen to be more appealing than traditional pretreatment techniques since it makes use of naturally occurring microbes or their enzymes. According to Sindhu *et al.,* (2016) and Millati *et al.,* (2011), biological pretreatment has a number of advantages over other pretreatment techniques, including low energy requirements, simple equipment and operation procedures, no or minimal inhibitor formation, low downstream processing costs, and no need to recycle chemicals after pretreatment.

3.9.4 Anaerobic Digestion of Microalgae

The AD of microalgae biomass is the sequential internal redox process in which the organic parts of biomass decompose under the combined activity of many microorganisms. The four fundamental stages of AD for the synthesis of methane are hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Fig. 3.12) (Yang *et al.,* 2011). Each of these stages has a unique microflora that aids in the process as a whole (Yu and Schanbacher, 2010).

Thermal pretreatment enhanced 62% and 16% methane yield for *Chlorella vulgaris* and secondary sludge, respectively. On the other hand, primary sludge supported the highest anaerobic biodegradability (97%) and when combined with thermally pre-treated *C. vulgaris*, methane yields were higher (13–17%) than the ones expected theoretically. Thereby, this study showed that algae biomass is a potential cosubstrate for biogas production together with municipal wastewater sludge.

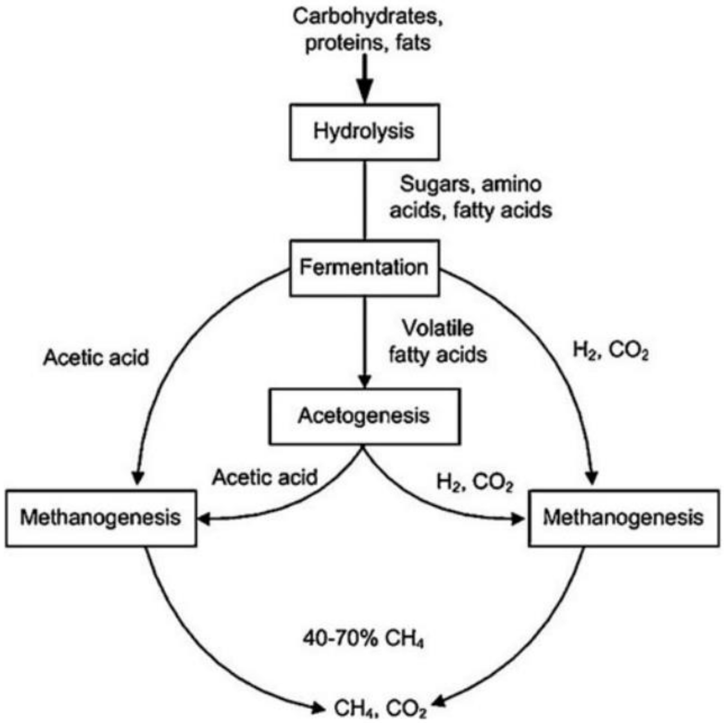


Fig. 3.12. Process flow of the degradation of organic material through anaerobic digestion. (Reproduced from Li *et al.,* 2011 with the permission from Elsevier, copyright 2011)

In the hydrolysis step, the microflora in the digester work to biodegrade the polymeric components of the microalgal biomass, including the carbohydrates, proteins, and lipids, producing simple substrates like glucose, fructose, amino acids, and long-chain fatty acids. Following the hydrolysis stage, the acidogenic phase of AD occurs, during which simple constituents are transformed into a mixture of short-chain volatile fatty acids (VFAs) and other minor metabolites (CO_2, H_2, and acetic acid). In the third phase, VFAs are transformed into acetic acid and H_2 by the action of acetogenic bacteria, which are then finally metabolized into methane in the methanogenesis stage by a variety of methanogenic bacteria (Li *et al.,* 2011).

As was previously mentioned, the end product of AD is a mixture of CH_4 and CO_2 known as biogas, and the proportions of these gases depend on the biochemical makeup and biodegradability of the biomass. In the case of carbohydrates (glucose, starch, and cellulose), the ratio of CH_4 to CO_2 is 50:50 (Eq. 5a), however a greater amount of CH_4 is produced if the substrates are protein or lipid, with estimates of the CH_4 to CO_2 ratios being 55:45 (Eq. 5b) and 70:30 (Eq. 5c), respectively.

$$C_nH_{2n-2}O_{n-1} \text{ (Starch or cellulose)} + nH_2O \longrightarrow 0.5CH_4 + 0.5CO_2 \quad \text{Eq. (5a)}$$

$$C_{10}H_{20}O_6N_2 \text{ (Proteins) } 3H_2O \longrightarrow 5.5CH_4 + 4.5CO_2 + 2NH_3 \quad \text{Eq. (5b)}$$

$$C_{54}H_{106}O_6 \text{ (Lipids) } 28H_2O \longrightarrow 40CH_4 + 17CO_2 \quad \text{Eq. (5c)}$$

By anaerobic digestion, it is possible to integrate algae cultivation to existing wastewater treatment plants (WWTP) for nutrient recycle and biomethane production.

According to study of Wang and Park (2015), they investigate two wildtype green algae, *Micractinium* sp. and *Chlorella* sp., for their growth in high nitrogen wastewater (mixture of sludge centrate and primary effluent wastewater) and subsequent anaerobic digestion under mesophilic conditions. *Micractinium* sp. produced more extracellular polymeric substances (EPS)-proteins than *Chlorella* sp. during cultivation, according to EPS analysis and extraction in both algae species. *Chlorella* sp. allowed a larger CH_4 yield on the volatile solids fed the digester (VS_{fed}) of 230 dm^{-3} kg^{-1} than *Micractinium* sp. (209 dm^{-3} kg^{-1}), according to anaerobic digestion of harvested algae, which revealed the opposite tendency.

These findings suggested that anaerobic digestibility and biogas yield were impacted by the various growth patterns of two species of algae with varying EPS expression levels. Algae's biogas production and ability to reduce volatile solids were both enhanced by co-digestion with waste activated sludge (WAS).

According to Mahdy *et al.,* (2015), they evaluated the feasibility of using microalgae biomass as feedstock for anaerobic digestion together with other biomasses (primary and secondary sludge) normally generated in WWTP. Raw microalgae biomass anaerobic biodegradability (33%) was higher than that of secondary sludge (23%).

3.10 Algae-based renewable energy production through Hydrothermal Liquefication (HTL)

Traditionally, high-lipid strains of microalgae have been subjected to energy-intensive thermal drying, solvent extraction, and transesterification to make biodiesel as part of the biofuel production process. These steps are expensive and necessitate the use of organic solvents that are hazardous to both people and the environment. Furthermore, lipid-rich strains typically have slow growth rates (resulting in reduced culture productivity), and the lipid mass fraction typically ranges between 20 and 50% (Chisti 2007), leaving a significant amount of the microalgae biomass unconverted.

In this situation, hydrothermal liquefaction seems to be a potential medium temperature and high-pressure thermochemical conversion method that converts the entire microalgae biomass into a liquid energy carrier (biocrude oil). In terms of converting microalgae, liquefaction is one of the most promising conversion methods, if not the most promising, due to the higher value of the primary product and the lower energy requirements compared to other technologies. The process conditions described in the literature typically range from 250 to 375 °C, 10 to 20 MPa, and 5 to 20% of microalgal mass in the slurry feed, which can be accomplished with just 12% of the energy required for their complete dewatering (Xu *et al.,* 2011).

Algae Biorefinery Concept approach, which is shown in Fig. 3.13, was presented by Garc'a Alba *et al.,* (2012) and is a method for a complete biorefinery process scheme. The four main steps are hydrothermal processing, fractionation, harvesting, and growth.

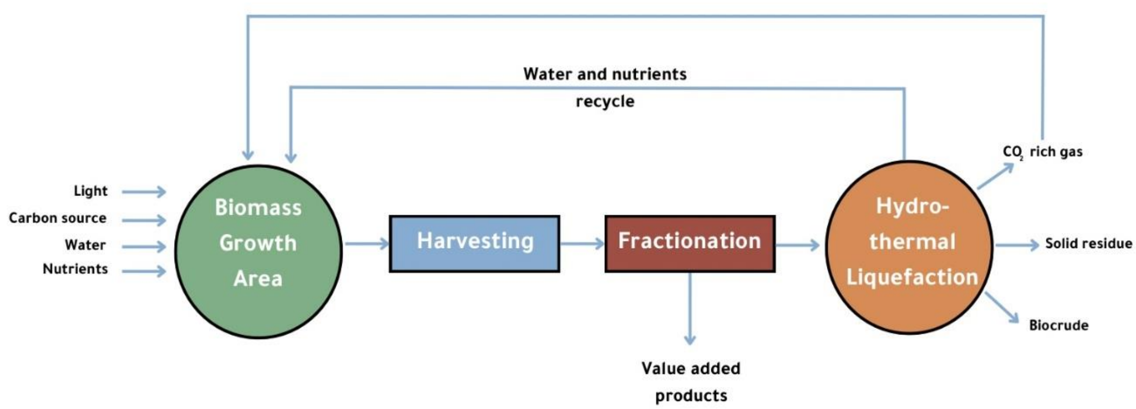


Fig. 3.13. Conceptual scheme for an algae biorefinery (adapted from Garcia Alba *et al.,* 2012).

For the growth step, there are numerous techniques available (Mata *et al.,* 2010; Patil *et al.,* 2008). Small fermenters or photobioreactors are typically utilized in laboratories, while open ponds or closed systems are frequently used in large-scale applications. The microalgae must be harvested after being cultivated in order to concentrate them at the required loading rate for the proposed thermochemical procedure.

A fractionation phase must be performed before the hydrothermal liquefaction in order to produce value-added products other than biofuels. Fractionation is typically thought of as combining extraction with a pretreatment stage. Typical pretreatment (fraction-disclosing) techniques include mechanical cell rupture, ultrasound, and PEF (pulse electric field).

Following fractionation, the entire biomass or any leftovers would go through the thermochemical conversion stage, generating a biocrude oil along with gas, aqueous, and solid phases. The aqueous phase would ideally contain a large proportion of the nutrients from the microalgae cultivation method and could be recycled to the growth area, while the other fractions may go through upgrading operations to maximize their use potential. As a way to get proteins as a value-added co-product while lowering the nitrogen content of the biocrude produced by a subsequent thermochemical conversion of the remaining biomass, the combination of fractionation and a thermochemical conversion step has been proposed (Garc'a Alba *et al.,* 2012). The overall yield of biocrude oil would be reduced as a result of this fractionation phase.

There is still some mystery around the mechanism of hydrothermal liquefaction. It is currently unclear how certain variables, such as temperature, the dosage of a heterogeneous or homogeneous catalyst, holding period, or ash content, affect some processes. According to the literature, repolymerization and hydrolysis compete for energy in hot compressed liquid water that is close to the critical point (374 °C and 22.1 MPa) (Garc'a Alba *et al.,* 2012; Zou *et al.,* 2010).

The first is more significant early in the process, when the microalgae are broken down and depolymerized into smaller molecules. These molecules could be very reactive, causing them to polymerize and create biocrude, gas, and solid compounds (Biller and Ross, 2011; Demirbaş 2000; Yang *et al.,* 2004). Repolymerization, condensation, and breakdown of the components from the various phases may take place when the reaction time or temperature is increased.

As a result, the yields of solid and gas may rise, whereas the yield of biocrude oil may fall (Zou *et al.,* 2009; Zhou *et al.,* 2010; Anastasakis and Ross, 2011). This is consistent with the finding that the viscosity of biocrude reduces as holding times rise (Minowa *et al.,* 1995). In Fig. 3.14, the HTL pathways are depicted schematically.

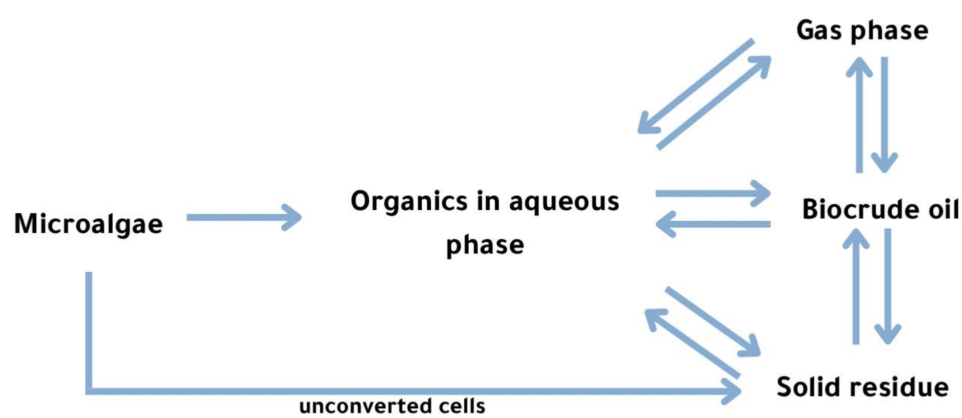


Fig. 3.14. Scheme of HTL kinetic pathways.

Recent researches have demonstrated that HTL can transform algal biomass from wastewater treatment plants into a variety of bioenergy products. (Brewer *et al.,* 2017; Chen *et al.,* 2014).

In the study of Arun *et al.,* (2018), through HTL, *Chlorella vulgaris* biomass grown in wastewater was converted into bio-oil that was then enhanced into transportation fuels. With a 15 g/200 mL biomass loading rate and a 3% wt. nano ZnO catalyst loading, the bio-oil yield was 29.37% wt. at 300 °C for 60 minutes. By using a catalyst, bio-oil's oxygen and nitrogen content were lowered, and its calorific value was enhanced (19.6 ± 0.8 MJ/Kg). Through liquid-liquid extraction (LLE), bio-oil was enhanced, and at 30 °C, dichloromethane solvent (18.2% wt.) produced a greater yield.

In another study, *Scenedesmus abundans* has been grown in domestic wastewater using a unique lab-scale hybrid loop airlift photobioreactor (HLALPBR). The greatest biomass content of 3.55 g L^{-1} and productivity of 209 mg L^{-1} d^{-1} were attained. Chemical oxygen demand, dissolved inorganic nitrogen, and dissolved inorganic phosphorus could all be eliminated from home wastewater by *Scenedesmus abundans* by 80.19%, 90.73%, and 86.31%, respectively. Later, 35.5 wt.% of bio-oil was produced from the hydrothermal liquefaction of algal biomass. According to a carbon mass balance across different product streams, bio-char, bio-gas, bio-oil, and post-HTL wastewater product streams, respectively, sequestered roughly 0.46, 0.15, 0.963, and 0.35 g of CO_2 for every g of algae, respectively (SundarRajan *et al.,* 2020).

Nostoc ellipsosporum, a microalga grown in municipal wastewater, was studied by Devi and Parthiban (2020) to determine the best conditions for growth in order to maximize biomass production, nutrient removal effectiveness, and bio-oil yield. As a growing medium, several mixtures of Fog's nutrition were combined with municipal effluent. *Nostoc ellipsosporum* was able to remove 87.59% of the nitrogen and 88.31% of the phosphate from wastewater thanks to the optimization of growth conditions and adaptation to wastewater. Additionally, the yield of bio-oil from the hydrothermal liquefaction of biomass was 24.62% at 300 °C.

3.10.1 Conversion mechanisms

Although the conversion mechanism is still not entirely known, some authors have attempted to offer potential conversion pathways for the various fractions of terrestrial biomass during HTL (Toor *et al.,* 2011; Peterson *et al.,* 2008; Behrendt *et al.,* 2008) and microalgae (Biller and Ross, 2011). According to Torri *et al.,* (2012), the main components of biocrude oil are primarily derived from the liquefaction of lipids and algae nans under mild conditions (less than 250 °C), whereas under more extreme conditions (300-375 °C), the conversion of carbohydrates and proteins is encouraged, which raises the nitrogen content of the biocrude oil.

3.10.1.1 Conversion of lipids

Triacylglycerols (TAGs), primarily having an aliphatic character, are the most common kind of microalgal lipids. A glycerol backbone is coupled to three fatty acids to make up its structure. One end result of the hydrolysis of TAGs is glycerol. Glycerol is transformed into methanol, acetaldehyde, propionaldehyde, acrolein, allyl alcohol, ethanol, and formaldehyde via HTL (Bühler *et al.,* 2002). Gas products, namely CO, CO_2, and H_2, are also produced. Although fatty acids can be transformed into long-chain hydrocarbons, they have higher thermal stability.

3.10.1.2 Conversion of proteins

Proteins are peptide-linked polymers of amino acids that rapidly hydrolyze under HTL conditions. However, due to further decarboxylation to create carbonic acid and amines, as well as deamination to create ammonia and organic acids, the yield of amino acids is minimal. These processes' byproducts may subsequently repolymerize into long-chain hydrocarbons, aromatic ring-type structures like phenols, or nitrogen heterocyclics like indole or pyrrole (Biller and Ross, 2011; Toor *et al* 2011; Peterson *et al.,* 2008).

3.10.1.3 Conversion of carbohydrates

Carbohydrates' direct breakdown products don't become biocrude oil. The fundamental process for liquefaction is thought to be the breakdown of carbohydrates into polar, water-soluble organics such organic acids (such as formic, acetic, and lactic), aldehydes, benzenes, and alcohols, all of which carry a significant quantity of oxygen (Biller and Ross, 2011; Toor *et al* 2011; Peterson *et al.,* 2008). The biocrude fraction then contains the bigger hydrocarbons that may be produced by the aldehyde- and benzene-type structures.

3.10.2 Hydrothermal Liquefication products

3.10.2.1 Biocrude oil

The hydrothermal decomposition of the components of microalgae (lipids, proteins, carbohydrates, and algaenans) yields biocrude oil, a black, viscous, and energy-dense liquid. Its energy content is 70-95% that of petroleum fuel oil

(Brown *et al.,* 2010) and it resembles heavy crude in composition (Ross *et al.,* 2010). According to Garc'a Alba *et al.,* (2012), the usual elemental composition (in mass fraction) of the biocrude oil produced by Desmodesmus sp. at 375 °C and 5 min of reaction time is N 6.3%, C 74.5%, H 8.6%, and O 10.5%. It contained 33.9% less oxygen than the initial microalgae cells and had a higher heating value (HHV) of 35.4 MJ kg^{-1}.The feedstock and production conditions seem to have a significant impact on the physical and chemical characteristics of biocrude oil. Gas chromatography analysis can define HTL oils to a certain extent, however the majority of the heavy chemicals do not elute into the column and are therefore left uncharacterized. This biocrude oil's high nitrogen content — typically about 5–7% — which causes significant NOx emissions during combustion—is the main issue.

3.10.2.2 Aqueous phase

Given its high richness in organic matter and nutrients, the treatment of the aqueous phase produced during HTL is crucial. $PO^{3-}{}_4$, NH_4, and CH_3COO^- are its primary ingredients, in addition to metallic cations such K^+, Na^+, and Mg^{2+} (Ross *et al.,* 2010; Ross *et al.,* 2011). The high concentration of organics in the aqueous phase might also be useful for heterotrophic strains as a carbon source. In addition to directly recycling this phase into the culture medium, gasifying it under supercritical conditions to create a fuel gas with a high hydrogen content is an intriguing alternative handling method. After this process, the bulk of the nutrients should ideally still be disseminated in the ensuing aqueous phase, allowing for their recycling into the growth stage.

3.10.2.3 Gas phase

20% of the original organics in the microalgae feedstock are yielded by the gaseous fraction (Garcia Alba *et al.,* 2012; Brown *et al.,* 2010; Jena and Das, 2011). H_2 and CO_2 are the primary gas byproducts of HTL. According to several authors, after the water's critical point is passed, the concentration of CO_2 drops while that of tiny hydrocarbons (CH_4 and C_2) rises. The low levels of CO described in the literature could mean that HTL primarily uses decarboxylation rather than decarbonylation to remove oxygen (Garcia Alba *et al.,* 2012) or that the CO generated quickly interacts to form CO_2 and H_2 through the water gas shift reaction (Elliott and Searlock Jr, 1983). If the microalgae slurry contains salts from the growth media, this second approach might be improved. The created CO_2 may be recycled into the microalgae production system, and the produced H_2 could be used to further hydrotreat the biocrude.

3.10.2.4 Solid residues

A solid residue with a high ash content and minimal hydrogen, sulfur, or nitrogen is produced by microalgal HTL as well. Nearly all of the writers report solid yields of less than 10% (Yang *et al.,* 2004; Minowa *et al.,* 1995; Ross and Biller, 2011). Due to their potential nutrient content, these solid residues are desirable as a soil amendment. Additionally, the residual ashes might be recycled as nutrients for microalgal development, while they could be used as feedstock for later thermochemical processes like pyrolysis or gasification that would yield alternative energy carriers.

Conclusion

Microalgae are a dominant group of prokaryotic and eukaryotic photosynthetic microorganisms that have capability to biological treatment due to their significant ability for bio-sorption of toxic heavy metals and the use of organic and inorganic nutrients from wastewaters, which are the major environmental pollutants.

Increasing demand of energy sources, global warming and pollution of water bodies are major global concern. As far as agricultural, industrial and municipal wastewater is concern, several conventional technologies are being employed for their treatment and disposal. But these technologies are not as effective as they are very costly and energy intensive. Microalgal-based bioremediation offers cost effective means of reutilization of nutrients for biomass production.

Algae biofuel is a possible replacement for petroleum-based fuels,due to its sustainability, renewability, and environmental friendliness. When compared to fossil fuels, algae can produce a lot of lipids and cut greenhouse gas emissions by 30–50%. Algae are also sustainable feedstock for producing a wide range of different biofuels such as biodiesel, bioethanol, bio-hydrogen, bio-oil and bio-syngas etc.

In summary, algae-based integrated technology for wastewater treatment and bioenergy production, and other high-value bioproducts are a sustainable approach to improve industrial feasibility and profitability and to satisfy the fast-growing energy demand of world.

Recommendations

Finding sustainable approaches to wastewater treatment is both a necessary and challenging endeavor. In comparison to current traditional wastewater treatment methods, the incorporation of microalgae into a variety of wastewaters can decrease the cost of wastewater treatment, get a lower footprint in terms of energy consumption, and provide environmental sustainability.

Moreover, integrated microalgal biorefinery not only solves environmental problems, but also acts as a producer which can produce high added-value bio compounds such as biofuel, biodiesel, and other valuable compounds.

Algae biofuel is a potential substitute for petroleum-based fuels. If microalgae bioenergy is to be developed into a substitute for fuel and used on a big scale, it is also imperative to find practical technology to enable economical, effective, and high-density growing of algae.

Although the technology for developing and producing algal energy is advanced, new strategies should be suggested to enhance culture and production. Evaluating the viability of large-scale production of algal biofuel requires a realistic technological and engineering assessment.

Overall, with effective adoption of these recommendations of, algae-based treatment could minimize the current economic barriers and creation inexpensive and sustainable biofuel.

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