Clean Energy Production Technologies

Series Editors: Neha Srivastava · P. K. Mishra

Pradeep Verma *Editor*

Micro-algae: Next-generation Feedstock for Biorefineries

Cultivation and Refining Processes

Springer

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Series Editors

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The consumption of fossil fuels has been continuously increasing around the globe and simultaneously becoming the primary cause of global warming as well as environmental pollution. Due to limited life span of fossil fuels and limited alternate energy options, energy crises is important concern faced by the world. Amidst these complex environmental and economic scenarios, renewable energy alternates such as biodiesel, hydrogen, wind, solar and bioenergy sources, which can produce energy with zero carbon residue are emerging as excellent clean energy source. For maximizing the efficiency and productivity of clean fuels via green & renewable methods, it's crucial to understand the configuration, sustainability and techno-economic feasibility of these promising energy alternates. The book series presents a comprehensive coverage combining the domains of exploring clean sources of energy and ensuring its production in an economical as well as ecologically feasible fashion. Series involves renowned experts and academicians as volume-editors and authors, from all the regions of the world. Series brings forth latest research, approaches and perspectives on clean energy production from both developed and developing parts of world under one umbrella. It is curated and developed by authoritative institutions and experts to serves global readership on this theme.

Pradeep Verma
Editor

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Dedicated to My Beloved Mother

Preface

The fast depletion of fossil fuels has forced to move out of reliance on fossil fuel for industrial and energy sector's needs. Due to its rich protein and lipid content, microalgal biomass has been considered as one of the suitable substrates for biorefinery. Attempts have been made to develop an integrated microalgae-based biorefinery. In microalgal biomass-based biorefinery, different biofuel biodiesel, bioethanol, bio-hydrogen, and value-added compounds such as carotenoids, fatty acids, and protein can be produced simultaneously. The microalgal based biorefinery has the potential for generation of bioelectricity and utilization of wastewater as a substrate, making the overall process self-reliant and helping in bioremediation. There is a need to have detailed information on microalgal diversity and its characteristic properties. There is also a need to have a better understanding of the cultivation and harvesting technologies for microalgal biorefinery application along with downstream processing for enhanced recovery of biofuel and value-added products. Thus, this book *Micro-algae: Next-Generation Feedstock for Biorefineries—Cultivation and Refining Processes*, part of Springer's book series *Clean Energy Production Technologies*, will attempt to provide an account on cultivation and refining aspects of microalgae-based biorefineries. The book will also provide an overview of microalgae as a suitable feedstock for the production of biofuel (bioethanol, biodiesel, bioelectricity) and value-added products (cosmeceuticals, essential chemicals, etc.). The advancements associated with the utilization of microalgae as a suitable microbial system for concurrent wastewater treatment and resource recovery via utilization of wastewater as a nutrient medium for microalgae cultivation will also be highlighted in several chapters of the book. The book also covers several aspects of the heterotrophic microalgal production system via the utilization of wastewater. The recent innovations and upgrades in large-scale microalgal production, harvesting, and extraction (enzymatic and biocatalytic pretreatment) are incorporated in the book. The book attempts to highlight new microalgae-based biorefinery approaches and how these approaches can be consolidated to design an integrated self-sustainable microalgae-based biorefinery. This edited book will be equally beneficial for researchers in the area of microalgal assisted biorefinery and bachelor's, master's, or young budding graduate students as a textbook.

Ajmer, Rajasthan, India
Pradeep Verma

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First off, I would like to convey my gratitude to the series editor, Dr. Neha Srivastava, and Prof. P.K. Mishra for considering the submission of this book entitled *Micro-algae: Next-Generation Feedstock for Biorefineries—Cultivation and Refining Processes* under the book series "*Clean Energy Production Technologies*." I am thankful to Springer Nature for accepting my proposal to act as editor for the current book volume. This volume of the book series was only possible because of the support from all the researchers and academicians who contributed to the book; therefore, I am thankful for their contribution. I would also like to thank my Ph.D. scholar, Dr. Bikash Kumar, currently working as Post doctoral researcher at IIT Guwahati, for providing me with all the necessary technical support and editorial assistance during the entire stage of book development. I am also thankful to the Central University of Rajasthan (CURAJ), Ajmer, India, for providing infrastructural support and a suitable teaching and research environment. The teaching and research experience at CURAJ has provided the necessary understanding of the needs of academicians, students, and researchers in a book that was greatly helpful during the development of the book. I am also thankful to the Department of Biotechnology for providing me funds through sponsored projects (Grant Nos. BT/304/NE/TBP/2012 and BT/PR7333/PBD/26/373/2012) for setting up my laboratory "Bioprocess and Bioenergy Laboratory."

I am always thankful to God and my parents for their blessings. I also express my deep sense of gratitude to my wife (Savita) and kids (Netra & Mohak) for their support during the development of the book and in life.

Contents

Editor and Contributors

About the Editor

Pradeep Verma completed his Ph.D. from Sardar Patel University, Gujarat, India, in 2002. In the same year, he was selected as a UNESCO Fellow and joined the Czech Academy of Sciences, Prague, Czech Republic. He later moved to Charles University, Prague, to work as a Post-Doctoral Fellow. In 2004, he joined as a visiting scientist at the UFZ Centre for Environmental Research, Halle, Germany. He was awarded a DFG fellowship, which provided him another opportunity to work as a Post-Doctoral Fellow at the University of Göttingen, Germany. He moved to India in 2007, where he joined Reliance Life Sciences, Mumbai, and worked extensively on biobutanol production, which attributed a few patents to his name. Later, he was awarded JSPS Post-Doctoral Fellowship program, and he joined the Laboratory of Biomass Conversion, Research Institute of Sustainable Humanosphere (RISH), Kyoto University, Japan. He is also a recipient of various prestigious awards, such as the Ron Cockcroft award by Swedish society and UNESCO Fellow, ASCR, Prague. Recently, for his contribution to the areas of fungal microbiology, industrial biotechnology, and environmental bioremediations, he was awarded the prestigious Fellow award for Mycological Society of India (2020), P.K. Jain Memorial Award (MSI), and Biotech Research Society of India (2021).

Prof. Verma began his independent academic career in 2009 as a Reader and Founder Head at the Department of Microbiology at Assam University. In 2011, he moved to the Department of Biotechnology at Guru Ghasidas Vishwavidyalaya (A Central University), Bilaspur, and served as an Associate Professor till 2013. He is currently working as a Professor (former Head and Dean, School of Life Sciences) at the Department of Microbiology, CURAJ. He is a member of various national and international societies/academies. He has completed two collaborated projects worth 150 million INR in the area of microbial diversity and bioenergy.

Prof. Verma is a group leader of the Bioprocess and Bioenergy Laboratory at the Department of Microbiology, School of Life Sciences, CURAJ. His areas of expertise involve microbial diversity, bioremediation, bioprocess development, lignocellulosic process, and algal biomass-based biorefinery. He holds 12 international patents in the fields of microwave-assisted biomass pretreatment and bio-butanol production. He has more than 73 research and review articles in peer-reviewed international journals and contributed to several book chapters (32 published, 11 in press) in different edited books. Prof. Verma has edited 07 books and is Editorial board member, guest editor and Reviewers to prestigious high impact journals.

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Chapter 1
Microalgae Biomass Biorefinery: A Sustainable Renewable Energy Feedstock of the Future

Rahul Kumar Goswami, Komal Agrawal, and Pradeep Verma

Abstract Energy plays a crucial role in development and industrialization and is fulfilled by using non-renewable fossil fuels. The drawbacks of fossil fuels are non-renewable as well as the generation of toxic pollutants to the atmosphere. Thus, it will be very difficult to fulfil the energy demands in the future. Looking to the current energy needs, researchers are focussing on an alternative to fossil fuels. Biofuels are the sustainable option for the fulfilment of energy demands made up by biomass of different substrates. However, their conversion process is high cost, and low production rate makes them non-compatible to fossil fuels. Microalgae biomass is considered a good substrate for the production of biofuel. It accumulates lipids and carbohydrate in their cell which is generally biorefined for the production of biofuels. The microalgal is a substantial candidate for biofuel production and fulfilment of energy demand to resolving the upcoming energy crisis. Thus, this chapter discussed the role of microalgae and biorefinery steps for the production of biofuels.

Keywords Energy · Biofuels · Microalgae · Lipids · Carbohydrates · Biorefinery

Abbreviations

CO_2	carbon dioxide
Ca	calcium
EDTA	ethylenediaminetetraacetic acid
FAME	fatty acid methyl ester
GHG	greenhouse gas
LED	light-emitting diode
IOC	inorganic carbon

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Mg	magnesium
N	nitrogen
NO	nitric oxide
NO_3	nitrate
NO_2	nitrite
NH_4^+	ammonium
OCs	organic carbons
PBR	photobioreactor
PUFAs	polyunsaturated fatty acids
RuBisCO	Ribulose-1,5-bisphosphate carboxylase
S	sulphur

1.1 Introduction

The population is increasing day by day which directly demands industrialization. Regulation of industries needs energy source which plays a vital role in social-economic development and the energy need is fulfilled by non-renewable fossil fuels (Chen et al. 2013). According to the Global Status Report, majorly 78.4% of energy is accomplished by these fossil fuels (coal, petroleum) (Kumar et al. 2020). But burning fossil fuel releases loads of toxic gases which threaten the environment (Chen et al. 2013). The burning of fossil fuel is the prime emitter of CO_2 which increases the emission of greenhouse gases (GHG), which leads to climate change and global warming (Chiaramonti et al. 2017). Thus, scientists are looking forward and searching for an alternative option for the fulfilment of energy, which are (i) renewable, (ii) non-toxic, (iii) cheap, (iv) do not release any secondary pollutant, and (v) are suitable for the environment (Chiaramonti et al. 2017). The alternative option is solar energy, hydropower and wind, and biofuels. Among these, biofuels are an ideal candidate for the fulfilment of energy. It is cheap, biodegradable, non-toxic, renewable, and sustainable for society (Mutanda et al. 2011; Chiaramonti et al. 2017). The biofuels are available in different forms like bioethanol, biodiesel, biogas, biohydrogen, etc., which are made by microbial fermentation, photolysis, transesterification, anaerobic digestion, etc. (Goswami et al. 2020b; Goswami et al. 2020c).

The biofuels are categorized into different generations, and it depends on the feedstock. In first-generation biofuels, mainly food crops are used, while in second-generation biofuels, non-edible food crops and lignocellulose biomass are used; in third-generation biofuels, algae biomass are used, whereas in fourth-generation biofuels, genetically engineered microorganisms (microalgae and cyanobacteria) are used (Agrawal and Verma 2022; Chiaramonti et al. 2017). The advantages and disadvantages of different generation biofuels are shown in Table 1.1.

The bottleneck of first- and second-generation biofuels provides the opportunity for third-generation biofuels. The third-generation biofuels are made up of the

Table 1.1 Different generation of biofuels, advantages, and disadvantages

Generation	Biorefinery process	Advantages	Disadvantages	References
First	Starchy food crops such as potatoes, corn, wheat, and sugarcane were used for fermentation to produce bioethanol Oil-producing crops such as soybean, sunflower, animal fat, etc. were used to produce biodiesel by transesterification	✔It is a conventional and well-established method ✔Used in different developed countries	✗Compete with food crops ✗Creates negative impression on food safety, water sacredness, global food markets ✗Use of agricultural land for production of these food crops	Berni et al. (2013) Medipally et al. (2015), Kumar et al. (2020)
Second	Utilizes waste materials, lignocellulose biomass, non-edible food crops such as *Jatropha*; agricultural waste biomass is used for the production of bioethanol	✔Use most abundant biomass for biofuel production ✔Not competes with food crops ✔Low cost compared to the first generation ✔More energy efficient	✗Required advanced biorefinery system ✗Processing rate is very slow ✗Not suitable for mercantile scale	Alam et al. (2012), Adenle et al. (2013)
Third	Utilize the microalgae and cyanobacterial biomass or their biomolecules such as lipids and carbohydrates	✔Biomass contains 50–60% of lipid ✔Does not compete with food crops ✔Requires no advanced technology ✔Cultivated in non-arable land ✔Produces different types of biofuels ✔Captures CO_2 from the environment	✗Required water for cultivation ✗Harvesting of biomass is a major issue ✗High biorefinery cost	Goswami et al. (2020a)
Fourth	Genetically modified organisms capture environmental pollution and produce high biomolecules	✔Captures a high amount of CO_2	✗Society not accepting genetically modified organisms	Ben-Iwo et al. (2016)

biomass of microalgae or cyanobacteria. Microalgae are photosynthetic, unicellular microorganism which contains lipid and carbohydrate in their cells, which can be used to produce biofuels (Mehariya et al. 2021a, b). The microalgal-based biofuels are eco-friendly, are renewable, and can be cultivated in non-farming land (Bhatt et al. 2014). Thus, this chapter discusses the screening and selection of microalgae strains showing high biomass productivity that contain lipid and carbohydrates in their cells, which can be used for the production of renewable biofuels. This chapter also provides the information about harvesting and biomass conversion (biorefinery) process for the production of biofuels.

1.2 Microalgae Overview

Microalgae are eukaryotic, unicellular, phototrophic microorganisms, ranging between size 0.2 and 100μm or even higher (Chen et al. 2013; Bhatt et al. 2014; Choo et al. 2020). They are ubiquitous microorganisms found in diverse environmental conditions (Saini et al. 2021; Bhardwaj et al. 2020; Agrawal et al. 2020). It can grow and survive in brackish water, freshwater, saline water, and wastewater (Rawat et al. 2011; Daroch et al. 2013; Chen et al. 2015). Microalgae lack roots, stems, and leaves (Bhatt et al. 2014; Goswami et al. 2020a), but they possess photosynthetic activity. The mechanism of photosynthetic is discussed in the empirical eq. 1 (Zhou et al. 2017). Microalgal cells could grow autotrophically (by capturing atmospheric CO_2 in the presence of lights), heterotrophically (grow in dark conditions by utilizing inorganic-organic carbon source from the environment), and mixotrophically (absence and presence of light) (Mehariya et al. 2021a). Microalgae required simple nutrients for their growth; it does not require external carbon sources. It could survive in different pH, temperatures, salinity, and nutrient (Hu et al. 2008; Brennan and Owende 2010; Gong and Jiang 2011). The microalgal cells fix the atmospheric CO_2 from the environment or as well in pollutant industries. It helps in the mitigation of CO_2 and utilizes it for the regulation of their metabolism (Stephens et al. 2010). Some microalgae have the ability to remediate wastewater. Microalgae utilize wastewater as a nutrient source for growth and development, and their biomass can be further utilized for biofuel production (Goswami et al. 2020b). The strains *Chlorella pyrenoidosa*, *Chlorella vulgaris*, *Chlorella minutissima*, and *Scenedesmus* sp. are reported for the remediation of different wastewater, and their biomass is used for biodiesel production (Zhou et al. 2020; Goswami et al. 2020b).

Furthermore, microalgae cells have a maximum productivity of biomass compared to other conventional agriculture crops such as soya bean, corn, etc. Microalgae have four growth phases: lag phase, exponential or log phase, stationary phase, and lysis or decline phase. The microalgal cells contain 80% dry weight of biomass (Bhatt et al. 2014). Microalgal cells contain different types of bioactive compounds, which have great commercial values. Its biomass contains 20–40% lipids, 10–20% carbohydrates, 30–50% protein and carotenoids, and up to 5% nucleic acids (Choo et al. 2020; Goswami et al. 2021a); also the concentration of the content may vary from species to species. The biomass of microalgae can be used for the production of value-added molecules and biofuels (Mutanda et al. 2011). This ideal behaviour or wide application of microalgae attracted researchers for the production of biofuels.

$$6CO_2 + 6H_20 \overset{\text{Light energy (hv)}}{\rightarrow} C_6H_{12}O_6 + 6O_2 \tag{1}$$

1.3 Microalgae Potential for Biofuel Production

Microalgal biomass contains carbohydrates and lipids which are essential compounds for the production of biofuels (Choo et al. 2020). In comparison with plants, microalgae can grow faster and have a high biomass production rate (Goswami et al. 2022a; Agrawal and Verma 2022; Goswami et al. 2021b). Microalgal-based biofuels cannot interfere with food security (Mutanda et al. 2011). Microalgae can utilize flue gas (CO_2) from a polluted environment and increase their biomass, which can then be utilized as feedstock for biofuel production (Chaturvedi et al. 2020). Scientists reported that around 40,000 different microalgae were identified, which mainly consists lipid in their cells (20–50%) in their total biomass (Zhu 2015). Mostly *Chlorella* sp., *C. vulgaris*, *C. protothecoides*, and *Chlamydomonas reinhardtii* are used for the production of microalgal-based biofuels, due to their highest productivity rate, and contain maximum lipid contents inside their cell as well as contain highest carbohydrates in their cells (Mutanda et al. 2011; Chen et al. 2013; Li et al. 2013; Medipally et al. 2015). Several types of biofuels are produced by using microalgae biomass, such as liquid biofuel (biodiesel and green hydrocarbon), fermentative biofuels (bioethanol and biobutanol), gaseous biofuel (hydrogen, methane), and solid biofuel (biochar) (García-Casal et al. 2009; Choo et al. 2020). Many reports suggest that low lipid-containing microalgae strain can grow faster than high lipid-containing microalgae (Chen et al. 2015). Several reports suggest that under unfavourable conditions, lipid productivity increases inside the cells. So, different stress conditions will be provided to microalgae for lipid and carbohydrate production. These lipids are transesterified into industrial important biofuels (Chen et al. 2015). Some microalgal cells contain a high amount of carbohydrates in cell walls (cellulose and soluble polysaccharides) and in plastids contain starch. This carbohydrate is converted into fermentable sugar, which is further fermented into bioethanol with the help of anaerobic microbes. Using microalgae biomass (carbohydrate) has benefits as it does not contain lignin and hemicelluloses, which can minimize the biorefinery process cost in biofuel production (Choo et al. 2020). In the below subsection, different microalgal biofuels are summarized.

1.3.1 Microalgae-Based Biodiesel

Most microalgae contain a high amount of lipid in their cell; this microalgal lipid is used for the production of biodiesel. The fatty acid composition may vary from species to species and environmental conditions (Chini Zittelli et al. 2006; Rodolfi et al. 2009). The lipid production by microalgal cells was determined by a conventional method such as gravimetric or solvent extraction method, which are further identified by fatty acid methyl ester (FAME). The physical quality of fatty acids and triglycerides can be determined by a number of double bonds, chain length, and the number of fatty esters (Mittelbach and Remschmidt 2004; Ramos et al. 2009). The important parameters of good fuels (biodiesel) include highest cetane number, more viscosity, oxidative stability, lubricity, ignition quality, and combustion heat (Miao and Wu 2006). The harvested lipid is transesterified into biodiesel with the help of different acids or solvents (especially methanol or n-hexane) (Chaturvedi et al. 2020; Goswami et al. 2020b). Different microalgae strains reported for biodiesel are summarized in Table 1.2.

1.3.2 Production of Bioethanol

Carbohydrates are chief products derived from photosynthesis and carbon fixation metabolism (Calvin cycle) (Mehariya et al. 2021a). The microalgal carbohydrate is either present in cell walls in the form of cellulose, pectin, and other polysaccharides or present inside plastids in the form of starch (as reserve materials). In microalgae biomass, carbohydrate composition varies from species to species. The production of bioethanol will require screening of ideal microalgal strains, which show the highest carbohydrates productivity suitable for biofuel production (Rangel-Yagui et al. 2004; Rismani-Yazdi et al. 2011). The microalgae species which contain glucose as a carbohydrate will be the most ideal feedstock for the production of bioethanol (Wargacki et al. 2012). Therefore, microalgae are suitable for bioethanol production, but their cultivations and harvesting technology are high cost, which is not economically feasible for large-scale production. The different microalgae strain used for bioethanol production and their carbohydrate content is summarized in Table 1.3.

As in previous sections already discussed, the two major biomolecules (lipid and carbohydrates) are considered for biofuel productions. However, rather than biomolecules, whole algal biomass is also used in the production of biofuel (shown in Fig. 1.1). Several microalgae species are also reported for the production of other biofuels such as biohydrogen, biomethane, biobutanol, and syngas. The biobutanol is produced via two-stage fermentation (acidogenic and solvogenic) of biomass or sugar molecules. Biohydrogen is produced by photo or dark fermentation by utilization of biomass with the help of bacteria which contain hydrogenase enzyme. Furthermore, biogas in other important bioenergy products is produced by anaerobic digestion of the microalgal biomass. For the enhancement of biomass and

Table 1.2 Different microalgal species and their total (%) lipid productivity

Microalgal species	Lipid (%)	References
Dunaliella primolecta	23	Mutanda et al. (2011)
Nannochloris	20–35	Mutanda et al. (2011)
Chlorella sp.	50	Medipally et al. (2015)
B. braunii	80	Medipally et al. 2015
C. vulgaris	50–58	Wang et al. (2014)
C. sorokiniana	19–22	Mata et al. (2010)
Nannochloropsis oculata	53.2	Krishnan et al. (2015)
C. protothecoides	58.4	Mata et al. (2010)
C. vulgaris	45.68	Huang and Su (2014)
N. vigensis	19.29	Aravantinou et al. (2013)
Dunaliella tertiolecta ATCC30929	70	Takagi and Karseno (2006)
Nannochloropsis oculata	16	Converti et al. (2009)
Chlamydomonas reinhardtii	25.25	Hill and Feinberg (1984)
Scenedesmus obliquus	31.4	Rawat et al. (2011)
C. saccharophila	18.10	Colman et al. (2002)
Scenedesmus sp. *LX1*	33	Kurano et al. (1995)
Auxenochlorella protothecoides	28.9	Wang et al. (2010)
Chlorella sp.	26.99	Kumar et al. (2010)
Scenedesmus sp.	30.09	Kumar et al. (2010)
Chlorella emersonii	63	Scragg et al. (2002)
Nannochloris sp.	50	Huesemann and Benemann (2009)
Phaeodactylum tricornutum	57	Gong and Jiang (2011)
Pavlova Salina	31	Gong and Jiang (2011)
Neochloris oleoabundans	35–65	Gouveia and Oliveira (2009)
Scenedesmus rubescens JPCC GA0024	73	Matsunaga et al. (2009)
C. minutissima	57	Gouveia and Oliveira (2009)
Dunaliella tertiolecta	71	Minowa et al. (1995)
Picochlorum sp.	25	Pereira et al. (2013)
C. vulgaris SAG 211-11b	42.1 ± 2.6	Selvarajan et al. (2015)
Micractinium pusillum LC-11	32.3 ± 6.7	Selvarajan et al. (2015)

biomolecules different optimization conditions and factors are required which are summarized in the below section.

1.4 The Key Factor in the Microalgae Biorefinery Process for Biofuel Production

1.4.1 Screening of Microalgal Strain for Biofuel Production

An important parameter for biofuel production is the selection of an appropriate algae strain (Selvarajan et al. 2015). The main attention focused on microalgae

Table 1.3 Different microalgae species and their total carbohydrate productivity

Microalgal species	Carbohydrate productivity	References
Chlorella vulgaris IAM C-534	37.0	Hirano et al. (1997)
Chlorella vulgaris CCAP 211/11B	55	Illman et al. (2000)
Chlorella vulgaris P12	41	Dragone et al. (2011)
Chlorella vulgaris	55	Brennan and Owende (2010)
Chlamydomonas reinhardtii UTEX 90	60	Hirano et al. (1997)
Chlamydomonas reinhardtii IAM C-238	55.0	Kim et al. (2006)
Nannochloropsis sp.	11	Hu and Gao (2003)
Scenedesmus obliquus	38	Ho et al. (2012)
Scenedesmus obliquus	49	Ho et al. (2013b)
Chlorella vulgaris FSP-E	51	Ho et al. (2013a)
Chlorella vulgaris	18.6	Fathi et al. (2013)
Chlorella vulgaris A-1123	37.8 + −1.4	Piligaev et al. (2015)
Chlorella vulgaris FSP-E	51.0 + −0.7	Piligaev et al. (2015)
Dictyosphaerium ehrenbergianum CCAP IL-2	40.3 ± 6.1	Selvarajan et al. (2015)
Micractinium sp. *IL-3*	41.5 ± 8.1	Selvarajan et al. (2015)

which exhibit highest productivity rate, grow in harsh conditions, as well as grown in all cultivation conditions (phototrophic, heterotrophic, and mixotrophic mode), required minimal nutrients for their growth, and capable of production of biomolecules (lipids and carbohydrates) (Makareviciene et al. 2011; Aravantinou et al. 2013; Selvarajan et al. 2015). Species that have relatively high lipid content are striking for biodiesel production (Rodolfi et al. 2009), whereas for bioethanol production, carbohydrates are attractive molecules that can be present inside the microalgae cells. Some microalgal strains showed extraordinary achievement for the production of biofuels such as *Chlorella vulgaris*, *Scenedesmus obliquu*s, *Chlamydomonas reinhardtii*, and *Picochlorum* sp. (de la Vega et al. 2011; Choo et al. 2020). The collection of microorganisms are influenced by many parameters such as (i) where the sample was collected, (ii) the growth condition of the cell, and (iii) if the organisms cells are damaged it will not survive. Therefore, temporal collection techniques should be adopted in sampling collection sites (Anandraj et al. 2008; Barrera Bernal et al. 2008; Mutanda et al. 2011). For the mass culture, the selected microbes should be optimized in the local climatic conditions. The selected strain must be grown in local climatic conditions as well as in low-nutrient conditions (Mutanda et al. 2011). It is suggested that before the selection of species, initially checking the harvesting cost of a particular microalgal species (Borowitzka 1997), whereas in screening stages, selecting an appropriate microalgal strain which is capable in the production of high lipid contents (Mutanda et al. 2011) are required. Moreover, high biomass, lipid productivity, and carbohydrate productivity may be increased by genetic modification of microalgae strain, optimizing the cultivation

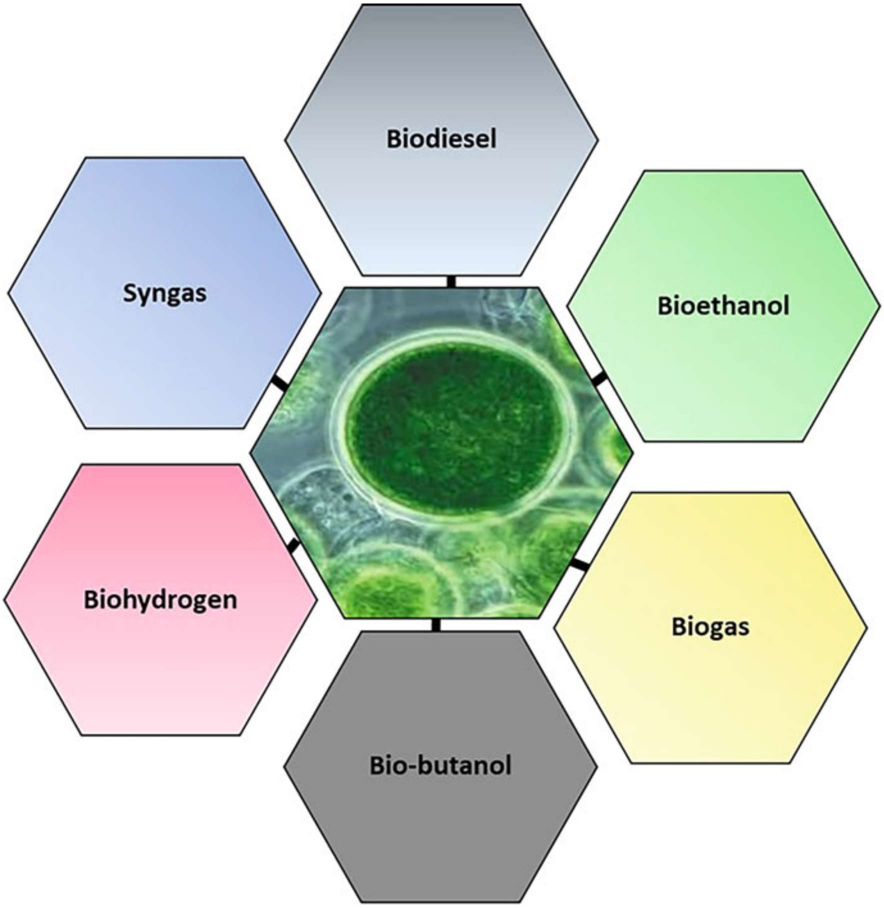

Fig. 1.1 Different types of biofuel produced using microalgae

condition (carbon source, nutrients, light intensity, etc.) as well as maintaining a low-cost cultivation system which makes it more economically feasible for large-scale biofuel production (Choo et al. 2020).

1.4.2 *Physiological Parameter Affecting Microalgae Biomass Production*

1.4.2.1 Light

It is a key factor for microalgae cells for growth and development. Microalgae utilize light as an energy source for CO_2 fixation. Optimum light is an important factor for their growth; if the light intensity is low, the RuBisCO does not function properly, which regulates the normal metabolism of algae cells. But if the light intensity is high, it can allow photoinhibition and hinder the growth of microalgae (Zhu 2015). The different reported literature suggested that the optimum light intensity was 3000–5000 lux depending on the microalgae species. The microalgae cell utilizes sunlight naturally, but in the laboratory, artificial light was provided by LED tube light (cool fluorescent light). Continuous illumination in microalgae can provide better growth performances, but several reports suggested that the optimization of the day-night cycle can also enhance the biomass production and it can vary from species to species (Zhao and Su 2014). The wavelength of light is directly correlated with the molecule production inside microalgae cells. Some literature showed the effect of wavelength on the lipid production inside the microalgal cells. Severes et al. (2017) suggested that different wavelengths of light (red and blue) may enhance biomass production rate in *Chlorella* sp. and reported that red light wavelength can double the dried lipid productivity inside the cells (Severes et al. 2017), whereas Monika and his co-worker studied the growth behaviours of *Chlorella* sp. on different light wavelength colours (red, yellow, and white), and they found that red light wavelength enhances the growth and lipid production in microalgae (Rai et al. 2015). It was reported that light colours also affect the lipid content and fatty acid profile in microalgae, and they conclude that saturated fatty acid compositions are the same in blue, green, and white light and they are reduced in the presence of red light (Alishah Aratboni et al. 2019).

1.4.2.2 Temperature

Temperature is a key factor for the growth of microalgae cells; slight temperature changes may not harm the growth rate. Moreover, if the temperature increases to the optimum level, it reduces the growth rate or inhibits the growth that leads to loss of biomass (Larsdotter 2006; Zhu 2015). The ideal growth temperature for microalgae species is 20 °C–30 °C. In humid climate conditions, the temperature is generally increasing because of lack of water evaporation. Some reports state that few species of microalgae also survive in low temperatures (15 °C) or below freezing (Zhu 2015). The temperature is maintained in media or cultivation systems by using different water-cooling techniques (Zhu 2015). Temperature also plays a crucial role in lipid productivity in microalgae cells; it varies from species to species. Earlier published reports have suggested that the enzymes of lipid metabolic pathways are highly sensitive to temperature (Dickinson et al. 2017). Some reports showed that when the temperature rises, microalgae cells accumulate saturated fatty acids, whereas in low temperature, it produces or accumulates unsaturated fatty acids. Menegol et al. (2017) reported that microalgae *Heterochlorella luteoviridis* at 22 °C accumulate PUFA (40.7%), but when the temperature shifted to 22 to 27 °C, it enhances the lipid accumulation (52.9%) of fatty acids (Menegol et al. 2017).

1.4.2.3 Carbons

Microalgae assimilate inorganic or inorganic carbon (IOC) in photosynthesis. Microalgae fix CO_2 present in the atmosphere (Goswami et al. 2022b; Prakash et al. 2021). This carbon further assimilates into sugar molecules (Goswami et al. 2020a). The IOC utilizes normally in the form of CO_2 and HCO_3^-, which are generally present in the atmosphere, whereas some microalgae species show heterotrophic mode and utilize OCs or organic acid, sugar, acetates, or glycerol, e.g., *C. pyrenoidosa*, *C. vulgaris*, etc. (Goswami et al. 2020a). This carbon source can be chemo- or photoheterotrophically assimilated. Some microalgae species can utilize both OC and IOC (mixotrophic); it can utilize the carbon dioxide from the atmosphere, and after that, it also utilizes organic carbons present inside the media. It has the ability to shift from phototrophic to heterotrophic. The atmospheric CO_2 can be provided to algal cultures through aeration by using different techniques, or it can be naturally fixed by alga cells; if CO_2 is present in low amount in the atmosphere, it is necessary to provide extra carbon source for their growth (Larsdotter 2006). The carbon requirement differs from species to species (1 to 5% CO_2). Industrial domestic wastewater contains lots of OC and IOC carbon source, and it can affect the growth of microalgae; if the concentration is low, it raises the pH, and if the pH increases, the carbon changes into carbonate ions, which cannot be assimilated by microalgae cells, whereas if the carbon concentration is high, it decreases the pH, which hindered the microalgae growth (Zhu 2015). The appropriate CO_2 supply is an ideal factor to enhance carbohydrate productivity inside cells. Some reports suggest that increasing the concentration of CO_2 may improve the percentage of carbohydrates accumulation. According to Xia and Gao, 186 mol/L of CO_2 enhance the carbohydrates productivity in *C. reinhardtii* (3.19 to 7.40%) and *C. pyrenoidosa* (9.30 to 21%) (Xia and Gao 2005; Chen et al. 2013). According to Montoya et al., a high concentration of CO_2 (8% v/v) increases the fatty acids and lipid productivity up to 0.029 g/L/day (Ortiz Montoya et al. 2014). But Ying et al. show that CO_2 concentration of 0.02 g/L can inhibit the growth of *Dunaliella salina* (Alishah Aratboni et al. 2019). Moreover, *C. pyrenoidosa* shows maximum lipid production of 0.10 g/L/day at a concentration of 5% CO_2 (Alishah Aratboni et al. 2019).

1.4.2.4 Nitrogen

Nitrogen (N), phosphorus (P), sulphur (S), magnesium (Mg), and calcium (Ca) are macronutrients, which are important factors for the growth of microalgae cells. Nitrogen is the second most important nutrient required for microalgae growth. It consists of 10% of total biomass (Larsdotter 2006; Chen et al. 2013; Ho et al. 2014). In the atmosphere, nitrogen is present in many forms like NO (nitric oxide), NO_2^- (nitrite), NO_3^- (nitrate), and NH_4^+ (ammonium). The optimum concentration of nitrogen in wastewater or the media influences the growth of microalgae, while the maximum concentration of nitrogen can hamper the growth (Kumar et al. 2010).

Nitrogen is important for the synthesis of proteins, amino acids, chloroplast, several types of enzymes, and co-enzymes (Turpin 1991; Chen et al. 2013). In nitrogen depletion conditions or a low level of nitrogen, several microalgae could renovate proteins and peptides into lipids or carbohydrates as energy storage molecules (Chen et al. 2013). According to Illman et al. (2000) the cultivation of *C. vulgaris* in a low nitrogen-containing medium enhances carbohydrate productivity up to 55% (Illman et al. 2000), whereas D'Souza and Kelly, (2000) reported that microalgal species of *Tetraselmis suecica* in nitrogen-limiting condition may enhance the cellular carbohydrates productivity (10-57%) (D'Souza and Kelly 2000). However, some reports suggest that there is a competition between lipids and carbohydrates productivity under environmental stress conditions because of metabolic pathways associated with the formation and breakdown of energy-rich molecules (e.g., carbohydrates and lipids) which are closely linked to each other, whereas degradation of starch provides the initial precursor molecule for the production of acetyl CoA, which is the precursor of fatty acid synthesis (Chen et al. 2013). However, metabolic modification is required according to the need of molecules. If starch is needed, then block the precursor of lipid biosynthesis (Radakovits et al. 2010).

1.4.2.5 Phosphorus (P)

Phosphorus, as another important macronutrient, is utilized by microalgae. Phosphorus is present in the form of orthophosphate and polyphosphates. Microalgae cells utilize inorganic orthophosphates (PO_4) as macronutrients (Ruzhitskaya and Gogina 2017). Microalgae cells require energy for the consumption of organic orthophosphate. Microalgae cells have the ability to store the phosphorus in the form of polyphosphate in their volutin granules and utilize polyphosphate when phosphorus is not available in media (Larsdotter 2006). In wastewater, phosphorus is present in a maximum concentration in the form of polyphosphates and orthophosphate. So, phosphate dilution is required for microalgae.

1.4.2.6 Sulphur (S)

Sulphur is a macronutrient that is present in the media in the form of sulphate or oxides of sulphur. In the media, the concentration is above 100 ppm that causes growth inhibition in microalgae. Some species of microalgae also survive at this concentration, but their lag phase becomes longer. A higher concentration of sulphur in the wastewater or media can reduce the microalgae biomass (Kumar et al. 2010). Sulphur oxides affect the microalgae by decreasing the media pH (Matsumoto et al. 1997). However, pH can be adjusted by adding buffer in the media, but it can increase the overall cost of cultivation.

Other macro- and micronutrients are required in trace amount, e.g., manganese, copper, boron, nickel, molybdenum, zinc, and cobalt. This trace element regulates the metabolism of microalgae. Additionally, some species of microalgae required vanadine, selenium silicon, sodium, and chelating agent EDTA (Fogg and Thake 1987; Larsdotter 2006).

1.4.2.7 Salt Stress

Salt plays an ideal role in the physiological and biochemical pathways of growth, development, reproduction, and metabolism of important biological molecules such as lipids, proteins, and carbohydrates in microalgal cells. Salt stress is a good enrichment approach for the accumulation of lipid inside microalgae cells (Mohan and Devi 2014). It causes osmotic pressure in microalgae cells, which generates stress that leads to and triggers the modification in metabolic pathways. These changes allow the adaption of microalgae cells, and that saline flocculation significantly increases the lipid contents in microalgae (Alishah Aratboni et al. 2019).

1.5 Microalgal Biomass Biorefineries for Biofuel Production

The biorefinery process is a major stage of biofuel production from microalgae. It includes cultivation and harvesting of biomass by using different methods and lastly processing, extraction, and separation of biofuels from crude microalgal cells. The different biorefinery steps are shown in Fig. 1.2.

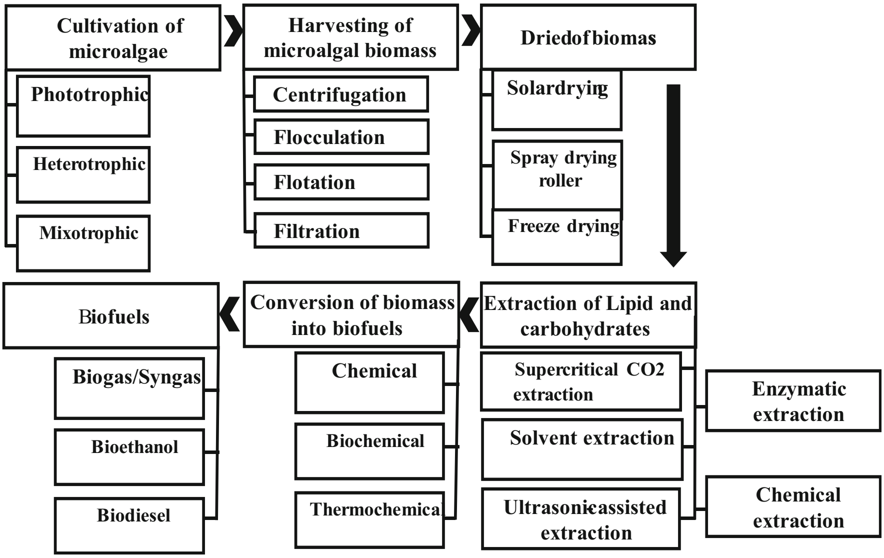

Fig. 1.2 Schematic diagram of biorefinery steps and techniques used for biofuels production (Chaudhary 2013; Chen et al. 2013, 2015; Medipally et al. 2015; Zhou et al. 2017)

1.5.1 Cultivation of Microalgae

Microalgae cultivation mode is of generally three types, that is, phototrophic, heterotrophic, and mixotrophic. It depends on the species, environmental condition, and biotic and abiotic factors (Chen et al. 2013; Bhatt et al. 2014; Choo et al. 2020). So, this section will provide a brief account of different cultivation modes.

1.5.1.1 Phototrophic Mode of Cultivation

Microalgae have the highest photosynthetic potential compared to higher plants. In phototrophic mode, microalgal cells only required water, sunlight, and CO_2 (which is present in the environment). It is the light-dependent mode of cultivation (Zhao and Su 2014). In this mode, microalgae can capture the CO_2 with help of sunlight and regulate their metabolic pathways. It fixes the CO_2 through Calvin cycles (three-step process): (1) carboxylation, (2) reduction, and (3) regeneration (Zhao and Su 2014). This cultivation is performed inside the open pond and enclosed photobioreactors (Medipally et al. 2015). These types of cultivation systems are low cost and widely or commercially used for microalgae biomass production. The open pond system is the oldest and simplest mode of microalgae cultivation, mostly used for the commercial production of valuable microalgae. This cultivation system is practiced since the 1950s. Currently, 98% of commercial algal biomass was produced by this system (Borowitzka 1999; Fulton 2004). There are several types of open pond, which are generally different based on shape, area, and material which are used for the construction of ponds. Some open ponds are simple which are made up of concrete materials (circular ponds or unmixed ponds), whereas some types of pond system are modified by adding paddle. This paddle helps in the mixing of media or cells inside the ponds (raceways stirred paddle ponds, circular pond with rotating arms) (Borowitzka 1999). In this system, raceway pond system is the most commonly used system for microalgal cultivation. The open pond systems do not compete in the arable land. The maintenance of this type of system is easy, but the major problem of these system is the contamination of other wild microalgal strains (Chisti 2008), whereas in enclosed photobioreactor systems, they are made up of glass tube, or transparent tubes, or transparent materials. Some common types of PBR are used for commercial production of microalgal biomass such as annual, flat panel reactor and tubular bioreactors (Carvalho et al. 2006; Chisti 2006). In this reactor the external factors such as pH, temperature, light intensity, and other factors are manually maintained.

1.5.1.2 Heterotrophic Cultivation

It lights an independent process. Microalgae cells utilize organic carbons as a carbon source for their growth and development. The organic carbon sources are provided through media in the form of glucose, glycerol, sodium acetate, etc. (Bhatnagar et al. 2010; Zhou et al. 2017). Some microalgae strains show the highest cells density in light depletion condition, or their growth is inhibited by the continuous supply of lights. So, the heterotrophic mode (light-independent mode) is more compatible with this type of microalgae. The organic carbon which is provided to microalgae should be very small so it can easily pass towards the cell walls. This organic carbon molecule transforms into lipids and other valuable molecules via hexose monophosphate shunt (Zhou et al. 2017). Few microalgal strains show photography mode, where light is required as an energy source. The main reason for the downfall of heterotrophic mode cultivation is the growth of other microorganisms due to the presence of organic carbons and moreover, some of the microalgae requires light as energy source (Chen 1996). Furthermore, glucose used as a major organic carbon source for the establishment of cultivation mode which increases the cost of cultivations. Only a few types of microalgae could possess this type of cultivation, e.g., *C. minutissima* (Bhatnagar et al. 2010), *C. protothecoides* (Shi et al. 1999), *C. zofingiensis* (Ip and Chen 2005), *C. vulgaris* (Liang et al. 2009), *Crypthecodinium cohnii* (Couto et al. 2010), and *Schizochytrium limacinum* (Johnson and Wen 2009).

1.5.1.3 Mixotrophic Cultivation

Microalgae follow both light-independent (heterotrophic) and light-dependent (phototrophic) pathways for growth and development. This condition is called mixotrophic. In this type of cultivation, microalgae utilize both organic carbons and atmospheric carbon from the environment (Medipally et al. 2015; Zhan et al. 2017). In the absence of light, microalgae can consume organic carbon which presents in media, whereas in the presence of light, microalgae photosynthesize and capture atmospheric CO_2. The main benefits of this mode of cultivation are biomass production which is higher compared to other cultivation modes. According to the report of Pulz and Scheibenbogen (2007), the *C. vulgaris* show twofold biomass production in mixotrophic mode, or it is total of both heterotrophic and autotrophic mode production. The mixotrophic mode of cultivation is a two-stage cultivation method that follows the diurnal cycle. Mixotrophic cultivation decreases the effect of biomass lost during dark respiration. Therefore, biomass production will be increased, which suggests that the mixotrophic cultivation mode is good for biofuel production (Medipally et al. 2015) (Table 1.4).

1.5.2 Harvesting of Biomass

After cultivation of microalgae, harvesting is a major step; microalgae cell is separated from the media. This stage is dewatering and harvesting of microalgal biomass. Microalgal biomass is concentrated and dried and released for the next stages where biomass can be transesterified to produce biofuels. Harvesting is a very

Table 1.4 Microalgae showing phototrophic, heterotrophic, and mixotrophic growth and their lipid productivity (%)

Mode	Microalgae cells	Lipid productivity	Reference
Phototrophic	*Chlorella vulgaris*	50–58	Mata et al. (2010)
	Chlorella protothecoides	57.8	Mata et al. (2010)
	Chlorella sorokiniana	22.0	Mata et al. (2010)
	Dunaliella tertiolecta	–	Grierson et al. (2009)
	Synechococcus sp.	–	Grierson et al. (2009)
	Tetraselmis chuii	–	Grierson et al. (2011)
Heterotrophic	*Chlorella protothecoides*	0	Chen and Walker (2011)
	Chlorella vulgaris	23	Liang et al. (2009)
	Chlorella sorokiniana	23.3	Zheng (2013)
Mixotrophic	*Chlorella sorokiniana*	–	Wang et al. (2014)
	Chlorella protothecoides	58.4	Wang et al. (2014)
	Chlorella vulgaris	22.0	Wang et al. (2014)
	Chlamydomonas sp.	–	Ogawa and Aiba (1981)
	Chlorella sp.	–	Ogbonna and Tanaka (1998)
	Chlorella globosa	–	Bhatnagar et al. (2011)

crucial stage, and currently, it is a challenging area of biofuel technology. Microalgae have a small size and low density which creates the problem and increases the total capital cost. The problem is the release of lipid from the intracellular space, which is an energy-extensive step. The simple method of harvesting biomass is gravity sedimentation, but it is time-consuming and creates a low density of harvested algal cells (Zhou et al. 2017). The major techniques currently use for microalgae cell harvesting are described briefly in the subsection (Kang et al. 2005), such as centrifugation, flocculation, filtration, and flotation (Uduman et al. 2010; Abdelaziz et al. 2013; Zhou et al. 2017).

1.5.2.1 Centrifugation

Centrifugation is broadly used for harvesting and separation of microalgae cells from the media. It separates the biomolecules based on particle size and density separation. Its efficiency is more than 95% (Salim et al. 2011). Generally, all types of microalgae cells can be separated by this technique, but it depends on the microalgal species. It is widely used in small- and large-scale microalgal culture systems (Milledge and Heaven 2013). It is also effective in large-scale culture harvesting, but that increases the operational cost which is not economically feasible (Salim et al. 2011; Zhou et al. 2017). On a large scale, it is only used for harvesting industrial important value-added products (PUFAs, secondary metabolites, cosmetic and pharmaceutical products) (Goswami et al. 2022a; Kapoor et al. 2021). Mostly disc-stack centrifuge is used in industries or commercial plants for harvesting of value-added algal metabolites and biofuel compounds. It is made up of a relatively shallow cylindrical bowl which contains the number of closely metal cones (stacks) that rotate and separate the biomass from the media (Milledge and Heaven 2013).

1.5.2.2 Flocculation

It is harvesting by the formation of microalgae aggregates, which destabilize algae cells from water or media (Brennan and Owende 2010; Chaudhary 2013). It is an advanced method of harvesting algal cells, it can harvest large quantities of microalgae cell suspension, and it is compatible with a broad variety of microalgae (Uduman et al. 2010). Flocculation may occur naturally in some microalgae; it can be flocculated in environmental stress, such as changes in pH and oxygen and changes in nitrogen concentration. However, it only occurs in some microalgae cells (Uduman et al. 2010). Flocculation can be artificially induced by applying physical or chemical methods. In chemicals methods, different chemicals are added, which allow the flocculation. Organic (starch or chitosan) or inorganic (alum, lime) compounds are used for creating flocculation (Pulz and Scheibenbogen 2007; Chaudhary 2013). Microalgae cells surface negative charges because of functional group ionization on the microalgal cell wall. This can be neutralized by adding positively charged cationic polymers and electrodes. But the flocculants are algae specific, and their recovery makes it problematic (Oswald 1988; Grima et al. 2003; Shen et al. 2009). However, an ideal flocculant may be inexpensive, easy for recovery and recycling, and non-toxic (Grima et al. 2003). The University of America has suggested that cationic polyelectrolytes are more effective for flocculating freshwater microalgae (Granados et al. 2012). Naturally, in some microalgae, flocculation can also be achieved by changing the pH (Grima et al. 2003). Shifting of pH 11–12 induces the flocculation in *Chlorella* sp. (Rai et al. 2015). But these processes are only achieved in few microalgae because it also disrupts the cells and changes the colour of algae cells which is not good from an economic point of view and creates problems for large-scale production (Benemann and Oswald 1996; Milledge and Heaven 2013). The newly developed fungi-assisted bio-flocculation technique has been developed. It is a more efficient technique that harvests 98% of biomass. These techniques may be applied in large-scale production unit because it is economically feasible (Uduman et al. 2010; Zhou et al. 2017).

1.5.2.3 Flotation

It is an effective separation and harvesting process of microalgae cells from the media. It is fast compared to the normal sedimentation process. In this, some chemical or synthetic polymer is used for the creation of floats. The addition of chemicals can cause secondary pollution, corrosion, and toxicity, which is not economically viable (Uduman et al. 2010). It is also created by adding small air bubbles, which removes microalgae cells from water or media (Shelef et al. 1984; Edzwald 1993). The air bubble is provided through a sparger.

1.5.2.4 Filtration

Filtration is a technique that is used for harvesting microalgal cells. In this technique, the liquid from media is passed through filter and biomass is accumulates on the filter layer and forms microalgal biomass cake. Different size ranges of filters are used for the harvesting of algal cells (Grima et al. 2003). It is classified based on filter pore size. In macrofiltration the pore size ranges between 0.1 and 10μm, whereas in microfiltration the pore size ranging between 0.2 and 2μm, and in the reverse osmosis filter the pore size is very small (<0.001um). There are different types of filter that can be used such as microfiltration, ultrafiltration, dead-end filtration, and vacuum filtration. For filtration of *Chlorella* sp., mostly microfiltration is used (Edzwald 1993), whereas for larger size cells or flocculated cells, microfiltration is used. Ultrafiltration is another option for the recovery of very small size microalgae cells. Yet filtration is a time-consuming and costly process, which increases the overall operational cost of biofuel production (Sánchez et al. 2008; Mata et al. 2010; Milledge and Heaven 2013). Therefore, designing simple low-cost harvesting techniques required might reduce the overall operational cost of biofuel production.

1.5.3 Processing, Extraction, and Conversion of Biomass for Biofuel Production

1.5.3.1 Drying of Algal Biomass

Microalgae biomass is dried by using different techniques. A variety of methods were developed for drying algal cells such as solar drying, spray drying, roller drying, and freeze-drying. For the drying process, external energy is required, but in the solar drying method, only solar energy is required which is accomplished by direct sunlight, but it is weather dependent and may cause denaturation of an organic compound due to overheating. However, it is a less expensive technique for drying the algal cells and requires larger areas (Oswald 1988; Brennan and Owende 2010). Freeze-drying, roller drying and spray driving have been widely used in the food industry. It is used for drying the microalgae which containing food metabolites in their biomass such as *Dunaliella* sp. (contain β-carotene) and *Haematococcus pluvialis* (contain astaxanthin) (Grima et al. 2003). In this method, spray drying has been mostly used for drying highly valuable algal products. However, it needs external energy and is expensive, and as a result of which, it is not frequently used for biofuel production (Oswald 1988; Grima et al. 2003; Brennan and Owende 2010). Freeze-drying is a good method for drying highly important microalgal cells; it cannot disrupt or deteriorate the microalgal metabolites, but it is a more expensive technique. Solar drying is a good low-cost technique for drying the algal cells for biofuel production.

1.5.3.2 Extraction of Lipid and Carbohydrate from Microalgal Biomass

Lipids and starch are biomolecules that are used for the production of biofuels. Their extraction process is different from the extraction of sugar compounds from microalgae and requires saccharification techniques (Chen et al. 2013), whereas for lipid extraction, different extraction techniques were used such as supercritical CO_2 extraction, solvent extraction, enzymatic extraction, ultrasonic-assisted extraction, and osmotic shock extraction (Medipally et al. 2015). Ultrasonic-assisted extraction, supercritical CO_2 extraction, and osmotic shock extraction are generally used in lab-scale extraction, whereas mostly enzymatic and solvent-based extraction is used for lipid extraction on large scale. The extraction techniques are depending on the types of lipids, cost, efficiency and toxicity, and handling. The supercritical and osmotic shock method is not useful in industries because of the high processing cost, whereas enzyme-based lipid extraction is commercially viable yet it is costly, so new modification is required in those techniques which reduces processing cost (Medipally et al. 2015).

Different strategies are used to produce sugar from microalgae biomass, such as alkaline treatment, enzyme treatment, and mineral acid treatment (Van de Vyver et al. 2010; Chen et al. 2013). In enzymatic treatment, different enzymes are used such as cellulases (cellulose-degrading enzyme) and amylase (starch-degrading enzyme). Enzyme-based hydrolysis has more advantages than chemical hydrolysis. This high glucose yield was obtained, without the formation of any toxic compounds (Cara et al. 2007; Choi et al. 2010). Chemical saccharification is a fast process, but it requires high temperature, acid, alkali, and pressure. Due to this, 5-hydroxymethylfurfural is produced, which is an inhibitor of the fermentation process. But this problem can be overcome by maintaining proper reaction conditions. This method is widely acceptable for saccharification of microalgal biomass containing carbohydrates. It is a simple, cheaper, and intensive method yet sugar yield is lower than enzymatic treatment (Chen et al. 2013).

1.5.3.3 Biomass to Biofuel Conversion Techniques

The biomass conversion is depending on microalgal species, their carbohydrate, and lipid contents. The conversion techniques are classified into three categories: chemical, biochemical, and thermochemical (Medipally et al. 2015). The microalgal content and conversion process produce different types of biofuels such as biodiesel, bioethanol, biofuels, and biomethane (shown in Fig. 1.3). The different conversion techniques which obtain biofuels are described briefly in the below subsections (Chen et al. 2015).

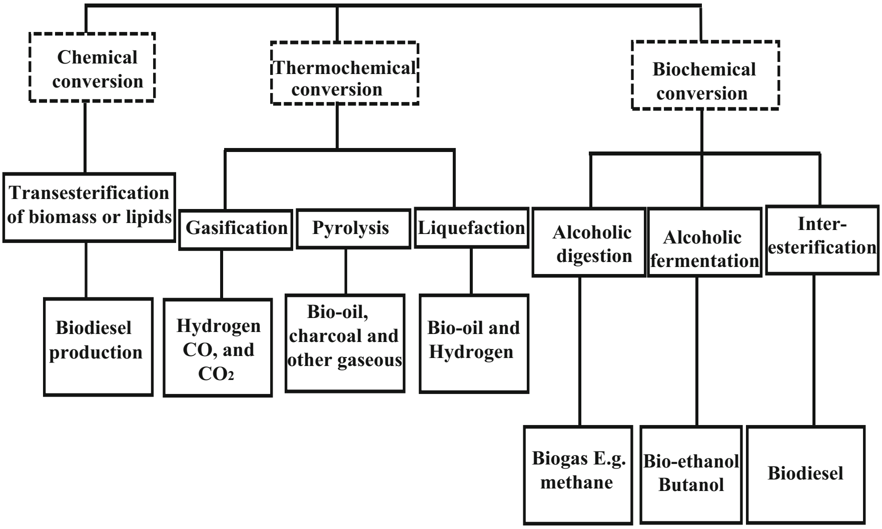

Fig. 1.3 Schematic diagram of microalgal biomass conversion technology of biofuel production (Tsukahara and Sawayama 2005; Medipally et al. 2015)

1.5.3.3.1 Chemical Conversion

Production of biodiesel is achieved by chemical conversion of microalgae biomass. Transesterification is a delicate process that requires proper conditions; it depends on the types of fatty acids, the molar ratio of alcohol to oil, water content, and mainly type of catalyst that is used (Medipally et al. 2015). In this method, the triglyceride (lipid) reacts with mono-alcohol in the presence of acidic and alkaline catalysts to generate a mixture of fatty acid and glycerol. Many reports showed that microalgal lipid can be used for the production of biodiesel by the process of transesterification. Two approaches have been used in the transesterification process: the two-stage process or conventional process and the in situ single-stage process or direct transesterification process. In the conventional transesterification method, lipid has been extracted from microalgae biomass via mechanical or chemical methods and then diverted to transesterification and purification steps. In direct transesterification method or in situ method, it involved lipid extraction and transesterification simultaneously (Wen and Ben 2009; Zeng et al. 2011; Velasquez-Orta et al. 2012; Milano et al. 2016). Johnson and Wen (2009) performed both methods to produce biodiesel from microalgae *Schizochytrium limacinum*; in conventional transesterification method, it resulted in 57% of crude biodiesel (% of total dry biomass) and in FAME of 66.37%, whereas in chloroform-based direct transesterification method, it resulted in 66% of crude biodiesel (% of total dry biomass) and in FAME of 63.47% (Wen and Ben 2009; Milano et al. 2016). Then another chemical method is esterification. The esterification approaches are a reversible reaction process. In this method, the free fatty acids are converted into alkyl ester through an acid catalyst.

The main goal of this technique is to reduce the formation of foam and improve and enhance the conversion of biodiesel. *Monoraphidium contortum* microalgae were used as feedstock for lipid esterification, and results showed that the conversion into methyl esters is averagely 91.7% (Razzak et al. 2013).

1.5.3.3.2 Biofuel Production Via Thermochemical Conversion

Thermochemical conversion is a method of thermal decomposition of microalgae biomass into biofuel products. Conversion is categorized into different types: gasification, pyrolysis, and liquefaction (Tsukahara and Sawayama 2005). Advantages of Thermochemical conversion are (i) better nutrition recovery, (ii) smaller carbon footprint, (iii) short processing time, (iv) no emission of fugitive gas, (v) work well with either wet or dry biomass, (vi) capable in handling various feedstock and blends, pathogens and pharmaceutical active compound are eliminated (Razzak et al. 2013).

Gasification is a thermochemical process in which microalgae biomass has fractionally combusted and formed syngas at a high temperature ranging between 800 and 1000 °C with suitable pressure (Milano et al. 2016). The syngas has low caloric value and can be directly used as fuel for engines and turbines (Razzak et al. 2013), whereas thermochemical liquefaction converts wet microalgae biomass via high pressure ranging between 5 and 20MP and temperature ranging between 200 and 350 °C with catalyst. The main advantage of gasification process is its ability to convert wet biomass into energy. So, it reduces the time required for drying the biomass. But drawbacks of this process are initial cost is too high due to the complex design of the fuel-feed system and reactors (Brennan and Owende 2010). Furthermore, pyrolysis is another thermochemical process that converts biomass into bio-oil, charcoal, and other gaseous products in the absence of air/oxygen. In this process, the temperature is used at ranges between 350 and 550 °C (Goyal et al. 2008). It can be further classified into three classes: conventional pyrolysis, fast pyrolysis, and flash pyrolysis. Pyrolysis has the ability to be used for large-scale biofuel production, especially the fast pyrolysis process is more feasible and effective to produce good quality and high yield of fuel oils (Demirbaş 2006).

1.5.3.3.3 Biochemical Conversion Method

In the biochemical conversion process, microalgal biomass is converted into biofuels or biogas. This process is classified into anaerobic digestion, alcoholic fermentation, and interesterification. Biochemical process is beneficial for the environment and is a less energy-consuming process, but it is not used in large-scale production because of slow processing efficiency and slow reaction steps (Zhou et al. 2017). In the anaerobic digestion microalgae, biomass is transformed into organic compounds, and then organic compounds are converted into biogas or biomethane (Wen and Ben 2009; Kobayashi et al. 2013). This process involved CO_2 and methane production;

this gaseous biofuel can be used directly for generating power gas engines or upgraded into natural gas quality biomethane or as cooking fuel. Anaerobic digestion is appropriate for all organic materials. The selection of digestion system depends on the basis of moisture content of microalgae and carbon and nitrogen ratio in microalgal biomass. The presence of the highest concentration of nitrogen will lead to ammonia inhibition of digestion (Brennan and Owende 2010; Razzak et al. 2013). Currently, anaerobic digestions are commonly used in macroalgae species such as *Chlorella sorokiniana*, *Macrocystis pyrifera*, *Sargassum*, and *Laminaria* biomass to produce biogas (Wen and Ben 2009; Kobayashi et al. 2013). The second biochemical process is alcoholic fermentation. Fermentation is a process of converting organic sugar (starch, cellulose, monomer of sugars like glucose, fructose, etc.) into bioethanol. The microalgae biomass contains starch or other sugar molecules; firstly it is hydrolysed into simple sugar by enzymes or by the chemical or mechanical process before diverting to yeast fermentation to produce bioethanol. Then ethanol is purified by using a distillation process for the removal of water and other impurities (McKendry 2002). *C. vulgaris* is a microalgae that has the highest starch content which can be used for bioethanol (Brennan and Owende 2010). Third biochemical process is the interesterification reaction where the enzyme is used to produce fatty esters. This can be achieved by using high-activity enzyme produced from *Candida antarctica*, which would produce triacetin and long-chain fatty acid methyl or ethyl ester. In recent years, there is a growing interest in interesterification used in biodiesel production where it yields highly pure product, and the by-products can be easily removed. However, the cost of the enzyme is high which makes the process not economically feasible at the current stage (Milano et al. 2016).

1.6 Future Challenges in Microalgal Biofuel Production

Microalgal biofuels are commercially feasible, and their conversion process is simple. But it has some downfalls such as low production of lipid and carbohydrates. The many microalgal sizes are very low and create a problem for harvesting. The harvesting process may increase the operation cost of biofuel production (Medipally et al. 2015). The cultivation process is simple, but the biomass production is low, whereas use of carbon substrate for biomass production in media also allows to grow other microorganism. So, it required proper sterile conditions. The major challenges that arise for microalgal biofuel production are the biorefinery process. Algae producing more lipids and more carbohydrates should be selected for biofuel production. The microalgal cell condition should be optimized. Microalgal biomass conversion for biofuel production is a good approach for the future for resolving the energy crisis, yet more researches are required. The cultivation cost may be reduced by using naturally atmospheric carbon and wastewater as a nutrient supplement. This approach have dual profits; (i) cleaning the environment (ii) the production of biofuel. Furthermore, the major problem of microalgal biorefinery can be overcome by designing a simple low-cost biorefinery system for biofuel production.

1.7 Conclusion

The microalgal-based biofuel system is a good alternative energy source; it can produce all types of biofuels (biodiesel, bioethanol, biobutanol, biogas, etc.) and can resolve the energy crisis. Yet many problems are arising for their cultivation and biorefinery process. The cultivation condition should be optimized and should select the high biomass production microalgae. Wastewater and atmospheric CO_2 should be used to reduce the cost of cultivation. The main problem which arises during the biofuel production is biorefinery (especially in harvesting), which can be sorted out by designing low-cost integrated biorefinery system or bioreactors. The microalgal biomass also contains other highly valuable biofuels which need to be extracted from biomass and integrated with their biorefinery system to minimize the total operational cost of biofuel production.

Conflict of Interest All authors approve the submission and declare that there is no conflict of interest.

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Chapter 2
Biorefinery Approach for Sustainable Biodiesel and Bioethanol Production from Microalgae

Lukapriya Dutta, Julie Baruah, and Eeshan Kalita

Abstract Microalgae-derived biofuels and bioethanol present an opportunity for the production of sustainable fuels in the face of rapidly depleting fossil fuel reserves. Fossil fuels are known to present a multitude of challenges in terms of environmental pollution due to combustion and environmental depredation stemming from extraction and refinery processes. Nonetheless, fossil fuels remain the fuel of choice due to their competitive pricing and industrial predominance. Microalgal biofuels in contrast are yet to attain commercial acceptance, owing to the toilsome and extravagant extraction procedures for yielding a single by-product. The advent of biorefinery processes has led to the development of sustainable technologies for the production of numerous downstream products from fuel feedstocks. Microalgae being rich in proteins, long-chain polyunsaturated fatty acids (PUFA), polymeric carbohydrates, and antioxidants pigments are most appropriate for integration into biorefinery processes. A slew of by-products can thereby be extracted sequentially or simultaneously alongside the biofuels, leading to significant reductions in operational costs. This chapter presents a broad overview of the evolution of microalgae-based biorefinery with a special emphasis on biorefineries associated with biodiesel and bioethanol production. It also provides an insight into the recent advances leading to future opportunities for microalgae-derived biofuel production.

Keywords Biorefinery · Microalgae · Biodiesel · Bioethanol

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Abbreviations

PBR	photobioreactor
SHF	separate hydrolysis and fermentation
SSF	simultaneous saccharification and fermentation
FAME	fatty acid methyl ester
FAEE	fatty acid ethyl ester
HVED	high-voltage electric discharge
CA	carbonic anhydrase
DHA	docosahexaenoic acid
EPA	eicosapentaenoic acid
GLA	γ-linolenic acid
PUFA	polyunsaturated fatty acid
IoT	internet of things
DSS	decision support system

2.1 Introduction

An ever-increasing worldwide energy demand, as well as global warming caused by the use of fossil fuels, emphasizes the importance of researching and implementing alternative clean, green, and sustainable energy options. Among various other renewable energy possibilities, bioenergy production from microalgae has gained widespread attention in recent years (Mishra et al. 2019; Goswami et al. 2021a). Gradual scientific research has established microalgal biomass to be the viable source, towards the generation of biofuels such as biodiesel and bioethanol in an environment-friendly manner (Ismail et al. 2020). Biofuels can be classified differently according to their feedstock. Most food crops are considered as the feedstock for first-generation biofuel production. Organic wastes, lignocellulosic, and municipal wastes are the feedstocks for second-generation biofuel. However, algal biomass being non-food competitors is considered to be superior over other feedstock for third-generation biofuel production and has gained attention for higher environmental performances (Elshobary et al. 2021; Gambelli et al. 2017; Agrawal et al. 2020).

Microalgal biomass predominantly consists of protein, lipid, and carbohydrates. While plenty of other bioactive compounds, vitamins, and micro- and macronutrients, pigments and sterols can also be produced from it following certain procedures (Koutra et al. 2020; Mehariya et al. 2021). In comparison to terrestrial plants, microalgae can rapidly grow by using a small amount of water and nutrients and also has the ability to grow in wastewater environments by utilizing the excess nutrients. Besides, it can sequester carbon dioxide released from the atmosphere and industrial gases (Bhardwaj et al. 2020). Therefore, microalgae have been proposed as a sustainable feedstock for biorefinery applications (Bhalamurugan et al. 2018).

The concept of biorefinery incorporates multiple processes that transform biomass into energy and value-added products, increasing its benefits and profitability while decreasing environmental waste (Brasil et al. 2017). Another key concept is the production of biofuels using biomass transformation and processing equipment. Microalgal biorefineries include two basic stages: upstream processing and downstream processing. It also includes several conversion technologies, namely, biochemical conversion, transesterification, thermochemical conversion, microbial photosynthetic fuel cell, etc. Depending on the type and amount of feedstock, desired product, and economic considerations, the conversion process is being selected and implemented (Chew et al. 2017).

Apart from providing enormous prospects in the field of research, insufficient productivity strategy, and small-scale extraction, the purifying process of microalgal biomass has become a limiting issue in reaching a broad societal level. Likewise, increasing productivity while lowering production costs, in the face of increasing populations with expanding energy requirements is also a major challenge (Schiano di Visconte et al. 2019). It is reported that a large-scale biomass cultivation strategy for value-added products has not been executed yet. Despite having advanced technologies, the cost of producing microalgal biofuel is still two times higher than that of producing fossil fuels (Brasil et al. 2017). One approach to address this challenge through recent scientific advances is microalgal biomass cultivation using wastewater where excess nutrients of wastewater are being utilized as nutrients and a growth medium for microalgae (Prakash et al. 2021). It reduces the cost of production and the number of nutrients required for cultivation while purifying the wastewater in the process (Agrawal and Verma 2022). Genetic engineering of microalgal strains is another approach that can enhance the lipid content as well as other biomolecules of interest that can be used in a higher yield of biorefinery-based products (Chaturvedi et al. 2020; Kumar et al. 2020). Microalgal lipids and carbohydrates play a pivotal role in biodiesel and bioethanol production (Ghosh et al. 2017). Pre-treatment of feedstock followed by lipid transesterification and carbohydrate fermentation are the determining steps involved in these processes. However, biodiesel and bioethanol production has not received desirable commercial success, and hence there is extensive ongoing research to develop competitive and comprehensive biorefinery strategies (Karpagam et al. 2021).

This chapter highlights the importance of the microalgal biorefinery concept for addressing the global energy crisis by producing sustainable biofuels. Also, it gives special emphasis on different processes involved in biodiesel and bioethanol production. Another objective of this chapter is to study the limiting factors of microalgal biorefineries providing insights into recent advances and future opportunities to overcome these factors.

2.2 Concept of Microalgal Biorefinery

Biorefinery is a facility that is largely based on the concept of converting biomass-derived compounds into multiple high-value, marketable, and profitable products. It is similar to conventional oil refineries, the basic difference being the crude feedstock (Bhattacharya and Goswami 2020).

Microalgae are unicellular eukaryotic photoautotrophic organisms that can convert light energy, water, and carbon dioxide into microalgal biomass (Table 2.1). Microalgal biomass has higher contents of protein (6–70%), carbohydrate (4–64%), lipid (2–50%), nucleic acid (1–6%), and nutrients like nitrogen and phosphorous (De bhowmick et al. 2019). They can be cultivated in an open pond or closed system such as a bioreactor. One of the most promising advantages of microalgal biomass cultivation is the ease of handling, lower energy consumption, and reduced overall expense for the whole process. Microalgae can grow using environmental water and CO_2 and have a shorter life cycle as compared to the terrestrial plant (Bhattacharya and Goswami 2020; Goswami et al. 2021b). Owing to their higher photosynthetic rate, the biorefinery framework using microalgae to generate bio-based products and biofuels has numerous potential towards sustainability and economic profitability (Khoo et al. 2020).

2.2.1 *Microalgae Cultivation System*

Microalgae can be found in natural ecosystems such as ponds, rivers, and oceans; however, it cannot be used readily for biofuel production as it does not provide a huge amount of biomass. To meet the industrial requirements and achieve production capacities, microalgae have to be cultivated and harvested on a large scale where inorganic and organic chemicals have been conventionally used. Despite the efforts for microalgal biomass cultivation, the constraints associated with the optimization of harvesting and propagation have restricted it from realizing the economic reality

Table 2.1 Examples of some of the microalgal species and their targeted biofuel

Microalgae species	Targeted biofuel	Source
Botryococcus braunii	Biodiesel	Moreno-garcia et al. (2017)
Dunaliella tertiolecta	Biodiesel	Norjannah et al. (2016)
Chlorococcum humicola	Bioethanol	Deprá et al. (2018)
Spirulina sp.	Bioethanol	Culaba et al. (2020)
Scenedesmus dimorphus	Biodiesel	Figueroa-Torres et al. (2020)
Chlamydomonas reinhardtii	Bioethanol	Suparmaniam et al. (2019)
Chlorella vulgaris	Bioethanol	Jambo et al. (2016)
Nannochloropsis gaditana	Bioethanol	da Maia et al. (2020)
Porphyridium cruentum	Bioethanol	Phwan et al. (2018)
Chlorella protothecoides	Biodiesel	Peng et al. (2020)

(Suparmaniam et al. 2019). Both open ponds with a paddle wheel for disseminating water and nutrients throughout the microalgal cells and closed systems are used in far-reaching industrial microalgae cultivation practices. Open pond cultivation system remains constantly exposed to the environment, which significantly increases the chances of contamination while being more economical to operate. Closed systems like photobioreactors lower the possibility of contamination in culture systems but are more cost-intensive (Fulbright et al. 2018). The open and closed microalgal cultivation systems are discussed in detail in the following section:

2.2.1.1 Open Cultivation System

To fulfill the demand for futuristic methodologies, the cultivation of microalgae in larger quantities is necessary. Cultivation of microalgae in an open pond is one of the oldest experimental approaches and is largely accepted for mass-scale cultivation as it possesses certain advantages. The chief advantage of open cultivation is its easy handling and cost-effectiveness using sunlight as a source of energy; the operation costs are low for this technique (Jerney and Spilling 2018). Microalgae cultivation can be carried out in natural water (lakes, lagoons), open or covered constructions, or in man-made basins. Nutrient supply is commonly used from the surface runoff water, and new approaches for wastewater cultivation are being implemented to reach the nutrient requirements (Tan et al. 2018). Raceway ponds and circular ponds are usually considered strategies for open cultivation systems (Fig. 2.1) (Suparmaniam et al. 2019).

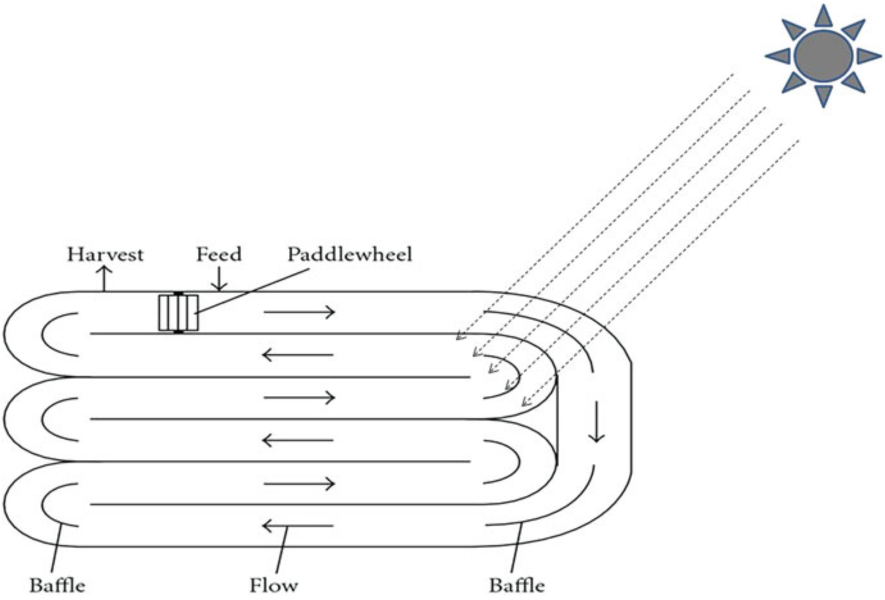

Fig. 2.1 Diagrammatic illustration of a raceway pond (Dickinson et al. 2017)

The majority of open ponds are built in a circular or raceway pattern, where raceway ponds with a shallow approximate depth of 0.3 m for optimal exposure to sunlight to improve the growth of microalgal cells are constructed. The construction feature of these ponds includes highly inexpensive materials, where the walls of the pond are constructed with either concrete or rammed earth with plastic lining. Throughout the cultivation cycle, a paddlewheel is used to keep the system running continuously to eliminate the possibility of deposition. In the front space of the paddlewheel, a feeding port is located through which the continual nourishing with nutrients and microalgal broth is processed (Faried et al. 2017). Calipatria, California, is said to have the world's largest raceway-based biomass generating plant (USA). In this plant, *Spirulina* sp. is grown in large quantity contributing to the food industry (Kiran et al. 2014).

The circular pond is another form of open pond system where a central pivot rotating agitator is present for the mixing of culture. A greater impact of construction expense of this kind of pond makes it non-recommendable for commercial use (Lee et al. 2018).

Microalgae being photoautotrophic, carbon sequestration is one of the important functions of these microorganisms. Carbon sequestration refers to the process of microalgal cells absorbing CO_2 from the atmosphere and storing it as food reserves. The primary advantage of employing microalgae is that captured carbon does not need to be disposed of because it can be converted into lipids, carbohydrates, and other biomolecules (Mondal et al. 2017). Extracellular carbonic anhydrase (CA) enzyme facilitates the process of carbon sequestration in microalgae (Sültemeyer 1998). In the case of microalgal cell culture, CO_2 fixation is determined by light intensity and cell density. Thus closed cultivation system is regarded as ideal for monitoring light utilization efficiency of microalgal cells in order to enhance carbon sequestration (SundarRajan et al. 2019). Recently, microalgal screening, cultivation, and reactor system development are all being studied in order to improve carbon trapping even more (Xu et al. 2019).

Apart from the aforementioned benefits, the open cultivation system has numerous drawbacks that must be considered. Most importantly the risk of extraneous contamination of the culture medium and evaporation largely affects the biomass yield (Chew et al. 2018). Additionally, large spaces are required for the construction of the entire functional setup for an open pond. Another important limitation of this technique is its uneven sunlight distribution where the sunlight can reach up to only a certain depth which eventually affects the culture condition of the biomass (Faried et al. 2017; Suparmaniam et al. 2019; Tan et al. 2018).

2.2.1.2 Closed Cultivation System

Closed cultivation systems were being introduced to lower the contamination, by increasing the ability to control the culture condition. Usually, mass-scale cultivation of microalgal biomass is processed through this technique. The term closed system itself refers to photobioreactors that have no direct exchange of gases with the outer environment. Usually, gas exchange is performed through the gas filter in order to reduce contamination inside the culture suspensions (Yen et al. 2019). A photobioreactor is a closed, semi-closed, or open vessel made of transparent and water-resistant materials. PBRs can provide an ideal environment for microalgae cultivation, and different PBRs have been introduced in past years that have the potential to reach the commercial need of microalgae cultivation (Ting et al. 2017). PBRs can present the desired condition to microalgal growth by providing proper light and temperature, carbon source and nutrients, suitable pH, etc. (Sun et al. 2016). Elevated concentrations of CO_2 gas are regarded to be the main source of inorganic carbon for the cultivation process of photoautotrophic microalgae. It is pumped into the slurry of algal cells in the form of bubbles through the gas aerator in PBRs. By interchanging the boundaries of the gas-liquid phase, these bubbles eventually dissolved in the cell suspension. Photoautotrophic microalgae use this carbon to produce complex organic molecules almost rapidly (Zhao et al. 2015; Zhou et al. 2017). Sedimentation and O_2 deposition in the cell suspension are prevented by efficient mixing of the cell suspension. The optimized light conditions and nutrients supply in PBRs can help in proper cell growth along with lipids and pigments deposition in cells (Guo et al. 2015; Liao et al. 2017). It is reported that synergic efforts of light, CO2, and nutrients determine microalgal growth. These three factors are crucial in mass-scale microalgal biomass production. The design and construction of PBRs generally considered a single factor, but all the three factors and their impacts are strongly interrelated in a multiphase photobioreactor (Fu et al. 2019). Different photobioreactors for closed system cultivation are:

- Vertical tube photobioreactors
- Horizontal tube photobioreactors
- Stirred tank photobioreactors
- Flat panel photobioreactors (Chew et al. 2018) (Fig. 2.2)

Despite having great progress in implementing photobioreactors for large-scale biomass cultivation, more efforts are required to reduce the limitations of this concept. High equipment cost and process cost in PBRs design are some of the major barriers to its further development. Finding a more rigid and economic strategy in PBR constructions is necessary to enhance its cultivation efficiency (Yen et al. 2019; Goswami et al. 2021b).

From single-cell microalgae to multiproduct biorefinery concept, the whole approach includes a robust continuous process. After cultivation, it includes different stages and conversion methods depending on the end product. An overall flow diagram for the biorefinery concept is depicted here (Fig. 2.3).

2.3 Microalgal Biorefinery for Biodiesel Production

The production of biofuel can be a potential solution for the alarming environmental challenges that arise due to the substantial use of fossil fuels. In industries, fossil fuel has gained major attention; however, they are less environmental friendly and

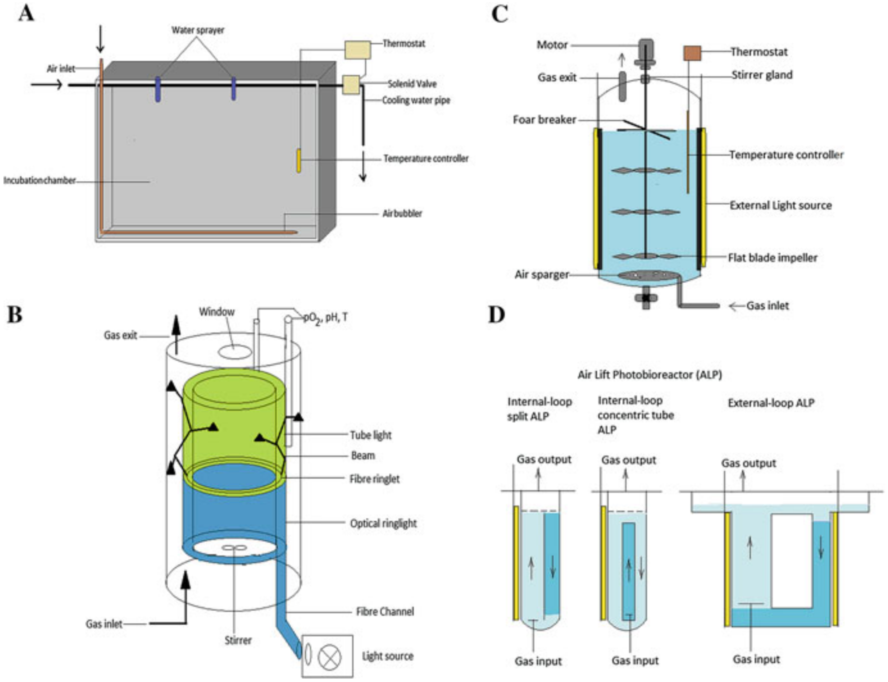

Fig. 2.2 (**A**–**D**) A brief representation of photobioreactors of various types used in microalgal closed cultivation (adapted from Mondal et al. 2017)

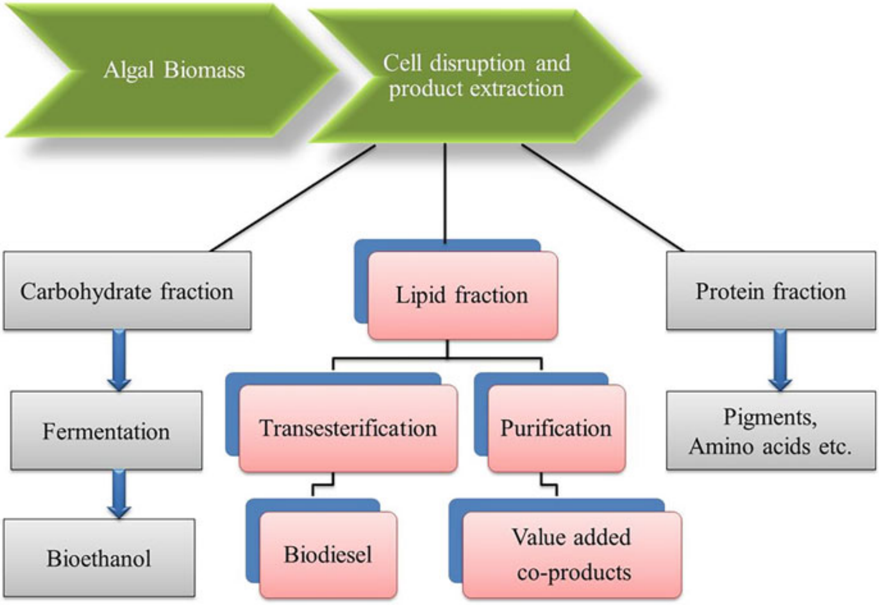

Fig. 2.3 A brief overview of microalgal biorefinery approach

non-renewable. In comparison to this, biofuels are one form of alternative renewable energy. Biofuels are classified into three generations as the first generation, the second generation, and the third generation, depending on the feedstock utilized, i.e., food sources, lignocellulosic biomass, agriculture waste, and algal biomass, respectively (Peng et al. 2020). Recent exploration in the field of research has recognized microalgal biofuel production strategy as a direct energy conversion process to produce biofuels. Photoautotrophic algae have the ability for biomass accumulation. This organic biomass is the raw material for producing biohydrogen, biodiesel, bioethanol, etc. (Rodionova et al. 2017). The use of microalgae in biofuel production by converting its oil content into biodiesel is a way to vanish the limiting factors associated with second-generation biofuels. Primary considerations of biodiesel production are the selection of proper microalgae strain, cultivation process, harvesting and pre-treatment process, extraction, and transesterification. Strain selection is crucial because different strains differ greatly in their cellular contents and growth behavior such as high-value products, lipid, fat content, and ideal growth circumstances (Dickinson et al. 2017). According to reports, the dry weight of microalgal species biomass like *Botryococcus braunii* and *Chlorella vulgaris* contains 50% of lipids and has a higher biodiesel generation potential (Najafi et al. 2011). Upstream processing and downstream processing are the two main stages of biodiesel production. Upstream processing usually deals with the microalgal strain selection and factors associated with the cultivation of microalgae (Chew et al. 2017).

2.3.1 Upstream Processing

Microalgae with an elevated amount of lipids are good candidates for biodiesel extraction. Upstream processing starts with the selection of suitable microalgal species and their cultivation by optimizing several growth parameters. Temperature, nutrients, pH, light intensity, CO_2, etc. can stimulate the accumulation of valuable components in the microalgal cells (Fig. 2.4) (Yew et al. 2019). *Nannochloropsis oculate*, *B. braunii*, and *Chlorella vulgaris* are some high lipid-containing microalgal species that are used in biodiesel production (Cornejo-Corona et al. 2016). The growth of microalgae is primarily driven by nutrients like nitrogen and phosphorus, and increased nutrient availability ensures larger biomass production. According to various reports, the growth rate is also affected by the source of illumination, and artificial lighting such as LED lighting is more effective than natural lighting (Koyande et al. 2019).

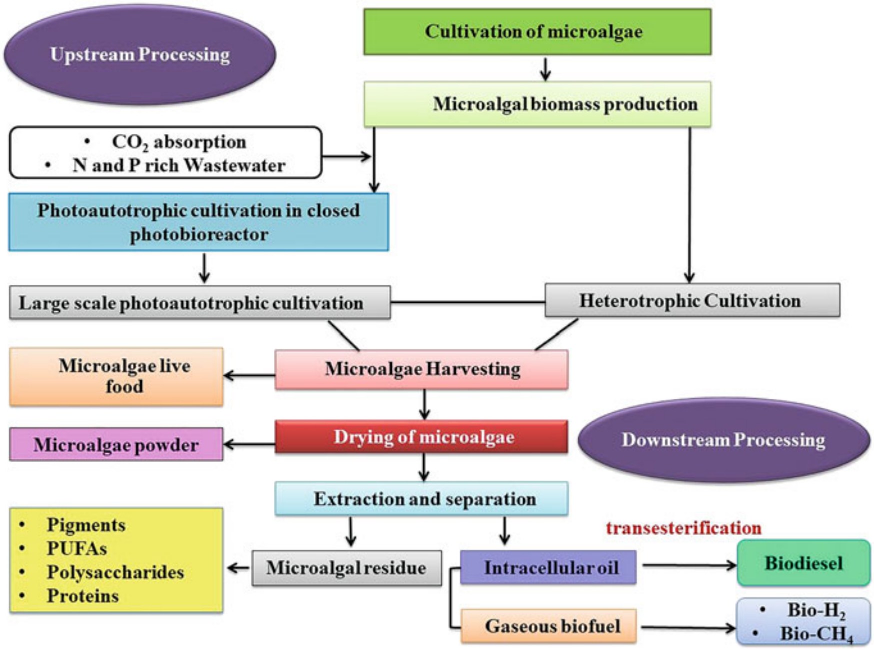

Fig. 2.4 Overall representation of upstream and downstream processing (adapted with permission from Wang et al. (2021))

2.3.2 *Downstream Processing*

Downstream processing of microalgae is a sequential process that includes microalgae harvesting, extraction of product and purification, etc. (Lam and Lee 2015). According to previous statistics, the downstream processing stage accounts for 60% of overall biodiesel output (Kim et al. 2013). Therefore, an economic, simplified integrated approach of downstream processing is required to be implemented (Lam and Lee 2015; Goswami et al. 2021c). A brief discussion about downstream processing is stated below.

2.3.2.1 Harvesting

Harvesting is a sequential process in which different downstream techniques are used to concentrate the biomass by removing water content from the microalgae growing medium. Microalgae harvesting can be done by following conventional as well as advanced methods (Table 2.2). Appropriate method selection is based on the energy consumption and cost depending on the cell size and density. Conventional methods like filtration, centrifugation, sedimentation, and electric methods are

Table 2.2 Microalgae harvesting technique and their characteristics

Harvesting technique	Characteristic
Centrifugation	Centrifugal force is a major driven force for sedimentation of microalgae
Filtration	Filtration of biomass is processed through pores
Flocculation	By adding substance to the culture for sedimentation of cells
Flotation	Microbubbles are used to carry microalgae to the surface

commonly used. Among them, centrifugation is highly used for the microalgae harvesting process (Vasistha et al. 2021).

Different advanced harvesting techniques are also introduced; they are advantageous over the conventional ones. Flocculation is the most convenient and effective process for harvesting microalgae. Developments in research fields have led to the advancement of flocculation processes. Currently, nanomaterial-driven flocculation techniques are considered an efficient and reusable method. The flocculation process enhances the particle size of suspension by aggregating and gradually increasing the settling rate (Japar et al. 2017).

2.3.2.2 Cell Disruption

Microalgal cell disruption plays an important role in biorefinery-based techniques. The cell wall acts as a barrier to the release of intracellular components of the cell. Cell disruption techniques degrade the cell wall and release the inner cellular components to be used for further processing. Cell disruption techniques can be distinguished as:

- Biochemical cell disruption (enzymatic and alkali/heat treatment).
- Mechanical cell disruption (bead milling and high-pressure homogenization).
- Physical cell disruption (pulse electric field and ultrasound treatment).

Cell disruption is preferred to be performed in mild conditions in order to maintain the native state of the inner cell components (Lam and Lee 2015).

The selection of cell disruption processes depends on the types of microalgae cells as microalgae show diversity in their cellular compositions. According to previous studies, some algae species like *Scenedesmus* sp. have a multilayer cell wall that makes them highly resistant to chemicals and biological disruption (Yew et al. 2019). Because they lack a cell wall, *Pavlova* sp. and *Dunaliella* sp. are more susceptible to chemical and biological pre-treatment procedures (Dunker and Wilhelm 2018). Advances in research have increased the interest in a combined pre-treatment approach that can enhance the level of cell disruption. The effectiveness of a combination of mechanical, chemical, and thermal pre-treatment methods in cell disruption is being investigated (Tsapekos et al. 2016).

2.3.2.3 Extraction of Lipid

The extraction of lipids is a prerequisite for biodiesel generation since microalgae show a significantly higher content of lipids in comparison to other sources. Different strains of microalgae differ in fatty acid composition and fatty acid profile of lipids, depending on the medium composition, cultivation process, illumination intensity, aeration rate, etc. (Muhammad et al. 2021). Although lipids are not soluble in water, they get dissolved in organic solvents. Lipids are divided into polar and nonpolar groups based on their head group's polarity. Microalgae use polar lipids to produce cell membranes, while nonpolar lipids are used as an efficient source of energy (Subhash et al. 2017). Lipid extraction from microalgae is categorized into two main methods, i.e., chemical and mechanical (Mubarak et al. 2015). Usually, organic solvents and superficial fluid extraction methods are used in chemical extraction. Nonpolar solvents dissolve polar lipids, while polar solvents dissolve nonpolar lipids. Lipids can be dissolved by various solvents like methanol, acetone, ethanol, chloroform, benzene, butanol, n-hexane, etc. Among them, hexane, methanol, and chloroform have proven to have great promises for the extraction of lipids (Bundhoo 2018). Two of the most used solvent extraction processes are Folch's technique and Bligh and Dyer's technique. Lipids are extracted by blending chloroform with methanol in a 2:1 proportion, along with the cell homogenization step equilibrated with one-fourth volumes of saline solutions (Folch 1957). Bligh and Dyer's method use 1:2 (v/v) chloroform and methanol as lipid extraction solvent (Blight and Dyer 1959). According to earlier studies, 94.9% of lipid is obtained from *Nannochloropsis* sp. using Folch's procedures in its liquefied biomass (Wu et al. 2017). Earlier reports state that Bligh and Dyer's methods can be employed to yield more than 95% of crude lipids (Yew et al. 2019). For batch production of lipids in industries, a continuous solvent method is used through a process known as Soxhlet extraction. In order to reload the biomass with fresh solvents, this process requires continual evaporation and precipitation cycles of the solvent. A mixture of hexane: alcohol or fresh hexane is commonly used as solvents in this process (Sati et al. 2019).

Extraction of lipid is done using a variety of mechanical methods in both pilot and commercial scales. Mechanical extraction does not necessitate the use of a solvent; besides they use expeller press procedures, bead mills, microwave-assisted pyrolysis extraction, ultrasound-assisted extraction, etc. Mechanical processes are said to be more effective since they are not dependent on the type of microalgae used. They are less likely to cause contamination in the lipid product (Kumar et al. 2020). For cell rupture and extraction of intracellular cell compounds, the high-voltage electric discharge (HVED) approach was recently introduced. In this technique, an electric voltage charge is used to destruct the cell wall and thus release the intracellular components (Li et al. 2019).

2.3.2.4 Transesterification

After purification and separation of impurities and solvents from the desired products, lipid contents undergo a transesterification reaction to produce biodiesel. Among different techniques like microemulsion, pyrolysis, and blending, transesterification reaction is widely used, as biodiesel obtained through this process can directly be used in engines (Fig. 2.5) (Miao and Wu 2006). Transesterification requires three moles of triglycerides of algal lipid content to react with methanol or ethanol, and this reaction yields one mole of glycerol and three moles of fatty acid methyl ester (FAME) or fatty acid ethyl ester (FAEE). Atmospheric pressure and temperature of 60 °C to 70 °C are considered ideal for transesterification reactions (Bhatia 2014). Three main transesterification techniques for biodiesel production are non-catalyzed reaction, chemical-catalyzed reaction, and enzyme-catalyzed reaction. Among them, enzyme-catalyzed reactions are widely used. The use of catalyst can boost biodiesel production through a transesterification reaction. Lipase enzyme is generally used for biodiesel production. This enzyme can convert algal biomass-extracted oil into biodiesel in the form of fatty acid alkyl ester and glycerol as a by-product (Norjannah et al. 2016). Besides enzyme catalyst, base catalyst is used in the mass production of biodiesel in industries. NaOH and KOH are the major base catalysts, as they can give a higher yield of biodiesels within a very short span of reaction time (Amini et al. 2017). Sulfuric acid is an acidic catalyst that can replace the usage of base catalysts (Zhang et al. 2016 from Yew). To overcome the requirements of catalysts, a superficial transesterification technique was introduced. Besides having advantages in comparison to the catalyst-driven transesterification reactions, a detailed study about this technique is still awaiting (Patel and Shah 2015).

2.3.3 Other Valuable Coproducts

Microalgae are a remarkable source of biofuels and other bioactive high value-added compounds. Lipids and pigments are examples of bioactive compounds having

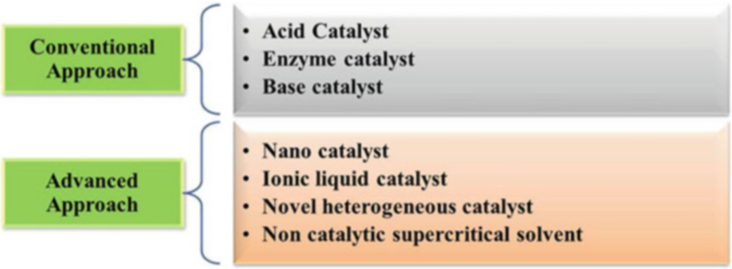

Fig. 2.5 Transesterification approach for biodiesel production

various clinical properties (Mondal et al. 2017). According to Ubando et al. (2014), the cost of biofuel can be reduced by manufacturing higher value-added coproducts along with the biofuel of interest. An integrated approach for heat and electricity generation from pre-treated biodiesel feedstock is one such important illustration. After lipid extraction, biomass can be anaerobically digested to produce methane, which can be utilized to generate heat and energy and further turned into methanol (Ubando et al. 2014). Microalgal lipids having more than 20 carbons in their structure provide valuable products to nutraceutical industries and the pharmaceutical sector. Polyunsaturated fatty acids like omega-3 and omega-6 are generally found in microalgae species like *Parietochloris incise*, *Nannochloropsis* sp., *Phaeodactylum tricornutum*, *Thalassiosira*, etc. High-value fatty acids such as docosahexaenoic acid (DHA), γ-linolenic acid (GLA), eicosapentaenoic acid (EPA), etc. can be obtained prior to the transesterification step of biodiesel production and can be sold as a supplement of omega-3 fatty acid in their pure form (Dickinson et al. 2017). Likewise, glycerol is a coproduct of biodiesel production that has numerous applications in the cosmetics industries (Hu et al. 2018; Kumar and Bharadvaja 2020). Reports state that PUFA from marine alga can be obtained as a by-product from the lipid extraction processes like Bligh and Dyer extraction, sonication and solvent extraction, etc. Recovery of these valuable compounds from ocean fishes is currently restricted in view of marine resource depletion and has motivated the by-product recovery from microalgal feedstock (Chew et al. 2017).

2.4 Microalgal Biorefinery for Bioethanol Production

Bioethanol being a nontoxic, biodegradable, sustainable, clean fuel, the global production of bioethanol is increasing rapidly from 2000 to 2020. First generation of bioethanol was produced from food crops that raised many concerns regarding food security. Gradual efforts have emerged third-generation bioethanol from microalgae as an advantageous one in place of previous forms (Phwan et al. 2018). Microalgae are used in bioethanol production due to the presence of higher carbohydrate content and lower lignin content. Bioethanol is produced by biochemical conversion, followed by fermentation processes, and the degree of pre-treatment and types of fermentation strategies can have a great impact on bioethanol production (Peralta-Ruiz et al. 2018). Pre-treatment methods break down the carbohydrate into simple sugars and then convert it into bioethanol by the most important process of fermentation (Fig. 2.6) (Chng et al. 2017 from Phwan et al. 2018).

2.4.1 *Pre-Treatment*

Pre-treatment methods can disrupt the cell wall of microalgae by releasing intracellular carbohydrates of the cell. Starch-rich microalgal cells are needed to be

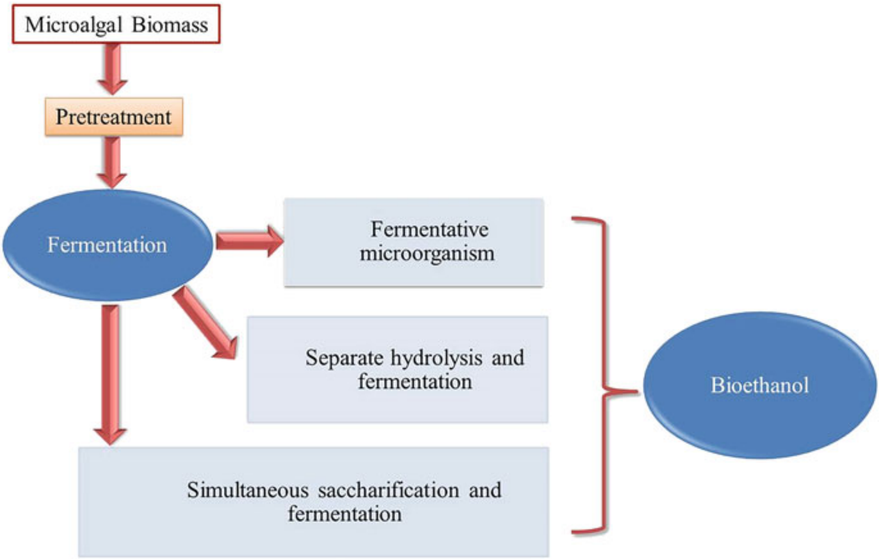

Fig. 2.6 An overview of fermentation processes and bioethanol production

pre-treated and broken down for the modifications of inner carbohydrates to attain bioethanol by converting the obtained glucose content (da Maia et al. 2020). The starch content of microalgae is composed of amylose and amylopectin which have higher molecular weights, and these molecules remain in their crystalline form which is difficult to hydrolyze through enzymes action. Hence different pre-treatment methods are used to alter their structure in addition to methods like chemical and mechanical treatments for releasing the intracellular carbohydrates into simpler forms for bioethanol production (Velazquez-Lucio et al. 2018; Phwan et al. 2018). Chemical hydrolysis is the most common method for hydrolyzing polysaccharides into simple sugars. A wide range of acids can be used among which sulfuric acid is the most common. Enzymatic hydrolysis followed by saccharification releases simple sugars such as glucose and mannose from the microalgal cell. Enzymatic hydrolysis is gradually gaining attention for microalgal biofuel production, as they are more susceptible to enzymes (Jambo et al. 2016). In comparison to chemical hydrolysis, enzymatic hydrolysis does not require a sophisticated setup for completion of the process, nor does it release any toxic substances while providing a higher yield of carbohydrate (Velazquez-Lucio et al. 2018; Milano et al. 2016). Bacteria and fungi can produce cellulase enzymes, some examples being *Bacillus*, *Clostridium*, *Thermomonospora*, *Cellulomonas*, *Erwinia*, *Ruminococcus*, *Bacteroides*, *Microbispora*, and *Acetovibrio* for bacteria and *Penicillium*, *Streptomyces*, *Fusarium*, *Trichoderma*, *Humicola*, *Schizophyllum* sp., *Phanerochaete*, etc. for fungi (Hill et al. 2006).

2.4.2 Fermentation

Aerobic and anaerobic microorganisms such as bacteria and fungi, can be used for the fermentative conversion of the microalgal starch and cellulose content into bioethanol and other value-added products since they perform energy synthesis as a prime requirement for their survival (Bibi et al. 2017; da Maia et al. 2020; Özçimen et al. 2015). *Saccharomyces cerevisiae* is the most commonly utilized strain for fermentation conversion due to their higher ability for bioethanol production and lower accumulation of by-products, etc. (Jambo et al. 2016). Besides, *P. stipites*, *Kluyveromyces fragilis*, *K. marxianus*, *E. coli*, and *Klebsiella oxytoca* are some other examples of microorganisms used for fermentation. Some microorganisms like *Chlamydomonas moewusii*, *C. vulgaris*, *Spirulina*, etc. can expel ethanol produced by anaerobic fermentation inside the cell even in absence of light. These microalgae accumulate starch in large quantities (Demirbas 2010; de Farias and Bertucco 2016; Phwan et al. 2018). The primary metabolic pathway of ethanol production is in glycolysis where glucose is converted into two molecules of pyruvate that is reduced anaerobically into ethanol (Bai et al. 2008; da Maia et al. 2020). For the generation of bioethanol, the most common methods are:

- Separate hydrolysis and fermentation (SHF).
- Simultaneous saccharification and fermentation (SSF).

The hydrolysis and fermentation reactions occur separately in the SHF method, and separate reactors are required to carry out both the reactions under optimum conditions of pH, temperatures, culture conditions, etc. However, hydrolysis and fermentation occur simultaneously in the same reactor in the case of SSF workflows. Reports had stated that SSF is more advantageous than SHF due to higher yields of bioethanol (Lam and Lee 2015).

2.4.3 Other Valuable Coproducts

Microalgae’s high carbohydrate content can produce bioethanol and other very useful commercial chemicals as a part of the comprehensive approach for lowering costs and reducing residual waste output (da Maia et al. 2020). Carbohydrates of microalgae can be converted to methane and biohydrogen by a process of anaerobic digestion (Zhu 2015). In addition to this, it has a great impact on the food, cosmetics, nutraceutical, and pharmaceutical industries. Carbohydrate polymers like alginate, glucan, laminarin, and mannitol are extensively used in paper, pharmaceutical, and textile industries as they can act as stabilizing agents. Another biopolymer, agar, is commonly utilized in the food industry for its stabilizing and gelling properties (Özçimen et al. 2015). According to previous studies, *Porphyridium cruentum* accumulates substantial amounts of extracellular carbohydrates that can be widely used in the skincare and cosmetic industries (Leu and Boussiba 2014). Some sulfated polysaccharides like carrageenans, agarans, and fucoidans can act as bioactive compounds like antioxidants, anticoagulants, and antitumor agents (Dickinson et al. 2017). Besides value-added by-products, pigments of microalgae play an important role in deriving different industrial products. Astaxanthin is a xanthophyllic red pigment with multiple applications in the food, feed, and nutraceutical industry. It can act as an antioxidant, anticancer, and anti-inflammatory agent. Another xanthophyllic compound is lutein, which is widely used to treat neurodegenerative disorders in view of their antioxidative properties (Kumar and Bharadvaja 2020).

2.5 Future Opportunities of Microalgal Biorefinery in Biofuel Production

Gradual development in the field of scientific research has influenced largely human minds to search for new strategies. So far biomass-derived components were utilized for the production of biodiesel and bioethanol. However, path-breaking ideas are emerging from different researches which mainly focus on inserting genes in strains like *E. coli* to break down the cellulosic mass thereby giving a continuous source of sugars for bioethanol production (Niphadkar et al. 2018). Although the importance of the third-generation biofuels has already been established, the scale-up to commercial capacities and optimized performances are currently the biggest challenges. Advances in genetic engineering and genomic research have turned the focus towards the development of microalgal species with enhanced growth, productivity, and content accumulation for high value-added products to utilize these improvements and make biofuel as an economic reality; the biorefinery approach is most suitable (Eppink et al. 2017).

Metabolic engineering is one such emerging approach that converts microalgal cells into efficient cell factories by selecting proper metabolic pathways and enzyme targets, etc. High-throughput genomic, transcriptomic, proteomics, and metabolomics approaches could enhance metabolic engineering more accurately for biofuel production. However, these potential approaches still could not emerge as an economic possibility due to various limitations (Callegari et al. 2020). Microalgae cultivation and upstream processing are highly costly; therefore, a new cultivation system, i.e., wastewater cultivation, is newly introduced and gaining attention everywhere. The dewatering step in biodiesel production is highly laborious as well as requires a great amount of cost which needs to be lowered in the future to increase productivity. Likewise, research should be a focus for a new and cost-effective strategy for the microalgal lipid extraction process for biodiesel production (Yew et al. 2019). A new concept for the fourth-generation biofuel places emphasis on photosynthetic solar fuel production from algae and cyanobacteria. Metabolic engineering is the basis of this approach which uses photosynthesis to produce ready-to-use chemicals and other products. However, inadequate research studies regarding metabolic engineering and fourth-generation biofuels prevent this highly efficient approach to be implemented properly (Acheampong et al. 2017; Phwan et al. 2018).

After computers and the internet, the internet of things (IoT) is regarded as the third wave of the global information industry, capable of establishing connections between people, systems, and services (Perwej et al. 2019). Microalgal biorefinery has progressed to the point where IoT is pervading conventional methodologies, enhancing their potential and providing for better control over the processes and yields. The current integration of IoT in microalgal biorefineries opens up a lot of possibilities for low-cost upstream and downstream processing. Several achievements have already been made including a highly automated screening process for microalgal strain selection, lipid extraction for biodiesel production, preliminary studies on microalgae microrobots, low-cost biosensors development, integration of biosensors in PBRs, etc. Different growth parameters like temperature, light intensity, pH, and nutrient concentrations can be optimized and monitored through specific sensors connected with the internet. The microalgae production plants are monitored through these wireless sensors, and internet connection helps in transferring real-time data to IoT platforms for further processing through a modeling and decision support system (DSS). However, to improve the performance of these models, the IoT concept along with its benefits and drawbacks must be thoroughly investigated before newer and more relevant models with futuristic methods can be developed (Giannino et al. 2018; Wang et al. 2021).

2.6 Conclusion

Biofuel is a potential alternative to fossil fuel that has advantages over all the alarming limitations of fossil fuels. Among all other feedstocks, microalgae had proved to be the ones that can meet every possible shortcoming of previous ones. Microalgal biomass has been identified as a viable raw material for biodiesel and bioethanol synthesis because of its increasing amount of lipids and carbohydrates. Mainly biochemical conversion such as transesterification and fermentation are used for the production of biodiesel and bioethanol. However, higher cost cultivation, as well as upstream and downstream processing of microalgae, is hindering the biofuel production to make available commercially on a large scale. Therefore newer prospects are emerging such as wastewater cultivation of microalgae, metabolic engineering, fourth-generation biofuel, etc. However insufficient research data regarding these approaches barely makes them available thus fulfilling economic benefits. A proper way to study all these factors covering all hindrances to present biofuel as a sustainable alternative of fossil fuel by removing alarming energy crisis and environmental issues is needed in the coming future.

Competing Interests All the authors declare that they have no competing interests.

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Chapter 3
Microalgal Promise to the Next Generation: A Dual Potential Perspective as Cosmeceuticals and Biofuels

Arun Kumar Rai and Saurav Anand Gurung

Abstract The gradual rise in the human population and an ever-increasing demand for high-value products and alternative fuels has pushed industries into discovering new bioactive compounds and process technologies. Microalgae, an emerging bioresource with distinctive metabolite composition and an efficient growth rate, thus offer a unique platform to meet these demands of sustainable and alternative sources of food and energy. Till date several species of microalgae have been evaluated for their application as cosmeceuticals, pharmaceuticals, nutraceuticals, and biofuels owing to the major bioactive profiles including proteins, polysaccharides, polyunsaturated fatty acids (PUFAs), functional pigments, and vitamins. In addition, the prospect of genetically modified microalgae holds a greater promise to the future for high-value products and third-generation biofuels.

Keywords Cosmeceuticals · Biofuels · Bioactive ingredients · Genetically modified microalgae

Abbreviations

PUFAs	polyunsaturated fatty acids
CAGR	compound annual growth
US	United States of America
MAAs	mycosporine-like amino acids
HA	hydroxy acids
PMNS	polymorphonuclear leukocyte
mg	milligram
ml	milliliter
kDa	kilodaltons
EPA	eicosapentaenoic acid
DHA	docosahexaenoic acid

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TAGs	triacylglycerols
MUFAs	monounsaturated fatty acids
SFAs	saturated fatty acids
MGDG	monogalactosyl diacylglycerol
DGDG	digalactosyldiacylglycerol
MW	molecular weight
UV	ultraviolet radiation
FADD	Fas-associated death domain
MMPs	matrix metalloproteinases
CDP	*Chlorella*-derived peptide
ACE	angiotensin-converting enzyme
CaO	calcium oxide
CO_2	carbon dioxide
CME	*Caulerpa microphysa* extract
FAME	fatty acid methyl ester
V/V	volume per volume
Mg/gm	milligram per gram
cc/g	cubic centimeter per gram
AFDW	ash-free dry weight
MJ/kg	megajoules per kilogram
SEM	scanning electron microscope
PSII	photosystem II
CRISPR	clustered regularly interspaced short palindromic repeats
SSF	saccharification and fermentation
SHF	separate hydrolysis and fermentation
CH_4	methane

3.1 Introduction

The cosmetic industry is predicted to exceed more than US$ 390 billion by 2025, with a compound annual growth rate (CAGR) of 4.3% from 2016 to 2022. Changing lifestyles, the rising economy, and the ineluctable health benefits from natural ingredients are the major factors impacting the thriving trend in the global cosmetics market. Growing awareness about the ill effects of toxic constituents and the developing need for a green lifestyle has further shifted the oversight towards natural products derived from cosmetics. Cosmetics and cosmeceuticals are increasingly using active ingredients from natural sources which present great biodiversity of desired and safe components. Microalgae have come a long way as a unique potential with several primary bioactive compounds of cosmeceutical, pharmaceutical, and nutraceutical importance such as proteins, polysaccharides, pigments, lipids, and vitamins (Agrawal and Verma 2022; Andrade 2018; Morais et al. 2015). Owing to a range of bioactive compounds displaying antioxidant,

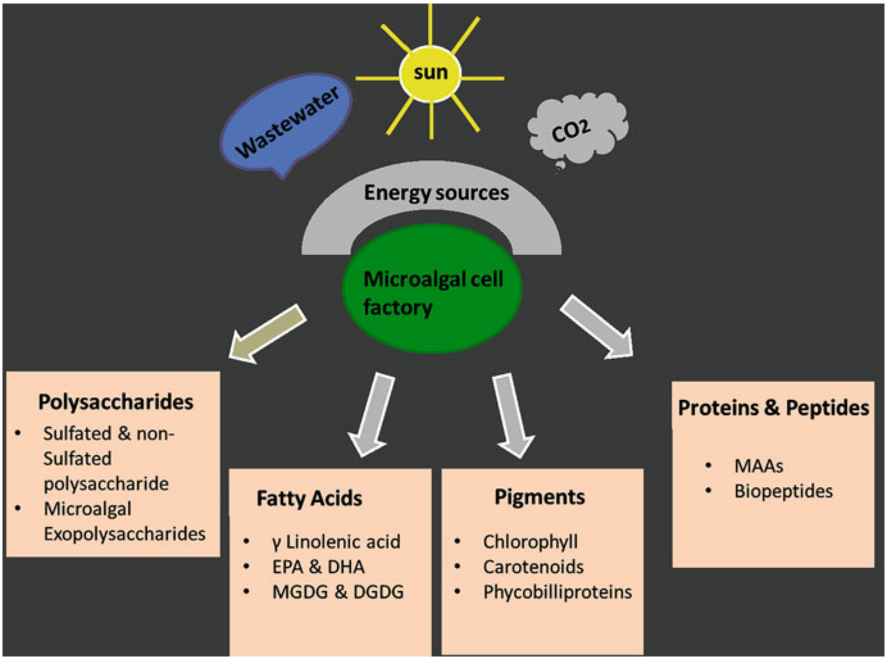

Fig. 3.1 Bioactive components from microalgae with cosmeceutical potentials

immunomodulatory, and anticancer activities, microalgae are currently explored for utilizing these properties in cosmetic application including anti-aging, sunscreens, wound healing, and anti-acne treatments (Mehariya et al. 2021; Yarkent et al. 2020; Pangestuti and Kim 2011). Numerous microalgae have prospected for cosmeceutical properties, for example, *Spirulina platensis* extracts showed wound healing activities in HS2 keratinocyte cells (Gunes et al. 2017), and *Spirulina* extract loaded PCL nanofiber improved skin regeneration by increasing fibroblasts viability and modulating (reactive oxygen species) ROS levels. Furthermore, antiproliferative and anticancer properties of *Spirulina* and *Chlorella* are also widely reported (Cha et al. 2008; Czerwonka et al. 2018; El-fayoumy et al. 2021; Fayyad et al. 2019; Kyadari et al. 2013). Notably, application of microalgae in thalassotherapy as a marine cosmetic for the therapeutic and revitalizing schemes are novel frontiers in microalgal biotechnology (Mourelle et al. 2017) (Fig. 3.1).

Another distinct application of microalgal bioresource includes the demanding area of biofuels. In the coming years, depletion of fossil fuels as well as concerns of global warming further necessitates an urgent requirement for alternative energy sources (Kotasthane 2017). In comparison to crop-based biofuels, Microalgal derived biofuels have several advantages as follows: (1) It reduces pressure on fertile agricultural land which can be instead used for agricultural practices to nourish the growing population. (2) Unlike lignocellulose-rich crop biomass which requires several pretreatment processes, microalgae with low levels of lignocellulose provide a cost-effective raw material for biofuel extraction. (3) Adaptability of microalgae in extreme conditions and wastewater provides feasible bioenergy source as well as scope for greener technological advancements. (4) Finally the prospect of genetically modified microalgae and strain optimization studies can further enhance the bio-products development in area of biodiesel, bioethanol, biogas, and bio-oil (Bhardwaj et al. 2020; Molinuevo-Salces et al. 2019; Adegboye et al. 2021; Goswami et al. 2021b; Hannon et al. 2010). In this chapter we briefly discuss the wide range of bioactive compounds with potential role in cosmeceuticals as well as capture the overview of different microalgal species involved in cosmeceuticals and biofuels generation. Additionally, we also discuss numerous bio-products that have potential role in biofuel development.

3.2 Microalgae as Cosmeceuticals

In view of the changing "greener" lifestyles and the growing awareness for natural ingredients, microalgae emerge as the popular choice for the cosmeceutical industry. The potential of microalgae as a rich source of pigments, proteins, vitamins, minerals, polysaccharides, and fatty acids provides a direct benefit for human health, especially as a cosmetic product. Microalgal bioresource offers a unique platform for novel bioactive molecules in the cosmeceutical industry, a combination of cosmetics and pharmaceuticals, i.e., possessing health benefits. Microalgae represent a diverse group of unicellular prokaryotic (Prochlorophyta and Cyanophyta) and eukaryotic (Chlorophyta, Bacillariophyta, Phaeophyta, Rhodophyta, and Chrysophyta) microorganisms characterized by a wide range of bioactive metabolites with antioxidant, anti-inflammatory, and immune-modulatory properties. Extracts from microalgae such as *Arthrospira*, *Chlorella*, *Nannochloropsis*, *Spirulina*, and *Dunaliella* have already been established as an ingredient in skincare compositions (Stolz and Obermayer 2005). Exsymol (Monaco) used protein-rich extract from *Spirulina platensis* with anti-aging properties. *Spirulina* firming masks produced by Optimum Derma Aciditate (Lithuania) improve skin moisture balance and immunity (Chakdar et al. 2012). Dermochlorella D, an amino acid concentrate from *Chlorella vulgaris* produced by Codif (France), promotes activation of collagen synthesis and reduces stretch marks and vascular imperfections (Ryu et al. 2015). The cosmeceutical value of microalgae is conferred due to the feasibility of growing them in extreme conditions and also due to the wide range of chemicals with favorable biological activity (Mobin and Chowdhury 2019). Although a plethora of microalgae have been identified and utilized in industrial operations for cosmeceuticals, yet these represent only a percentage of their substantial diversity. To date more than 25,000 novel bioactive compounds have been discovered from different oceanic regions of the world (Blunt et al. 2016). These bioactive metabolites are categorized into different major groups, viz., polysaccharides, fatty acids, pigments, proteins, peptides, etc. (Table 3.1).

Table 3.1 The cosmetic activity of major microalgae with their unique bioactive composition

Microalgal species	Cosmetic activity	Active ingredients	References
Spirulina platensis	Anti-aging, anti-acne, wound healing	α- and γ-linolenic acid, C-phycocyanin	Ragusa et al. (2021), Gunes et al. (2017)
Chlorella fusca, *Chlorella minutissima*, *Chlorella vulgaris*, *Chlorella sorokiniana*, *Chlorella ellipsoidea*	Anti-aging, control of inflammatory process and pigmentation, protection against sun damage	Chlorophyll, carotenoids, sporopollenin, and mycosporine-like amino acids	Agustina et al. (2021), Chatzikonstantinou et al. (2017), de Andrade et al. (2017), Kwang et al. (2008), Caicedo et al. (2020)
Nannochloropsis gaditana, *Nannochloropsis oceanica*	Anti-aging, oxidative stress response	Violaxanthin, fucosterol, antityrosinase, zeaxanthin	Kim (2014), Ma et al. (2015), Letsiou et al. (2017), Hyun Min Kim et al. (2019), Yarkent et al. (2020), Park et al. (2021)
Dunaliella salina	Antioxidants	β-Carotene	Kane et al. (2016), Silva et al. (2021), Tran et al. (2014), Yarkent et al. (2020)
Haematococcus pluvialis	Antioxidant, skin conditioning, and protection	Astaxanthin	Vieira et al. (2021), Guerin et al. (2003), Zakaria et al. (2021), Ruiz-Domínguez et al. (2019), Marino et al. (2020)
Coelastrella striolata var. *multistriata*	Antioxidants	Canthaxanthin	Abe et al. (2007)
Scytonema sp.	Antioxidants	Mycosporine-like amino acids (MAAs)	Vega et al. (2020)
Anabaena vaginicola	Antioxidant and anti-aging agent	Lycopene	Hashtroudi et al. (2013)
Scenedesmus obliquus, *Scenedesmus rubescens*	Anti-inflammatory and anti-aging	*Scenedesmus* extracts	Cengiz and Sevilay. (2019), Campiche et al. (2018)
Porphyridium sp.	Anti-inflammatory and antioxidant	Sulfated polysaccharides	Matsui et al. (2003)) Arad and Levy-Ontman (2010), Arad and van Moppes (2013), Tannin-Spitz et al. (2005)
Botryococcus braunii	Antioxidants	Violaxanthin, β-carotene, lutein, and zeaxanthin	Koller et al. (2014)

3.2.1 Polysaccharides

Polysaccharides are the most abundant biochemical molecule made up of thousands of monosaccharide units. These polymeric compounds can be categorized as homo-polysaccharides or hetero-polysaccharides based on the single or different monosaccharide unit that makes the compound. The glycosidic bonds that frequently occur in these compounds are β-1,4 or β-1,3 and α-1,4, α-1,2, or α-1,6 linkages. The α-glucans (starch and floridean starch) have been reported in green, glaucophyte, charophyte, cryptomonad, dinophyte, red microalgae, and cyanobacteria (Mobin and Chowdhury 2019). Similarly, the β-glucans (chrysolaminarin and paramylon) have been found in euglenophytes, haptophytes, *Chlorella* genus, *Skeletonema* diatom, and also *Porphyridium* and *Nostoc flagelliforme* (Mourelle et al. 2017). The cosmetic action provided by β-1,3-glucans includes blood cholesterol levels reduction, antioxidant activities, as well as immune stimulator (Koller et al. 2014). Xia et al. (2014) discovered a novel β-glucans, chrysolaminarin, named CL2 obtained from *Odontella aurita* that showed strong antioxidant activities. Until recently, at an industrial level, Algatech has licensed their rights to the production of β-1,3-glucans from fermented microalgae *Euglena gracilis*. High concentration and purity of the biochemical in *Euglena gracilis* compared to common sources in the market such as oats, mushrooms, and yeast make them potential and effective bioresource with powerful health benefits (for an example see https://www.algatech.com/algatech-product/bioglena-beta-glucan). In a cosmetic formulation, use of hydroxy acid (HA) is promoted to provide skin moisturization; however, since it can be only extracted from plants and animals, the cost is relatively high. Studies have shown the application of polysaccharides from marine algal species like *Saccharina japonica* and *Chondrus crispus* can provide a much more cost-effective alternative to hydroxy acid (Wang et al. 2015). As reported by several authors, water-soluble polysaccharides from *Spirulina platensis* are another potential raw material for the cosmeceutical industry with strong antioxidant activities (Chaiklahan et al. 2013; Kurd and Samavati 2015).

Microalgal exopolysaccharides are complex glycopolymers that protect microorganisms against extreme environmental stress conditions like dehydration as it allows them to retain cellular water. It is thus widely accepted that this property of the microalgal exopolysaccharide can be utilized for moisturization and hydration of the skin (Stoyneva-Gärtner et al. 2020). Although only a few studies have been conducted on these lines, extracellular sulfated polysaccharide from red microalgae *Porphyridium* sp. has drawn prominent interest based on their remarkable nutritional, therapeutic, and cosmetic activity. Estee and Companies (2003) reported the in vitro and in vivo anti-inflammatory activity of polysaccharide material from *Porphyridium* sp. The bioactive sulfated polysaccharide prevented the polymorphonuclear leukocyte (PMN) migration involved in skin inflammation. In another study, the antioxidant properties of sulfated polysaccharide extract from *Porphyridium* sp. have been reported. At a concentration of 2 mg ml^{-1} and 10 mg ml^{-1}, *Porphyridium* polysaccharide gave antioxidant activity of 45% and 90%, respectively (Tannin-Spitz et al. 2005). Furthermore, the sulfated polysaccharide from *Porphyridium* sp. is also believed to be an excellent alternative to hyaluronic acid as a bio-lubricant with antioxidant activities (Raposo et al. 2013a). Based on the significant cosmetic properties, sulfated polysaccharides thus make a suitable candidate as a cosmetic bioresource (Arad and van Moppes 2013). Notably in a study conducted on polysaccharide, extract of *Caulerpa microphysa* (Chlorophyta) suggested excellent anti-inflammatory and wound healing with good moisture-holding capacity. *Caulerpa microphysa* extract (CME) showed complete inhibition of β-hexosaminidase, a critical inflammatory mediator in allergic reactions thus suggesting the anti-allergic potential of CME (Lee et al. 2021). Table 3.2 presents a list of microalgal polysaccharides and their specific features and cosmetic action.

3.2.2 Fatty Acids

Traditionally, microalgae are considered a rich source of lipids, especially the long unsaturated fatty acids such as γ-linolenic acid, arachidonic acid, stearidonic acid, and ω-3 fatty acids: eicosapentaenoic acid (EPA) (ω-3 C 20:5) and docosahexaenoic acid (DHA) (ω-3 C 22:6), etc. (Mobin and Chowdhury 2019). The total lipid production in microalgae may reach up to 30–70% of their dry weight depending on the species type (Balasubramaniam et al. 2021). Microalgal species like *Chlorella emersonii*, *Dunaliella tertiolecta*, *Nannochloropsis* sp., *Porphyridium cruentum*, *Botryococcus braunii*, and *Neochloris oleoabundans* show high lipid content of more than 60% of their dry weight. Freshwater microalgal species like *Chlorella minutissima*, *Chlorella protothecoides*, *Chlorella vulgaris*, *Scenedesmus dimorphus*, and *Scenedesmus obliquus* and marine microalgal species like *Dunaliella salina*, *Nannochloris* sp., *Crypthecodinium cohnii*, *Isochrysis galbana*, *Nitzschia* sp., *Phaeodactylum tricornutum*, *Schizochytrium* sp., and *Skeletonema costatum* display moderate lipid content in the range of 40–60% of their dry weight. Microalgae that display lower lipid content, i.e., below 40% of their dry weight, include *Arthrospira maxima*, *Spirulina platensis*, *Dunaliella primolecta*, *Chlorella sorokiniana*, *Ankistrodesmus* sp., *Chaetoceros muelleri*, *Cylindrotheca* sp., *C. cohnii*, *Ellipsoidion* sp., *Euglena gracilis*, *Haematococcus pluvialis*, *Monodus subterraneus* UTEX 151, *Monallanthus salina*, *Oocystis pusilla*, *Pavlova salina*, *Thalassiosira pseudonana*, and *Tetraselmis suecica* (Mobin and Chowdhury 2019; Maity et al. 2014). Evidently, two genera of microalgae, viz., *Dunaliella* and *Chlorella*, show differential total lipid content at a species level: *Dunaliella tertiolecta* (more than 60%), *Dunaliella salina* (40–60%), and *Dunaliella primolecta* (less than 40%) and also *Chlorella emersonii* (more than 60%), *Chlorella vulgaris* (40–60%), and *Chlorella sorokiniana* (less than 40%). A greater emphasis must be thus given to the microalgal strains with high lipid content to empower cost-effectiveness for economic production.

The unique fatty acid profiles from microalgae can be broadly categorized into polar and neutral lipids. The polar lipids are mainly constituted of membrane lipids

Table 3.2 Microalgal polysaccharide features and their cosmetic activity

Microalgae species	Polysaccharide type	Major polysaccharide features	Cosmetic activity	References
Porphyridium sp. and *Porphyridium aerugineum*	Sulfated exopolysaccharide	MW 2000–7000 kDa; major sugars: xylose, glucose, and galactose	Antioxidants	Burg and Oshrat (2015)
Navicula sp.	Sulfated exopolysaccharide	MW 17 kDa; major sugars: Glucose, galactose, rhamnose, xylose, and mannose	Antioxidants	Fimbres-Olivarria et al. (2018)
Isochrysis galbana	Sulfated exopolysaccharide	MW 15.934 kDa; major sugars: Mannose, glucose, galactose, and rhamnose	Antioxidants	Sun et al. (2014a, b)
Cylindrotheca closterium	Sulfated exopolysaccharide	Major sugars: Glucose, galactose, xylose, mannose, and rhamnose	Not described	Staats et al. (1999)
Sarcinochrysis marina	Sulfated polysaccharide	MW 2595 kDa; major sugars: L-arabinose, D-fructose, and glucose	Antioxidants	Sun et al. (2014a, b)
Phaeodactylum tricornutum	Sulfated polysaccharide	MW 449 kDa; major sugars: Glucose, glucuronic acid, and mannose	Anti-inflammatory and immunomodulatory	Guzmán et al. (2003)
Pavlova viridis	Sulfated polysaccharide	MW 3645 kDa; major sugars: rhamnose, D-fructose, glucose, and mannose	Antioxidants	Sun et al. (2014a, b)
Pavlova viridis	Sulfated polysaccharide	MW 386.96 kDa; major sugars: D-fructose, glucose, and mannose MW 54.99 kDa; major sugars: D-fructose, glucose, mannose, and L-rhamnose	Immunomodulatory	Sun et al. (2016)
Chlorella stigmatophora	Sulfated polysaccharide	MW 22 kDa; major sugars: Glucose, glucuronic acid, xylose, and ribose/fucose	Anti-inflammatory and immunomodulatory	Guzmán et al. (2003)
Spirulina sp.	Sulfated polysaccharide	MW 200 × 103 g/Mol; major sugars: Acofriose and rhamnose	Anticancer	Senni et al. (2011)
Parachlorella kessleri	Non-sulfated polysaccharide	MW 3.05 × 105 and 9.84 × 104 g Mol − 1 with α-L-rhamnan and xylogalactofuranan in different ratios	Immunomodulatory	Sushytskyi et al. (2020)

Dictyosphaerium pulchellum and *Dictyosphaerium tetrachotomum*	Non-sulfated exopolysaccharide	Galactose, xylose, unidentified 2-OMe-hexose and fucose, and glucuronic acid	Immunomodulatory	Halaj et al. (2019)
Aphanothece halophytica	Non-sulfated exopolysaccharide	MW 2000 kDa; major sugars: glucose, fucose, mannose, arabinose, and glucuronic acid	Not described	Li et al. (2001)
Chlorella pyrenoidosa	Non-sulfated polysaccharide	MW 188 and 1020 kDa; major sugars: Arabinose and galactose	Immunomodulatory	Suárez et al. (2006)

such as phospholipids and glycolipids in a microalgal cell. On the contrary neutral lipids are localized in the cytoplasm as triacylglycerols (TAGs) (Yao et al. 2012). Long-chain polyunsaturated fatty acid (PUFA) usually belongs to polar lipids, whereas a neutral lipid consists of monounsaturated fatty acids (MUFAs) and saturated fatty acids (SFAs) in the triacylglycerols (TAGs). Based on the percentages of characteristic fatty acids such as C 16:0/C16:1 and eicosapentaenoic acid (EPA) (ω-3 C 20:5) for neutral (TAGs) and polar fatty acids, respectively, fatty acid profiles of different microalgae can be compared. *Nannochloropsis oceanica* fatty acid percentage values in terms of r^2 of C16:0 and EPA were 0.94 and 0.97, respectively. Similarly, *Chlorella pyrenoidosa* r^2 values for C18:1/ C18:3 with TAG content were 0.91 and 0.99, respectively. This method of correlation thus allows researchers to precisely quantify TAGs in microalgae (Shen et al. 2016). Several species of microalgae have been explored for their rich lipids content and their subsequent application in the cosmetic industry. Gamma-linolenic acid from *Spirulina platensis* functions as the potential bioactive compound with anti-aging, anti-wrinkle, collagen synthesis, anti-inflammatory, and antioxidant activities (Hoseini et al. 2013). *Porphyridium cruentum* is an excellent source of polyunsaturated fatty acids (PUFAs) that provides photoprotection against the high intensity and UV light as well as prevents skin dehydration. ω-3 PUFAs, namely, eicosapentaenoic acid (EPA, 20:5 ω3) and docosahexaenoic acid (DHA, 22:6 ω3), from *Isochrysis galbana* show anti-inflammatory effect. Furthermore, several extracts of lipids, PUFAs, and pigments from *Isochrysis galbana* have been claimed to have skin and hair care properties (Abdoul-latif et al. 2021; Bonfanti et al. 2018). Microalgal squalenes have been reported to have moisturizing, antioxidant, and anti-aging properties. A large number of cosmetic products, viz., lipstick, lotions, eye pencil, eye shadows, eye makeup remover, and perfumes, utilize the squalene in their formulations. *Botryococcus braunii*, *Schizochytrium mangrovei*, and *Thraustochytrium* sp. are a few microalgae species that produce these thriving molecules. Microalgal galactolipids, i.e., monogalactosyl diacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG), present another unique bioactive resource with cosmeceutical applications based on its anti-inflammatory activities. Bruno et al. compared dose-dependent anti-inflammatory activity of MGDG and DGDG and concluded that eicosapentaenoic acid (EPA) presence in MGDG further enhanced the activity (Bruno et al. 2005). Microalgal species such as *Chlorella minutissima*, *Chaetoceros*, *Cyclotella*, *Ellipsidion*, *Isochrysis*, *Monochrysis*, *Monoraphidium*, *Nannochloris*, *Nannochloropsis*, *Nitzschia*, *Phaeodactylum*, *Porphyridium*, *Skeletonema*, and *Thalassiosira* are the potential sources of the galactolipids (Asraful et al. 2020).

3.2.3 Pigments

Pigments are another widely explored bioactive resource from the microalgal biomass. Fat-soluble pigments such as chlorophyll and carotenoids including β-carotene, astaxanthin, and fucoxanthin as well as water-soluble phycobiliproteins such as allophycocyanin, phycocyanin, phycoerythrin, and phycoerythrocyanin are the most ubiquitous in the microalgal cell factory. *Agardhiella*, *Arthrospira*, *Chlorella*, *Dunaliella*, *Haematococcus*, *Muriellopsis*, *Nannochloropsis*, *Nostoc*, *Phaeodactylum*, *Porphyridium*, *Polysiphonia*, *Scenedesmus*, and *Spirulina* are major microalgal genera that produce these wide ranges of bioactive pigments. They are commonly present in cyanobacteria (blue-green algae), Rhodophyta (red algae), and unicellular eukaryotic algae (cryptomonads). Phycobiliprotein's properties as an antioxidant and free radical scavenging activities have drawn major attention in cosmetics as well as in food, pharmaceuticals, and biomedical applications. Furthermore, microalgal pigments also exhibit anti-inflammatory, antiangiogenic, antiviral, antiobesity, antidiabetic, anticancer, anti-osteoporotic, and neuro- and hepatic-protective activities (Pailliè-Jiménez et al. 2020). At present Dainippon Ink and Chemicals Inc. (Japan) utilizes *Arthrospira* spp. with a brand name of Linablue as a food colorant as well as cosmetic applications (Saini et al. 2021; Morocho-Jácome et al. 2020). Algenist (CA, USA) introduced a novel vitamin C from *Spirulina* which enhances skin tone, smoothens texture, and prevents photoaging (for an example see BLUE ALGAE VITAMIN C™ Skinclarity Brightening Serum (algenist.com)). Because of their pH and heat stability characteristics, phycobiliproteins are used as a natural colorant in eyeliners, lipsticks, and other cosmetic formulations (Balboa et al. 2015).

There is an increasing demand for carotenoids derived from microalgae considering their pharmaceutical and cosmetic role. *Chlorella*, *Dunaliella*, *Haematococcus*, and *Muriellopsis* are the routine sources for carotenoids. Astaxanthin extracted from *Haematococcus pluvialis* is reported to have antioxidant biological activity as well known to reduce skin pigmentation. A clinical study on two human subjects, 30 women aged 20–55 years old and 36 men aged 20–60 years old, concluded astaxanthin's role in improving elasticity, skin texture, moisture content of corneocyte layer, and corneocyte condition as well as reducing skin wrinkle and age spot size in both subjects (Goswami et al. 2021c; Tominaga et al. 2012). In another study, astaxanthin was found to significantly suppress transepidermal water loss and wrinkle formation thus preventing photoaging caused due to UV-A radiation (Komatsu et al. 2017). AstaPure is a commercial product of astaxanthin extracted from *Haematococcus pluvialis* utilized by Algatech (Israel) as topical creams and emulsions because of its cosmeceutical activity on skin health, eye health, and immunity ((Morocho-Jácome et al. 2020), also see AstaPure Natural Astaxanthin l astaxanthin manufacturer l bulk astaxanthin (algatech.com). Fucoxanthin mainly found in *Cercis siliquastrum*, *H. fusiformis*, *L. japonica*, *Undaria pinnatifida*, and *Sargassum fulvellum* also offers antioxidant properties. Fucoxanthin extracted from *Phaeodactylum tricornutum* showed protection against oxidative damage (Kawee-ai et al. 2013). A study reported the anti-pigmentary activity of fucoxanthin by inhibiting tyrosinase, a key enzyme in melanogenesis, and also preventing the UV-B radiation-induced skin pigmentation (Shimoda et al. 2010).

The most common carotene produced by the halotolerant microalga *Dunaliella salina* is β-carotene, i.e., up to 10% of its dry weight biomass. Apart from pro-vitamin A activity, β-carotene is also accepted as a cosmeceutical because of its properties like antioxidants, anticancer, anti-inflammatory, and immune modulators (Raposo et al. 2013b). Guruvayoorappan and Kuttan (2007) demonstrated the antiangiogenic activity of β-carotene on B16F-10 cell lines. The study showed that β-carotene prevented neovascularization, i.e., the formation of tumor-directed capillaries by tailoring the expression of proinflammatory cytokines such as matrix metalloproteinase 2 (MMP-2) and MMP-9 and also preventing nuclear translocation of the transcription factor. In another study, β-carotene exhibited antioxidant and anti-inflammatory activity on *H. pylori*-infected human gastric epithelial AGS cell lines. *H. pylori*-infected cell lines showed increased expression of matrix metalloproteinases (MMPs), key molecules in metastasis and cancer invasion. However, on treatment with β-carotene, the expression of MMPs was significantly controlled primarily by reducing the ROS levels in the cell line (Bae et al. 2021).

3.2.4 Proteins and Peptides

Mycosporine-like amino acids (MAAs) are water-soluble, low molecular weight (0.188 to 1.05 kDa) bioactive resources found in cyanobacteria, microalgae, macroalgae (chlorophyta and Rhodophyta), and several other marine organisms (Wada et al. 2015). Oligosaccharide-linked mycosporine-like amino acids were first reported in *Nostoc commune* which also showed significant photoprotection against UV-B radiation. These molecules have a characteristic UV-A and UV-B absorption, a critical feature for cosmeceutical application as sunscreens (Bohm et al. 1995). Schmid et al. (2006) characterized MAAs-based sunscreens (Helioguard 365) extracted from *Porphyra umbilicalis* in in vitro and in vivo conditions and demonstrated the anti-aging and antioxidant properties of the biochemical. Helioguard 365 containing porphyra-334 and shinorine showed protection from irradiation-induced DNA damage and prevented lipid peroxidation ultimately providing smoothness and firmness of the skin. In another study porphyra-334 extracted from *P. yezoensis* showed significant antioxidant properties with ROS production inhibition as well as induced type I collagen and elastin suggesting a role as an anti-wrinkle or anti-aging agent (Ryu et al. 2014). A commercial product named HELIONORI® with mycosporine-like amino acids (MAAs) as an important ingredient is derived from *Porphyra umbilicalis*. The cosmetic product protects against UV-A-induced skin damage and DNA damage and also prevents premature photoaging ((Figueroa 2021), e.g., see HELIONORI® - GELYMA)). Until recently, MAAs has been reported in *Chlorella*, *Pseudochlorella*, *Stichococcus*, *Apatococcus*, *Bracteacoccus*, *Coccomyxa*, *Elliptochloris*, *Pabia*, *Prasiolopsis*, and *Pseudococcomyxa* (Stoyneva-Gärtner et al. 2020).

Biopeptides are low molecular weight (3 kDa) short peptides derived from plants, animals, and microalgal sources. *Chlorella* and *Spirulina* are a few microalgal genera that are currently explored for biopeptides with cosmeceutical properties. Chlorella-derived peptides showed inhibitory activity towards UV-B-induced matrix metalloproteinase-1 (MMP-1) involved in the photoaging process and also restored collagen and TbRII (transforming growth factor, TGF-β, receptor) preventing further damage to the skin (Chen et al. 2011). In another study, *Chlorella*-derived peptide (CDP) prevented UV-C-induced cytotoxicity and DNA damage through inhibition of caspase-3 activity and Fas-associated death domain (FADD) expression (Shih and Cherng 2012). Sadeghi et al. (2018) reported spirulina-based peptides with antimicrobial and anticancer properties. In this study spirulina-based peptides (<3 kDa peptide fraction) showed prominent dose-dependent inhibition of SW480 cells (human colon adenocarcinoma cell), and the same fraction also demonstrated significant antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. Furthermore a phycobiliprotein-derived bioactive peptide, SpirPep1, attained significant (angiotensin-converting enzyme) ACE inhibitory activity thus demonstrating anti-tumor and antihypertensive activity (Anekthanakul et al. 2019).

3.3 Microalgae for Biofuel Production

Due to environmental concerns including increasing greenhouse emissions and the world energy crisis, a need for an alternative source of energy with CO_2 mitigation potential has arisen. Microalgae provide a unique feedstock for biofuels production that can outcompete the traditional fossil fuels and crop-based biofuels providing a sustainable approach to the energy crisis as well as increasing the efficiency of biofuel production. In developing countries like India with 120 million tonnes of petroleum consumption per year, at least 21% of the agricultural land for biofuels production is necessary (Mondal et al. 2017). However, with microalgal bioresource, less than 2–3% of the agricultural land would be required considering the high oil yield productivity in terms of area utilized. Compared to the oil yield of crops such as caster, maize, oil palm, and physic nut which range from 172 to 5366 oil liters/hectare/year, microalgae with high oil yield can produce up to 136,900 oil liters/hectare/year (Medipally et al. 2015). Significantly microalgal land use efficiency for biofuel production was 338 times greater than corn biodiesel (Lum et al. 2013). Furthermore the CO_2 capture efficiency of microalgae is estimated to be 10–50 times higher than that of terrestrial plants (Li et al. 2008). Studies report CO_2 capture from many microalgal species such as *Chlorella kessleri*, *Chlorella emersonii*, *Chlorococcum littorale*, *Galdieria partita*, and *Synechococcus* PCC7942. The photoautotrophic potential of microalgae thus provides an added advantage to the utilization of greenhouse gas like CO_2 in the upstream application for biofuels like bioethanol, biodiesel, biogas, biohydrogen, bio-oil, and syngas.

3.3.1 Biodiesel

Major biodiesel standards specification like US specification (ASTM D6751), European biodiesel specification (EN 14214), and Indian biodiesel specification define biodiesel as a mixture of monoalkyl esters of long-chain fatty acids (Mondal et al. 2017). Transesterification is a primary process for the conversion of triglycerides (oils) generated from microalgae to biodiesel using different catalysts such as alcohols, acids, and enzymes. Tang et al. (2011) reported *Dunaliella tertiolecta* as a potential feedstock for biodiesel production extracted using methanol and chloroform. Fatty acid methyl ester (FAME) profile showed the significant percentage of methyl linolenate (C18:3), methyl palmitate (C16:0), methyl oleate (C18:1), and methyl linoleate (C18:2) with the highest cell density at 2–6% of CO_2 concentration. A study conducted on biodiesel production from *Spirulina platensis* using acid and methanol reported a viscosity value of 4.8 in accordance with the EN 14214 standard thus affirming its quality as a biodiesel substitute to fossil-derived fuels (El-Shimi et al. 2013). Investigation on the choice of the solvent for lipids extraction in *Nannochloropsis* sp. showed chloroform/methanol (1:2 v/v) with the highest percentage yield of 60.37% (Rahmanpour and Shariati 2015). Extraction process is one of the major factors affecting biodiesel yield apart from the choice of solvents. A study on microalgae *Schizochytrium limacinum* validated that direct transesterification of microalgal biomass using methanol and sulfuric acid along with chloroform, hexane, and petroleum ether produced higher biodiesel yield (10–20%) compared to indirect extraction-transesterification method using chloroform and methanol as extracting agent and additional sulfuric acid as transesterification agent (Johnson and Wen 2009). In another study using *Scenedesmus* sp. for in situ or direct transesterification process, a higher biodiesel production was recorded for alkaline catalyst (55.07 ± 2.18%) than acidic catalyst (48.41 ± 0.21%) (Kim et al. 2014). Nutrient limitation is another factor that can impact the efficiency of biodiesel production in microalgae by lipid accumulation, i.e., converting excess carbon into storage lipids. In one study nitrogen limitation increased the biodiesel yield in *Chlorella* sp. and *Desmodesmus quadricaudatus*. For *Chlorella* the unsaturated fatty acids increased from 37.92% to 41.6% in nitrogen-free medium, and similarly for *Desmodesmus quadricaudatus*, saturated fatty acids increased from 51.62% to 66.92% in nitrogen-free medium (Shafik et al. 2015).

3.3.2 Bioethanol

On account of low ligno- and hemicellulose content in microalgae compared to lignocellulosic biomass observed in conventional crops, microalgae with high levels of carbohydrates provide an appropriate choice for bioethanol production. Bioethanol production from biomass requires processes such as pretreatment, saccharification or enzymatic hydrolysis, fermentation, and distillation. Mild pretreatment is required for microalgal biomass owing to the absence of lignocellulose in algal biomass. During the process of pretreatment and saccharification, the complex polysaccharide is broken down into fermentable monomeric sugars. Several methods of pretreatment and saccharification are employed, i.e. physical or mechanical (ultrasonication, high-pressure homogenization, autoclave, bead beating), chemical (acidic hydrolysis, alkaline hydrolysis, and supercritical CO_2), or enzymatic treatment (Agrawal et al. 2020; Kumar et al. 2020a; Phwan et al. 2018; Behera et al. 2015). Pretreatment studies using alkaline hydrolysis produced a maximum reducing sugar concentration of 88 mg/g dried biomass and 81 mg/g dried biomass in *Chlorella* sp. and *Tetraselmis suecica*, respectively (Behera et al. 2015). In another study, microalgae *Chlorococcum infusionum* was used for alkaline pretreatment which produced the highest glucose yield of 350 mg/g dried biomass with the highest bioethanol yield of 0.26 g/g dried biomass (Harun et al. 2011). Khan et al. (2017) reported that calcium oxide (CaO) treatment before acid and enzymatic hydrolysis significantly doubled the yield of monomeric sugars in *Microcystis aeruginosa*. Furthermore, a high temperature of 120 °C (autoclave disruption treatment) combined with acidic treatment showed the highest disruption and sugar extraction efficiency for microalgae *Scenedesmus obliquus* (Miranda et al. 2012). Jeon et al. (2013) investigated the ultrasonic pretreatment prior to microbial fermentation for bioethanol feedstock production in *Scenedesmus obliquus* YSW15. The results demonstrated that ultrasonic treatment at 15 min duration increased the dissolved carbohydrates concentration at 0.12 g/g dried biomass further increasing the bioethanol yield through microbial fermentation. Scanning electron microscope (SEM) images confirmed that rupture of microalgal cell wall allowed fermenting microbes to ingress the microalgal cellular system thus further enhancing the treatment efficiency. After pretreatment and saccharification, microbial fermentation process then converts the fermentable monosaccharides into bioethanol and other bio-products. Several fermenting microorganisms have been utilized for the process, viz., yeast and fungi such as *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Kluyveromyces fragilis*, *Kluyveromyces marxianus*, etc. as well as bacteria such as *Escherichia coli*, *Klebsiella oxytoca*, and *Zymomonas mobilis.* A study on bioethanol production from red microalgae *Porphyridium cruemtum* depicted that simultaneous saccharification and fermentation (SSF) was a better method than separate hydrolysis and fermentation (SHF). A bioethanol yield of 65.4 and 70.3% using *Saccharomyces cerevisiae* KCTC 7906 was achieved for seawater *Porphyridium cruemtum* and freshwater *Porphyridium cruemtum*, respectively (Miyamoto et al. 1979). Using *Escherichia coli* as a microbial fermenting organism, three marine algae *Chlorella vulgaris*, *Chlamydomonas reinhardtii*, and *Undaria pinnatifida* were explored for bioethanol production. The highest bioethanol yield of 0.4 g/g biomass was observed for acidic and enzymatic pretreated *Chlorella vulgaris* biomass (Lee et al. 2011). Many other fermenting microorganisms such as *Saccharomyces bayanus*, *Saccharomyces cerevisiae* S288C, *E. coli* KO11, and *Zymomonas mobilis* have been utilized for fermentation treatment for microalgae such as *Chlorococcum* sp., *Chlamydomonas reinhardtii* UTEX 90, *Chlorella variabilis*, and *Chlorella vulgaris* FSP-E, respectively, at different pretreatment procedures and fermentation conditions (Phwan et al. 2018).

3.3.3 Biogas

Owing to the presence of polysaccharides such as agar, alginate, carrageenan, laminarin, and mannitol and low cellulose content, microalgae present a superior bioresource for biogas extraction. Biogas production occurs through several anaerobic fermentation steps involving primarily conversion of insoluble high molecular weight organic molecules into soluble organic fraction. In the subsequent steps, volatile fatty acids and alcohols are released by acidogenesis; these volatile organic compounds are then converted into acetic acid and hydrogen and finally conversion of acetic acid and hydrogen into methane and carbon dioxide gas. The entire process of anaerobic fermentation requires microorganisms for biodegradation such as acidogenic bacteria, acetogenic bacteria, and methanogens (Behera et al. 2015). It is essential that the hydrolysis of the microalgal cells must be efficient enough to increase the biodegradation of microalgal biomass. A study on anaerobic biodegradation of *Scenedesmus obliquus* and *Phaeodactylum tricornutum* under similar experimental conditions showed that different microalgae have different biodegradation efficiencies owing to their unique physiology and thermal tolerance (Zamalloa et al. 2012). Several works have been reported for the methane and biogas yield from microalgae such as *Scenedesmus obliquus*, *Chlorella vulgaris*, *Nannochloropsis salina*, *Spirulina maxima*, *Phaeodactylum tricornutum*, *Isochrysis galbana*, etc. (Jankowska et al. 2017; Goswami et al. 2021d). A study on methane gas production from *Spirulina maxima* reported that mechanical and thermochemical treatments had a positive effect on acidogenic bacteria; however, it had no subsequent effect on the methanogens (Samson and Leduy 1983). Similarly, thermal pretreatment of *Scenedesmus* sp. showed a significant anaerobic biodegradability of 48% when the temperature was increased from 70 to 90° C thus establishing the effect of higher temperature on the biodegradation efficiency (González-Fernández et al. 2012). Another condition that increases the yield of biogas like methane from microalgae was a reduction of sodium concentration in the biodegradation mixture. Santos et al. (2014) described a 71.5% increase in methane yield by removing inhibitory sodium in the anaerobic digestion mixture in microalgae *Isochrysis galbana*. Another important variable that affects the yield of methane biogas includes the inoculum/extract (I/S) ratio. A consistent I/S ratio of 1 produced effective specific methane productivity of 0.304–0.557 L CH4/g volatile solids in five microalgae, viz., *Chlorella vulgaris* UTEX 395, *Phaeodactylum tricornutum* CCMP 632, *Nannochloropsis* sp., *Nannochloropsis salina*, and *Nanofrustulum* sp. (Zhao et al. 2014).

3.3.4 Biohydrogen

As an alternative energy and fuel source, hydrogen is advantageous as its combustion only releases water thus mitigating CO_2 levels and reducing greenhouse emissions (Nagarajan et al. 2021). Hydrogenases are the main enzymes involved in biohydrogen production in microalgae. These enzymes accept electrons through many sources like photosynthesis; an approach of direct photolysis is applied where the electrons released during photolysis of water are utilized by the enzymes for biohydrogen production. Also, other approaches include indirect photolysis; in this mechanism electrons released during the fermentative metabolism of stored carbon catalyze the biohydrogen production (Limongi et al. 2021; Goswami et al. 2021a; Nagarajan et al. 2021). A study on thermophilic algae *Mastigocladus laminosus* described the inhibition of hydrogen production yield due to hydrogen consumption by oxygen and carbon monoxide. Conclusively at the higher temperatures, the decrease in oxygen concentration enhanced the biohydrogen yield by 50% (Miyamoto et al. 1979). Kose and Oncel (2014) further enhanced the biohydrogen production in green microalgae *Chlamydomonas reinhardtii*. Using genetically engineered *Chlamydomonas reinhardtii* with mutations in D1 protein, a higher biohydrogen yield rate was generated 1.3 ± 0.5 mL/L.h compared to the wild strain with twofold lower yield rate of 0.57 ± 0.2 mL/L.h. The mutated D1 protein blocks PSII repair system preventing the generation of oxygen and subsequently increasing the activity of oxygen sensitive hydrogenases. Apart from *Chlamydomonas reinhardtii*, biohydrogen production is also reported in *Chlorella fusca*, *Chlamydomonas moewusii*, *Chlorococcum littorale*, *Lobochlamys culleus*, *Scenedesmus obliquus*, and *Tetraselmis subcordiformis* (Limongi et al. 2021).

3.3.5 Bio-Oil and Syngas

Bio-oil or biocrude oil is liquid fuel derived from organic biomass at anaerobic and high-temperature conditions (Behera et al. 2015). Pyrolysis is the main method for bio-oil production as it provides high-temperature conditions (400–600 °C) in the absence of oxygen. Microalgae *Chlorella protothecoides* has been investigated for biocrude oil production with highest yield of 52% produced at a temperature of 500 °C for a relatively short time of 5 min (Peng et al. 2000). Hydrothermal liquefaction is generally at a lower temperature (250–350 °C) under high-pressure conditions. In a study, a maximum biocrude yield of 65 wt% ash-free dry weight (AFDW) was produced at 350 °C in 5 min for halophytic microalga *Tetraselmis* sp. (Eboibi et al. 2014). Furthermore, in a study on high-protein high-ash microalgae, *Cyanobacteria* sp. and *Bacillariophyta* sp., a significant bio-oil yield of 21.1% and 18.21% per dry biomass weight was observed at 325 °C for 45–60 min (Huang et al. 2016). At a temperature of 350 °C, *Nannochloropsis* sp. generated the highest bio-yield of 43% weight at a holding time of 60 minutes (Brown et al. 2010).

A study investigating optimum thermochemical liquefaction operations in *Spirulina platensis* reported a remarkable biocrude quality at par with the petroleum crude. At 350–380 °C, the biocrude product has similar energy properties with density of 34.7–39.9 MJ/kg compared to that of petroleum (42.9 MJ/kg) (Jena et al. 2011). The thermochemical conversion of microalgal biomass can be directed to bio-oil as well as syngas production through hydrothermal liquefaction and hydrothermal gasification, respectively (Barreiro et al. 2013). Gasification is operated at high temperatures of 800–1000 °C which converts biomass into combustible gas mixtures like carbon dioxide, carbon monoxide, methane, hydrogen, and nitrogen. In a study, hydrothermal gasification of *Spirulina platensis* produced syngas mixtures of methane, carbon dioxide, carbon monoxide, and hydrogen. Using ruthenium catalyst in supercritical water, it was then possible to separate the methane from syngas producing a methane-rich biogas that can be further explored for biofuel properties (Stucki et al. 2009). Duman et al. (2014) reported catalytic steam gasification of *Nannochloropsis oculata* and seaweeds including *Fucus serratus* and *Laminaria digitata* which produced maximum hydrogen gas yields of 413 cc/g, 937 cc/g, and 1036 cc/g algal residue. The syngas produced hydrogen as the major component with carbon monoxide and methane in trace amounts. The method thus offers promising means to maximize hydrogen production from micro- and macroalgal biomass.

3.4 Future Perspectives

To attain the promising utilization of microalgal bioresource, persistent efforts are being carried out in the genetic modification of microalgae. Genetic engineering of microalgae aims to provide novel strain with exclusive features like high lipids yield, greater biomass accumulation, higher expression of bioactive compounds, superior CO_2 capture, and possible role in wastewater treatment. Several steps are required in the genetic manipulation of microalgae. The choice for genetic transformation techniques such as electroporation, particle bombardment, etc. is an important barrier to strain improvement. *Chlamydomonas reinhardtii* has been utilized in the past for genetic transformation through electroporation. Apart from transformation, efficient expression of our choice gene through codon optimization and promoter selection is also remarkably important (Chaturvedi et al. 2020; Barrera and Mayfield 2013). Barrera and Mayfield (2013) investigated overexpression of malic enzyme in *Phaeodactylum tricornutum* generating genetically improved transgenic cell with 2.5-fold higher lipid yield as well as higher biomass, thus suggesting novel prospects in biodiesel production. In another study enhanced lipid content of 46.4–52.9% was achieved in *Chlorella ellipsoidea* by overexpressing GmDof4 transcription factor from soybean (*Glycine max*) (Zhang et al. 2014). Couso et al. (2011) reported the stable increase in carotenoids production by overexpression of exogenous phytoene synthase gene in the *Chlamydomonas reinhardtii*. In recent times genetic improvement of the microalgal bioresource is currently pursued by the development of gene-editing tools like clustered regularly interspaced short palindromic repeats (CRISPR/Cas9) (Kumar et al. 2020a, b). CRISPR/Cas9-based technology was utilized for the expression of omega-3 fatty acid desaturase (fad3) gene in *Chlorella sorokiniana* and *Chlorella vulgaris* FSP-E. Higher lipid content of 46% (w/w) in *C. vulgaris* FSP-E was achieved using the novel genetic tool (Couso et al. 2011). Success of genome editing has also been reported in *Nannochloropsis oceanica*, *Phaeodactylum tricornutum*, and *Chlamydomonas reinhardtii* (Vazquez-Villegas et al. 2018). Although genetic tools provide a propitious opportunity for better products development from microalgae, it must be also stringently regulated and monitored to prevent compromise on human health and safety issues. In silico simulation studies show that upon escape to the natural habitat, these genetically superior microalgae with excellent growth kinetics can cause the formation of harmful algal blooms, creating a nutritional challenge to zooplanktons and ultimately giving rise to ecological imbalance (Flynn et al. 2013). Thus as more industries are exploring genetically modified microalgae for commercial applications, a need to monitor appropriate regulation and risk assessment has arisen. A new greener technology with minimal environmental pollution as well as highly regulated road map for the microalgal industry is thus the need of the hour.

3.5 Conclusions

The enormous bioactive compounds in microalgae provide a plethora of opportunities for several applications including biomedical, cosmetics, nutraceuticals, and biofuels. Prominent among the bioactive components include polysaccharides, fatty acids, pigments, proteins, and peptides. Till date, several interesting cosmetic formulations have been developed utilizing the unique metabolites present in microalgae. A wide range of health benefits with antioxidants, immune-modulatory, and anticancer activities provides a much needed drive to the cosmeceutical industry. The prospect of next-generation fuels such as biodiesel, bioethanol, biogas, etc. has allowed industries to explore the microalgal diversity. Although numerous microalgal species have been utilized for several applications, many are yet to be characterized. Industries have utilized several approaches to amplify the yield such as nutrient limitation, photochemical parameters, and genetic modification. Favorably numerous microalgae have shown promising yield thus providing scope for future developments.

Competing Interests All the authors declare that they have no competing interests.

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Chapter 4
Microalgae-Based Technologies for Removal of Textile Wastewater

Rahul Kumar Goswami, Komal Agrawal, and Pradeep Verma

Abstract Textile industries are the world's fastest emerging industries that utilize a large amount of water in different processing stages. It is an industry that generates a huge amount of wastewater and pollutants in the ecosystem of the earth. It contains a mixture of dyes, heavy metals, and heavy nutrient load which increases the biochemical oxygen demand (BOD) and chemical oxygen demand (COD) of the polluted water, and it needs to be treated before being discharged into the ecosystem. The conventional physicochemical treatment process is costly, is energy extensive, and generates a huge amount of sludge. Thus, an alternate biological remediation process is required. The microalgae-based wastewater remediation technology or phycoremediation is effectively employed in the treatment of textile wastewater. The microalgae utilize the nutrient load from the textile wastewater and increase their biomass. The microalgal biomass has great market value and can be used for the production of diverse kinds of bioenergy products. Thus, the present chapter deals with the composition of textile wastewater, possible conventional treatment methods, the role of microalgae in the phycoremediation of textile wastewater, and their mechanism. Furthermore, the chapter also provides fruitful knowledge about recent microalgal-based integrated technology used in the remediation of textile wastewater.

Keywords Textile wastewater · Dyes · Microalgae · Phycoremediation · Bioenergy products

Abbreviations

BOD	biochemical oxygen demand
COD	chemical oxygen demand
CO_2	carbon dioxide

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Cr-PSU-NFW	polysulfone nanofibrous web for entrapment of *Chlamydomonas reinhardtii*
DR-31	Disperse Red 3B
HRAP	high rate algal ponds
MFC	microbial fuel cell
M-MFC	microalgal-based fuel cells
NPs	nanoparticles
PBRs	photobioreactors
TDS	total dissolved solids
TWW	textile wastewater

4.1 Introduction

Industrialization has a significant role in the development of a country. Textile industries are an important traditional industry and are emerging as the largest manufacturing sector globally (Wu et al. 2017). The textile industries generate approximately 1 trillion dollars yearly sales and contribute up to 7% of worldwide export and boost the global economy (Lellis et al. 2019; Bhatia et al. 2020). The textile industries use various types of raw materials such as woolen, synthetic fibers, polyester, and cotton. The textile industries consume more water as compared to other industries. For example, approximately 200 L of wastewater is generated after producing 1 kg of fabric. This type of wastewater contains a variety of dyes, salts, and toxic compounds which cause serious environmental problems (Bhatia et al. 2020). The presence of dyes and other compounds has carcinogenic properties, and these compounds pollute the natural water bodies. Furthermore, textile wastewater also reduces the clarity of water and gas solubility and eventually is toxic to aquatic animals and plants (Wu et al. 2017). Thus, proper treatment is required before the discharge of these wastewaters into water bodies (Goswami et al. 2020b). Many conventional physical and chemical treatments have been used and developed; however, these methods are not significant due to expensive processes at the commercial level and generate a huge amount of sludge and are difficult to dispose of/discard (Daneshvar et al. 2007; Bhattacharya et al. 2017). Furthermore, these methods do not detoxify and decolorize the textile dye effectively and show limited applicability (Bhattacharya et al. 2017). Thus, the development of green sustainable technology is required which reduces the pollutants and carbon footprints from textile wastewater treatment and maintains the ecological balance of the earth (Behl et al. 2019). The conventional biological process such as bioremediation of textile wastewater is gaining attention and adaptability by the industries. The bioremediation of pollutants using bacteria, fungi, and other phototrophic microorganisms is suitable for the industrial level (Agrawal and Verma 2019a, b, 2020a, b; Chaturvedi and Verma 2015; Chaturvedi et al., 2013; Verma and Madamwar 2002a, b). However, the lower efficiency of these systems and tolerance level of pollutants towards microorganisms are the major bottleneck of the biological system (Behl et al. 2019). Furthermore, the produced biomass of these species is not utilized for any other purpose. Thus, microalgae have wide applications in the treatment of different wastewater and are designated as phycoremediation (Goswami et al. 2020b; Bhardwaj et al. 2020; Agrawal et al. 2020). Microalgae can completely utilize the textile pollutants as a nutrient source and their produced biomass which can be utilized for biofuels and other bioproducts production (Chaturvedi et al. 2020; Goswami et al. 2020a). Furthermore, the treated wastewater can be reused for industries or farming (Behl et al. 2019; Goswami et al. 2020b; Agrawal and Verma 2021a). Microalgae are eukaryotic microorganisms that perform photosynthesis in the presence of light and fix the carbon dioxide from the environment and regulate its metabolism for their growth (Goswami et al. 2020c; Mehariya et al. 2021). However, the conventional phycoremediation techniques are not appropriate due to their lower treatment efficiency (Behl et al. 2020), but integration or advanced modification of the system might help to reduce the pollutants from textile wastewater and that help to maintain the concept of circular bioeconomy. Thus, this chapter deals with the sources and process and composition of textile wastewater, different treatment processes, the role of microalgae in textile wastewater treatment, and their mechanisms. Furthermore, the chapter also provides knowledge about the integration of microalgae with different advanced technology.

4.2 Sources and Steps Involved in Textile Wastewater Generation

The textile industries are categorized into dry and wet fabric industries. The dry textile industries generate solid waste materials, whereas a high amount of wastewater is generated from wet textile industries (Bhatia et al. 2020). Water consumption and wastewater generation in textile industries depend on the types of raw materials, chemicals, or the steps involved in fabric processing (Holkar et al. 2016; Fazal et al. 2018). The textile industries consume 100–1000 gallons of water every day which generates a huge amount of wastewater (Fazal et al. 2018). Different industries have different steps processes. The foremost process of the textile industry is (1) pretreatment (sizing, desizing, scouring or bleaching, mercerization), (2) dyeing, (3) printing, and (4) finishing (Siddique et al. 2017). In different steps, water and diverse kinds of chemical, dye, and salt are used which generates different types of waste effluent shown in Fig. 4.1 (Bhatia et al. 2017, 2020). The process of sizing requires diluted mineral oils or mineral acid for the exclusion of starch residue and polyvinyl alcohol, and resultant effluent has a high ~300–450 ppm biological oxygen demand (BOD) (Paździor et al. 2019). In the bleaching stages, natural colors are removed by using different chemicals such as peracetic acid, hydrogen peroxide, hypochlorite, sodium chlorate, and chlorite (Paździor et al. 2019; Bhatia et al. 2020). Then mercerization is another pretreatment stage in which 18–24% NaOH was used

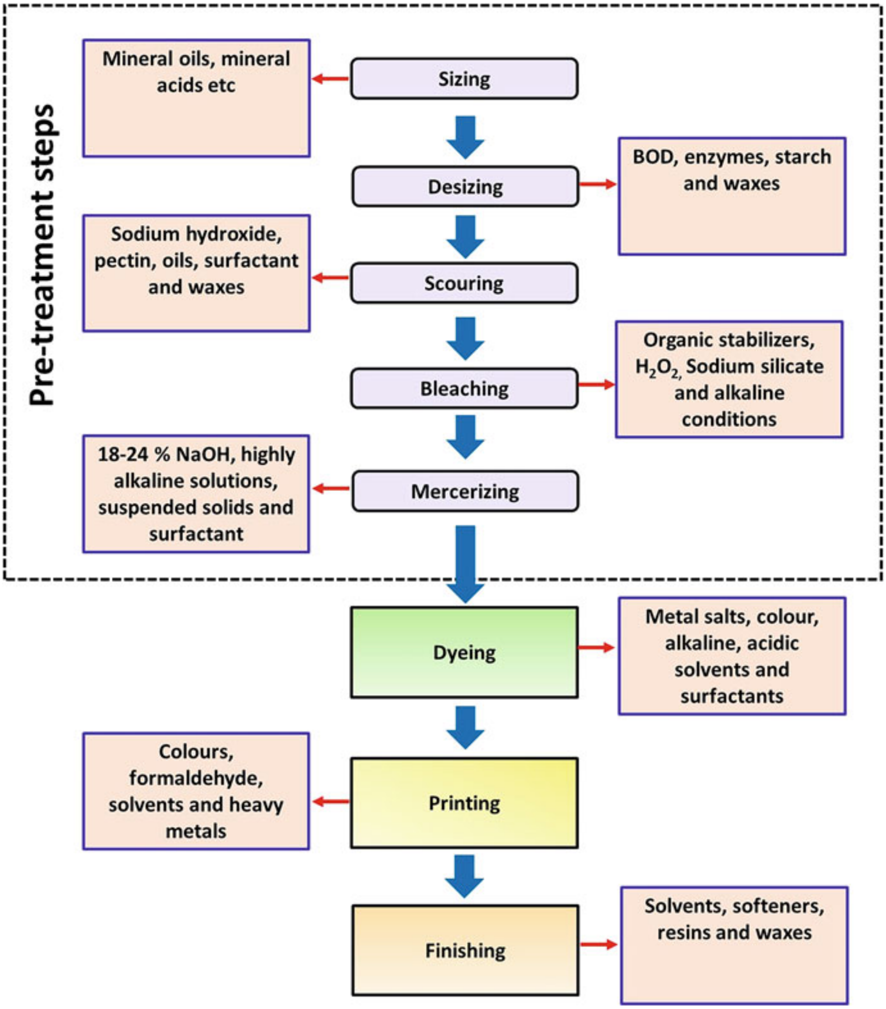

Fig. 4.1 The main pollutant arises in different steps of textile wet processing of fabric adapted from Holkar et al. (2016)

to vivify a shine or polish to the fabric; apart from NaOH, some other highly alkaline solutions are also used (Bhatia et al. 2017). The continuous mercerization process generates effluents that contain different types of highly alkaline compounds such as sodium hydroxide, suspended solids, and surfactants which are used. Furthermore, it also contains different loads of organic carbon (Paździor et al. 2019). In the dying stages, varieties of dyes such as auxochromes (hydroxyl, amine, sulfonate, and carboxyl) and chromophores group (carbonyl, azo, quinoid, etc.) are used for the dyeing of fabric (Bhatia et al. 2020). The dyeing of clothes also depends on the type of fabric; for coloring of polyester and cotton fibers, disperse and reactive dyes are used, respectively (Bhatia et al. 2020). Azo dye is a reactive dye commonly used in the textile industry and covers up to 70% of all dyes. The dyeing with reactive dyes required alkaline pH which is achieved by the addition of NaOH (~1.5 g/L) and electrolytes such as sodium sulfate or sodium chloride. The effluent release after dyeing has high pH and chloride content which have low biodegradability (Paździor et al. 2019). Washing after dyeing or additional rinsing or neutralization of dye also requires water. The effluent of these steps contains diluted dyes, surfactants, organic acid, formic acid, and acetic acids (Paździor et al. 2019). Printing and finishing are the last steps in textile industries that improve the waterproofing, softening, and antibacterial properties of the fabric fibers (Bhatia et al. 2020). In printing and finishing stages the different solvents, waxes and resins are used. However, a low amount of waste effluent is generated during these processes that contains high pollutants or contaminant load (COD ~130–200 g/L), mixtures of organic solvents, waxes, and resins paste (Paździor et al. 2019; Bhatia et al. 2020). Furthermore, cleaning of textile machines after the process also generates a huge amount of wastewater (Paździor et al. 2019).

4.3 Chemical Composition of Textile Dye

The previous section discussed the different stages of chemicals used in textile industries and their effluent composition. It is estimated that worldwide textile industries used ~100,000 types of dyes, which cause severe toxicity to the environment. The textile or dye industries create 7×10^5 tons of organic dye annually, in which ~40% are not disposed of in inappropriate manners (Andrade and Andrade 2018). The main composition of textile dye is present of organic dyes, phosphate (phosphorus, orthophosphate), COD, BOD, nitrogen (ammonia, nitrates), mineral oils, acids, alkalis (NaOH, sodium hypochlorite), trace heavy metals (cadmium, magnesium, iron, arsenic lead, chromium, nickel) salts, etc. (Andrade and Andrade 2018; Fazal et al. 2018; Yaseen and Scholz 2019). In textile wastewater consists of BOD (80–6000 mg/L), COD (150–12,000 mg/L), total suspended solid (15–8000 mg/L), total dissolved solids (1500–12,000 mg/L), nitrogen (10–80 mg/L), phosphate (10–300 mg/L), sulfate (600–1000 mg/L), NaOH (~10 mg/L), Na_2CO_3 (20 mg/L), NaCl (~300 mg/L), oil and grease (10–50 mg/L), and different heavy metals (<10 mg/L) (Yaseen and Scholz 2019). The dyes are categorized into natural and synthetic. The natural dyes are easy to digest or remediate; however synthetic dyes are harmful and difficult to dispose of in the environment. The synthetic dyes are mainly alienated to their chemical structures such as azo, sulfur, anthraquinone, triarylmethane, and phthalocyanine (Yaseen and Scholz 2019). The dye effluent of textile industries is highly carcinogenic in nature and high in color, pH, COD, BOD, pH (Yaseen and Scholz 2016), heavy metals, and temperature (Yaseen and Scholz 2019). The main metals found in dye effluents are cobalt, chromium, and copper. The concentration of dyes in textile industries ranges from

10 to 800 mg/L which depends on the fabric dyeing methods, types of fabric, and types of dye used (Yaseen and Scholz 2019).

4.4 Different Approaches Used in the Treatment of Textile Wastewater

Textile wastewater is a mixture of pollutants which are already discussed in the previous section. The presence of complex pollutants requires proper treatment before discharge into the environment. Several conventional physiochemical approaches such as homogenization, adsorption with activated carbon, ion exchange, flocculation, membrane filtration, coagulation and sedimentation, irradiation and electrochemical destruction, reverse osmosis, nanofiltration, etc. have been employed to treat wastewater (Siddique et al. 2017; Fazal et al. 2018). A conventional chemical method such as oxidative process, ozonation, H_2O_2 with irradiation, organic solvent or salt treatment (NaOCl), AI_2 $(SO_4)_3$, $FeSO_4$, $FeCI_3$, and lime (Siddique et al. 2017) too has been used. However, the conventional physical and chemical method cannot completely remove the pollutants from textile wastewater, as it is a costly process, is energy-intensive, is less efficient, and produces a high amount of sludge (Fazal et al. 2018). The advanced oxidation process is another method that has been used for the treatment of textile wastewater and consists of Fenton reaction (H_2O_2 with Fe^{2+}), photocatalysis, etc. However, these techniques are more efficient but require a high amount of energy (electricity) or chemicals for the treatment of textile wastewater (Siddique et al. 2017; Paździor et al. 2019; Agrawal and Verma 2021b).

The conventional biological processes consist of (1) living organisms that remediate textile wastewater and used as a nutrient source, (2) anaerobic digestion or biodegradation, (3) aerobic degradation, (4) enzyme-based treatment, and (5) utilization of dead cells or biomass or compounds which can use as adsorbent (Siddique et al. 2017). The biological treatment method such as bioremediation is an emerging technology that has been used for the treatment of textile wastewater. In this method, different microorganisms, e.g., bacteria, fungi, yeast, and algae, have been used. The treatment is generally performed in bioreactors with proper agitation or aeration, but recently wetlands are also used for treatment purposes (Paździor et al. 2019). Furthermore, a large amount of sludge is produced by these methods which are a major drawback. The biological methods are a cost-effective process, but initially, the investment cost is high; furthermore, it only treats the biodegradable compounds that exist in textile wastewater (Paździor et al. 2019). Microalgae-based phycoremediation of textile wastewater is a promising concept as compared to other treatment methods (Fazal et al. 2018). Microalgae not only remove toxic compounds from wastewater but also used nutrients from wastewater for growth and generate various value-added compounds (Mehariya, et al. 2021). More details about phycoremediation are discussed below section.

4.5 Role of Microalgae in Textile Wastewater Treatment

Microalgae are eukaryotic microorganisms present in the aquatic environment. Naturally, it fixes CO_2 in the presence of sunlight and possesses photosynthesis (Goswami et al. 2021). Furthermore, it has grown in a different mode such as autotrophic (naturally photosynthesis process), heterotrophic (consumes organic or inorganic carbon source in the absence of light), and mixotrophic (consumes high nutrient load in the presence of light). The mixotrophic ability of microalgae makes them a potent candidate for phycoremediation of wastewater treatment (Singh et al. 2016; Fazal et al. 2018; Goswami et al. 2020b). The microalgae are cultivated in an open pond system (circular and raceway pond) and a closed system using photobioreactors. The microalgae are auspicious microorganisms as compared to other microbes because it cannot only remediate the wastewater but it also produces biomass. The nutrient removal efficiency of microalgae is more than 90% which depends on which type of microalgae strains used for the phycoremediation process (Goswami et al. 2020). The textile wastewater contains numerous dyes, phosphates, organic carbons, nitrates, and other essential nutrients which are the main nutrient components for microalgae growth, and their effects are summarized in Table 4.1. The textile wastewater provides an opportunity to make cheap cultivation media for microalgae. Several types of research have been established for the treatment of textile wastewater. The microalgae biomass contains lipid, carbohydrates, protein, and other pigments which have several applications and great market value. The carbohydrates and lipids are generally considered for biofuel production such as biodiesel, bioethanol, biohydrogen, biobutanol, and biomethane (Chaturvedi et al. 2020; Goswami et al. 2020a, b). The conceptual scenario of the microalgae phycoremediation process of textile wastewater and their application are shown in Fig. 4.2. The growth of microalgae in textile wastewater also depends on the presence of nutrient loads, dyes, and other growth factors. The presence of pollutants above the permissible limits in textile wastewater inhibits the growth of microalgae (Fazal et al. 2018). The presence of high carbon, nitrogen, and phosphorus content in textile wastewater can enhance biomass productivity, whereas starvation of nitrogen and phosphorus content enhances the lipid accumulation in microalgae (Bhatt et al. 2014). Mostly different strains of *Chlorella* strains show significant decolorizing efficiency in textile wastewater remediation, and it removes 63–69% of dyes (Chu et al. 2009). Similarly, microalgae *Chlorella pyrenoidosa*, *Chlorella vulgaris*, and *Oscillatoria tenuis* biodegrade the azo dye into simple aromatic amines and decolorize the colored textile wastewater (Forgacs et al. 2004; Noel et al. 2014). Furthermore, Cheriaa et al. (2009) used *Chlorella* sp. for biodegradation of different textile dyes. The *Chlorella* sp. degradation efficiency is quite impressive in different dyes such as direct blue (79%), Remazol brilliant orange (75.3%), crystal violet (72.5%), and indigo (89.3%). Moreover, El-Kassas and Mohamed (2014) cultivated *Chlorella vulgaris* in textile wastewater. The result showed the *Chlorella vulgaris* removed COD 70%. Rather than *Chlorella* sp., other microalgae are also involved in the remediation of textile wastewater and dye which are shown in Table 4.2.

Table 4.1 Different textile wastewater nutrient which effects the growth of microalgae adapted from (Das 2015; Fazal et al. 2018)

Source of nutrients	Effect on microalgae growth
Carbon	• Key components for the growth • Especially organic carbon (glucose) in dark conditions improves growth rate • It also allows microalgae to grow in mixotrophic mode and produce maximum biomass • However, the above permissible concentration of carbon source may cause feedstock inhibition and inhibits the growth of microalgae
Nitrogen	• It is a key nutrient required for the synthesis of nucleic acids, proteins, or amino acids • It exists usually in the form of nitrates, ammonia • Starvation condition promotes lipid accumulation inside microalgae cells
Phosphorus	• It is the foremost compound required for energy metabolism • It supports the formation of nucleic acids, ATPs, amino acids, and lipids • Inorganic phosphate plays a key role in the phosphorylation process
Magnesium	• Works as enzyme activator compounds • Central atoms in chlorophyll • Help in the synthesis of nucleic acids and protein
Potassium	• It regulates osmoregulation in cells • Help in the photosynthesis process • Help in ion exchange in cell growth membranes
Iron	• Key protein compounds help in the synthesis of protein cytochrome and ferredoxin • A high concentration of iron can reduce the growth of microalgae
Chromium	• Responsible for oxygen release • High concentration can affect the cell machinery • Act as a photosynthesis inhibitor
Copper	• Improves the yield of biomass • Improves the oil content in microalgae • Helps in enzyme activation
Molybdenum	• Its deficiency inhibits nitrogen uptake • It contains nitrogenases and nitrate reductase

4.5.1 Mechanism of Microalgae-Based Phycoremediation

Microalgae-based phycoremediation occurs in bioaccumulation, bioconversion, and biosorption process. In bioaccumulation process, microalgae accumulate the nutrients present in the textile wastewater, and later it is used as a nutrient source, whereas in the bioconversion process, microalgae consume the dye or other nutrient substances and convert into simple or other products. The bioconversion occurs due to the presence of different degradable enzymes inside microalgae cells. In the biosorption process, microalgae work as a biosorbent where it can adsorb the dyes from textile wastewater (Chu et al. 2009). This mechanism also involves different cell walls or cell membrane receptors and enzymes. Moreover, dead microalgae cells also work as biosorbent (Fazal et al. 2018). Microalgae have a high surface area and

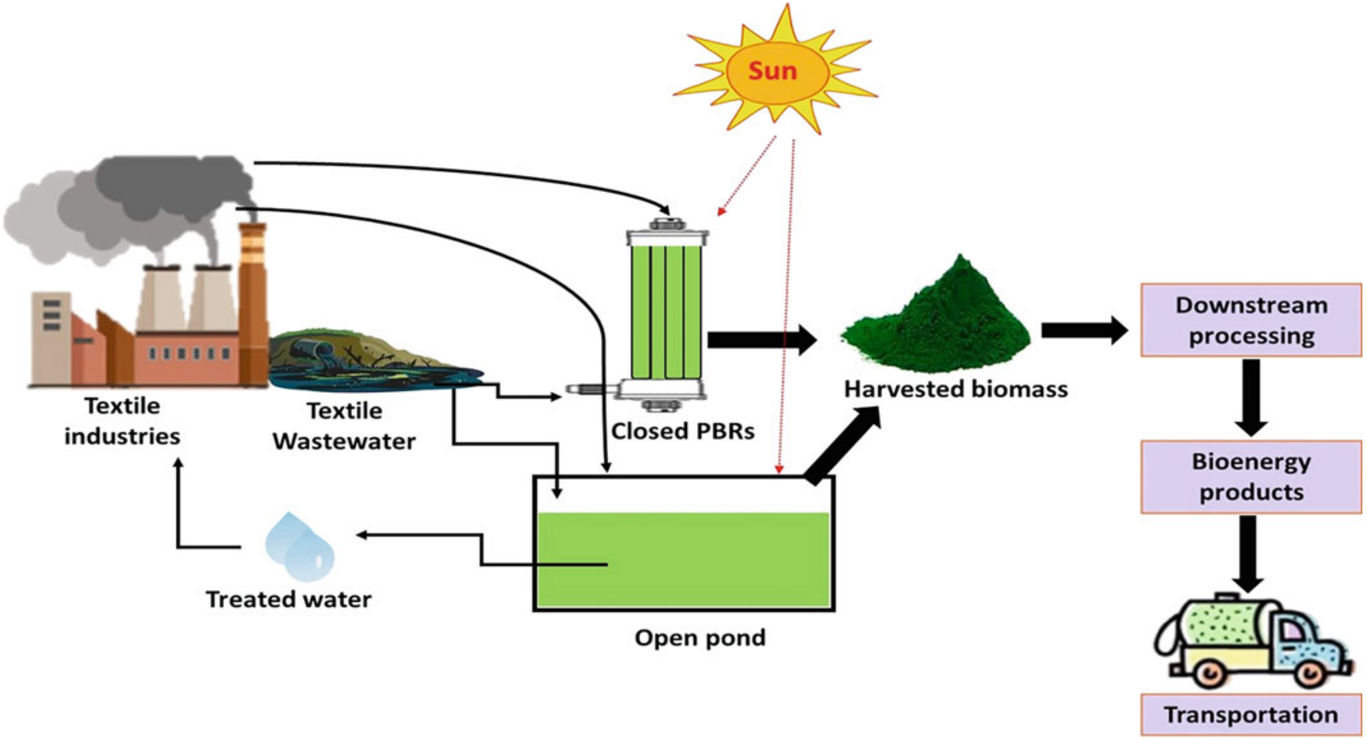

Fig. 4.2 Conceptual illustration of textile wastewater utilization for bioenergy production

Table 4.2 Phycoremediation of textile wastewater dyes from different microalgae strain

Microalgae	Dyes/textile wastewater (TWW)	Biosorption capacity (%)	Decolorization efficiency (%)	COD removal (%)	Biomass productivity/lipid content	References
Chlorella pyrenoidosa	Methylene blue	21.3	90	na	na	Pathak et al. (2015)
Chlorella sp.	Methylene blue and methyl orange	na	99.9	na	na	Seo et al. (2015)
Chlroella vulgaris	Tectilon yellow 2G	na	63–69	na	na	Acuner and Dilek (2004)
Chlorella pyrenoidosa	DR-31	30.53	96	82.7	na	Sinha et al. (2016)
Gloeocapsa pleurocapsoides	FF sky blue	90	na	na	3.34 mg/L/d	Parikh and Madamwar (2005)
Chlorella vugaris	TWW	20.8	80	na	8.114 mg/L/d	Pathak et al. (2015)
Neochloris sp.	TWW	na	na	34.5	0.109 mg/L/d	Gopalakrishnan and Ramamurthy (2014)
Chlorella vulgaris	TWW	na	77	69.9	0.0019 mg/L/d	El-Kassas and Mohamed (2014)
Desmodesmus sp.	Textile azo dye (DR 31)	36	90–92	na	11 %	Behl et al. (2019)
Chlorella sp. G23	TWW	na	50	<90	16.6 %	Wu et al. (2017)
Scenedesmus sp.	Starch TWW	na	~90	89.5	9800 kg/7 d	Lin et al. (2017)
Cosmarium sp.	Malachite green	na	89.1	na	na	Daneshvar et al. (2007)
Chlorella sp.	TWW dye	na	43	na	na	Kumar et al. (2014)
Isochrysis galbana	TWW dye	na	55	na	na	Kumar et al. (2014)
Chlorella vulgaris	Methylene blue	na	na	na	na	Krishna Moorthy et al. (2021)
Spirulina platensis	Methylene blue	na	na	na	na	Krishna Moorthy et al. (2021)
Chlorella sorokiniana 246	Color removal	na	70	na	na	Oyebamiji et al. (2019)

Chlorella sorokiniana 1665	Color removal	na	60	na	na	Oyebamiji et al. (2019)
Micratinium sp.	Color removal	na	62	na	na	Oyebamiji et al. (2019)
Chlorella sp. CB4	Color removal	na	62	na	na	Oyebamiji et al. (2019)
Chlorella sp. KU211 a	Color removal	na	63	na	na	Oyebamiji et al. (2019)
Chlorella sp. KU211 a	Color removal	na		na	na	Oyebamiji et al. (2019)
Chlorella vulgaris	Brazilwood	na	99.5	na	na	Abd Ellatif et al. (2020)
Chlorella vulgaris	Orange G	na	99.5	na	na	Abd Ellatif et al. (2020)
Chlorella vulgaris	Naphthol Green B	na	98.5	na	na	Abd Ellatif et al. (2020)
Chlorella vulgaris	Blue dye Green dye	na	63.89 and 45.71	na	na	Raymond and Kadiri (2017)
Sphaerocystis schroeteri	Blue dye Green dye	na	63.87 and 60	na	na	Raymond and Kadiri (2017)
Chlorella variabilis	sodium dodecyl sulfate-containing TWW	na	na	na	74.96 ± 2.62 g/(m^2/d)	Bhattacharya et al. (2017)
Immobilized Chlorella sp. Wu-G23 (G23)	TWW	na	77.9	80.2	na	Wu et al. (2021)

na Not available

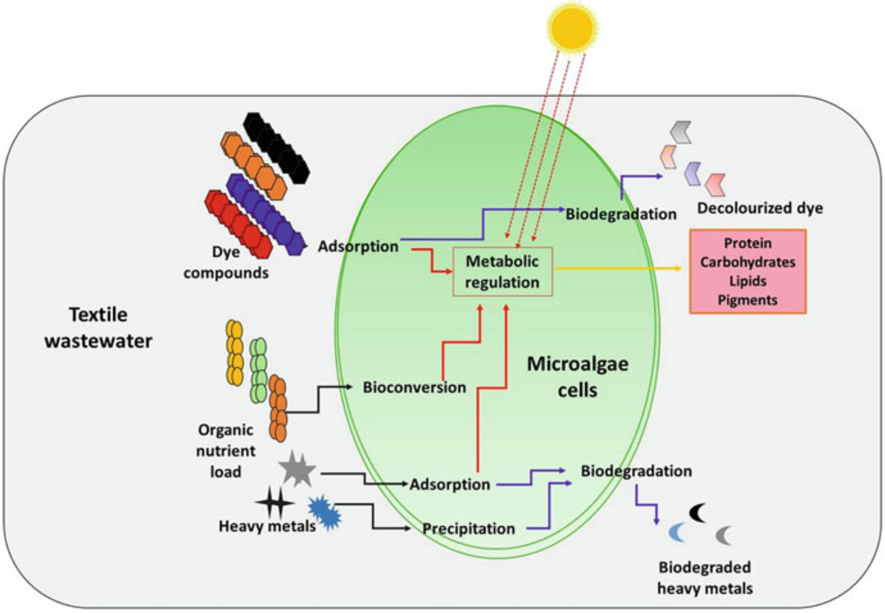

Fig. 4.3 Mechanism of textile wastewater remediation by microalgae

strong affinity of binding which increase the binding efficiency towards azo dye (Pathak et al. 2015). The microalgal cells consist of carbohydrates and proteins, which deliver functional groups to bond with basic dyes and metals. The mechanism of microalgae-based textile wastewater removal is shown in Fig. 4.3. The composition of microalgae cells and types of strains are important factors before starting the phycoremediation process (Fazal et al. 2018).

4.6 Recent Microalgae Technologies for the Treatment of Textile Wastewater and Their Future Prospects

4.6.1 Raceway Pond Cultivation System

The raceway pond cultivation system is an open cultivation system that is mostly used in the mass cultivation of microalgae. It is also called the high rate algal ponds. The size of the raceway pond is 1–200 ha. It consists paddlewheel for recirculation of media or better mixing of inoculum. The large-sized raceway ponds are low cost, but initially they required a large area. Furthermore, the biomass productivity rate is low compared to other cultivation systems (Fazal et al. 2018). However, this system can be suitable for large-scale phycoremediation of textile wastewater. Lim et al. (2010) collected the textile dye from a garment factory located in Senawang Industrial

Estate, Negeri, Sembilan, and investigated the use of *Chlorella vulgaris* UMACC 001 for bioremediation of the textile wastewater and textile dye such as Supranol Red 3BW, Lanaset Red 2GA, and Levafix Navy Blue EBNA in HRAP system. This study suggested that the *Chlorella vulgaris* showed maximum color removal efficiency from textile dyes (~47%) and produces 106.67 ± 5.77–203 ± 15.28 mg/L of biomass. The integration of raceway pond microalgae cultivation system for phycoremediation of textile wastewater is the significant approach. However, more investigation is required before assembling in textile industries for dye removal.

4.6.2 Closed Cultivation System

A closed cultivation system is a controlled artificial system more appropriate technique for the removal of dyes, contaminants, or heavy metals from textile wastewater. In this system, different PBRs are used such as tubular PBRs, flat panel PBRs, and column PBRs (Fazal et al. 2018). The major advantage of these systems is having high removal efficiency compared to other cultivation systems. The biomass productivity of algae is also high. Furthermore, closed bioreactors generally produce high cell growth efficiency due to ease to control and have lower contaminations risks (Wu et al. 2021).

Most of the batch mode textile wastewater removal experiments proceed in this system. The tubular PBRs are the most suitable and can be used for outdoor cultivation of microalgae and remediation of textile wastewater, but the initial installation cost is high, and overheating and toxic accumulation of oxygen fouling are the major issues (Fazal et al. 2018). Aragaw and Asmare (2018) cultivated microalgae in closed flask PBRs for the study of dye decolorization. This study showed that microalgae removed COD (91.50%), BOD (91.90%), TDS (89.10%), and color removal efficiency up to 82.60%. Similarly, Sinha et al. (2016) cultivated *Chlorella pyrenoidosa* NCIM 2738 in a continuous cyclic photobioreactor for removal of diazo dye Direct Red 31 from wastewater. The result showed that microalgae *C. vulgaris* NCIM 2738 decolorized 96% dye with 40 mg/L concentration at pH 3. Furthermore, it also reduced the amount of BOD (56.44%), COD (82.73%), phosphate (19.88%), sulfate (54.54%), and TDS (84.18%).

4.6.3 Nanoparticles or Immobilized Algal Cell Treatment System

Recently, green synthesis of green metal nanoparticles using microalgae is gaining attention from the scientific community towards the treatment of wastewater. The green synthesis of NPs is non-toxic, cost-effective, eco-friendly, and biocompatible compared to other synthesis methods. The metal accumulation efficiency of microalgae-based NPs provides an opportunity for the removal of heavy metal from textile wastewater (Goswami et al. 2020b). Furthermore, synthesized silver nanoparticles from microalgae also integrate with the photocatalytic dye degradation method. For example, Rajkumar et al. (2021) synthesize silver nanoparticles using *Chlorella vulgaris* biomass and studied the removal of methylene blue via photocatalytic decolorization process with synthesized silver nanoparticles under the presence of sunlight irradiation. This study showed 96.51% photocatalytic decolorizing efficiency.

Immobilization technology is a worthy approach for wastewater treatment. The microalgal biomass are entrapped into the polymer matrix or attached on the outward surface of a solid carrier such as alginate to form microalgae-based polymeric matrix granules in cross-linking solution. The immobilized algal cells have more wastewater removal efficiency and gain attention towards the treatment of different wastewater (Leong and Chang 2020; Wu et al. 2021). This approach is also used in the treatment of textile wastewater or dye. For example, Wu et al. (2021) studied the efficiency of immobilized *Chlorella* sp. Wu-G23 (G23) to treat textile wastewater. This method shows significant efficiency towards the removal of COD (70.8%), nitrates (80.2%), and decoloring efficiency (77.9%) from textile wastewater. Rather than alginate, other cross-linking solutions have also been used for entrapping microalgae biomass such as polysulfone nanofibrous web. San Keskin et al. (2015) used polysulfone nanofibrous web for entrapment of *Chlamydomonas reinhardtii* (Cr-PSU-NFW) and studied the removal ability of Remazol black 5 and Reactive blue 221 dye. The result showed that Cr-PSU-NFW shows decolorization efficiency (30.2 ± 0.23%) against reactive blue 221 and (72.97 ± 0.3%) against Remazol black 5.

4.6.4 Co-Cultivation (Consortia) of Microalgae with Microalgae or Other Robust Microorganisms

The development of consortia for the treatment of different wastewater was well developed. However, treatment of textile wastewater has not been researched yet (Kumar et al. 2018). However, some recent studies suggested that consortia of microalgae with microalgae, bacteria, and fungi show remarkable benefits towards the treatment of textile wastewater. Kumar et al. (2018) cultivated mixed microalgae consortia for the treatment of textile wastewater via fed-batch operation. This study showed that the consortia of microalgae can decolorize the color of textile wastewater ranging from 68 to 72%. This finding might be a help to develop the consortia of microalgae for the treatment of textile wastewater (Kumar et al. 2018). Furthermore, Mubashar et al. (2020) studied the consortia of *C. vulgaris* (microalgae) with *Enterobacter* sp. MN17 to the treatment of textile wastewater and removal of heavy metals and colors. This investigation suggested that the consortia of bacteria with algae significantly enhance the removal efficiency of COD (74%), color (70%), and different heavy metals (79–93%). Rather than bacteria, fungi consortia with algae also show significant removal efficiency of textile wastewater nutrient load. For example, Tang et al. (2019) studied the removal/decolorizing efficiency of Disperse Red 3B (an anthraquinone dye) and nutrient load from textile dyes effluent by making the consortia of *Aspergillus* sp. XJ-2 (fungi) and *Chlorella sorokiniana* XJK. This investigation showed that the consortia of these microorganisms show remarkable decolorizing efficiency and significantly remove the COD (93.9%), total phosphorus (83.9%), and total ammonia nitrogen (87.6%). The development of consortia for the treatment of textile wastewater is a win-win approach. However, more pilot-level investigation is required on the mercantile scale.

4.6.5 Microalgal-Based Fuel Cells (M-MFC) for the Treatment of Textile Wastewater

Microbial fuel cell (MFC) is an emerging technique used to treat wastewater and generate bioelectricity. This system was mostly established with a bacterial system. However, recent studies suggested that it can also be applied with microalgae for the treatment of different wastewater. Rather than wastewater treatment or bioelectricity generation, this system is also used in the production of biohydrogen (Jaiswal et al. 2020). The MFC is the sequential biochemical or biocatalytic reaction. It was divided into two compartments: anode or cathode, and these are separated by an ion-exchange membrane. The sequential catalytic reaction produces protons and electrons by degradation of wastewater present in this system. Then the electron undergoes to the anode surface through the electrical mediator. After that, the electron subsequently moves to the cathodic compartment through the outer circuit and creates current flow in the direction of the cathodic compartment to the anodic compartment (Jaiswal et al. 2020). Several types of microbial fuel cells are a design incorporated with microalgae for bioelectricity generation such as microbial carbon capture cells, photosynthetic algal microbial fuel cells, sediment microbial fuel cells, etc. (Arun et al. 2020). Furthermore, it is also divided into the number of compartments. For example, single-chamber MFC is using microalgal biocathodes. This system is used for the biodegradation of textile dye wastewater to the generation of bioelectricity. This experimental investigation showed that it generated 123.2 ± 27.5 mW m^{-3} of bioelectricity and showed significant removal efficiency of color, COD, and heavy metals (Logroño et al. 2017). The major drawbacks of this system are low electricity generation. However, more successive investigations are required before its establishment for the treatment of textile wastewater.

4.7 Conclusion

Microalgae are well-suited microorganisms for the treatment of textile wastewater. Microalgae have been reported for their COD, BOD, and nutrient removal efficiency as well as the decolorizing ability (<90%) from textile wastewater. Furthermore, the produced biomass of microalgae can be used for the production of different bioenergy products, and treated water can be reused for textile industries. The integration of recent technologies with microalgae-based phycoremediation such as designing of MFCs and using low-cost PBRs and consortia can enhance the removal efficiency of dye present in textile wastewater. However, more mass level treatment is required before assembling into textile industries. But microalga-based phycoremediation is a sustainable win-win approach for the treatment of textile wastewater.

Conflict of Interest All authors approve the submission and declare that there is no conflict of interest.

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Chapter 5
Treatment of Textile Waste Effluents Using Microalgae: A Suitable Approach for Wastewater Remediation and Lipid Production

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Abstract Industrialization plays a pivotal role in global economic development and is perceived to be one of the major causes of water pollution worldwide. One such industry that contributes to contaminating waters is the textile industry that utilizes water in all its operations. In India alone, these textile industries release several tonnes of wastewaters every day. These released wastewaters are often untreated and are a concoction of harmful contaminants such as metals, dyes, phenols, and detergents. Besides this, energy supply is another major problem that is rising at an alarming rate. Fossil fuels, considered as the main source of energy globally, are rapidly depleting due to their limited availability, leading to fuel shortage globally. Thus, it has become imperative to find sustainable ways of averting the consequences caused by these ascending shortages. In this context, simultaneous applications of microalgae in phytoremediation and the production of sustainable biofuels can be considered a feasible endeavour. Microalgae can resourcefully multitask between bioremediating wastewaters, breaking down complex organic and inorganic contents, and subsequently generating valuable biomass after remediation to be used in bioenergy production. The current chapter provides detailed insight into the existing knowledge concerning textile dye wastewater treatment by microalgal-based systems. Furthermore, it also highlights the potential of algal biomass generated after remediation that can be utilized in the production of lipid as a biofuel precursor.

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5.1 Introduction

The textile industry is a significant part of the Indian economy, as it represents 2% of the gross domestic product (GDP), 8% of incomes created from customs, and around 14–16% through complete exports in the country (Restiani and Khandelwal 2016). The textile industry is also the second biggest generator of employment, following the agricultural sector, and serves in connecting with around 35 million jobs in both rural and metropolitan areas of the country (Restiani and Khandelwal 2016). Moreover, factors like readily available skilled labour, the wide spectrum of dyeable fabrics, and constantly growing large network of national and international markets with ever-changing needs add to making India a leader in the global textile sector (Restiani and Khandelwal 2016).

The textile industries also ravenously consume water, widely using water in each step all through the textile processing activities (Yaseen and Scholz 2019). With the development of several small, medium, and large textile units quickly across the country, the textile industry is presently one of the major modern polluters. More than 1,00,000 variations of coloured pigments and dyes are utilized by textile industries around the world, of which roughly 2,80,000 tons are released yearly in the effluent (Sinclair 2015). In India alone, the textile sector discharges 16×10^8 L of water each day as waste (Ghaly et al. 2014). The released effluents are often untreated and contain hazardous toxins like hydrolysed unfixed dyes, surfactants, salts, and sulphides (Fazal et al. 2018). Although dyes are the major component of textile effluent, the released effluent is also categorized by its high levels of chemical oxygen demand (COD), varying pH levels, high temperature, and critical levels of nitrogen, phosphorus, hazardous heavy metals, and other toxicants (Lellis et al. 2019; Kumar et al. 2021). Thus, the release of these wastewaters to the environment causes detrimental effects on not only the flora and fauna of the aquatic ecosystem but also on human health (Lellis et al. 2019). Although several conventional physicochemical processes are available for effluent treatment, they have certain limitations as they are considerably expensive, require proper sludge handling and disposal, and often lead to the generation of toxic by-products (Rajasulochana and Preethy 2016). Therefore, there is a tremendous demand and interest to develop cost-effective eco-friendly substitutions for the efficient remediation of effluents. In this context, an alternate arrangement is to follow a biological approach, which is economic and utilizes eco-friendly organisms like bacteria, algae, and fungi for the efficient remediation of the coloured effluents (Englande Jr. et al. 2015).

Concurrently, the world today is also facing a global energy emergency which is brought about by abrupt limitations of petroleum and petroleum-based derivatives (Daneshvar et al. 2018b). Despite their restricted stock, the interest in energy use of petroleum derivatives is expanding constantly. Production of energy from “negative-esteem” effluents has garnered interest among researchers globally, which can simultaneously address the world's energy and water contamination crisis (Daneshvar et al. 2018a).

Microalgae can be considered as a self-supportable option, answering both these issues of water contamination and fuel scarcity, in an inexpensive and eco-friendly manner. Microalgae are one of nature's most significant naturally occurring assets, which grow rapidly and produce a large variety of useful metabolites like lipids, proteins, sugars, and other value-added products (Behl et al. 2020). Thriving well in effluents, microalgae can endure a harsh toxic environment by utilizing the organic and inorganic compounds present in the effluent for their growth, thereby remediating these sites in return (Behl et al. 2019; Agrawal and Verma 2021). Additionally, algal biomass produced after remediation can be further employed in the production of biofuels. Microalgal determined biodiesel, which is rich in C16–C18 unsaturated fatty acids, might be considered as an alternate for clean and sustainable energy (Behl et al. 2020).

The current chapter provides detailed insights into using the existing knowledge concerning textile dye wastewater and conventional treatment technologies employed to treat these coloured wastewaters and elaborates on the advantages of employing biological systems, especially microalgae. Furthermore, it also underlines the potential of utilizing algal biomass generated after remediation in the production of lipid as a biofuel precursor and the production strategies employed to scale up algal-based remediation to an industrial scale for its economic viability.

5.2 Composition of Textile Dyeing Wastewater

The fundamental composition of the textile dyeing effluents remain the same, i.e. the presence of pigments and coloured dyes, harsh organic and inorganic compounds, solvents, and detergents; however, their configuration fluctuates depending upon the type of textile process being carried out (Takahashi and Kumagai 2006). These textile effluents, then released into the environment, have higher temperatures and record varying pH levels ranging from an acidic pH 4 to being severely basic at pH 12 (Fazal et al. 2018). Effluent streams produced during different phases of the dyeing processes are ultimately blended to what we know as textile waste effluent as illustrated in Fig. 5.1.

The textile effluent generated after the preparation stage mainly consists of sizing agents, cleaving enzymes, soaps, and detergent and bleaching agents (Yaseen and Scholz 2019). The generated effluent has low biodegradation potential and is majorly responsible for the presence of organic content in the textile effluent. Moreover, around 50% of the dye applied along with the concoction of auxiliary chemicals also gets exonerated in the wastewater (Jargalsaikhan et al. 2021). The effluent is also rich in chemicals such as caustic soda, salt, acetic acid, and hypochlorite. Table 5.1 depicts various input materials applied in the textile dyeing and finishing processes.

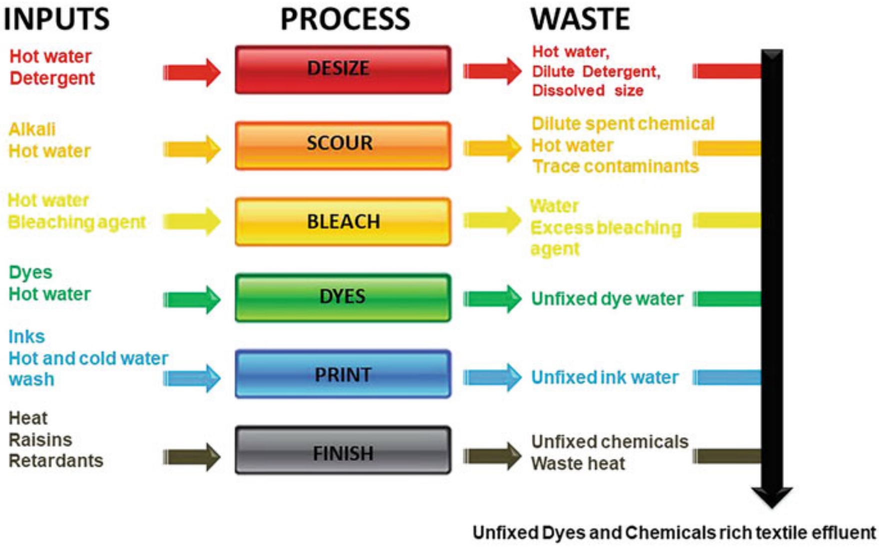

Fig. 5.1 A flow diagram of the textile processing stages in textile dyeing and finishing industries and the effluent released

Table 5.1 Different input materials utilized in various textile dyeing and finishing processes

Material	Components
Water	
Fibre	Cotton, wool, silk, nylon, rayon
Acids	Acetic acid, formic acid
Alkalis	Sodium hydroxide, potassium hydroxide, sodium carbonate
Bleaching agents	Hydrogen peroxide, sodium hypochlorite, wax
Dyes	Acidic, basic, direct, disperse, vat, reactive, Sulphur
Salts	Sodium chloride
Sizing agents	Starch, polyvinyl alcohol, carboxymethyl cellulose
Stabilizers	Sodium silicate, sodium nitrate, organic stabilizers
Surfactants	Fatty amine ethoxylates, fatty alcohol ethoxylates
Auxiliary finishers	Fire retardants, fabric softeners, handle modifiers, mordents

5.2.1 Dye Inputs

Naturally available pigments and dyes have been used by skilled artisans since ancient times as an approach to convey their craft, talent, and creativity (Adinew 2012). Synthetic dyes were first accidentally synthesized by William Perkin in 1856, while he was experimenting to fabricate quinone, an antidote for malaria (Rahman 2016). However, it was only at the beginning of the twentieth century that an upsurge in the synthesis of several thousands of new dyes was observed. However, these days the extensive use of synthetic dyes is common especially in various textile dyeing and printing industries (Singh et al. 2017). These stable organic and organic amalgams provide more binding ability, intense colouration on the fabric, and resistance than most available textile dyes (Fazal et al. 2018).

Apart from organic components, synthetic dyes principally bear colour, as they easily absorb light in the visible spectrum. These dyes consist of at least one chromophore (colour-inducing groups), a delicate framework of alternating single and double bonds that displays electron resonance and is responsible for the organic nature of the colour (Bafana et al. 2011). Furthermore, various synthetic dyes also consist of auxochromes that act as colour helpers. Auxochromes can either be carboxylic acids, sulphonic acids, amino acids, or hydroxyl groups. These auxochromes indirectly aid in determining the stability of the dye and dye intermediates (Benkhaya et al. 2020).

Dyes are mostly classified based on their chemical types and application. Azo dyes are one of the most predominant, accomplished, and adaptable categories of synthetic dyes employed in the textile dyeing and finishing industry (Bafana et al. 2011). One of the fundamental characteristics of these azo dyes is the presence of one or more azo bonds (–N=N–) that link various aromatic complexes. Other vital functional groups such as hydroxy (–OH) and amino group (–NH–) are also found in these dyes (Benkhaya et al. 2020). Azo bonds can easily be separated by biological and chemical processes which lead to the liberation of aromatic compounds, believed to be more toxic and mutagenic than their mother compounds (Sinha et al. 2016). Azo dyes are largely the most preferred and dominant choice to colour fabrics owing to low manufacturing cost and ease of access (Kumar and Chowdhury 2018). Some of the commercially available azo dyes being used are described below.

1. *Acid dyes*: these water-soluble anionic dyes are mostly applied in an acidic medium. Both polyamide and proteinaceous fibres contain a cationic charge which gets activated during the dyeing process. These dyes have little to no compatibility with cationic polymers cellulose and polyester-based fabrics since they are unable to form anionic bonds (Bafana et al. 2011).
2. *Disperse dyes*: these hydrophobic dyes require amalgamation with polymeric matrices to develop a stable solution and are manufactured for fabrics like polyester. These dyes have zero compatibility towards hydrophilic cellulose fires such as cotton, organza, and cellophane but can be easily applied to fabrics such as polyethylene, terephthalate, and cellulose acetate (Bafana et al. 2011).
3. *Basic dyes*: these hydrophilic dyes require amalgamation with a mordant which forms an insoluble complex with the dye and easily colour onto fabrics like linen, cotton, polyester, and acrylics. They are unsuitable for other fibres as they are not fast to light and easily lose colouration. They mostly undergo an after-treatment process on its surface to prevent the colour from washing off (Bafana et al. 2011).
4. *Direct dyes*: these water-soluble dyes generally have an azo bond and a high atomic weight and are ideal for fabrics where hydrogen bonds are generated during the dyeing process. Direct dyes are used to dye fibres such as wool, nylon, rayon cotton, and silk and can be dyed directly onto the fabric. Direct dyeing is fundamentally natural dyeing that omits the use of medium dyes and binders

Table 5.2 Percentage of unfixed dyes lost in the effluent for various varieties of dyes when applied to different fibres

Fibres	Dye variety	Binding potential (%)	Lost in effluent (%)
Acrylic	Basic	95–100	0–5
Cellulose	Disperse	90–100	0–10
	Pigment	99–100	0–1
	Vat	80–95	5–20
Nylon	Acid	80–90	10–20
	Direct	90–95	5–10
Polyamide	Acid	80–95	5–20
	Direct	70–95	5–30
	Reactive	50–90	10–50
Polyester	Sulphur	60–90	10–40
	Metal complexes	90–98	2–10
Polypropylene	Spun dyed	90–95	5–10
Wool	Reactive	85–93	7–15
	Metal complexes	98–99	1–2
Cotton	Reactive dyes	30–40	60–70

during the dyeing process. Colours made by direct dyeing are not as bright as basic dyes but have improved light resistance and extensive washing (Bafana et al. 2011).

5. *Vat dyes*: these polycyclic hydrophobic compounds are a mixture of indigo, carbazole, and anthraquinone and are used to dye fabrics such as cotton, rayon, silk, linen, wool, and nylon. These dyes have high colour fastness (Bafana et al. 2011).
6. *Reactive dyes*: these dyes generate covalent bonds which lead to very high colouration and fastness and are used to dye cellulosic fabrics, cotton, silk, and fleece. These dyes contain a reactive group that binds directly with the hydroxyl or amino group present in the fabric (Bafana et al. 2011).
7. *Sulphur dyes*: these low-cost dyes are the reason behind deep colouration in fabrics like cotton, rayon, and linen and possess high fastness to light. A major drawback of these dyes is their ability to make the fabric fragile, thereby breaking its structure.

Not all dyes bind to the fabrics during the dyeing process. Table 5.2 describes the percentages of unfixed dyes in various fabrics. Reactive dyes when applied to cotton fabric have the least binding potential (Bafana et al. 2011). A major percentage (52%) of the textile fibre industry consists of cotton dying leading to the vast discharge of coloured effluents in the environment.

5.3 Hazardous Impact of Azo Dyes on the Environment

In India alone, the textile industry releases 42×10^7gallons of water as waste every day. These released wastewaters often go untreated and contain many harmful pollutants such as dyes, metals, phenols, detergents, and hazardous chemicals (Brüschweiler and Merlot 2017). The azo dyes used in textile dyeing are assimilations of latent dyes and other added chemicals which increase the binding affinity of the dye to the fabric (Dutta et al. 2018). The final textile wastewater, when being released into the environment, contains most or no biodegradable mixtures and is usually rich in auxiliary finishers and poses a major challenge for the environment (Kiran et al. 2017). The discharge of such chemical-rich coloured textile wastewater into natural aquatic ecosystems has harmful effects on the flora and fauna of the aquatic ecosystem (Hasanuzzaman 2016). Additionally, the presence of toxic wastewater can further worsen the oxygen content of the water due to the low penetration of sunlight. Furthermore, they would also increase the biological oxygen demand (BOD) of the body of water, leading to a greater lack of oxygen levels (Holkar et al. 2016). Consequently, the aquatic life present in the ecosystem would deteriorate dramatically. In addition, the inclusion of various toxic compounds and equally dangerous by-products brought in by the wastewater also demolishes the aquatic fauna in the water body and the adjacent soil (Kiran et al. 2017).

Although azo bonds present in the dye, when cleaved, lighten the colour of textile wastewater, it should be noted that the mere removal of the colour from the wastewater does not guarantee the cessation of adverse effects of the wastewater (Madhav et al. 2018). Toxic chemical compounds can lead to other problems such as eutrophication. Little or no sunlight available for photosynthesis can subsequently alter or hinder the oxygen transfer mechanism that takes place at the water-air junction, which would affect marine life in the water and impair the water's self-purification mechanism (El-Sheekh et al. 2009). This wastewater, when flowing into the arable land surrounding the aquatic ecosystem, can clog the pores of the soil and reduce productivity of the soil. If the wastewaters are discharged into sewers and rivers, it will affect the quality of the drinking water and make it unsuitable for human use (Periyasamy et al. 2019).

Therefore, azo dyes can be considered as one of the leading causes of environmental toxins, and the release of these colourful heterogeneous compounds has adverse effects on bodies of water, soil fertility, aquatic fauna, and the overall integrity of the ecosystem (Rahman 2016). The use of such water for industrial and recreational facilities is restricted by the presence of rich organic and inorganic coloured chemical compounds and is gradually becoming a global problem, making its treatment a mandatory requirement before its release (Sarkar et al. 2017).

5.4 Discharge Standards

The permissible wastewater discharge limit values for the textile dyeing industry in India are set by the Central Pollution Control Board (CPCB), which also monitors and checks other industrial wastewater discharges (Sarkar et al. 2017). The minimum standards for the textile processing industry by various governing bodies are listed in Table 5.3. Since these are minimum permissible limit values, the state emission control boards are authorized to tighten them according to the environmental damage they cause. Depending upon the disposal method, these standards are defined as continental surface water, on land for irrigation, public sewerage, and marine disposal. The industry receives instructions and is required to treat its wastewater in accordance with the standards set by the CPCB before it is released into the environment.

The wastewater from the textile industry is neutral to strong alkaline due to the use of caustic soda and other detergents, which are used in large quantities in textile dyeing (Ghaly et al. 2014). Biochemical reactions occurring in aquatic organisms are temperature-dependent, and releasing high-temperature wastewater into bodies of water can intensify these chemical reactions (Ali et al. 2019). Furthermore, higher biological oxygen demand (BOD) of the textile wastewater is usually recorded due

Table 5.3 Permissible wastewater discharge limit values for textile dye bath effluents set up by various pollution control bodies in India

	Permissible limits		
Parameters	CPCB	WHO	BIS
pH	6.5–8.5	6.5–8.5	6.5–8.5
Temperature	>5 °C RT	40	–
Electrical conductivity (EC) (µs/cm)	1000	–	600
Biological oxygen demand (BOD) (mg/L)	30	100	100
Chemical oxygen demand COD (mg/L)	250	10	250
Total suspended solids (TSS) (mg/L)	100	100	100
Total dissolved solids (TDS) (mg/L)	2000	2000	2100
Total solids (mg/L)	2200	2100	2200
Hardness (mg/L)	200–500	200	500
Chloride (mg/L)	250–1000	250	600
Turbidity (NTU)	10	–	10
Alkalinity (mg/L)	200–600	–	200–600
Carbon dioxide content (CO_2) (mg/L)	100	–	–
Water stability	Non-corrosive, non-scale forming		
Cadmium (ppm)	1.0	2.0	1.0
Chromium (ppm)	2.0	2	2.0
Lead (ppm)	0.01	0.01	0.1
Arsenic (ppm)	0.1	0.2	0.2

CPCB Central Pollution Control Board, *WHO* World Health Organization, *BIS* Bureau of Indian Standards.

to the usage of rubber, starch, and enzymes in the finishing process of fabrics. Elevated BOD is harmful to aquatic animals such as fish and microorganisms (Jaishankar et al. 2014). Additionally, higher chemical oxygen demand (COD) of the textile wastewater has been observed due to the use of detergents, softeners, impurities, and a high concentration of xenobiotic compounds that are not harmful and are influenced by the microflora (Rasalingam et al. 2014). Increased concentration of total soluble solids (TSS) can be attributed to the release of undissolved solid particles, which are removed from the fabric during the washing process (Rasalingam et al. 2014). A high TSS causes an increase in turbidity and decreases light penetration, which suppresses the photosynthetic activity of phytoplankton and green aquatic flora. Elevated total dissolved solid (TDS) values are due to the use of common salt (NaCl) in the pre-treatment and preparation processes. The number of dissolved solids in the wastewater gives an idea of the severity of the contamination (Noonpui and Thiravetyan 2011). A higher concentration of TDS gives rise to colour, increases the salt content, and is also odour-causing in wastewater (Jaishankar et al. 2014). Chloride in wastewater comes mainly from raw water taken for dyeing. Chlorine-based chemicals act as fixatives for some azo-based dyes. The chlorine content in the wastewater from textile dye baths also increases due to the water softening process, in which sodium chloride is used to refill the softeners. The enhanced chloride content in the water ($>$250 mg/L) gives the water a salty taste and can be harmful to human health if consumed (Samchetshabam et al. 2017). As the concentration of various ions, dyes, and other pollutants increases, the water becomes cloudier and more polluted. An increase in turbidity reduces the rate of photosynthesis, which reduces the lifespan of the water body. The wastewater from the collected textile dye baths is mostly corrosive in nature due to the presence of weak and strong bases such as carbonates and hydrogen carbonates, which are used in dyeing processes (Ghaly et al. 2014).

5.5 Types of Wastewater Treatments

As gathered from the above section, we already know that textile wastewater is a heavy concoction of various hazardous chemicals, salts, surfactants, mordents, and sizing agents. Thus, their treatment becomes mandatory for their safe disposal into the environment. In this context, several technologies are available for effluent treatment. These technologies are broadly classified into physical, chemical, and biological treatment methods.

5.5.1 Physical Treatment Methods

In this phase, physical processes are used to purify the wastewater; processes such as sieving, sedimentation, and skimming are used to remove the solids without the use of chemicals. One of the most important physical wastewater treatment techniques is sedimentation, in which insoluble/heavy particles are suspended from the wastewater (Musa and Idrus 2021). As soon as the insoluble material settles on the bottom, the pure water can be separated. Another effective physical water treatment technique is ventilation. This process consists of circulating air through the water to provide oxygen. Filtration, the third method, is used to filter out any contaminants. A special type of filter is used to pass the wastewater and separate the pollutants and insoluble particles it contains (Sharma and Bhattacharya 2017). The sand filter is the most widely used filter (Sharma and Bhattacharya 2017). Some of the primary physical treatment methods are:

1. *Screening*: This mandatory first step involves the removal of large floating and non-biodegradable constituents such as papers, plastics, containers, wood, and rags. Proficient removal of these solids protects the downstream equipment and parts of the treatment plant from any possible blockage and damage (Siddique et al. 2017).
2. *Grit chambers*: These are large sedimentation basin situated at the front of a wastewater treatment plant and is used to separate sand, eggshells, and other non-putrescible materials that may lead to clogging or abrupt wear and tear of pumps in the treatment plant (Wang et al. 2011).
3. *Flotation*: Flotation eradicates dispersed or suspended substances from wastewater, using very fine gas bubbles, which pass the substances to the surface and are subsequently separated using a cleaning device. For textile wastewaters, flotation processes are used to separate oils, fats, and finely suspended solids and particles. The smaller the micro-bubbles, the better the deposit of particles or droplet function. Nowadays, effluent technologies have advanced to dissolved air flotation, a method proven to be economically efficient (Wang et al. 2011).
4. *Sedimentation*: sedimentation employs gravity to separate solid particles in sedimentation basins. Sludge is accumulated at the bottom of the tank and is episodically removed. The conversion reactions decompose and putrefy the resulting sludge to generate digester gases, which in their split form, such as biogas, are converted into electricity and can be employed to meet the increasing global energy demand (Gupta et al. 2016).
5. *Equalization*: Certain industrial wastewater releases undesirable waste in a short period of time. Discharging such highly polluted toxicants into adjoining water bodies can cause further damage; thus, these wastewaters are blended with other wastewater and are gradually released into the ecosystem, thereby avoiding immediate shocks to the ecosystem and the treatment plant. This process is known as equalization (Gupta et al. 2016).

5.5.2 Chemical Treatment Methods

This treatment involves using chemicals to treat wastewaters. Chlorine is the most used oxidizing chemical that eradicates bacteria. Another commonly used oxidizing agent is ozone. Techniques such as neutralization employ the addition of either acids or bases to the wastewater binging it to its natural pH of 7 (Sharma and Bhattacharya 2017).

1. *Neutralization*: The neutralization process adjusts the pH of the generated wastewater before its release into the ecosystem. The wastewater is mixed with either acids or alkali into the effluent as per the requirement after physical treatment methods like precipitation and flocculation to neutralize the industrial effluent (Holkar et al. 2016).
2. *Adsorption and chemisorption*: Adsorption refers to the accumulation of substances on the surface of a solid. It is a physical process wherein the molecules are attached to the interface of the surface by van der Waals forces (Holkar et al. 2016). Chemisorption, on the other hand, is a chemical reaction occurring between the adsorbate and the adsorbent, resulting in the formation of new chemical bonds between the two. In contrast to adsorption, chemisorption is not a reversible process (Gupta et al. 2016). For wastewater treatment, adsorbents such as activated carbon are used to bind soluble compounds which cannot be adequately removed either by physical or biological treatment methods such as precipitation, microbial bioremediation, and flocculation (Gupta et al. 2016). Adsorbents doped with activated carbon can be used to remove arsenic and other heavy metals. Granulated iron hydroxide is another ideal candidate for the removal of toxic metalloids present in textile wastewater (Gupta et al. 2016).
3. *Precipitation*: During precipitation, soluble substances are separated from liquids using suitable measures. Heavy metals, for example, are converted into insoluble metal hydroxides, carbonates, or sulphur-containing compounds. Anions are often precipitated as calcium, iron, and aluminium salts (Yaseen and Scholz 2019). Fluoride ion separation can also be achieved by precipitation with milk of lime. For the efficient treatment of textile wastewater, the addition of salts such as iron (II) sulphate, iron (II) chloride, and aluminium chloride reduces the phosphate concentration in the wastewater (Bunce et al. 2018). Phosphate precipitation can be integrated into the biological cleaning process at the same time or used as a separate treatment method (Bunce et al. 2018; Yaseen and Scholz 2019).
4. *Flocculation*: The suspended or colloidal particles in the wastewater are converted into very fine particles by flocculation (Jan et al. 2015). Appropriate chemicals and flocculants help agglomerate these particles and create macro flakes that can easily settle out (Brandt et al. 2017). Furthermore, flocculation also improves the sedimentation properties from the wastewater and discharges the resulting sludge. Iron and aluminium salts are often used in flocculation (Ratnayaka et al. 2009; Siddique et al. 2017).
5. *Ion exchange*: These chemical compounds swap ions from one solution into another. In cation exchange processes, for example, calcium ions are exchanged for sodium ions. As soon as the ion exchanger is exhausted, it must be regenerated (Jaishankar et al. 2014). The ion exchange process is fundamentally based on the principle of displacement; the higher the ion charge, the stronger the bond

between the ions and the exchange matrix (Madhu and Chakraborty 2017). Ion exchangers, which are suitable for removing heavy metals and anions in textile wastewater, are often used as monitoring filters after the precipitation and flocculation processes. In addition, they promote the softening of the water, thus changing the salt content in the water and desalinating it (Bhatia et al. 2017).

6. *Electrochemical processes*: The electrochemical processes are comparatively a newer approach to treat wastewaters and were introduced in the mid-1990s (Chen 2004). The USP of this method is non-consumption of chemicals and the formation of zero sludge during the treatment (Chen 2004). Furthermore, non-toxic metabolites are generated by this process. Dyes rich in recalcitrant chemical compounds can be competently treated by electrochemical processes (Chen and Hung 2007).
7. *Advanced oxidation processes*: Advanced oxidation processes (AOP) involve the in situ production of highly reactive free radicals such as hydroxyl radicals (· OH), hydrogen peroxide (H_2O_2), ozone (O_3), and oxygen radicle (O_2·), which can easily oxidize intricate organic compounds present in the textile wastewater (Deng and Zhao 2015). Unlike their counterparts, hydroxyl radicals are nonselective and can readily attack organic compounds, formulating them into simpler and less toxic intermediates (Ameta et al. 2018). A major advantage of advanced oxidation processes is their "green" and "eco-friendly" property, as they do not transfer pollutants from one phase to another or generate copious amounts of toxic or dangerous sludge (Ikehata et al. 2008). Different combinations of heterogeneous and homogeneous methods used in AOPs are:

   - *Photochemical irradiation*: Photocatalysis is an emerging technology that combines semiconductors, light, and/or oxidants for the degradation of organic compounds into water and carbon dioxide molecules. Over the last few decades, photocatalysis using titania (TiO_2) has become an immensely popular AOP approach for the treatment of textile effluent without the addition of any toxic chemicals (Ikehata and Li 2018).
   - *Fenton and photocatalytic processes*: Among AOPs, photo-Fenton processes have been extensively investigated due to the possibility of using a renewable energy source (i.e., solar energy) and a low concentration catalyst that can be readily employed to treat textile wastewater (Ameta et al. 2018).
   - *Ozonation*: Ozonation is employed mainly for the removal of complex and intricate toxic chemicals present in textile water. Ozonation readily degrades the organic and inorganic components present in textile wastewater into simpler non-toxic compounds (Deng and Zhao 2015).

5.5.3 Biological Treatment Methods

Biological processes are commonly the most preferred option for treating textile wastewater because they have a low environmental impact and are also inexpensive as compared to other treatment methods (Yerramilli et al. 2005). Biological treatments require little or no addition of chemicals and use a fair amount of energy. Biological treatment fundamentally exploits the microorganisms' capability to biotransform toxic and hazardous chemical complexes (mainly azo dyes) and then further use it as a source for growth (Behl et al. 2019). Owing to properties like increased stability and complexity of chromophores, azo dyes are difficult to break down (Sinha et al. 2016). Several studies over the past two decades have confirmed the partial or complete biodegradation of dyes by microorganisms (Behl et al. 2020; Daneshvar et al. 2018a; Sinha et al. 2016). Researchers have identified several types of microorganisms that not only degrade azo dyes easily but can also effectively treat textile wastewater at the same time. These microbes can "adapt" or acclimate to toxic environments and naturally become resistant strains over time. The presence of enzymes such as azo reductase, oxidases, and dismutase in these microorganisms is critical in converting a wide variety of toxic compounds into less harmful configurations (Rao 2006). The challenge is to identify promising microorganisms that are equipped with such enzymes that easily break down azo dyes and other pollutants present in wastewater, use organic and inorganic pollutants for their growth, and, in return, remediate these sites (Vikrant et al. 2018). Many microorganisms have been identified, including bacteria, fungi, yeast, actinomycetes, and algae, which, under suitable environmental conditions, can discolour various azo dyes and even completely mineralize them (Hirooka et al. 2003; Roy et al. 2018).

5.5.3.1 Wastewater Treatment Using Fungi

Fungi are eukaryotic heterotrophic microorganisms that produce various proteins, enzymes, and metabolites such as carotene, lovastatin, lactic acid, and protease (Adrio and Demain 2010; Deshmukh et al. 2016). Bioremediation using fungi is an attractive alternative, as fungi readily adapt to various carbon and nitrogen sources (essential for growth) and can completely decolourize and mineralize dyes (Kaushik and Malik 2008; Agrawal and Verma 2020). Different types of fungi that have the potential to decolourize dyes are described in Table 5.4**.**

5.5.3.2 Wastewater Treatment Using Bacteria

Bacterial decolourization is a relatively faster method of microbial treatment than fungal decolourization (Khan et al. 2012). While most fungal species decolourize dyes after a few days of incubation, bacterial decolourization occurs within hours (Khan et al. 2012). Azo dyes are carcinogenic in nature due to the reductive bioconversion of the azo bonds into aromatic amines, which are also toxic to flora and fauna and require further oxidation. In addition, decolourization by bacteria can be easily manipulated, as several bacterial species can easily adapt to changes in pH, temperature, and nutrient availability (Chen 2006; Franciscon et al. 2012). Furthermore, the enzymes present in bacteria undergo a conversion reaction in which the

Table 5.4 Textile dye decolourization by various species

Microorganism	Dye	Dye concentration (mg L^{-1})	Decolourization (%)	Incubation period	References
Fungus					
Acremonium kiliense	Malachite green	5	95.4	72 h	Youssef et al. (2008)
Aspergillus Niger	Reactive red 120	40	74.2	4 d	Ibrahim et al. (2018)
Coriolus versicolor	Acid orange II	10	55	1 d	Srikanlayanukul et al. (2008)
Funalia trogii	Reactive blue 19	100	96.3	8 h	Fazli et al. (2009)
Irpex lacteus	Reactive orange 16	150	85.8	2 d	Svobodová et al. (2007)
Lentinus polychrous	Indigo carmine	20	97.6	3 h	Sarnthima et al. (2009)
Penicillium ochrochloron	Cotton blue	50	93	2.5 h	Lekhak et al. (2008)
Pycnoporus sanguineus	Trypan blue	20	70	24 h	Annuar et al. (2009)
Thelephora sp.	Orange G	50 μM	33.3	9 d	Kuppusamy et al. (2003)
	Crystal violet	5	37	7 d	Kuppusamy et al. (2003)
Bacteria					
Bacillus sp. *VUS*	Navy blue 2GL	50	94	48 h	Dawkar et al. (2009)
	Crystal violet	5 μM	100	1 h	Ayed et al. (2009)
Enterobacter cloacae	Reactive black 5	1000	35.63	120 h	Wang et al. (2009)
Yersinia sp.	Reactive red 195	30	87	48 h	Jirasripongpun et al. (2007)
Serratia sp.	Reactive red 195	30	46	48 h	Jirasripongpun et al. (2007)
Enterococcus gallinarum	Direct black 38	22–250	71–85	24 h	Soni et al. (2015)
Kocuria rosea	Malachite green	50	100	5 h	Parshetti et al. (2006)
Micrococcus glutamicus	Scarlet red	150	100	36 h	Saratale et al. (2010)
Agrobacterium radiobacter	Crystal violet	10	100	8 h	Parshetti et al. (2011)
Proteus vulgaris	Scarlet red	150	100	30 h	Saratale et al. (2009)
Pseudomonas sp. *SUK1*	Reactive red 2	1000	95	18 h	Saratale et al. (2009)
Shewanella decolorationis	Crystal violet	50	100	28 h	Li et al. (2014)

Algae					
Nostoc linckia	Crystal violet	200	72	1.5 h	Mona et al. (2011)
Spirogyra rhizopus	Acid red 247	100	100	100 min	Özer et al. (2006)
Cosmarium sp.	Malachite green	10	87.2	7 h	Daneshvar et al. (2007)
Synechocystis sp.	Reactive red 11	100	55	7 d	Liang et al. (2018)
Cystoseira barbatala	Methylene blue	10	90	6 h	Ozudogru et al. (2017)
Pithophora sp.	Malachite green	30	89	3 h	Kumar et al. (2006)
Chroococcus minutus	Amido black 10B	100	55	26 d	Parikh and Madamwar (2005)
Spirulina platensis	Reactive red 120	100	99		Devi et al. (2015)
	Reactive blue 19	100	85	330 min	Devi et al. (2015)
Chlorella pyrenoidosa	Methylene blue	50	90	145 min	Lebron et al. (2018)
	Direct red 31	40	100	186 min	Sinha et al. (2016)
Chlorella sp.	Methylene blue	10	99.9	4 h	El-Sheekh et al. (2009)
	Methylene orange	200	70	10 d	Totiya and Sibi (2020)
Scenedesmus quadricauda	Remazol brilliant blue R	46	75	6 h	Kulkarni et al. (2018)
Spirogyra	Synozol reactive	46	85	21 h	Khalaf (2008)
	Reactive yellow 22	25	92	6 d	Totiya and Sibi (2020)
Chlorella vulgaris	Tectilion yellow 2G	400	63–69	14 d	Acuner and Dilek (2004)
Desmodesmus sp.	Methylene blue	20	98	6 d	Al-fawwaz and Abdullah (2016)
	Malachite green	20	98	8 d	Al-fawwaz and Abdullah (2016)
Desmodesmus armatus KB-1	Direct red 31	50	100	6 d	Behl et al. (2019)
Chlamydomonas sp. *TRC-1*	Violet coloured effluent	100	100	7 d	Behl et al. (2020)

azo bonds are reductively cleaved, resulting in the production of colourless, non-toxic, and oxidized amines (Chen 2006). Table 5.4 illustrates different types of bacterial species that are involved in dye removal.

5.5.3.3 Wastewater Treatment Using Algae

Algae are photosynthetic organisms that are inhabitants of both marine and freshwater systems. Their photosynthetic mechanism is like that of terrestrial plants, but microalgae are generally more efficient at converting solar energy into biomass (Benedetti et al. 2018; Parlevliet and Moheimani 2014). They are the source of oxygen which is considered as a primary step in the food chain in aquatic systems. Algae have fostered a wide variety of polymers that can easily remove the metals of interest, even when they are present in the environment in very low concentrations (Sambusiti et al. 2015). It is this ability of microalgae to absorb toxic metals that make them a perfect candidate for bioremediation. Algae can efficiently use excess nitrogen, phosphorus, and other metals present in industrial wastewater for their growth and generate sufficient biomass production (Benedetti et al. 2018; Mehariya et al. 2021a; Goswami et al. 2021a). Since microalgae are photosynthetic in nature, they do not require an additional source of carbon unlike other counterparts such as bacteria, fungi, and yeast, which require an initial nutrient input as an energy source (Monteiro et al. 2012). Several species of macro and microalgae have been identified that can efficiently degrade a variety of colourants (Christenson and Sims 2011) (Table 5.4). Most types of microalgae break down azo dyes by degrading azo bonds and producing simpler aromatic amines and other colourless intermediates (Francisco et al. 2010). These aromatic amines are naturally highly toxic and are further broken down by microalgae into simpler, non-toxic compounds, carbon dioxide and water (Christenson 2011).

In the last few years, the performance of several species of microalgae has been evaluated for their capacity to eliminate pollutants from textile dyeing wastewaters (Yaseen and Scholz 2019). The most normally utilized species that have shown maximum potential in remediating textile wastewater are *Chlorella*, *Chlamydomonas*, *Desmodesmus*, *Oscillatoria*, *Scenedesmus*, and *Spirulina* (Fazal et al. 2018). Studies conducted on *Chlorella* sp. developed in various colour wastewaters have demonstrated variable decolourization efficiencies, ranging from 72% to 90% (Ayele et al. 2021). Likewise, *Scenedesmus quadricauda* and *Cosmarium* sp. have demonstrated efficient removal for Remazol Brilliant Blue R and malachite green, individually (Ahmad et al. 2015; Daneshvar et al. 2007). Therefore, many microalgal strains can be utilized to treat textile wastewater containing a wide variety of colours. However, the constituents and their fixations of wastewater can antagonistically influence the cell growth. High centralizations of micronutrients (Cu, Zn, and so on) and the presence of complex natural chemicals can hamper with the biomass productivity of certain species. The presence of even small quantities of dyes in the wastewater can restrict the penetration of light and prevent photoautotrophic growth (Premaratne et al. 2021).

Bioremediation potential also depends upon other factors such as environmental conditions and the type of microalgal species employed (El-Kassas and Mohamed 2014). Several studies have been successful in treating textile wastewater because the isolated species were already well acclimatized to the harsh environmental conditions and could efficiently proliferate (Behl et al. 2020). Additionally, genetic and metabolic engineering can also be employed for the development of microalgal strains for improved biodegradation of dyes (Fayyaz et al. 2020).

Most species of *Chlorella* have exhibited the efficient potential to bioremediate textile effluent because of their robust nature and the potential to multiply under brutal ecological conditions (Lim et al. 2010; Wang et al. 2016). Among the genus, *Chlorella vulgaris* has been widely used for the treatment of textile effluent majorly consisting of pink coloured direct red 31 dye. The study demonstrated that *C. vulgaris* was effective in accomplishing significant removal efficiency of chemical oxygen demand (87.0%). Furthermore, the resilience of the *C. vulgaris* to colour and low pH conditions was significant in the improvement of this exceptionally effective process (Sinha et al. 2016). In another study carried out, *C. vulgaris* could effectively remove 83% of Congo red dye after 96 h of incubation (Hernández-Zamora et al. 2015).

The efficiency of bioremediation is also dependent upon the high complexity of organic and inorganic components of the textile effluent which are difficult to break down (Jamee and Siddique 2019). A potential alternative procedure to moderate this is to weaken textile effluent and subsequently diminish the concentration of these contaminants which might decrease the efficiency of bioremediation. Certain textile dyes and substantial metals can hinder the development of microalgae if present in high quantities (Singh and Arora 2011).

The utilization of immobilized microalgae is likewise an expected strategy to successfully treat textile effluents, as exhibited in various investigations in the literature (Abdel-Raouf et al. 2012; Premaratne et al. 2021). Immobilization of microalgae would be beneficial as far as improving nutrient availability and cost-effectiveness of the remediation process is concerned. *Chlorella* sp. immobilized in sodium alginate showed higher biomass development and efficient reduction in COD (75%) and ammonium (90%) levels in the textile effluent with 60% dye removal efficiency (Premaratne et al. 2021). This could be credited to the adsorption of supplements to the immobilized globules because of the ionic interactions between immobilization lattices and nutrients. In a separate study, immobilized *S. quadricauda* has proved effective in the total decolourization of indigo blue dye. The best removal was accomplished at the most reduced biomass concentration (0.1 g L^{-1}), which was ascribed to the availability of an increased surface area for absorption per algal cell unit (Chia et al. 2014). Immobilized *C. pyrenoidosa* alginate beads were additionally utilized in a study by Sinha et al. (2016), wherein complete decolourization of DR-31 dye was observed (Sinha et al. 2016). The development of immobilized algal-based matrices is a potential answer for the high energy prerequisite and cost-efficacy of microalgae cultivation.

One more forthcoming methodology to upgrade the microalgae-based treatment of textile effluent is to use microalgae-bacteria consortia, which might be valuable in diminishing the pollution chance and speeding up the treatment interaction (González-González and de Bashan 2021; Lim et al. 2010). It has been accounted for that some photosynthetic microscopic organisms are likewise equipped to removing colour from wastewater (Katheresan et al. 2018). In this way, a microalgae photosynthetic microorganism consortium can upgrade the treatment of textile effluent. Be that as it may, an extra examination is needed to research the possibility of microalgae-microbes consortia for the treatment of textile effluent (González-González and de Bashan 2021).

5.5.3.3.1 Mechanism of Bioremediation by Microalgae

Various mechanisms of bioremediation are known, such as biosorption, bioaccumulation, biotransformation, biomineralization, bioleaching, and microbe-metal interactions (Dixit et al. 2015).

5.5.3.3.1.1 Biosorption

Biosorption utilizes biological materials to remove contaminants through various mechanisms such as absorption, adsorption, ion exchange, and precipitation. Figure 5.2 demonstrates various mechanisms of biosorption employed by microalgae in bioremediation. It is dependent on various factors such as environmental conditions, biosorbent, constituents to be biosorbed, and metabolic processes. Both adsorption and absorption are included in biosorption. The pollutants to be removed by biosorption can either be organic, inorganic, soluble, or insoluble compounds (González et al. 2011). Furthermore, highly mobile metal ions of magnesium and potassium can also be easily eliminated by biosorption. This process removes heavy metals, phenolic compounds, and pesticides, besides a variety of

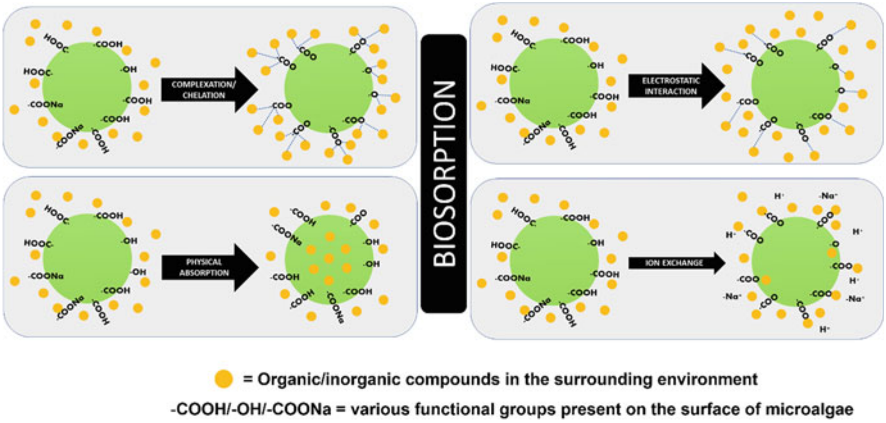

Fig. 5.2 A schematic representation of the various mechanisms of biosorption followed by microalgae during bioremediation

textile dyes (Dixit et al. 2015; Goswami et al. 2021b). Biosorption by microalgae has several advantages and disadvantages. It is an environmentally friendly and inexpensive method of removing pollutants. Moreover, algae have the potential to effectively remove pollutants even in low concentrations and do not lead to the production of toxic sludge after bioremediation (Nigam et al. 2018). Microalgae also offer suitable opportunities for biosorption of metals so that they can be recovered efficiently. This biological biosorbent can be recycled again and have shown excellent removal efficiency and can also be used in situ (González et al. 2011). However, there are certain drawbacks. The biomass of dead algae raises concerns about its toxicity at the site of biological remediation (Kaur and Bhatnagar 2002). Since biosorption occurs in living microorganisms, there is no influence over the biological characteristics of these biosorbents, and an early saturation (of biosorbents) is observed (Ummalyma et al. 2018).

5.5.3.3.1.2 Bioaccumulation

As already mentioned, absorption is a two-step process: the primary step, i.e., biosorption, is a standard reversible physiochemical process where in a sorbate is bound to the sorbent of biological origin (Nigam et al. 2018). The second phase, i.e., bioaccumulation, deals with the removal of ions bound to the cell surface in the first passive stage, which is identical to biosorption. These ions are then carried into the cell usually by an active transport system requiring additional cell energy (Fig. 5.3). A variety of sewage and other industrial effluents can be easily treated through biosorption and bioaccumulation (Sulaymon 2014).

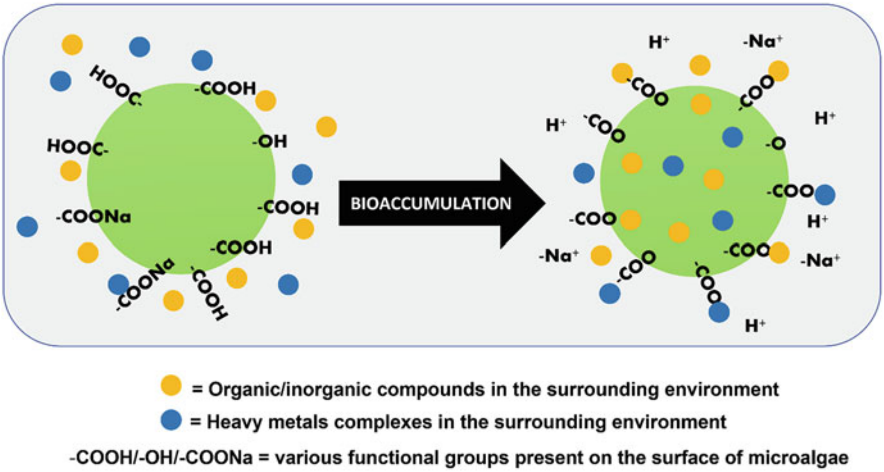

Fig. 5.3 A schematic representation of bioaccumulation mechanism followed by microalgae during bioremediation

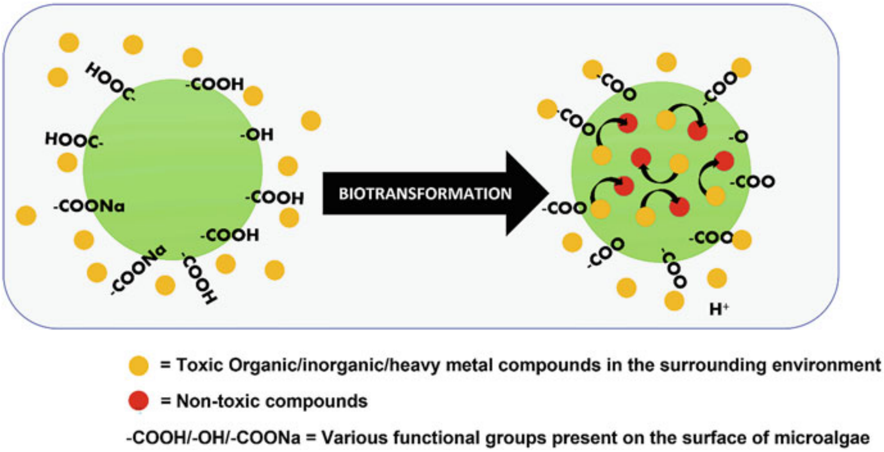

Fig. 5.4 A schematic representation of biotransformation mechanism followed by microalgae during bioremediation

5.5.3.3.1.3 Biotransformation

The role of microorganisms in the biotransformation of heavy metals into non-toxic forms is a well-understood concept for bioremediation of metal-contaminated sites (Nigam et al. 2018) (Fig. 5.4). These methods use natural microbial catabolic diversity to break down, convert, or enrich a wide variety of compounds, including hydrocarbons (oils), polyaromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and metals (Dixit et al. 2015). Algae are important components of the aquatic freshwater environment and can potentially remediate arsenic-contaminated water in wetlands through adsorption and biotransformation of inorganic arsenic (Mitra et al. 2017).

5.5.3.3.2 By-Products Generated by Microalgal Biomass After Bioremediation

In addition to the potential for bioremediation, microalgae biomass can generate several value-added compounds such as lipids, carbohydrates, proteins, vitamins, and antioxidants as well as other bioactive compounds and proteins (Bhalamurugan et al. 2018; Kothari et al. 2017; Mehariya et al. 2021b) (detailed in Fig. 5.5). However, the primary metabolites secreted by microalgae are essential to the physical integrity of the cell as they are used for the development and maintenance of the cell. The secondary metabolites, on the other hand, are essential for cell survival (Madhav et al. 2018). These metabolites (both primary and secondary) find commercial applications in the manufacturing of biofuels, nutritional supplements, cosmetics, and the pharmaceutical field (Khan et al. 2018; Goswami et al.

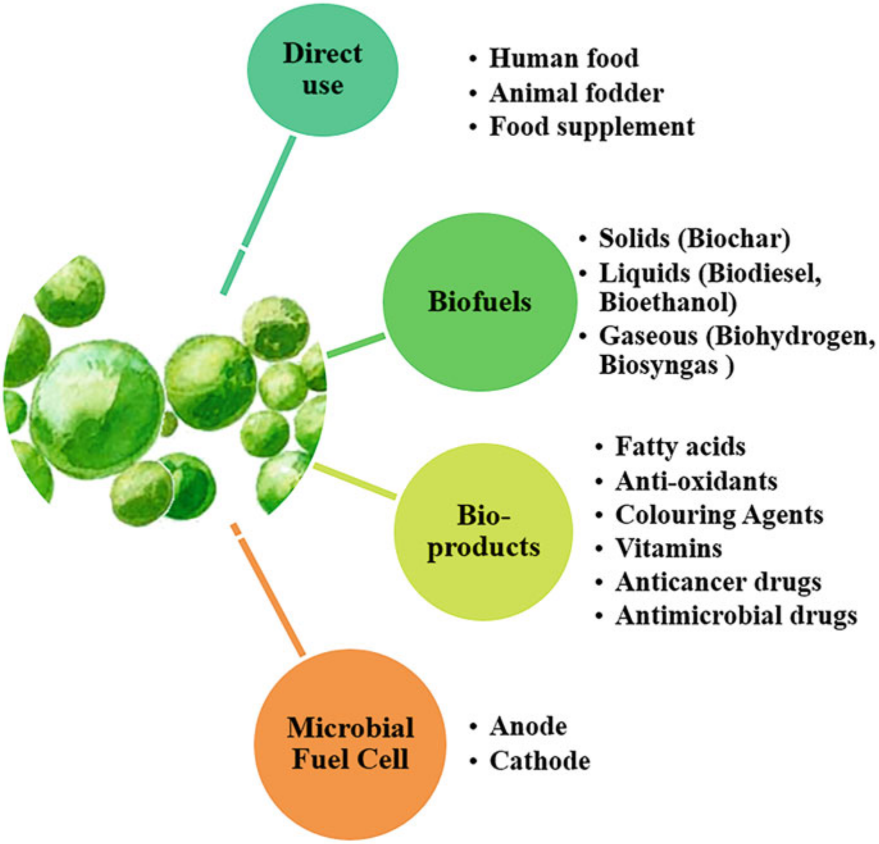

Fig. 5.5 Microalgae can convert atmospheric CO_2 into carbohydrates, lipids, and other valuable bioproducts using light. Microalgae biomasses are potentially rich sources for biofuels and bioactive compounds

2021c). Algae are being studied as a raw material for renewable fuels such as biohydrogen, bioethanol, and biodiesel (Wang and Yin 2018). Currently, bioethanol and biodiesel are the main biofuels available, most of which are derived from food crops, making food supplies difficult (Hood 2016). Biodiesel derived from microalgae appears to be the only renewable biofuel source that replaces petroleum-derived fuels without affecting food supplies (Chisti 2008; Islam et al. 2013). Algae-based biofuels and petroleum-based fuels have similar chemical and physical properties (Chisti 2007). Biodiesel from microalgae, obtained by transesterification, rich in C16 and C18 fatty acids, can be used as an alternative to clean and sustainable energy (Behl et al. 2020; Khanra et al. 2018; Rajkumar et al. 2014). The non-lipid fraction of microalgae, which mainly consists of proteins and carbohydrates, can also be administered to methane and ethanolic fuels (Rodionova et al. 2017). In addition to the production of primary metabolites (carbohydrates, proteins, and lipids), microalgae are also rich in the secretion of new biologically active secondary metabolites that are used in the pharmaceutical and nutraceutical sectors (Holkar et al. 2016).

5.5.3.3.3 Potential Role of Biofuels Produced from Microalgae

As of late, the liquid biofuels utilization sector has shown rapid development globally and is driven by approaches focussed on the accomplishment of energy security and release of greenhouse gasses (Brennan and Owende 2010b). First-generation biofuels have been for the most part extricated from food and oil crops such as rapeseed oil, sugarcane, sugar beet, and maize (Khan et al. 2018). However their impact to meet the general energy requirement is restricted because of limited availability of food and feed stocks, utilization of arable land, absence of standard agricultural practices, high water, manure prerequisites, and a requirement for protection of bio-variety (Khan et al. 2018). This has also brought up relevant issues on their capability to replace petroleum derivatives and the supportability of their generation. At present, about 1% (14 million hectares) of the world's accessible arable land is utilized for the creation of biofuels, giving 1% of worldwide vehicle powers (Moore 2008). In other words, expanding that to approach 100% is unreasonable and can have a serious effect on the world's food supply as well. The switch to the second generation of biofuels, also known as advanced biofuels, utilizes manufacturing opportunities from various types of non-food plant materials and animal waste used especially as a source of fuel. These second-generation biofuels provide even greater advantages in terms of environmental performance, restored overall energy efficiency, widely available feedstocks, and easy integration into the existing fuel supply and distribution system. However, they are not suitable for producing biodiesel. Second-generation biofuels require extensive processing to generate ethanol. It may take several years for switch grass to reach harvest density. Furthermore, this conversion technology has still not been used to its maximum potential scales for commercial exploitation and development (Khan et al. 2018).

The fuel of the third generation is the most energy-intensive since it surpasses the raw materials of previous generations by several times in energy output. The raw materials for this fuel are algae and photosynthetic microorganisms. Unlike fuels of the first generation, the second and third generation have a very complex production process, since the second requires a large number of chemical transformations, and, accordingly, this process carries large energy costs, and the raw materials for the third-generation algae and other microorganisms require a very complex structure, storage, and content.

Third-generation biofuels are energy-efficient, as they can easily outperform the raw materials required by their predecessors by a few times in energy yield. Raw materials required for third-generation biofuels are photosynthetic microorganisms. Microalgae could meet these conditions and therefore make vital contributions to meet the global energy demand while at the same time giving natural advantages. Microalgal-derived biofuels have the following advantages:

- Microalgae can be cultivated throughout the year, therefore generating larger oil yields than that of best oil seed crops. For example, microalgae generate a biodiesel yield of 12,000 L/ha as compared to 1190 L/hectare generated by rapeseed (Schenk et al. 2008).
- Microalgae need less than water than other crop harvests for their growth and thus reduce the load on freshwater sources (Dismukes et al. 2008).
- Microalgae can be grown efficiently on non-arable land using saline-based water, restricting the associated environmental challenges (Searchinger et al. 2008).
- Microalgae have fast growth potential, and numerous microalgal species have oil content ranging from 20 to 50% of the dry load of biomass. Certain species have exponential growth rates that can double up the biomass in periods as short as 3.5 h (Chisti 2008);
- Microalgal biomass also helps to bio-fixate waste carbon dioxide thus improving the air quality (1 kg of dry algal biomass can utilize about 1.83 kg of CO_2) (Chisti 2008).
- Microalgae can efficiently utilize organic and inorganic contaminants (particularly nitrogen and phosphorous) present in wastewater for their growth, providing a simultaneous solution towards bioremediation and biofuel production (Mohsenpour et al. 2021).
- Microalgal growth, development, and cultivation do not require the use of pesticide or any harmful chemicals (Mohsenpour et al. 2021).
- Microalgae can also produce other essential compounds like proteins, and the remaining biomass after oil extraction can be utilized as feed or compost or fermented to generate ethanol or methane (Maurya et al. 2016);
- The biochemical composition of the algal biomass can be modified by altering growth conditions to maximize the oil yield (Alishah Aratboni et al. 2019).

  Despite its exceptionally high potential as a biofuel precursor, numerous challenges restrict the advancement of algal biofuel innovation to commercial scalability and feasibility (Alishah Aratboni et al. 2019). These drawbacks include:

- Careful determination of species to balance prerequisites for biofuel generation and extraction of important value-added by-products (Khan et al. 2018).
- Accomplishing higher photosynthetic efficiencies through the development of production systems (Hannon et al. 2010).
- Developing and improving strategies for single-species growth and CO_2 dissemination losses (Qari et al. 2017).
- Potential for negative energy balance that accounts for water pumping, harvesting, and extraction (Hannon et al. 2010).
- Scarcity of commercial cultivation plants due to lack of prerequisite information (Qari et al. 2017).

5.5.3.4 Biofuel Productivity Factors

Major factors that play a pivotal role inefficient biofuel production and its economic viability include biomass productivity (selection of potential strains, photosynthetic efficiency, and lipid productivity) and harvesting costs (Borowitzka 1997; Brennan and Owende 2010a). Photosynthetic productivity is more significant for autotrophic algal species; for heterotrophs, the use of sugars is more significant.

5.5.3.4.1 Strain Selection

The determination of suitable algal strains is pivotal for the success of biofuel production from microalgae (Behl et al. 2020). Ideal algal strains for efficient biofuel production should (Behl et al. 2019):

1. Be robust and potentially be able to tolerate or easily adapt to high levels of stress.
2. Possess high biomass productivity.
3. Have a high lipid production rate.
4. Potentially dominate over wild strains in open cultivation systems.
5. Utilize minimal nutrients for its growth.
6. Potentially survive temperature changes.
7. Efficiently secrete a variety of value-added by-products.
8. Have high photosynthetic efficiency.
9. Demonstrate self-flocculation attribute.

At present, no known algal strain is equipped to meet all these necessities simultaneously. Research conducted by De Morais and Costa (2007) found that green microalgae (*Scenedesmus obliquus* and *Chlorella kessleri*) isolated from effluent treatment sites close to a power plant had the potential for fixation of CO_2, even though biomass productivities were lower as compared to closed system photobioreactors (Morais and Costa 2007). In another study conducted by Yoo et al. (2010), three microalgal species (*Botryococcus braunii*, *Chlorella vulgaris*, and *Scenedesmus* sp.) were cultivated under high CO_2 levels, and it was inferred that functional specificity was a significant factor in species determination; *B. braunii* was found to be the most apt candidate for biodiesel production, whereas *Scenedesmus* sp. was found to be appropriate for CO_2 moderation (Yoo et al. 2010). The isolation of autochthonous strains for biofuel production should be considered as an essential parameter, although these strains may not be the ideal for the production of lipids, and hence a need for genetic engineering may be required to manipulate lipid levels (Alishah Aratboni et al. 2019).

5.5.3.4.2 Photosynthetic Efficiency

Photosynthetic efficiency (PE) is the negligible part of light energy that is fixed as compound energy during photoautotrophic growth (Alishah Aratboni et al. 2019). Only the photosynthetic active radiation (PAR), i.e., wavelengths in the range of 400–700 nm, that address 42.3% of energy from the light range is caught (Brennan and Owende 2010b). This captured energy is utilized in the Calvin cycle to generate carbohydrates by using water molecules and carbon dioxide. This reaction can be demonstrated as:

$$6\ CO_2 + 12\ H_2O + \text{photons} = C_6H_{12}O_6 + 6\ O_2 + 6\ H_2$$

At least eight light photons (quanta) are needed to produce one mole of base sugar (CH_2O), one O_2 particle, and one H_2 moiety (Vasudevan and Briggs 2008). The average energy content of solitary quanta is approximately 218 kJ per mol; hence, the total potential light energy caught by photosynthesis is 1744 kJ per mol of CH_2O. Considering that the energy contained in one mole of CH_2O is around 467 kJ (1/6 of the energy content of glucose), the proficiency of sunlight to chemical energy conversion is roughly 27%. But since only PAR (42.3%) is utilized during photosynthesis, maximum PE is assessed at 11.3% (Bolton and Hall 1991).

Due to such affecting variables, most terrestrial plants accomplish PE levels commonly between 1% and 2% (Vasudevan and Briggs 2008). Algal species, on the other hand, owing to their simple cell structure, demonstrate significantly higher PE values than those terrestrial plants. Studies conducted by Doucha and Lívanský and Hase et al. on *Chlorella* sp. recorded PAR-based PE values to be 7.05%, 6.48%, and 6.56%, individually (Doucha and Lívanský 2006; Hase et al. 2000). *Synechococcus* sp. was found to have a PE of somewhere in the range of 2% and 4%, while *Chlorella sorokiniana* with a PE of 8.66% and *Chlorophyta* sp. with a PE of 4.15% demonstrated essentially higher qualities for microalgae as compared to terrestrial plants (Hase et al. 2000; Wu et al. 2008). Different investigations have proposed that significantly more elevated levels of PE can easily be achieved by microalgae (Masojídek et al. 2021). For instance, Hall et al. and Acie'n Ferna'ndez et al. recorded PE values of 15% and 21.6% for the microalga *Phaeodactylum tricornutum*, individually (Fernández et al. 1998; Hall et al. 2003). Other researchers have reported 20% PE for *Chlorella* and 19% PE for *Tetraselmis suecica* (Laws et al. 1986). In general, the illustrated evidence proposes that microalgae are an effective biomass asset for biofuel creation (Minowa et al. 1995).

5.5.3.4.3 Lipid Productivity

While numerous microalgae strains normally have high lipid content (20–50% dry weight), it is feasible to expand the lipid concentration by optimizing growth determining factors (Brennan and Owende 2010a; Hannon et al. 2010). Factors such as nitrogen level, light force [26,110], temperature, saltiness, CO_2 fixation, and harvesting methodology can be altered to enhance lipid accumulation (Zhu et al. 2016). However, enhancing the lipid accumulation does not necessarily result in an increment in lipid productivity as biomass efficiency and lipid accumulation are not related (Zhu et al. 2016). Lipid accumulation is the convergence of lipids inside the microalgae cells without considering the overall biomass production. Lipid productivity, on the other hand, considers both the lipid concentration inside cells and the biomass generated by these cells and is a more valuable indicator of the possible expenses of biofuel production. Studies focussed on the segregation of high lipid content in microalgae have demonstrated that they can be successfully developed in huge open pond cultivation systems for efficient biodiesel production (Alishah Aratboni et al. 2019; Mutanda et al. 2020; Zhu et al. 2016). The study also highlighted an increment in oil accumulation by algal cells due to an inversely proportional relation between nitrogen scarcity and oil productivity. The best strategy for improving microalgae lipid accumulation is to restrict the nitrogen supply, which not only results in efficient lipid accumulation but also brings about a progressive conversion in lipid composition from free unsaturated fats to triacylglycerol (TAG) (Mutanda et al. 2020). TAGs are more valuable for transformation to biodiesel. Lipid accumulation in microalgae happens when a supplement (commonly nitrogen) is depleted from the medium or turns into a growth-limiting factor. Cell proliferation is forestalled; however, accumulation of carbon continues which is then converted into TAG lipids and is stored inside existing cells (Sun et al. 2018).

5.6 Conclusion

Coloured effluents produced from textile industries often contain a bevy of harmful contaminants such as dyes, metals, and phenols and are known to cause a detrimental impact on both the aquatic ecosystem and human health as well whenever released without sufficient treatment. Microalgae can readily break down these hazardous organic and inorganic chemicals into smaller compounds utilizing them for its growth and biomass generation, thereby remediating these wastewaters in return. Over the last few years, microalgae have gained a lot of attention owing to their efficiency potential in the treatment of textile effluents as compared to conventional treatment methods. Microalgae can efficiently degrade dye either through biodegradation or biosorption. Moreover, microalgal remediation can also lead to an additional benefit of producing important biomass that can be handled into bioproducts, biofuels, and bioenergy production. However, there are only a few studies reported in the literature based on the treatment of textile effluent; thus further studies are required in this context that can confirm the feasibility of microalgal-based remediation at an industrial scale. Microalgal remediation can easily be integrated with the existing treatment technologies to evaluate its economic feasibility. Achievable yields of both biomass and lipids combined with the generation of value-added products, if carried out carefully, can be subjugated and could enhance algae's economic viability.

Mass cultivation by phototrophic cultivation systems is the best as far as net energy balance is considered. However, productivity may vary significantly when contrasted with heterotrophic production. Harvesting algal biomass represents the most elevated extent of energy input during biomass cultivation, yet at present, there are no standard harvesting procedures.

Additionally, certain factors that play a pivotal role in efficient biofuel production and its economic viability include biomass productivity, mainly related to the selection of potential strains, photosynthetic efficiency, and lipid productivity as well as harvesting costs. The isolation of autochthonous strains for biofuel production should be considered as an essential parameter, although these strains may not be ideal for the production of lipids, and hence a need for genetic engineering may be required to manipulate lipid levels. Future research needs to focus on synergizing the robustness of microalgae capable of bioremediation with their multifarious traits, leading to industrial applications as biofuel or bioenergy feedstock.

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Competing Interests All the authors declare that they have no competing interests.

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Chapter 6
The Multifaceted Microalgal Approach to Wastewater Treatment to Generate Energy and Essential Chemicals

Sreestha Dinesh Parambath, Yaseera Bhombal, Ashutosh Kumar, Meenakshi Singh, Murthy Chavali, and K. Chandrasekhar

Abstract The biological state-of-the-art purification of wastewaters has gained momentum in recent times. The microalgal capability to reduce N and P contaminants, as well as chemical oxygen demand (COD), is implemented in wastewater treatment processes. This green microalgal strategy to integrate wastewater treatment and to achieve better energy efficiency mainly depends on the purpose, scalability, nutrient uptake of algal species and economic feasibility. Therefore, the microalgal approach is sustainable as compared to conventional methods of wastewater treatments because it generates no toxic waste and can grow in limited resources to meet the soaring energy demand of the world. In this chapter, we discuss the successful trials on pretreatment methods employing microalgae to treat a variety of wastewaters based on a different selection criterion. Further, we focused on different microalgae cultivation systems with an emphasis on their benefits and drawbacks. Then, a brief evaluation of the microalgae biorefinery technologies was done to generate renewable energy and high-value chemicals. Lastly, the

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challenges faced in integrated microalgal wastewater treatment processes were outlined for wide-scale applications on bioenergy production.

Keywords Microalgae · Wastewater treatment · Biorefinery technologies · Bioenergy · Sustainable cultivation system

Abbreviations

BOD	biological oxygen demand
COD	chemical oxygen demand
MBWT	microalgae-based wastewater treatment
OP	orthophosphates
GS/GOGAT	glutamine synthetase/glutamine oxoglutarate aminotransferase
GDH	glutamate dehydrogenase
PWWs	piggery wastewaters
HRAP	high-rate algal pond
WWT HRAP	wastewater treatment HRAP
CSYE	cattle standing yard effluent
WSP	waste stabilisation ponds
PBR	photobioreactor
ATS	algal turf scrubbers
AMD	acid mine drainage
MFCs	microbial fuel cells
EAB	electrochemically active bacteria
ASP	activated sludge process
PMFCs	photosynthetic microbial fuel cells
SMFCs	sediment microbial fuel cell
EET	extracellular electron transfer
RNG	renewable natural gas
CNG	compressed natural gas
LNG	liquefied natural gas
LBM	liquefied biomethane

6.1 Introduction

Over the years there has been an exponential increase in the population magnitude, accompanied by escalating demand for water. According to estimates, the world might face a 40% water deficiency as it touches 2030 (Sun et al. 2016). The fast-paced urbanisation and industrialisation activities prey on natural resources, generating contamination of water resources by excess accumulation of carbonaceous compounds, nitrogen and phosphorus to high degrees. This can result in deleterious effects on the dissolved oxygen as well as interfere with the well-being of flora and fauna because of the high biological oxygen demand (BOD) and chemical oxygen demand (COD). Due to the paucity of technologies for reclaiming the used wastewater, there is an urge to improve the techniques to reuse water and recover nutrients using natural green algal cultivation (Pacheco et al. 2021). Microalgae's binding affinity with metals provides a great advantage of treating wastewater in an advanced and sustainable manner. It is possible because of its mass production, rapid metal uptake potential, an affinity for polyvalent metal ions and high range of metal tolerance, asserting the superiority of microalgal biotechnology over traditional approaches for the extraction of heavy metals (García-Galán et al. 2020; Prakash et al. 2021). Conventional methods for nutrient removal like nitrification-denitrification, aerobic activated sludge-based treatment, phosphorus removal, etc. fail to meet the sustainability standards due to energy consumption, lengthy process, carbon emission, excess sludge discharge, instability and resource wasting (Ubando et al. 2021). This is where microalgae-based wastewater treatment (MBWT) comes into the picture for bioapplications.

MBWT has been attracting the attention of researchers over the years for being a promising and sustainable opportunity for advanced nutrient recovery and bioremediation of wastewater for reducing eutrophication when released to water bodies (Agrawal and Verma 2022). This accounts for the metabolic flexibility of microalgae to carry out mixotrophic, heterotrophic and photoautotrophic metabolism (Hu et al. 2018; Goswami et al. 2020). Research has proven its efficiency and feasibility as a supplement for tertiary treatment of agricultural, municipal and industrial wastewaters (Whitton et al. 2015). The initial research on microalgae was carried out in the 1950s for its commercial application and later found out the symbiotic relationship between microalgae and bacteria which explained how microalgae are protected from the toxic compounds in the wastewater (Goswami et al. 2021a). Microalgae through photosynthesis provide O_2 for the bacteria to metabolise carbon, nitrogen and phosphorus which can then be utilised by microalgae as nutrients for further photosynthesis (Goswami et al. 2021c). Additionally, the biomass recovered can be used as a feedstock for biorefinery, sustainable bioproducts and other activities due to the high lipid content (Mehariya et al. 2021; Mal et al. 2021; Enamala et al. 2018).

Microalgae-derived biofuels are considered as the third generation of biofuels capable of satisfying the present energy needs. The production is carried out in non-arable lands, i.e. brackish and wastewater, thereby reusing water for sustainable production (Goswami et al. 2021c). Besides biofuels, microalgae are also capable of producing valuable compounds including carbohydrates and proteins to be used as fertilisers (Jae-Hoon et al. 2016; Mehariya et al. 2021). Through this context, we focus on the different selection criteria based on which microalgae are selected for treatment. The different types of wastewater streams including municipal, agricultural and industrial along with their composition, characteristics and the pretreatment methods carried out are then elaborated (Table 6.1). Further, we hold a comparative study on the different apparatus for algal cultivation among the high-rate algal pond, photobioreactor, algal turf scrubber and hybrid system which are commonly studied and used commercially recently. Lastly, we look into the biorefinery applications of

Table 6.1 General characteristics of different wastewater types

Parameters	Municipal wastewater			Dairy wastewater			Industrial wastewater		
pH	–	7.8	10.9	8	–	9.3	8	3.85	7.45
N[a]	27.7	37	40	1.23	510	1180	1316	6.85	41.52
P[a]	1.8	9.8	2.1	0–20	76	188	28	38.12	37.79
S[a]	10.7	–	–	–	143	–	–	20.38	–
COD[a]	161.0	–	1378.2	1465	5200	8640	–	34,600	–
TDS[a]	–	4.7	8195	–	–	–	–	–	–
Alkalinity[a]	–	366	200–600	–	–	12,140	4691	–	–
Cu[a]	–	–	–	–	0.0015	–	1	–	–
Zn[a]	–	–	–	–	0.087	–	3	–	–
Fe[a]	–	–	–	–	0.728	–	11	0.013	–
Mg[a]	–	100	–	–	37.49	–	1	10.43	–
Cl[a]	139	240	200–1000	–	15.4	–	–	–	–
Microalgae	*Micractinium* sp.	*Chlorella vulgaris* (CS-41)	*Chlamydomonas* sp. TRC-1	*Chlorella* sp. Wu-G23	*Chlorella sorokiniana* AK-1	*Neochloris aquatica* CL-M1	*Tribonema* sp. *Synechocystis* sp.	*Ettlia* sp. YC001	*Scenedesmus* sp.
Reference	Piligaev et al. (2018)	Mohseni et al. 2021)	Behl et al. (2020)	Wu et al. (2021)	Chen et al. (2020)	Wang et al. (2017)	Chen et al. (2020)	Kam et al. (2017)	Yirgu et al. (2021)

Parameters	Agricultural wastewater			Piggery wastewater			Brewery wastewater		
pH	3.5	4.3	–	–	8.3	6.3	7.4	7.26	6.8
N[a]	265	1100	168.3	249.8	9.3	15.3	62.7	2.9	108.49
P[a]	88	180	32.9	28.3	1.6	9.8	152	7.9	82.88
S[a]	7.79	–	–	–	–	–	13	302	–
COD[a]	20,473	27,700	195	3909	92.5	456.3	1746	–	4000
TDS[a]	12,500	–	–	–	–	0.48	894	–	293.36
Alkalinity[a]	–	–	–	–	–	–	–	–	–
Cu[a]	–	–	–	–	–	–	–	0.03	0.01
Zn[a]	–	–	–	–	–	–	–	0.04	0.095
Fe[a]	–	–	–	–	–	–	–	1.1	3.12
Mg[a]	–	–	–	–	–	–	–	4.9	–
Cl[a]	–	–	–	–	–	–	–	248	33.98
Microalgae	*Chlorella pyrenoidosa*	*Chlorella sorokiniana* CY-1	*Chlorella vulgaris*, *Scenedesmus obliquus*	*Chlorella pyrenoidosa*	Mixed	*Scenedesmus* sp. ISTGA1	Mixed	*Chlorella* sp., *Scenedesmus* sp.	*Desmodesmus* sp., *chlorella* sp., *Scenedesmus* sp.
Reference	Giraldo (2020)	Cheah et al. (2018)	Viegas et al. (2021)	Mohan et al. (2020)	García-Galán et al. 2020)	Tripathi et al. (2019)	Hemalatha et al. (2019)	Labbé et al. (2017)	Pandey et al. (2019)

[a] mg/L

the algal biomass including bioelectricity, bio-oil, biohydrogen, biomethane and biochar along with its challenges.

6.2 Selection Criteria for Potential Microalgae

6.2.1 Tolerance to High Toxicity

Microalgae are a potential candidate in the bioremediation of pollutants due to their versatile metabolic diversity and ability to transform with respect to the environment (Deng and yee Tam 2015). The tolerance to toxic substances is achieved in one way through microalgae-bacteria consortia. According to a study conducted, it was found that the species *C. vulgaris* has the potential to adapt and be resistant to the effects of xenobiotic orthophosphates (OP) (Kurade et al. 2016). Through phytotoxicity tests, we get information on the toxicity of a compound with respect to the growth inhibition of microalgae (Xiong et al. 2016). Higher concentrations of organic compounds can interfere with the biological functions of the microalgae leading to the breakdown of its cell membrane. In the study of biodegradation of diazinon by *C. vulgaris*, we see that lower concentrations stimulated the growth of the species but had detrimental effects when the concentration was raised. This can be related to the decreased biomass content in the microalgal culture. After 6 days of cultivation, the species overcame the toxic shock and presented significant growth and breaking down of diazinon (Kurade et al. 2016).

Understanding ammonia tolerance and utilisation is very important in the case of phytoremediation of wastewater using microalgae. At pH values above 9.2, free ammonia dominates, while at lower pH, ammonium ions (ammonia dissolved in water) predominate. Ammonia gas diffuses freely across the membrane, while ions are transported across the cell membrane by high- and low-affinity ammonium transporters (Nagarajan et al. 2019). Free ammonia at high concentrations can be toxic to microalgae as it can disrupt the oxygen-evolving complex of photosystem II (Drath et al. 2008) or can intervene in the proton gradient across the thylakoid membrane (Gutierrez et al. 2016). Therefore, for the existence of non-toxic ammonium ions, pH should be maintained at medium levels. According to the study, (González et al. 2008) pH should be maintained at neutral 7 for the functioning of oxygen release through photosynthesis by microalgae. The ammonium inside the cell is broken down by two mechanisms – the most common and the active one being the glutamine synthetase/glutamine oxoglutarate aminotransferase (GS/GOGAT) pathway which takes place in photosynthetic organisms or the glutamate dehydrogenase (GDH) pathway which occurs under heterotrophic conditions. Species of the Chlorophyceae (e.g. *Chlorella*) have the highest tolerance against ammonia at a concentration of 39,000 μM with 7600 μM being the optimal concentration for the maximum growth is observed. Some cyanophyceae members can have a tolerance level of 13,000 μM where 2300 μM is the optimal level for maximum growth (Collos and Harrison 2014).

6.2.2 *High Growth Rate*

To get the best output through microalgae wastewater treatment, there should be a good growth rate for the microalgae, and therefore understanding the factors influencing the rate is very important. The factors are broadly categorised as physical, chemical and biological which are overall influenced by operational factors like bioreactor specificities, mixing and dilution rate. Light and temperature are some examples of physical factors. The chemical factors include the availability of nutrients, and biological factors can be competition between species, infections etc. Nutrient and organic content along with the presence of any heterotrophic microorganisms in the secondary effluent can affect the growth of the microalgae (Lv et al. 2017). The selection of microalgae for the treatment also depends on the cell density and lipid content. The wastewaters contain high levels of nutrients such as N and P along with a spectrum of trace elements like K, Ca, Mg, Fe, Cu and Mn which are important for the growth and metabolism of the microalgae. Microalgae can efficiently metabolise excess phosphorus and store it as polyphosphate granules which helps in the growth of algae in case of the absence of adequate phosphorus. Therefore, the growth rate of algae will not respond immediately based on the concentration of phosphorus but have an immediate response to temperature and light. Also, to avoid the effect of micronutrients in limiting the growth of microalgae, these are added to the algal cultures along with a chelating agent like (Whitton et al. 2015).

According to the study conducted by Aravantinou et al. (2013), it was observed that marine species exhibited the highest growth rate when compared to freshwater species of microalgae. This is also confirmed by another study where marine algae are superior to freshwater algae in mixotrophic cultures (Cheirsilp and Torpee 2012). It was found that the growth rate of microalgae is not proportional to phosphorus removal. The P removal data states that greater intake is done by freshwater species than marine (Aravantinou et al. 2013). Temperature is an important factor where an optimum of 15–25 °C is ideal for algal growth and excess increase can decline the growth rate. Algae native to cold climates can get photoinhibited by high-temperature intensities which can be a constraint conducting outdoor wastewater treatment in cold regions. The cyanobacterium species *Anabaena variabilis* grows at an optimum pH of 8.2–8.4, and the rate decreases beyond pH 9 and will be unable to thrive. The pH of the system can increase due to CO_2 assimilation (Chevalier et al. 2000). The pH change can be controlled by turbulence and by promoting gas exchange between water and air. In certain cases, algae can produce compounds that are toxic to themselves during metabolism and inhibit their growth which is known as autoinhibition. Besides all these factors, biotic components can also influence the growth rate. Cyanobacteria can produce inhibitory compounds against eukaryotic algae, while the latter can produce antibacterial substances (Rojas et al. 2020).

6.2.3 High Nutrient Uptake

In general, microalgae have the potential to remove a large percentage of nutrients as well as environmentally hazardous substances including heavy metals (Ahluwalia and Goyal 2007; Goswami et al. 2021b). Recent studies have emphasised the importance of high-rate algal ponds (HRAPs) that can recover high rates of nutrients when integrated with additional CO_2 input (Sutherland and Ralph 2020). Agricultural and municipal wastewater canals are rich in nutrients, mainly nitrogen phosphorus and other micronutrients, and microalgae have the potential of rapidly and efficiently taking up these nutrients (90% of NH_4-N and PO_4-P) to produce rich biomass, thereby providing service to the environment. To achieve maximum efficiency in nutrient uptake, the selection of appropriate strains with maximum wastewater tolerance is a necessary criterion. In fact, the algae isolated from natural water bodies and WWT plants show better growth and nutrient utilisation, thereby adapting to the culture conditions and resulting in efficient bioremediation (Osundeko et al. 2014). Some of the commonly used strains for treatment include *Chlorella* sp. and *Scenedesmus* sp. according to some studies conducted by Sacristán de Alva et al. (2013) and Arbib et al. (2014) regarding their nutrient take-up efficiency. Uptake is the main mechanism by which nutrient is removed from the wastewater; hence, the growth rate is directly proportional to the nutrient removal. Also, their efficiency can be maintained only if the N/P ratio is properly ranged. For the growth of microalgae in open or closed systems, the secondary effluents from the wastewater should contain a relatively low concentration of inorganic nutrients (around 10–15 mg/L nitrogen and 0.5–1 mg/L phosphorus) so that the algae will grow well in the effluent as well as take up nutrients effectively alongside lipid accumulation (Al-Jabri et al. 2020).

The factors affecting the nutrient uptake of microalgae include the concentration gradient inside and outside the cell and the rate of diffusion through the cell wall. As the thickness of the unstirred layer of the water outside the cell increases, the diffusion rate decreases. Therefore, for the active transfer of nutrients, turbulence is very necessary. It is found that the more the diversity found among algal species (species richness), the higher is the rate of uptake of certain nutrients, i.e. they positively correlate (Ptacnik et al. 2008) concerning the rapid nutrient depletion of orthophosphates and inorganic nitrogen (Stockenreiter et al. 2016). The latest studies are focused on increasing the production of the biomass by including a community of species for cultivation (Kazamia Elena 2014) as the monocultures of the microalgal strain selected might not be a superior diverse community with regard to the production of lipids. In the examples of controlled cultivation, the lipid-biomass production and biomass-specific lipid content are high in the case of diverse communities when compared to their corresponding monocultures (Stockenreiter et al. 2011).

6.2.4 Scalability Approach

The scalability of microalgal growth systems is of prime importance when considering the commercialisation of biofuels based on microalgae (Quinn et al. 2011; Bhardwaj et al. 2020). Therefore, to understand the productivity potential of biofuel, one must structure and verify models to predict the productivity of microalgae at the industrial level while adding real locational characteristics (James and Boriah 2010). Interest around the potentiality of microalgae especially grew during World War II as the population rose and resource availability went downhill. Since previous times, *Chlorella* sp. was used widely due to its ability to prolific growth and adaptability to different environments. After understanding that microalgae-based biofuel production is not economically viable (Stephens et al. 2010), research has been focused on microalgae as a source of high-value products. However, studies conducted are mostly based on the laboratory scale and fail to detail the scaling up to the commercial level. Some previous literature that mentions scaling up include *Spirulina* (Richmond and Hu 2013) and *Dunaliella salina* (White and Ryan 2015) but fails to detail the constraints and its solutions.

Scaling up is usually carried out by a factor of 10 per step (10 ml to 100 ml to 1 L). The increase in the number of steps and time can not only increase cost but also the contamination. One feature that helps in scaling up to the commercial level is modifying the depth of the culture to increase the areal density even when biomass is low due to dilution. Another way is to utilise greenhouse or shaded pond cultures to manage the fluctuation in light and temperature (Borowitzka and Vonshak 2017). It is necessary to select the suitable strains ideal for high productivity outdoor cultivation. Borowitzka (2013) has done an extensive assessment on the algal strains and species compatible for large-scale commercial level cultivation. The potential algal strain should be tested under outdoor conditions at the early stage of the stain evaluation process. Outdoor cultures can also be used for strain selection by exploiting their in-pond evolution through phenotypic and genetic changes, commonly occurring in algal cultures to get the best strain with high productivity (Lakeman and Cattolico 2007; Chaturvedi et al. 2020). The key operational features to be considered while producing microalgal culture on large scale commercially are efficient production of inoculum in the shortest time, effective monitoring process to detect any problems in the culture so that early measures to remediate can be attempted, structuring an efficient operational protocol for managing the culture under different environments in the outdoor conditions to maintain productivity, quality of the product and reliability for a longer time and measure to recuperate from any operational or equipment breakdown to avoid culture collapse which disturbs the normal operation. All of these should be site and species specific for effective scaling up of microalgae in a profitable way (Borowitzka and Vonshak 2017; Satpati et al. 2022).

6.3 Characterisation of Wastewaters and Pretreatment Methods by Microalgae

Wastewater management has become an alarming issue worldwide owing to the ever-increasing population and industrialisation. The day-to-day domestic activities, as well as industrial production required for survival and sustainability, generate huge amounts of wastewater. The unrestrained discharge of wastewaters into the water bodies results in environmental pollution, making wastewater treatment a mandatory process. To treat these wastewaters efficiently, it is essential to categorise and characterise them initially. This characterisation helps decide the detailed procedure to be followed for wastewater treatment. Here, we have categorised the wastewater into six different types based on their source of origin as mentioned in Table 6.1.

6.3.1 Municipal Wastewater

The majority of municipal wastewater is generated by household and domestic activities. Municipal wastewater is continuously produced in vast quantities, which, with adequate treatment, might assist to offer a sufficient source of freshwater for long-term water reuse. Eutrophication is a big issue with municipal wastewater, as physical and chemical features such as COD, BOD, N_2, P, K, certain metals and microbial load combine to create an ideal eutrophic environment, which can lead to algal blooms. As a result, municipal wastewater must be treated to guarantee that it is low risk before being reused or disposed of in water bodies (Li et al. 2011). Many alternative treatments have been used to treat municipal wastewater, but because of the high nutrient levels, microalgae are being chosen over traditional treatment methods. Piligaev et al. (2018) studied a microalga named *Micractinium* sp. in the primary stage of wastewater treatment wherein the wastewater was autoclaved. The authors reported a removal ratio of 96% and 78% for nitrogen and phosphorus, respectively, with biomass productivity of 37 mg/L/day. A similar study conducted by Mehrabadi et al. (2017) displayed 100% removal ratios for both nutrients by the same microalgae where the wastewater was only filtered and not autoclaved. Also, the microalgae displayed biomass productivity of 177 mg/L/day. *Chlorella zofingiensis*, a protein-rich alga, was batch cultivated as well as cultivated using an outdoor wastewater culture system. The microalgae displayed efficient removal of nutrients indoor as well as outdoor, suggesting its practical application is feasible for bioenergy generation using municipal wastewater (Zhou et al. 2018). Mohseni et al. (2021) used *Chlorella vulgaris* using a unique bead immobilised microalgae method for the municipal wastewater, wherein the microalgae were immobilised into alginate beads. These immobilised microalgae displayed significant nutrient removal even under various stress conditions. *Scenedesmus* sp. has widely been used to treat domestic wastewaters. Two such *Scenedesmus* spp., i.e. *S. ISTGA1* and *S. obliquus*, have been used to treat municipal waste along with biodiesel production in two different studies (Eida et al. 2018; Tripathi et al. 2019). *Scenedesmus obliquus* was used to treat secondary municipal wastewater, wherein it utilised 95.2% of P and 78.5% of N, respectively (Eida et al. 2018). Similarly, microalgal strain, *Scenedesmus* sp. ISTGA1, efficiently utilised N and P. In addition, it also reduced heavy metals and organic contaminants after treatment (Tripathi et al. 2019).

6.3.2 Dairy Wastewater

Milk and milk product consumption have increased in recent years, so has the number of dairy-related businesses, as well as dairy wastewater. As a result, significant efforts have been made to limit the amount of dairy wastewater produced and to reduce the possible environmental impact. Milking parlour and dairy wash water, milk spillages, runoff from soiled yard areas, drainage water from building roofs, silage effluent and occasionally leachate from manure heaps make up dairy effluents. High BOD and COD, high pH, high ammonia nitrogen and residues of cleaning and disinfection chemicals define these effluents. Greater nitrogen and phosphorus levels cause eutrophication and must be removed before discharge (Monfet and Unc 2017). Treatment of dairy wastewater needs both physicochemical and biological methods for efficient effluent treatment. With respect to the characteristics of dairy wastewater, it can be suggested that a link can be established in both the dairy and microalgae industries through the recycling of nutrients from dairy effluents (DE) to cultivate microalgae. Hemalatha et al. (2019) used an undefined mixotrophic microalgae culture collected from Nacharam Cheruvu (lake) in Hyderabad. This mixotrophic culture displayed 90% removal of COD and moderate removal of the nutrient N and P as 65.5% and 73%, respectively. Similarly in another study, two different mixotrophic cultures of *Chlorella* and *Scenedesmus* sp. were used to treat dairy wastewater categorised into cattle standing yard effluent (CSYE) and milking parlour effluent (MPE). The former *Chlorella* mixotrophic culture efficiently reduced organic and ammonium content from the CSYE, while the latter grew better in MPE (Labbé et al. 2017). A screening experiment for biofuel stock production and dairy effluent treatment of some microalgae from the genera *Desmodesmus*, *Chlorella* and *Scenedesmus* was used for the treatment of simulated dairy effluent, out of which, the microalgae *Scenedesmus ASK22* sp. was found to be more promising for the SDE treatment as it displayed high COD (>90%), N (100%) and P (91.24%) removal efficiency and greater biomass production suitable as biodiesel feedstock (Pandey et al. 2019).

Patel et al. (2020) have studied microalgae cultivation in pretreated dairy whey, using *Chlorella protothecoides* which display a nutrient removal efficiency of approximately 99% and 91–100% for organic and inorganic pollutants, respectively. Another extraordinary study, conducted on *Chlorella vulgaris* CA1, extremophilic algae, was used for dairy wastewater pretreated with anaerobic digestion. The peculiarity of this microalga is it can tolerate high ammonia concentration and also displayed 96% and 74% removal of N and P (Pang et al. 2020).

6.3.3 Industrial Wastewater

Industrialisation plays a significant role in the country's development. Textile, culinary, dye and pharmaceutical sectors are just a few examples of industries that generate large amounts of wastewater. Depending on the type of industry, this effluent may contain heavy metals, dyes, preservatives and other harmful substances. The textile business is one of the world's fastest expanding and largest industries, with yearly sales of over 1 trillion dollars and up to 7% of global export (Lellis et al. 2019). The wide variety of synthetic dyes used to colour garments produces massive amounts of coloured effluent. Textile industries use more water than other sectors, resulting in highly contaminated effluent being discharged. Textile wastewater is harmful to aquatic life because it reduces light penetration, interferes with plant photosynthetic function and reduces microbial activity. To safeguard the environment and maintain sustainability, it is critical to treat textile wastewater with a variety of physical, chemical and biological treatments. Although physical and chemical procedures are effective, they are neither cost-effective nor environmentally friendly, and they frequently have downsides, such as sludge disposal. In the treatment of wastewater for nutrient recovery and other resource generation, such as biofuel and biochemicals, biological approaches are more favourable. Fazal et al. (2021) demonstrated bioremediation of textile wastewater using the microalgae *Chlorella vulgaris*, which could easily decolourise the methylene blue dye, reduce N and P up to 80% and also give valuable product biodiesel (9.12 mg/g). Similarly, a study employed *Chlorella variabilis* for treating textile effluent where the strain remediated the wastewater Al (100%), B (82.72%), Ca (45.66%), N (100%), Fe (100%) and P (78.17%) (Bhattacharya et al. 2017). Two studies employed immobilised microalgae for textile wastewater treatment using *Chlorella SPWuG23* and *Chlamydomonas* sp. *TRC-1* (Behl et al. 2020; Wu et al. 2021). Both studies used textile dyeing effluent as a substrate for microalgae growth. The former study using *Chlorella* sp. demonstrated different states of the microalgae (immobilised, suspended) in static and aerated conditions and found greater pollutant removal ability using immobilised microalgae (Wu et al. 2021). The latter study was a combined approach of treating textile wastewater as well as the production of bioelectricity (Behl et al. 2020).

The aerobic digestion of anaerobically pretreated wastewater was performed using microalgae *Tetraselmis indica* using a photobioreactor which reduced the content of P (70.03%), N (67.17%) and COD (66.30%) (Amit and Ghosh 2020). Mojiri et al. (2021) demonstrated efficient micropollutants pharmaceutical and personal care products (PPCPs) removal using a combinatorial approach and microalgae *Chaetoceros muelleri* which reduced the three main PPCPs CB2 (68.09%), SMT (64.08%) and TRA (693%). Telma Encarnação et al. (2020)

employed immobilised *Nannochloropsis* sp. to effectively remove paracetamol and ibuprofen, while the suspended cells removed olanzapine. Other than textile and pharmaceutical industries, microalgae have also been used for the treatment of effluent from food-based industries. Arashiro et al. (2020) utilised three different algae species, namely, *Nostoc* sp., *Arthrospira platensis* and *Porphyridium purpureum*, for remediating food industry wastewater up to 98%, 94% and 100% of COD, nitrogen and phosphate, respectively. In addition, valuable pigments like phycocyanin, Allu phycocyanin and phycoerythrin were also extracted. Effluents from the palm oil mill were treated with *Chlorella vulgaris* ESP.31, and *Chlorella sorokiniana* displayed the highest removal efficiencies with 62.07% nitrogen removal, 30.77% PO_4 removal and 47.09% of COD (Cheah et al. 2018). *Chlorella vulgaris* was also used for phycoremediation of heavy metal-contaminated industrial wastewater along with *Nostoc* and *Oscillatoria* which efficiently removes heavy metals like Zn, Cd and Cu and simultaneously reduced the NO_3 and SO_4 content.

6.3.4 Agriculture Wastewater

Agricultural and livestock operations are most likely the primary source of diffuse pollution in rural regions, affecting both surface and groundwater systems to meet food demands. The world’s growing population has led to more intensive animal farming operations and agricultural activities, resulting in increased use of veterinary medications (particularly antibiotics) in cattle farming and artificial fertilisers and synthetic pesticides in agriculture. Agricultural wastes can be either of organic nature from animal facilities (swine, dairy operations, aquaculture) or agricultural drainage with low organic but high nutrient content.

Intensive aquaculture development has increased recently. The most important factor in aquaculture is the high amount of water which ultimately leads to the generation of a high amount of aquaculture-based water. The wastewater is rich in nutrients due to feeding residues and excreta from the aquatic species; it also constitutes antibiotics and antimicrobial agents (Rosa et al. 2020). On a chemical basis, it consists of inorganic nitrogen, phosphorus, organic pollutants and other pollutants (Cheng et al. 2020). Ding et al. (2020) displayed treatment of such aquaculture wastewater using novel microalgae membrane bioreactors with an internally circulating fluidised bed and a *Platymonas helgolandica* var. *tsingtaoensis* microalga. This treatment system could efficiently remove nitrogen (especially ammonia) and phosphorus from the wastewater. Viegas et al. (2021) performed an extensive study using five different microalgae strain *Chlorella vulgaris*, *Scenedesmus obliquus*, *Isochrysis galbana*, *Nannochloropsis salina* and *Spirulina major* to remediate raw effluent from brown crab aquaculture. Interestingly, all the strains displayed total removal of N and P and COD ranging from 71.8% to 94%.

Poultry is a fast-growing agricultural sector that also creates large volumes of wastes. This waste includes residue feed, excreta, feathers and urine. Leachates with cleaning procedures enter into water generating poultry wastewater. This wastewater consists of nitrogen in the form of ammonia and nitrate, phosphorus, potassium and uric acid. In a study, the microalgae *Chlorella pyrenoidosa* was cultivated on poultry excreta leachate wastewater and displayed up to 80% removal of N and 88.57% of P removal (Mohan et al. 2020). In another study, the oleaginous microalgae *Scenedesmus obliquus* was used to heat the poultry wastewater and displayed almost 98% of N and 99% of P removal with a COD removal value of approximately 60% (Ferreira et al. 2018). The agricultural drainage water mainly consists of pesticides, chemicals, fertilisers, different salts and sometimes heavy metals. Other than the harmful chemicals, the water also consists of many macronutrients and micronutrients leached out of the soil into the water. Microalgae are known to grow in low-quality waters utilising N, P and bicarbonates. Thus, these microalgae also serve as good candidates for the treatment of surface runoff water. García-Galán et al. (2020) used microalgae-based bioremediation of pesticide-contaminated water using an undefined mixed culture of microalgae from a pilot HRAP. This mixed culture worked efficiently in decontaminating NH GT-N as well as a few pesticides. In another study, a filamentous algae *Cladophora* sp. was employed to treat organic liquid agricultural waste which demonstrated a reduction of N from 164.8 to 120.7 mg/L and reduction of P from 78.5 mg/L to 9.7 mg/L in 9 days (Flores-morales et al. 2021).

6.3.5 Piggery Wastewater

The global population is growing at an exponential rate, and by 2050, it is expected to reach 9.9 billion people. Globally, there has been a sharp increase in swine farms to meet the rising demand for food and protein resources. To reduce mortality and improve production, swine farming needs the use of veterinary antibiotics and steroid hormones. A prominent pork production farm, according to another report, generated roughly 1300 tonnes of wastewater each year. Due to the presence of high amounts of organic elements, nutrient contamination through piggery wastewaters (PWWs) is becoming a serious concern when disposed of without sufficient treatment. PWW, on the other hand, has high ammonium nitrogen and phosphorus content, which can be utilised as a nutrient supply for the culture of microalgae, which could be one of the greatest long-term solutions for pollution management and biofuel feedstock production (Bai et al. 2012). This could be a promising strategy for generating sustainable energy and mitigating global climate change in the future. *Neochloris aquatica* CL-M1 is used as a feedstock for biobutanol production (Wang et al. 2017). This microalga was cultivated on swine wastewater with a dual approach of treating wastewater and culturing microalgae biomass as a feedstock for biofuel production. Furthermore, the microalgae efficiently utilise nutrients and COD from the wastewater with reduction values of 73.5% for COD and 96.2% for N (Wang et al. 2017). *Scenedesmus obliquus* was used to treat swine wastewater generated from slaughterhouses using a bubble column reactor in a batch culture where it reduced the nitrogen in the wastewater by a value of 98%, whereas the PO_4

and COD values were not determined (Ferreira et al. 2018). Chen et al. (2020) have recently displayed treatment of swine wastewater using *Chlorella sorokiniana* AK-1 loaded sponge resulting in 90.1%, 97% and 92.8% removal efficiency for COD, N and P, respectively. *Tribonema* sp. and *Synechocystis* are auto-flocculation microalgal species used by Cheng et al. (2020) to remediate swine wastewater along with T-IPL pretreatment displaying a nutrient removal efficiency of 75.8–89.9% for NH_4^+, 71.4–72.7% for PO_4 and 55.6–68.6% for COD for the microalgae, *Tribonema* and *Synechocystis*, respectively. In another study, phytoremediation of swine wastewater for biogas generation was performed with the help of *Chlorella sorokiniana* decreasing the P and N content of the wastewater by 90% and 70%, respectively (Dinnebier et al. 2021).

6.3.6 Brewing Wastewater

Brewery industries, despite being an important component of the economy of the producing country, use a lot of water in the production process and then dump roughly 70% of it as effluent. When mixed with effluent, wastewater by-products such as yeast or wasted grains, which are created from two primary beer production steps (brewing and packaging), are the main contributors to pollution. Additionally, floor washing, bottle, tank and machine cleaning all contribute to the contamination of water bodies. This effluent contains significant levels of COD, N, P and other organic compounds, rendering it unfit for any beneficial application. Brewery effluent discharge into water bodies, whether untreated or partially treated, poses environmental concerns. This phenomenon causes environmental issues such as water scarcity, eutrophication and health-related issues in communities near discharge sites. Brewery industries must consequently treat and manage their wastewaters appropriately before final disposal into the environment (Lu et al. 2017). Conventional treatment procedures are associated with high operating and maintenance expenses, making them commercially unviable. Furthermore, excessive chemical use may result in ecological imbalances. Microalgae, a cost-effective and environmentally friendly water treatment technology, has been proposed as a solution to these issues.

The commonly used microalgae *S. obliquus* for wastewater treatment was also employed to treat brewery wastewater using a batch cultivation method outdoors. This experiment demonstrated 100% N removal, while around 60% P removal, the low value for P reduction was accounted to the low N/P ratio (Ferreira et al. 2019).

Ettlia sp. strain *YCOO1* was used for pretreatment of starch-enriched brewery wastewater obtained from a rice wine brewery, though the study did not report any nutrient reduction values (Kam et al. 2017). Another study employing microalgae from the same genus cultivated in a photobioreactor reported 100% NH_4-TN removal and approx. 70% of PO_4 removal from brewery effluent from an industry based in Ethiopia (Yirgu et al. 2021). Han et al. (2021) used a co-culture of three microalgae one from the genus *Scenedesmus*, *Scenedesmus* sp.366, and two from genus *Chlorella*, namely, *Chlorella sorokiniana* UTEX-1602 and *Chlorella* sp. L66. This co-culture displayed excellent nutrient removal values of 90.57%, 97.37% and 78.83% for N, P and CO_2, respectively. In addition, the co-cultivation of these microalgae fixed biological carbon at a rate of 32.09 mg/L/day. *Chlorella pyrenoidosa*, green algae along with its associated bacteria, was utilised to treat weak wort, a type of wastewater from the beer industry. The study demonstrated around 90.77–96.41% in N and 41.82–65.98% reduction in P, respectively, at a photon flux density value ranging from 150 to 430 (Giraldo 2020).

6.4 Comparative Account of Different Microalgae Cultivation Systems to Treat Wastewater

6.4.1 High-Rate Algal Ponds (HRAPs)

Wastewater treatment HRAPs (WWT HRAPs) are open raceway ponds of 30–40 cm in depth, which enhance natural biological treatment processes using low-power paddle wheels. HRAP initiates the treatment through a symbiotic relationship between bacteria and microalgae to yield dissolved nutrients as algal biomass which can be used as fertiliser and biofuel feedstock (Mehrabadi et al. 2015; Agrawal and Verma 2022).When compared with the conventional technologies used for wastewater treatments, especially in large cities, WWT HRAP makes use of low minimal capital and operational costs (Muga and Mihelcic 2008). These systems can substitute the electrical energy consumption with the solar energy captured through the algal photosynthesis, thereby saving 50% of the energy used by the mechanical systems (Craggs et al. 2014). The shallow depth aids in the photo-oxidation of organic contaminants, thereby efficiently disinfecting the system as compared to other wastewater treatment ponds (Davies-Colley 2005). Though the land area requirement for HRAP is large (1.7–2.7 ha/ml/day) and has variability in their treatment processes since it is a natural system, they are more efficient than the conventional waste stabilisation ponds (WSPs). HRAPs can efficiently remove nutrients in a frame of 4–8 days compared to WSPs which take about 30–60 days (Faleschini et al. 2011). The operational control variables for HRAP include organic loading rate, depth, mixing velocity and hydraulic retention time (Fig. 6.1).

The harvested biomass has a heating value of $18–22 \times 10^6$ MJ/tonne which makes it a potential feedstock for biofuel production. The system can virtually eradicate the disposal of sludge and put out minimal odour (Craggs et al. 2014). According to some studies conducted (Buchanan et al. 2018), HRAP shows better performance in the removal of heavy metals and pathogens except for orthophosphates. It should be noted that if the pH goes beyond 9, it can subside the nutrient removal utilising physicochemical processes such as volatilisation of ammonia and precipitation of phosphates unless overcome by increased algae assimilation. A disadvantage of this system is the necessity of a paddlewheel used for mixing

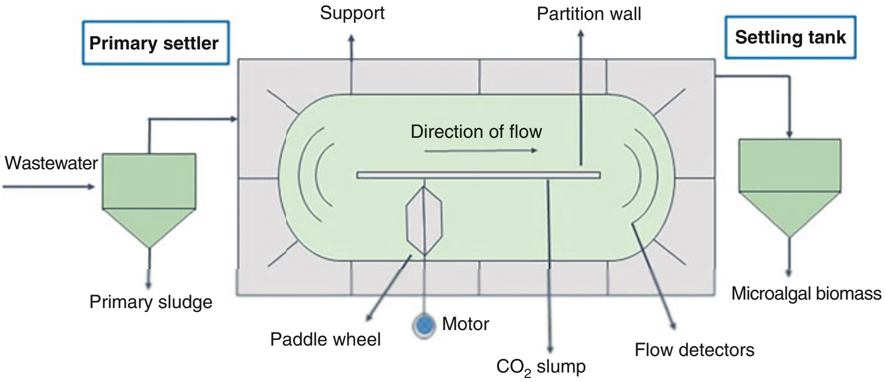

Fig. 6.1 A schematic diagram of high-rate algal pond (HRAP)

the system. This makes it strenuous in cases where electricity access is difficult. The problem can be mitigated by the usage of a small generator or powering the paddlewheel with solar (Shoener et al. 2014). When comparing photobioreactor (PBR) and HRAP, the former outperforms by producing high concentrations of algal biomass. But this is undermined by the severe biofouling experienced in PBR, which is not seen in HRAP along with its high construction cost and upscale challenge (Muñoz and Guieysse 2006). Therefore, HRAP poses a better alternative for other costly wastewater treatment setups and may provide a more flexible system providing many advantages as that of a bioreactor (Araki et al. 2001).

6.4.2 *Photobioreactor*

Microalgae cultivation is commonly carried out through two methods – open and closed cultivation systems. The latter is usually referred to as closed photobioreactors (PBRs) for the cultivation of specialised algal strains yielding specific biochemicals which shows great efficiency in quality due to its operation under controlled parameters, thereby overcoming the disadvantages of open cultivation systems like tanks and raceway ponds. It comprises a closed illuminated vessel where controlled biomass production takes place. PBR can be structured and optimised based on the chosen strain. They comparatively use less space with adequate light availability and minimise contamination (Fig. 6.2).

But outweighing its benefits are some downsides including biofouling, overheating, cleaning difficulties, benthic algae growth and accumulation of oxygen limiting algal growth. Economically, the installation and operation of PBR attract high capital costs (Muthu et al. 2016). Despite all these downsides, they have several other benefits—minimise contamination and promote axenic microalgal culturing, better control on the parameters like temperature, pH, CO_2 concentration and light, prevent evaporation and allow high cell concentrations and production of complex

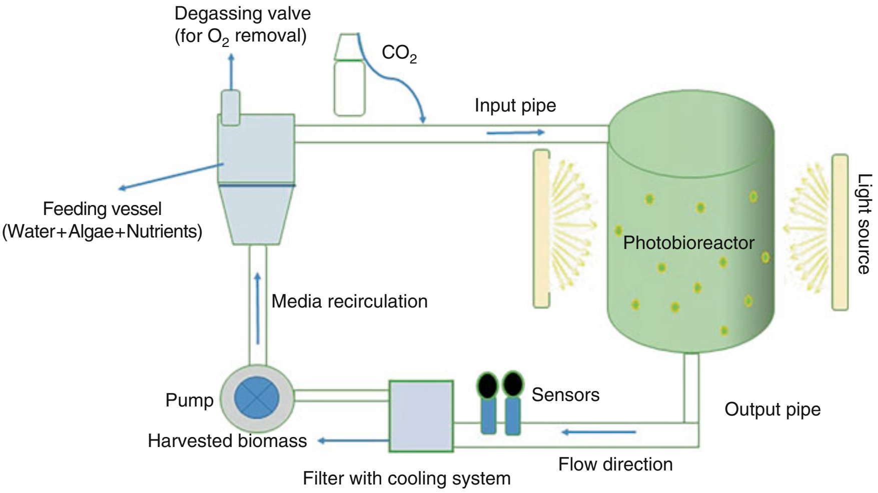

Fig. 6.2 A schematic diagram of photobioreactor

biopharmaceuticals. Various types of bioreactors are present which were modified from the basic tubular design to enable better light income and culture mixing (Muthu et al. 2016). Some of them are bubble column and airlift (coming under vertical tubular reactors), flat panel, horizontal tubular, helical-type and hybrid-type PBR. Among these, bubble column reactors have the advantage of having low capital, high surface area to volume ratio, steady vessel parts, better heat and mass transfer and efficiency in releasing oxygen and other residual gases. In terms of orientation, horizontal tubular reactors are advantageous due to their high light converting efficiency. They also have high photosynthetic efficiency and volumetric productivity when compared with flat panel or bubble column reactors.

Helical reactors require less land and finer CO_2 transfer to liquid from the gas phase. But the disadvantage of this which is similar to other reactors is biofouling, energy requirement for recirculating the culture using a centrifugal pump and the accompanying shear stress which hinders it from commercialising. It is essential to know the specifications such as light distribution, mass transfer, shear stress, scalability and biology of algae while developing a PBR. No single reactor is ideal and can satisfy all the conditions. So, hybrid reactors can be constructed to integrate the advantages of different reactors to be efficient in mass algal culture development (Singh and Sharma 2012). PBRs are more functional when worked with continuous cultures which are used for high biomass retrieval but cannot be used for lipid induction for biodiesel production (Mata et al. 2010). Despite the vast advances made in bioengineering and biotechnology promoting the use of PBR for algae cultivation, open systems are dominant at the industrial levels. This is due to reasons like unresolved technical troubles, exorbitant investment costs and production in photobioreactors. But there is the continuous optimisation of these systems done even now in the research field in terms of sustainability and environment friendliness.

6.4.3 Microalgal Turf Scrubber

An alternative to raceway ponds and open growth systems as algae cultivation systems is algal turf scrubbers (ATS) which are open flow attached growth systems used for tertiary treatment of municipal and agricultural wastewater (Kebede-westhead et al. 2003). ATS is an ecologically engineered device modelled from the tropical coral reefs which were considered to be highly productive. The units of ATS attained high primary productivity by integrating water flow and surge with high light intensity and frequent harvesting to maintain water quality in a variety of micro- and mesocosms such as estuaries, coral reefs and rocky shores. These are simple structures, and the biomass can be harvested using farm equipment (Pizarro et al. 2006). The system primarily includes an attached algal community forming a 'turf' grown on screens in a shallow basin (called 'raceway'). This turf accommodates a community of naturally occurring 'periphyton' which are defined as benthic organisms growing mixotrophically or photoautotrophically, including a range of bacteria, fungus, algae, detritus and the fauna supported by this matrix (Aston et al. 2018; D'Aiuto et al. 2015). As the water passes through, the algae uptake the nutrients biologically and produce oxygen utilising photosynthesis. In the end, the water is released back to the main body with less nutrient content and more dissolved oxygen content. The nutrients which were removed (scrubbed) are now stored as biomass in the algae and harvested once a week which is important in maintaining the higher growth rate of the algae and reducing the effects of the invertebrate micrograzers. Compared to other ecosystems, the biomass production rate of ATS is among the highest due to its high growth rate of algae aiding in nutrient scrubbing and oxygen production (Adey 2006). The nutrients removed include nitrogen, phosphorus and carbon dioxide which then undergoes chemical filtration and precipitation. In the long run, it helps in mitigating the eutrophication impact in natural waterways which can cause ecosystem disturbance and economic damage. This biomass can further be processed into compost material, feed for livestock and fuel products but at the cost of low-fatty acid content (Derose et al. 2019). These, along with high ash content, are the hurdles to be crossed for the commercialisation of ATS (Hoffman et al. 2017). Algae productivity can be limited by the interaction of temperature and light due to the usage of nutrient-rich water. Due to the flexibility of the design, ATS can be installed in rural areas to utilise the wastewaters from the rivers, lakes, etc. to provide numerous benefits which represent a sort of 'nutrient farming'(Hey et al. 2005). Other alternatives experimented with for ATS include constructed wetlands especially for treating dairy and animal manure. The wetland cells are placed after a lagoon or solids separator with the function of reducing BOD and N as they are considered to be effective in treating manure wastewater (Fig. 6.3).

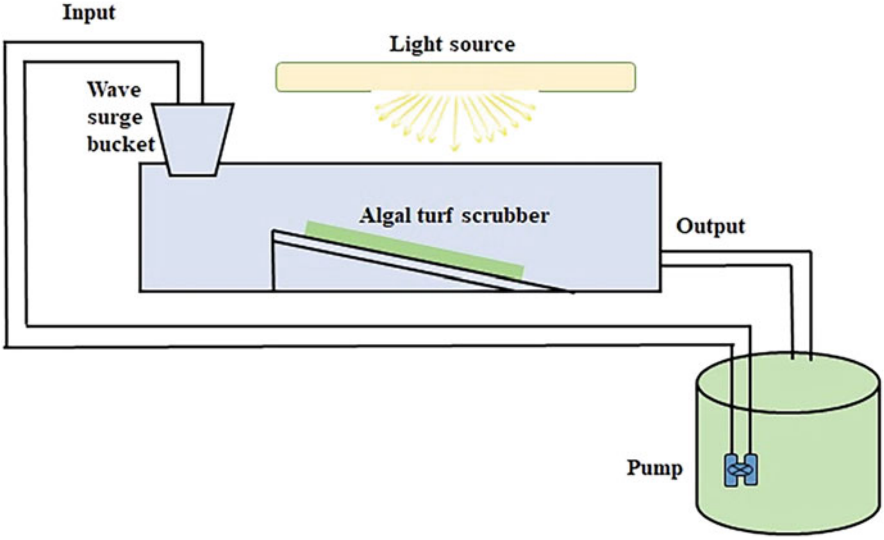

Fig. 6.3 A schematic diagram of algal turf scrubber

6.4.4 Hybrid System

The hybrid cultivation system involves a two-stage process generally integrating the closed photobioreactor and raceway pond, which are relative in production capacity. Hence it involves the features of both an open and closed cultivation system. It combines the benefits of both systems while curtailing some setbacks like the high capital associated with PBR and contamination in raceway ponds. The first stage of microalgae cultivation and biomass production takes place in PBR under sufficient nutrient conditions which is meant to decrease the hazard connected with the contamination of the cultivation by foreign species while maintaining cell multiplication. This is followed by the second stage in open raceway ponds to expose the cells to the stress of the nutrients and for lipid accumulation under nutrient-deficient environments (Fig. 6.4). A hybrid system is considered to be the most effective system for large scale cultivation (Huntley and Redalje 2006). The downstream processes applied include harvesting, centrifugation, drying, cell disruption, extraction and transesterification (Adesanya et al. 2014).

Two-phase hybrid systems are comparatively advantageous as they can separate the resulting algal biomass from the lipid accumulation stage (Schenk et al. 2008). According to a life cycle analysis, there is a minimised environmental impact by hybrid systems when compared with several open and closed systems (Vo et al. 2014). An example is a study conducted by Choi and Lee (2015) integrating the usage of calcined eggshell and microalgae hybrid system as an alternative to the large-scale treatment of effluent waters from acid mine drainage (AMD), and they were successful in removing heavy metals and neutralising the acidity. The average

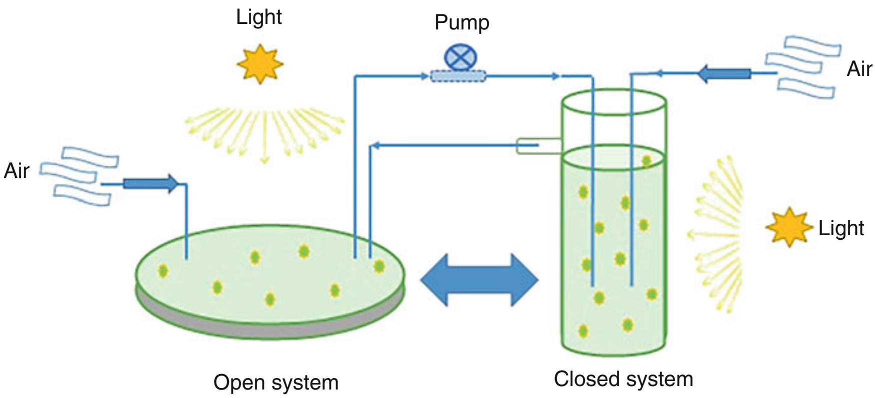

Fig. 6.4 A schematic diagram of the hybrid system

growth rate in a hybrid system was also prominently high when compared to other systems which are accounted to the fact that the biomass growth and lipid induction phases are independent of each other. This factor also helps in reducing contamination. Also, the biomass productivity due to solar irradiance was comparatively higher in hybrid systems, and they are a better candidate for the production of lipid-rich algae (Muthu et al. 2016).

There are many previous kinds of literature and studies mentioning the development of hybrid systems. Demirbas (2010) proposed that the integration of both closed and open systems is a logical solution for cultivating algae for biofuel at a low cost. Brennan and Owende (2010) demonstrated a two-stage hybrid cultivation system. The first stage involved PBR for reducing contamination and promoting active cell division, and the second stage included oil accumulation in open ponds for exposing the cells to nutrient stresses. Huntley and Redalje (2006) were able to successfully utilise a hybrid system for the cultivation of *Haematococcus pluvialis*. The hybrid system was valuable in cultivating *Nannochloropsis* sp. which had a capacity of oil production of 20 tonnes per hectare. But hybrid systems were not always able to combine the positives of both the systems in batch culture. They merely sectioned the cultivation into two stages, leaving behind all the shortcomings unattended.

6.5 Integrated Microalgae Biorefinery to Generate Energy and Essential Chemicals

The inquisitive nature of mankind in the context of energy requirements can be accredited for the development of technologies to generate electricity and fuel on account of industrialisation and economic growth that is driven by scientific progress. Therefore, satisfying energy demand using some of the non-renewable resources has created an alarming situation in the present time. Technologies are now being developed which focus mainly on renewable energy sources in concern of the present scenario of global warming, climate change, natural disaster, ozone depletion, fossil fuel depletion, food and water insecurities, species endangerment, etc. In the wake of these energy catastrophes, biorefinery technologies focusing on the exploitation of microalgae owing to its faster growth and carbon assimilation rate are emerging day by day in an attempt to supply renewable, sustainable, clean and cost-effective energy resources to the growing population (Chandrasekhar et al. 2020; Enamala et al. 2020).

6.5.1 Bioelectricity (MFCs)

Microbial fuel cells (MFCs) are the devices that produce electric power by deploying electrochemically active bacteria (EAB) also termed exoelectrogenic bacteria or electricigens as biocatalysts for the purpose to oxidise organic contaminants to produce electrons, protons and carbon dioxide at anode followed by a flow of electrons and protons through an external circuit and semipermeable membrane, respectively, at the cathode to reduce available oxygen into the water. However, maintenance of aeration or continuous oxygen supply in cathodic chambers is one of the important limitations of MFCs since they raise the overall cost of MFC technology besides accounting for the environmental cost (Ghadir Aly El-Chaghaby et al. 2022). This is responsible for the hindrance of applicability of this technology in the real world. This became the evidence of the potentiality of this promising technology which was a breakthrough moment that suddenly spiked the research work in MFC technology (Rahimnejad et al. 2015; Enamala et al. 2020).

Notably, all the exoelectrogens such as *Geobacter*, *Ochrobactrum pseudogrignonense*, *Pseudomonas*, *Clostridium*, *Shewanella*, *Geothrix*, *Geopsychrobacter*, etc. also play a prominent role in eliminating environmental carbon dioxide metabolically inside the MFC compartments (Jaswal et al. 2020). An organic compound derived from waste sludge serves the additional purpose of oxygen generation by respiring microbes to treat wastewater. This method is energy efficient and widely used in the activated sludge process (ASP). Treatment works through denitrification and reduction of toxic metals such as mercury, iron, copper, etc. and non-metals such as carbon, chlorine, sulfur and recalcitrant compounds followed by lowering of chemical oxygen demand (COD) rate. Examples include reducing toxic metals like iron present in wastewater into less toxic forms like ferric and ferrous cathode (Fadzli et al. 2021; Goswami et al. 2021b). The contaminants in the wastewater can also be represented as electron donor or electron acceptor (copper, iron, mercury, nitrate, perchlorate, etc.) which is used up by becoming a part of oxidation and reduction reactions at anode and cathode, respectively, catalysed by microbes through a process referred as bioremediation or phytoremediation in MFCs (Ucar et al. 2017; Chandrasekhar et al. 2018).

Table 6.2 Comparison of anodic chamber and cathodic chamber of MFC device

Anodic chamber	Cathodic chamber
The electrode is composed of different forms of carbon such as carbon paper, carbon cloth, carbon plate, etc.	The electrode is composed of platinum (Pt) or Pt black catalyst
Consists of analyte substrate or feed	Consists of catholyte
Bacteria feed on biomass and produce hydronium ions and electrons as a result of the oxidation of an organic substrate	Water is formed as a result of reduction of oxygen while combination reaction of electrons and hydronium ions
Anaerobic condition is maintained	Aerobic condition is maintained
Oxygen is kept separated to prevent harming the bacteria by their reaction with electrons and hydronium ions without them entering into circuits	Oxygen is continuously supplied for the further reaction of electrons and hydronium ions

Photosynthetic microbial fuel cells (PMFCs) are another term for MFCs integrated with photoautotrophic organisms such as certain bacteria, plants and algae located in a cathodic chamber for oxygen production during photosynthesis. Other types than PMFCs are dependent on artificial oxygen either supplied by water bubbling despite high energy consumption or directly from the air but only possible by the use of air-cathode in association with expensive catalysts. Both methods of oxygen supply establish concern regarding poor oxygen reduction ability on the cathode on account of poor contact of oxygen with the electrode and cost (Chandrasekhar and Ahn 2017). Table 6.2 represents a comparative analysis of PMFCs that also exists which only make use of photosynthetic bacteria for harnessing solar energy (Jaswal et al. 2020). MFCs also function with another approach for bioelectricity generation by deploying two types of bacteria, i.e. photoautotrophic and heterotrophic, together to exploit the benefits of the synergistic relationship between them. MFCs with this idea produce organic material or substrates by autotrophic bacteria photosynthetically which is subsequently made available to heterotrophic bacteria in the device itself. Apart from this, MFCs have also been operated using mixed microbial culture from domestic wastewater, brewery wastewater, natural microbial community, marine sediments and lake sediments (Tharali et al. 2016).

Apart from previously discussed models of MFCs, the recently developed MFCs technology called sediment microbial fuel cell (SMFC) is based on electrode simulation succession and possesses huge potential in solving environmental issues apart from power generation as they are handled in a large open environment. Yang and Chen (2021) studied SMFC and found it to be promising based on their ability of biodegradation and detoxification of waste material while also producing electricity. But as far as gaps are concerned, the mechanism and functions behind SMFCs are still needed to be explored. Moreover, SMFCs also seem promising in helping to explore more about ecology and other microbiological studies contemporarily. The fundamental design of MFC consists of a dual-chamber system equipped with a semipermeable membrane or proton exchange membrane (PEM) also known as polymer electrolyte membrane deploying mostly “Nafion” to separate anode and cathode chamber (da Costa 2018; Koók et al. 2021). The MFC chambers are composed of glasses as well as plexiglass and polycarbonate. Anode electrode is composed of carbon paper, carbon cloth, carbon fibre, graphite and graphite felt, while cathode either makes use of plain or platinum (Pt)-coated carbon electrodes immersed in water or ferricyanide that uses dissolved oxygen as electron acceptors (Tharali et al. 2016). However, catholyte like ferricyanide ([Fe(CN6)]3−) or permanganate (MnO4−) solutions that work effectively are not sustainable. Hence, there is the need to innovate better catholyte for ion exchange membrane or to upgrade the MFC technology to deal with such issues. Intriguingly, Tharali et al. (2016) also exhibited production of the power density of 2300 mW/m^2 which was almost 1.7 times greater comparatively than Pt/C cathodes just by plating the Pt/C cathodes with ionothermal carbon aerogel doped by nitrogen. Carbon electrodes are preferred over non-organic electrodes because they possess favourable characteristics such as chemical stability, non-toxicity, toughness, electrical conductivity, surface area specificity, corrosion resistance, durability, etc. while maintaining compatibility with microbial flora. Organic material such as leaves, corn stalks, grass, straw, manure, fruit, peels, vegetable waste, etc. is supplied to active microorganisms residing within the anodic chamber to undergo metabolic oxidation to subsequently release electrons and protons at the anode in anaerobic conditions (Fadzli et al. 2021).

The naturally organic compound produced by microbes during minerals reduction/oxidation responsible for extracellular electron transfer (EET) is also known as mediators. However, synthetic mediator such as naphthoquinone derivatives, ferricyanide and viologens is not in much demand due to a few demerits like toxicity, poor compatibility with PMFC, etc. Interestingly, besides these, contaminants from wastewater also perform the role of mediator in some rare situations (Ucar et al. 2017).

Electron mediators or comparatively more sustainable and potential shuttles facilitate the transportation of electrons to anode surface by different means such as bacterial nanowires or pili (Guang et al. 2020). Thus, electrons are transferred to the cathode via an external electric circuit for generating the current with the efficiency rate depending upon the biocompound synthesised by particular microorganisms during substrate reduction. For example, flavins secreted by *Shewanella oneidensis* MR-1 during substrate reduction were responsible for 80% (EET). This rate of efficiency is denoted by different terminologies such as Faraday efficiency, faradaic yield, faradaic efficiency, coulombic efficiency or current efficiency (Jaswal et al. 2020). Moreover, the role of mediators other than flavin such as cytochrome bd quinol oxidase secreted during reactions in increasing the rate of extracellular transfer of electrons (EET) from the cathode towards the acceptor at the anode by overcoming the resistance leading to elevated power density has also been reported in studies (Pankratova et al. 2018; Heydorn et al. 2020). However, the further challenge that lies in research is targeting the different kinds of naturally occurring mediators and also identifying the principle behind the improved rate of conduction of electrons in assistance of such mediators.

Apart from this, protons are also transported to the cathodic compartment facilitated by PEM. The simultaneous reaction of electrons and protons both brought from the anodic chamber reduces supplied oxygen to produce water in the cathodic chamber. Notably, biocatalyst or bacteria should be devoid of oxygen at the anode as it would interfere in electricity generation by reacting with them, and hence that MFCs are purposely designed with layers of glass wool and glass bead along with a facility of repetitive argon gas supply to separate oxygen from the bacterial region during feeding each time so that anaerobic condition can be maintained (Passos et al. 2016). In conclusion, future prospects of MFCs lie in finding the potential and nature of unexplored electricigens and upgrading the MFC technology with improved power density, Faraday efficiency, to maximise not only bioelectricity generation but also wastewater treatment rate by lowering chemical oxygen demand (COD).

6.5.2 Bio-Oil

The potentiality of microalgae versus other microbes is now being acknowledged as an economical, renewable, sustainable and pollution-free energy source that can be harvested daily for biofuel production in the present time. Microalgae for fractionation and biofuel production can produce ten times increased oil per acre compared to traditional biofuel crops. However, some of the developments made to extract liquid fuels from algae are still lagging in terms of expenditure to meet commercial demand. But progress in biofuel technology is displaying its future potential in solving the bottlenecks for large-scale production throughout downstream processing. Extraction is employed to crude algal oil, while biocrude oil is recovered via two steps methods that are hydrothermal liquefaction technology of microalgae followed by catalytic removal of heteroatoms (N, S, O) because algal species are rich in proteins (Mehariya et al. 2021).

Greater lipid content in microalgae such as *Schizochytrium limacinum* biomass favours them to stand over macroalgae for greater crude bio-oil production. In addition to factors like type, species and geographical location of algae, the composition of the bio-feed used is also responsible for determining the qualitative and quantitative parameters of bio-oil production. According to Biller et al. (2015), lipids were more responsible followed by protein and then carbohydrates in determining the yield of biofuel. Moreover, in addition to previously mentioned, algae own another characteristic on account of which the infrastructural expense for dealing the heat loss is budgeted (Xu et al. 2018). Microalgae specifically have greater water retention capacity compared to macroalgae as the latter contain higher carbohydrate content. Treatment is mandatory without which crude bio-oil cannot be used for the reasons of poor miscibility, poor viscosity, poor stability and large tendency to corrode due to which they can't be transported as fuel.

Hydrotreatment technologies were found to be effective in an attempt to fix the issues such as carbon deposition, limited heat transfer and unstable catalyst and ultimately increase the yield of crude oil besides other techniques such as emulsification, supercritical fluidisation, solvent addition, esterification, steam reforming, hydrocracking and zeolite cracking (Xu et al. 2018). Hydrothermal liquefaction is accredited for maximum bio-oil production rich in oxygen and nitrogen based on its ability to decompose proteins as well as carbohydrates besides just lipids as done in other extraction methods (Usami et al. 2020). Requirement of water in sub- or supercritical temperature (280–380°C) and pressure range of 7–30 MPa are maintained throughout the conversion of biomass into crude oil/bio-oil in hydrothermal liquefaction (Kiran Kumar et al. 2018).

Hydrotreatment is based on adding hydrogen to pyrolysis oil in a process called catalytic hydrogenation reaction followed by reduction of O, N and S as H_20, NH_3 and H_2S in the presence of a heterogeneous catalyst such as Ni/ZrO_2, etc. (Doukeh et al. 2021; Xu et al. 2018). Furthermore, ethanol, methanol and water are also added as oxygen-rich solvents are segregated in the latter procedure from fractionated bio-oil so that decomposition or reaction can be prevented, adding to the expense. Apart from this, the replacement of previously discussed solvents with tetralin is also promising in enhancing the yield of bio-oil by dealing with issues such as oxygen and carbon deposition within the solvents. Treatment of crude bio-oil by tetralin as an external source of hydrogen converted into naphthalene for 2 h with 10% weight reduction favoured lowering the H/C molar ratio of upgraded bio-oil at the temperature of 400°C (Xu et al. 2018). Hence, exploring the external source of hydrogen is still a challenge to compensate for hydrogen loss from crude bio-oil during hydrothermal liquefaction to produce hydrogen-rich upgraded bio-oil. Research is also required to understand the mechanism of external hydrogen in controlling the coke formation as well as estimating the yield of different microalgae under identical circumstances in hydrothermal liquefaction through experimentation.

6.5.3 Biohydrogen

The hydrogen produced utilising biomass is called biohydrogen. Biophotolysis, also known as water splitting photosynthesis, is the method to produce hydrogen and oxygen through the splitting of water as a result of the conversion of photonic energy into chemical energy assisted by microalgae through photosynthesis. Table 6.3 enlists a few successfully explored microalgae capable to produce biohydrogen in different culture conditions and carbon sources.

Biohydrogen as a cheap and clean energy source is an appealing option on account of depleting natural resources and global warming (Singh et al. 2020). The role of oxygen scavengers, nanoparticles and purple light sources is to maximise biohydrogen production while blue light for faster algal cultivation is well (Paramesh and Chandrasekhar 2020; Shanmugam et al. 2020; Ruiz-Marin et al. 2020). However, further experiments also need to be conducted in a consortium of diversified optimum conditions to conclude the best one to scale biohydrogen production at a commercial scale. Furthermore, the present study also focuses on the incorporation of genetically engineered microalgae for enhanced biohydrogen

Table 6.3 Biohydrogen productivity by different microalgae in different conditions

Microalgae	Carbon source	Conditions			H_2 rate (ml L^{-1} h^{-1})	Reference
		pH	Temp. (°C)	L.I. (μE/m^2/s)		
C. vulgaris	10 g /L glucose + $MgCl_2$ in sulfate-deprived media	7.5	30	140	60.4	Ruiz-Marin et al. (2020)
S. obliquus	10 g /L glucose + $MgCl_2$ in sulfate-deprived media	7.5	30	140	128	Ruiz-Marin et al. (2020)
Chlamydomonas reinhardtii UTEX 90	0.77 g starch/L + TAP media+2% CO_2	_	25	8	37.1	Kim et al. (2006)
C. Butyricum	0.77 g starch/L + TAP media+2% CO_2	_	30	8	5.72	Kim et al. (2006)
R. sphaeroides KD 131	0.77 g starch/L + TAP media+2% CO_2	_	30	8	5.72	Kim et al. (2006)
Tetraspora sp. CU255	4% (w/v) alginate matrix +0.125 mg DW mL^{-1} alginate + aerobic sulfur-deprived TAP medium	_	36	29	12.8 ± 0	Maswanna et al. (2020)

production by improving the rate of oxygen tolerance, increasing fermentative electron flow and elevating hydrogenase activity as well as microalgal immobilisation. For assistance, Lin et al. (2013) increased biohydrogen production up to nine times followed by knocking out of the PsbO gene linked with an oxygen-evolving complex of photosystem ll. Besides this, Xu et al. (2018) also reported three-time greater biohydrogen production as a result of higher oxygen tolerance under incorporating *E. coli* genes, i.e. catalase and pyruvate oxidase, into the genome of *Chlamydomonas reinhardtii.*

6.5.4 *Biomethane*

The conditioned or cleaned form of biogas free from non-metallic elements or other impurities such as H_2O, CO_2, H_2S or trace elements is termed as biomethane or renewable natural gas (RNG). The production of biomethane is based on a process of production of a sustainable, renewable and cleaner source of energy out of lignocellulosic biomass through anaerobic digestion, decomposition, gasification or other biogeochemical processes within biogas digester. Nevertheless, lignocellulosic recalcitrance (lignocellulose ability to prevent plant cell walls to be degraded or attacked by microorganisms), inhibitors and biochemical composition of lignocellulosic waste have been reported to affect the process of hydrolysis of microbes subsequently affecting bioconversion efficiency in producing biogas from organic (Ghimire et al. 2021). This is in the account of making structural arrangements like a covalent bond or sometimes hydrophobic bond by hemicellulose and lignin, respectively, with the cell wall. Besides this, a few other recalcitrant affecting biomethane production are minerals, pectin, protein cell wall, lipids, etc.

The biomethane as a fuel with the potential to substitute compressed natural gas (CNG) and liquefied natural gas (LNG) in the form of bio-CNG or bio-LNG, respectively, can be liquefied, transported and stored in the form of liquefied biomethane (LBM) for future purposes. Biomethane utilisation leading to the elimination of greenhouse emissions is one of the main benefits of biomethane. Furthermore, apart from their use as fuel to run vehicles, biomethane can also be used as a source of thermal energy for heating, cooking or other household or industrial purposes which can be stored also by virtue of poor ability to corrode compared to biogas (Koonaphapdeelert et al. 2020). In fact, several countries like Sweden and Italy have already accustomed to the use of biomethane for running their automobiles plus preparation also being done to integrate them with other infrastructure for their applications in daily life (Backman and Rogulska 2016).

Recalcitrance can be dealt with by different pretreatment methods such as physical treatment, chemical treatment, biological treatment, cell wall modification and employment of rDNA technology. Physical methods incorporate techniques like irradiance, comminution, liquid hot water treatment, steam explosion, etc. excluding microorganisms or chemicals, while chemical methods unlike physical methods are based on incorporating chemicals. These methods work by reduction of particle size, crystallinity, degree of polymerisation of cellulose to increase surface accessibility and digestibility by manipulations of their ultrastructure which will ultimately increase the biomethane yield. However, the particle size of the hemicellulosic substrate is excessively reduced, contributing to excessive production of inhibitors (volatile fatty acid) which is another factor responsible for reducing the biogas production (Hashemi et al. 2021).

6.5.5 Biochar

Biochar is characterised as a source of bioenergy in the form of organic matter processed through combustion in anaerobic conditions possessing physical and chemical features such as highly porous, higher surface area accounting for greater absorbency, greater charge density, greater water-holding ability and enriched with carbon. The process also termed pyrolysis employed for the formation of biochar has the potential to enhance the fertility of the soil by conditioning their biological, mechanical and physico-chemical characteristics such as cation exchange capacity, hydraulic conductivity, aeration capacity, porosity and structure. With this, biochar also plays a prominent role in combating global warming by lowering the emission of greenhouse gases such as nitrous oxide or methane followed by sequestration of carbon available in the soil. Biochar has tremendous commercial value because of its role in industries concerned with textile, fertiliser, food, cosmetics, wellness and waste management areas (Narzari et al. 2015; Çay et al. 2020).

The organic matter deployed as feedstock exists in different varieties. For instance, crop residues, tree clippings, sludges, poultry litter, lawn grass cuttings, vermicompost, leaves, wood products and other kinds of organic waste such as kitchen waste are utilised preferably in dried conditions to save energy required in pyrolysis. Also, organic source requirements may encourage deforestation, and hence emphasising the use of organic waste for biochar production rather than from forests is one of the challenges associated with biochar besides other challenges such as inspecting its durability and long-term impact on the soil ecosystem. Besides this, such soil is enriched with nutrients favoured by geochemical processes activated by biochar. However, these benefits are not stable and are greatly influenced by the kind of feedstock, temperature and pyrolytic conditions at which biochar was manufactured. Moreover, biochar also can decline these benefits with age. The increase in temperature is directly proportional to the increase in stability and aromaticity of biochar and inversely proportional to the yield of biochar. However, the favourable temperature for maximum yield of biochar varies between 350 °C and 800 °C, and this type of pyrolysis incorporated with low to moderate temperature and long residence time is addressed as slow pyrolysis (Siddiqui et al. 2016). Slow pyrolysis not only produces maximum biochar by retaining 50% carbon content from feedstock but also produces a wide range of important chemicals and gases such as coke, polyvinyl chloride ethylene dichloride, methanol, activated carbon and charcoal, syngas, etc. (Narzari et al. 2015). However, the other type, fast pyrolysis, often leads to gases and liquid products such as bio-oil production in anaerobic conditions and is often associated with moderate temperature and short residence time, which poses negative effects on the yield of biochar and contributes to the production of gaseous and liquid products.

6.6 Conclusion

The global expansion of energy needs has grown exponentially in the recent past, contributing enormously to economic growth while also creating serious environmental damage. The wastewater released from industrial, municipal, piggery, dairy, brewing applications, etc. involving physical, chemical and biological processes resulted in polluted water bodies. The natural algal flora in the waterbodies is capable to tolerate water toxicity of waste like antibiotics, steroids, reproductive hormones, analgesics, β-lactamines, antidepressants and detergents, as well as unspent solvent and heavy metals. This chapter highlights different cultivation types to upscale microalgae production to reduce the toxicity of wastewaters and save the environment from negative mutagenic consequences. Because selected microalgae can efficiently use wastewater as a growth substrate for valuable bioenergy and essential chemical generation, that will sustainably benefit human lives.

Competing Interests All the authors declare that they have no competing interests.

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Chapter 7
Heterotrophic Microalgal Production System Via Utilization of Wastewater in Microalgal Production

K. Nayana and K. Arunkumar

Abstract Several microalgal species have the ability to grow heterotrophically, exhibiting metabolic variety and adaptability. Compared to phototrophic circumstances, up to a 25 times increase in biomass concentration can be achieved under heterotrophic conditions, which has been shown in several studies. This benefit has resulted in the extensive demand for algae in waste bioremediation, for cleaner waters and valuable biomass that may be used as a feedstock for a range of valuable items, including food, feed, fertiliser, pharmaceuticals, and, more recently, biofuel. In order to make heterotrophic cultivation economically viable for high-volume, low-value commodities like biofuels, unconventional carbon sources such as food and agricultural waste and wastewater are advised. Because microalgae may change their metabolism in response to changing culture conditions, it is feasible to control and maximise the synthesis of desired chemicals. The current chapter summarises the most recent research on microalgae production systems that use carbon rather than light as an energy source.

Keywords Microalgae · Waste nutrients · Heterotrophic growth · Biomass · Biofuel

Abbreviations

BOD	biological oxygen demand
COD	chemical oxygen demand
PUFA	polyunsaturated fatty acids
TN	total nitrogen
TP	total phosphorous
TAG	triacylglycerol
HM	heavy metal
LED	light-emitting diode

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7.1 Introduction

Algae are natural sources of food and energy, as well as water refiners or purifiers and long-term answers to the world's climatic problems. Algae serve various purposes, including fuel production, CO_2 recycling, and animal and human food and feed. During space travel, algae serve as photosynthetic gas exchangers (Spolaore et al. 2006). In an integrated approach, they can create important pigments, carbohydrates, vitamins, lipids, proteins, and other useful substances, making them promising for current and future feedstocks and medicinal uses (Markou and Nerantzis 2013; Mehariya et al. 2021).

Growing microalgae does not necessitate the use of arable land. Furthermore, they have been demonstrated to survive in various wastewaters (Koller et al. 2014; Goswami et al. 2020). Microalgae have piqued the curiosity of researchers because they can generate at high rates all year and efficiently extract nutrients and HM from wastewater and use significant volumes of CO_2 simultaneously (Singh and Gu 2010; Goswami et al. 2021a). In the United States, wastewater quality enhancement technology was applied to produce methane from algal waste biomass (Pulz and Scheibenbogen 1998).

Due to its applicability and the ability to tolerate a wide range of nutrition sources, microalgal culture on wastewater effluents has gotten a lot of interest in recent years (Abdel-Raouf et al. 2012; Goswami et al. 2021b). Many developed and developing countries are working to improve the environmental quality of their water supply, mainly by nitrogen and phosphorus reduction in wastewater discharge. In the last few years, there has been a surge in interest in using mixotrophic microalgae in the treatment of sewage. This is due to their efficiency to consume organic and inorganic carbon, as well as inorganic nitrogen (N) and phosphorous (P) in sewage water for growth, resulting in a reduction in these components in the water. The major purpose of wastewater treatment before it is discharged into receiving systems is to greatly reduce the amount of carbonaceous (organic; typically determined as BOD) materials, as well as nitrogen (N) and phosphorus (P) components (Gray 2004; Grady et al. 2011; Goswami et al. 2021b). This is because large concentrations of these elements may have deleterious effects on dissolved oxygen (O_2) levels, trophic conditions, and, ultimately, aquatic animals and plants health.

The current objective is to transform our surroundings into a fully recyclable society, with trash avoided whenever possible and garbage used as a resource when it cannot be avoided. Ecosystems are impacted by the discharge of waste streams into the environment and natural water bodies, posing major sustainability issues for human society. Waste stream treatment seeks to greatly reduce the quantity of contaminants, such as carbonaceous organic, nitrogen, and phosphorus compounds, before they are discharged into the environment (Grady et al. 2011) as these materials, in high concentrations, can disrupt ecosystems, fauna, and flora, as well as endanger human health. Because autotrophic, mixotrophic, and heterotrophic metabolisms can be seen in microalgae, they are considered hardy and flexible microorganisms. As a result, these microalgae are utilised to treat a wide range of waste sources while also producing high-value products like pigments, proteins, PUFA, biofertilisers, food and feed, and biofuels that are industrially valuable for the production of pharmaceuticals and nutraceutical products and are good alternatives to biorefineries (Goswami et al. 2021d).

Moreover, this method makes use of the whole or portion of the microalgal biomass as well as the numerous products created by the cells, maximising the overall value of the process while also improving the environment (Lopes da Silva et al. 2019; Bhardwaj et al. 2020).

7.2 Microalgal Growth in Wastewater

In addition to being a renewable source of biomass, microalgae in wastewater treatment are a low cost and practicable solution for CO_2 bio-fixation (Almomani et al. 2019; Agrawal et al. 2020). Mixotrophic microalgae's ability to use organic and inorganic carbon, as well as inorganic nitrogen and phosphate, in wastewater to grow, resulting in reduced quantities of these components in the water, is the logic behind their usage to treat wastewater. The generation of O_2 by photosynthesis, which is required for heterotrophic bacteria to biodegrade carbon-rich substances, is the main benefit of using microalgae in sewage treatment. Figure 7.1 depicts microalgal-bacterial symbiosis in wastewater treatment.

7.2.1 Causes and Sources of Wastewater Production

Toilets, showers, kitchens, laundries, factories, and other wastewater sources are the most common. Every day, domestic families produce 200–300 L of wastewater per

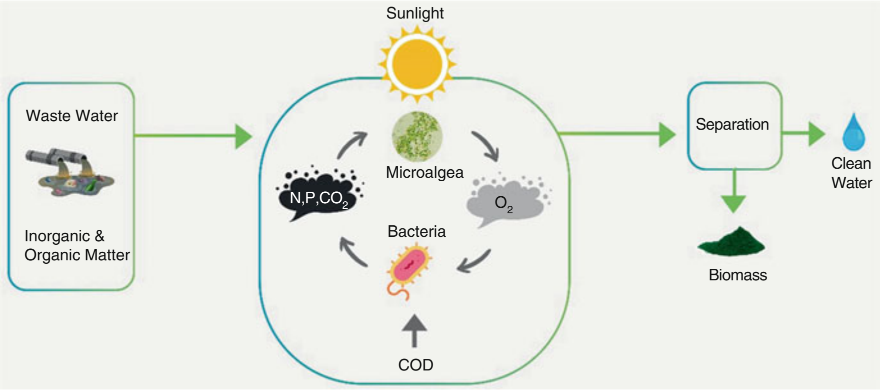

Fig. 7.1 Microalgal-bacterial association in wastewater treatment

person. In India, water contamination is the major cause of death, with a mean of around 580 people dying each year from cholera, dysentery, jaundice, typhoid, malaria, diarrhoea, and other diseases. Water contamination is a problem in developed countries, just like it is in developing countries. Due to rural expansion, the rate of waste discharge from provided water canal is increasing year after year. The same thing happens in cities due to a lack of attention to sewage channel construction or renovation, resulting in water pollution issues (Abdel-Raouf et al. 2012; Goswami et al. 2021c). A tiny leak in the sewage channels contaminates groundwater, making it unfit for human consumption and providing breeding habitat for numerous insects, including mosquitoes. Domestic trash, both treated and untreated, is also polluting farm drains. Agricultural activities such as watering, fertiliser usage, and application of several pesticides and insecticides consume the most water, with agricultural leftovers discharged into the environment as a substantial cause of water pollution for the ecosystem and aquatic life. Modern urbanisation causes water pollution due to fertiliser application, deforestation, soil erosion, man-made activities, ineffective waste management and treatment, landfills as more garbage is created, and so on. During the mining process, the release of some harmful toxic chemicals, metal debris, and sulphides from the rocks acts as a substantial source of water pollution, posing serious health risks. Oil spills caused by oil-carrying ships cause a vast number of hazardous pollutants to contaminate the sea and harm the marine community. The majority of deaths among aquatic animals and marine species are caused by water pollution caused by rising water temperatures due to global warming. The inappropriate disposal of nuclear waste created by radioactive material poses significant environmental risks.

7.2.2 Percentage of Nutrients in Wastewaters

Wastewater is made up of a complex combination of organic, inorganic, and synthetic components. Proteins, carbohydrates, lipids, amino acids, and volatile acids account for three-fourths of the organic carbon in sewage. Calcium, sulphur, sodium, magnesium, potassium, chlorine, phosphate, ammonium, bicarbonate, and HM are among the inorganic components (Lim et al. 2010). Microalgae can effectively treat wastewater from a variety of sources (municipal, agricultural, and industrial). Table 7.1 shows the usual N:P characteristics of various wastewaters.

There are many species in wastewater, both macro- and microscopic. The treatment need is determined by the number of any of these organisms in a wastewater body. Wastewater provides an ideal environment for microbial growth (Hu et al. 2012).

Table 7.1 Comparison of TN, TP, and some metal concentrations present in the wastewaters

Properties (mg/L)	Municipal wastewater	Concentrated municipal wastewater	Anaerobic digestion wastewater	Piggery wastewater
TN	27	56	537.26–702.73	2055
TP	5.04	15.8	72.62–111.58	620
Magnesium	0.088	16.5	23.83–58.26	213
Manganese	0.09	0.4	0.96–1.91	4.1
Potassium	20	45.7	22.38–68.15	2524
COD	31	–	1572.45–2265.37	37,643
Iron	0.12	0.05	6.83–15.35	169.2

Adapted from Salama et al. (2017).

7.2.3 Role of Microalgae as Wastewater Remediator

Research has looked into the biological removal of carbonaceous, nitrogenous, and phosphorus substances from wastewater effluents using microalgae. This has been done with a variety of microalgae on a variety of sewage water sources, including municipal, domestic, agricultural, brewery, refinery, and industrial effluents, with different treatment efficiency and microalgal population (Chiu et al. 2015). Microalgae play an important part in the treatment of household wastewater in ponds, as well as the treatment of different scale municipal sewage water in aerobic ponds (Abeliovich 1986; Oswald 1988). They improve nutrient, heavy metal, and pathogen elimination and deliver O_2 to heterotrophic aerobic bacteria for organic pollutant mineralisation, using CO_2 produced from respiration in the process (Munoz and Guieysse 2006).

Spirogyra has been found to effectively absorb radiophosphorus from wastewater (Mackenthum 1969). Several environmental conditions have a significant impact on algal cultivation. The efficiency of metal removal varies with algae species. According to several researches, individual algal species such as *Oscillatoria*, *Chlorella vulgaris*, *Chlamydomonas*, and *Scenedesmus chlorelloides* can draw out chromium, cadmium and copper, zinc, and molybdenum, respectively, from wastewater (Nakajima et al. 1981; Sen et al. 2013).

Scenedesmus obliquus was reported to effectively extract nutrients (carbon, nitrogen, and phosphorus) wastewater from piggery (Ji et al. 2013), while *C. pyrenoidosa* thrived well in water effluent discharged from dairy farms (Kothari et al. 2012). Other *Chlorella* species, such as *C. vulgaris*, have been identified to be good species for removing nitrogen, phosphorus, etc. from urban effluent (Gouveia et al. 2016). *Chlorella vulgaris* removed almost 88% BOD, 82% TN, and 54% TP from brewery effluent. *Chlamydomonas* sp., *Nanochloropsis* sp., *Dunaliella* sp., *Spirulina* sp., and *Botryococcus* sp. were among the microalgae species studied for their bioremediation capabilities (Cuellar-Bermudez et al. 2015).

7.2.4 Nutritional Mode and Factors Affecting Wastewater Growth

Microalgae can thrive in various wastewaters, including urban and industrial wastewaters, and agricultural leftovers (Bajhaiya et al. 2010). Microalgae can thrive in three different nutritional modes that rely on carbon uptake for biomass synthesis, depending on the species and environment: autotrophic, heterotrophic, and mixotrophic (Mata et al. 2010). Heterotrophic and mixotrophic modes grew at a somewhat faster rate than autotrophic modes. The nutritional mechanism differs from one species to the next. The autotrophic form of nutrition does not necessitate using a carbon source as a substrate, which relies on photosynthetically fixed sugars, whereas *C. protothecoides*, *Crypthecodinium cohnii*, and *Schizochytrium limacinum* all rely on an exogenously present carbon substrate. *Chlamydomonas reinhardtii* and *Chlorella vulgaris* are examples of mixotrophic nutrition, which combines autotrophic (photosynthesis) with heterotrophic (carbon substrate) feeding. Multiple metabolic pathways connected to this form of nutrition can be found in microalgae (Perez-Garcia et al. 2011). Algal biomass has a stoichiometric formula of $C_{106}H_{181}O_{45}N_{16}P$, with carbon (C) accounting for more than half of the total mass (Oswald 1988). Algae absorb CO_2 from the atmosphere and convert it to starch and lipids during photosynthesis. Aeration can be utilised to provide atmospheric CO_2 for experimental or industrial-scale algae cultivation. Algae can absorb organic carbon molecules like glucose, glycerol, etc. during heterotrophic growth. Nitrogen is the second most important macronutrient for algae growth. Ionic forms of nitrogen include NH_4^+, NO_3, NO_2, and others found in microalgae. Algae prefer to assimilate ammonium (NH_4^+) over other nitrogen sources, such as NO_3 and NO_2 (Bhaya et al. 2000). Phosphorus is a key macronutrient for algae, which prefers to absorb it in the form of inorganic orthophosphate (PO_4). Yan et al. found that depending on the wavelengths and intensities of the LED light, the performance and development of *Chlorella vulgaris* in synthetic wastewater changed dramatically. They also discovered that light intensity has a vital effect on microalgae growth, but only when the light intensity is optimal (Yan et al. 2013). The temperature between 20 °C and 40 °C was used to determine the best temperature for the microalga *Chlorella sorokiniana*'s maximum growth, and the favourable temperature was found to be around 38 °C. At 40 °C, however, growth was reported to be drastically reduced (Ugwu et al. 2007). Thus, physical and chemical parameters such as specific pH, the intensity of light, photoperiod, temperature, and biological elements affect the algae's nutrient removal efficiency in addition to the availability of nutrients (Delgadillo-Mirquez et al. 2016). In addition, biological factors like microbial communities and predation may play a role in open pond cultivation conditions (Fouilland 2012).

7.3 Effect of Waste Effluents on Microalgal Growth

Microalgae are remarkable in that they can photosynthesise like plants and use nutrients (nitrogen, phosphorus, organic, and inorganic carbon substrate) from wastewater while sequestering CO_2 and producing biomass for biofuel feedstock. Microalgal biochemical composition is influenced by environmental factors such as temperature, carbon availability, macronutrient, and micronutrient accessibility. As a result, the mode of cultivation, medium composition, and nutrient profile all significantly impact microalgae productivity and biochemical composition. Figure 7.2 indicates the advantage of using wastewater fertilisers to generate biomass of microalgae.

Some of the elements that influence the features and predominance of microalgae are wastewater properties and seasonal environmental conditions. Many study investigations have demonstrated that species of *Scenedesmus*, *Chlorella*, and *Chlamydomonas* effectively removed nitrates and phosphates in varying ranges from diverse wastewaters. Heavy metals and toxic chemicals are quickly eliminated by microalgae (Shao et al. 2018; Prakash et al. 2021). Table 7.2 shows some microalgae species growing in different waste nutrients.

Growing microalgae in waste nutrients is a green and long-term solution that removes or treats hazardous wastewater and produces a variety of valuable compounds that can be used in a variety of applications. Phytoremediation entails nutrient removal from many types of wastewater, toxic chemicals, and nutrient biosorbents via algae, CO_2 sequestration, and hazardous compound detection via biosensors based on microalgal (Lavoie and De la Noüe 1985).

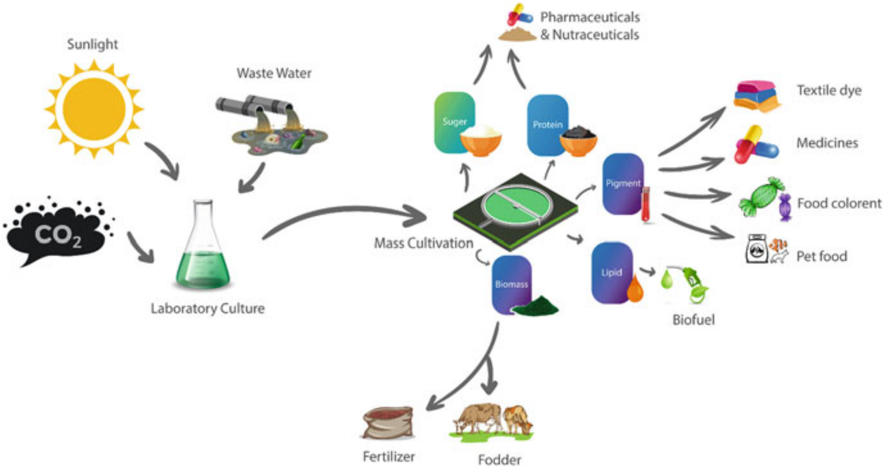

Fig. 7.2 Microalgal biomass and bioproducts generation using wastewater nutrients

Table 7.2 Some microalgal strains growing in different waste nutrients

Microalgae	Wastewater resource	Reference
Chlorella pyrenoidosa	Anaerobic-digested starch wastewater	Yang et al. (2015)
Chlorella vulgaris	Saline wastewater	Shen et al. (2015)
Scenedesmus obliquus	Brewery effluent	Mata et al. (2012)
Micractinium inermum NLP-F014	Domestic wastewater	Park et al. (2015)
Neochloris sp.	Dyeing industry effluent	Gopalakrishnan and Ramamurthy (2014)
Chlorococcum sp. RAP-13	Dairy wastewater	Ummalyma and Sukumaran (2014)
Chlamydomonas reinhardtii	Different stage wastewater	Kong et al. (2010)
Botryococcus braunii	Urban wastewater	Orpez et al. (2009)

7.3.1 Effect on Microalgal Biomass Production

Many microalgae species show promise for cultivation on a vast scale; however, there is a scarcity of data on industrial-scale biomass production and sewage water processing trials. Temperatures should be kept between 20 °C and 30°C throughout cultivation to ensure optimal microalgae development. In addition, microalgal development necessitates a large amount of various nutrients. Thus, industrial wastewater can be used to supplement nutrients (Abdel-Raouf et al. 2012). Microalgal growth is usually enhanced when the temperature rises to a certain point. Because of the fluctuating pH values in industrial effluent, direct production of microalgae is problematic. Despite this, most microalgal species can survive a wide range of pH tolerance levels (Zhu 2015). The majority of nitrogen in household wastewaters is NH4+, ammonium with nitrite, and nitrate in trace amounts. Because assimilation of NH4+ requires smaller amounts of energy than the transformation of nitrates and nitrites into structural nitrogen, this characteristic favours nitrogen intake by microalgae (Cai et al. 2013). Domestic wastewaters, on the other hand, have a carbon-nitrogen ratio (3.5:1) and a carbon-phosphorous ratio (20:1) that are too low for microalgae growth (carbon/nitrogen/phosphorous of 100:18:2) (Posadas et al. 2014). Carbon constraint occurs frequently during domestic sewage treatment using microalgae, reducing the rate of microalgal growth and, as a result, the efficiency of microalgae nutrient removal (Arbib et al. 2013). CO_2 supplementation (from biogas or flue gas) has been shown in recent research to boost the obtainability of carbon during wastewater processing, enhancing the yield of biomass (and assimilation of nutrients) while reducing a potential greenhouse gas emission.

7.3.2 Effect on Pigment Accumulation

Aki et al. in 2003 reported microalgae as a good source of astaxanthin pigment. Other pigments like lutein, phycocyanin, and phycoerythrin were also extracted from microalgal species (Aki et al. 2003; Fernandez-Sevilla et al. 2010; Khajepour et al. 2015). Microalgae produced in wastewater can also be utilised to make pigments, although one issue is the likelihood of the presence of hazardous metals and other contaminants in the biomass. Natural colourants derived from algal biomass, on the other hand, are a secure and effective substitute (Dufosse et al. 2005). Rodrigues et al. (2014) found that when agricultural and industrial wastes are used as a growth medium, *Phormidium autumnale* produces a high concentration of carotenoids, including all-trans-carotene (70.22 g/g), all-trans-zeaxanthin (26.25 g/ g), and all-trans-lutein (21.92 g/g). *Scenedesmus* sp., a microalga, can take nutrients from fermented swine effluent and produce roughly 2–3 times more lutein and astaxanthin than a control culture (Kim et al. 2007).

7.3.3 Effect on Lipid Production

Because microalgae typically contain a high proportion of high-value-added lipids like polyunsaturated fatty acids (PUFA) and a high number of intracellular oils, but a small concentration of protein and carbohydrate, lipids have been the most often recovered compounds from heterotrophic microalgal biomass thus far (Sajadian et al. 2018). The heterotrophic microalgal lipid concentration ranged from 9% to 69% depending on the species, growing circumstances, and media. *Chlorella* sp., ZTY4, is one of the microalgae that has shown heterotrophic growth on household waste and has the maximum lipid content (79.2%) (Zhang et al. 2013).

Neutral lipids, polar lipids, and sterols are all examples of microalgal lipids. There are two types of lipids produced by microalgae: structural and storage lipids. Under varied stress circumstances, as a storage lipid, microalgae can produce huge volumes of lipids. Lipids play different physiological roles within microalgal species, including membrane support for structural maintenance and signalling chemicals. Storage lipids are predominantly made up of glycerol esters of fatty acids or TAGs, as opposed to structural and signalling lipids (Yu et al. 2011; Chaturvedi et al. 2020).

7.4 Microalgal Biomass Applications

Microalgae are unique in that they may create a wide range of high-value products for a variety of applications, including medicines, nutraceuticals, cosmeceuticals, and food additives. Several heterotrophic microalgae species capable of growing on drainages have the capability to develop commercially essential products. These comprise lipids and pigments, both of which have potential in the pharmaceutical, nutraceutical, cosmetic, and food sectors (Saini et al. 2021). Furthermore, biodiesel can be made from the saponifiable lipid fraction (triacylglycerols).

7.4.1 Nutraceuticals and Pharmaceuticals

Proteins are one of the most abundant metabolites in microalgae biomass. Their output is influenced by a variety of factors such as species, growth phase, light quality, and environmental stress and dietary stress. *Spirulina* sp., as an example, is reported to produce almost 70% protein, according to the strain (Plaza et al. 2009). Traditional plants and animals commonly raised for sustenance may produce less protein per unit area compared to microalga *Dunaliella*. These proteins are nutraceutically important. In general, algal biomass provides more money through nutraceutical and health-related uses than its biofuel applications. As a result, using wastewater-grown microalgae to produce high-value goods from microalgae is a viable option.

7.4.2 Biofuel

Microalgal biomass has recently been found as a suitable raw material for biofuels such as biodiesel synthesis. Any raw material-to-energy conversion method that exists potentially employ microalgal biomass. Microalgae often contain 20–30% lipids, making them attractive for biodiesel generation (Chisti 2007; Agrawal and Verma 2022). *Nannochloropsis oculata* yields the most bioethanol, 3.68%, when grown in 75% municipal wastewater (Reyimu and Izsimen 2017). *Chlorella* sp. ZTY4 grown in domestic wastewater contains 79.2% lipid (Zhang et al. 2013), and *Micractinium reisseri* grown in municipal wastewater produces 40% lipid (Abou-Shanab et al. 2014).

7.5 Future Prospects

Microalgal cultivation in waste effluents has a number of benefits, which includes the provision of non-lignocellulosic biomasses, which reduces downstream lignin removal process hydrolysis costs and boosts biogas generation in less time. Microalgae biomass can also be made available at any time of year. A cost-effective large-scale microalgal biomass production concept is to combine waste effluent treatment and biomass-enhanced production utilising microalgae. This method of coupling can reduce the cost of culture media, encourage local businesses, and reduce the negative environmental impact. Microalgae use diverse forms of nutritional mode to sustain, such as phototrophic, heterotrophic, and mixotrophic modes, to successfully remove different types of contaminants from industrial effluent. The most promising applications for microalgae biomass include biodiesel and bioethanol in the energy sector, as well as aquaculture feed and biofertiliser. Because of increased urbanisation and industrialisation, scaling up of production of algal biomass utilising waste effluents will be necessary for the upcoming days. An integrated strategy of wastewater treatment and production of biomass utilising microalgae is now one of the most efficient methods for cost-effective and wide-scale biofuel production. However, to increase biomass yield to the desired quality, sophisticated, appropriate farming approaches must be expanded. In the future, the commercialisation of biomass generation utilising industrial effluent will be unavoidable. Researchers will gain a thorough understanding of the productivity of biofuel by identifying ideal wastewater components for lipid synthesis in microalgae. To use highly toxic wastewater, new microalgal species must be identified, cultured, and created using simple variation processes. For the integration of wastewater nutrient removal and biomass production, process parameters must be adjusted. Microalgae are small cell factories that serve as renewable, long-term, and cost-efficient biofuel sources. Microalgae have a lot of potentials when it comes to using wastewater for growth and producing a lot of biomass. Microalgal biomass is a biorefinery that can be used to make biofuels and a variety of value-added products. Microalgae cultivation in waste effluents can help to enhance water quality through a low-cost, environmentally friendly wastewater treatment method.

7.6 Conclusion

Urbanisation causes the massive release of household municipal wastewater, which leads to considerable environmental degradation. Wastewater is a complicated mixture of common organic and inorganic compounds that were released into the environment, resulting in true contamination. Eutrophication is caused by the release of organic molecules, as well as phosphates and nitrates. Growing microalgae can handle this serious problem with these wastes as nutrients. Microalgae (which includes diatoms) are photosynthesis apparatuses that transform carbon dioxide into bioenergy and valuable bioactive. Microalgae are found in a variety of natural environments. While most microalgae are photoautotrophic, some have been found to thrive in mixotrophic or heterotrophic environments.

Heterotrophic microalgae have a number of supremacy over autotrophic microalgae that may thrive on wastes with high COD loads while concurrently producing microalgal biomass, biofuels, and high value-added products including lipids, PUFA, and carotenoids, all of which have distinct commercial applications. Combining waste management with the production of heterotrophic microalgae could reduce overall treatment costs while also benefiting the environment. Microalgae have a wide range of cellular mechanisms that enable them to deal with the toxicity of heavy metals and nutrition loss. They are ideal for wastewater treatment because of their ability to absorb heavy metals. Microalgae are anticipated to be significantly superior to the current physicochemical procedures for heavy metal removal. Heavy metals like cadmium, chromium, mercury, lead, and zinc have been connected to serious health and environmental issues. However, more study is required to increase microalgal biomass and value-added product when wastes are used as a source of nutrients. Biomolecules such as carbohydrates and lipids from microalgal biomass produced in wastewater are better suitable for biofuel feedstock than food or feed. When numerous barriers connected with potentially costly procedures in biofuel production are overcome, microalgal biofuel technology can be upgraded from the laboratory to the pilot scale or commercial scale.

Competing Interests All the authors declare that they have no competing interests.

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Chapter 8
Recent Developments in the Enzymatic and Biocatalytic Pretreatment of Microalgae for Efficient Biofuel Production

Plabita Das, Julie Baruah, and Eeshan Kalita

Abstract The rapid rise in CO_2 emissions owing to fossil fuel combustion and increasing energy demands worldwide has fuelled the research for finding a viable substitute for fossil fuels. Microalgal biofuels, also known as 3G biofuels, hold the promise of being a sustainable yet economical substitute for conventional fossil fuels. The relatively untenable production of biofuels from food crops had its share of twofold complicit concerns regarding food security and economic viability. Microalgae on the other hand are compounded with benefits of CO_2 sequestration and rapid biomass accumulation at low energy inputs, only to be restricted by the scale of biofuel recovery. Pretreatment strategies for microalgae have been the key driver for efficient microalgal biofuel production through mechanical, thermal, and chemical procedures. With the advent of pioneering approaches entailing enzymatic and microbial pretreatments alongside the conventional approaches, productivity has further increased. In this chapter, we summarize the recent developments in the enzymatic and biocatalytic pretreatment methods for improving the yield of valuable products from microalgae. The chapter also discusses the various factors affecting the pretreatment process and provides an understanding of the future perspectives for exploring newer prospects.

Keywords Microalgae · Pretreatment · Enzymatic · Bacterial · Fungal

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Abbreviations

BP	biofuel production
EP	enzymatic pretreatment
BAP	bacterial pretreatment
FP	fungal pretreatment
SRF	soft-rot fungi
WRF	white-rot fungi
BRF	brown-rot fungi
1G	first generation
2G	second generation
3G	third generation
4G	fourth generation

8.1 Introduction

The rapid increase in the use of fossil fuels to meet global energy demands has resulted in an increase in CO_2 emissions since the nineteenth century, resulting in the current climate change catastrophe (Liu et al. 2021). As a result, the research focus over the last few decades has been on developing cost-effective and environmentally benign renewable energy alternatives to fossil fuels in order to avert this problem. Biofuels which are liquid or gaseous fuels derived from biomass have emerged as an efficient and environmentally sustainable alternative in this context (Sadvakasova et al. 2021). Biofuels typically include biogas, bioalcohol, biohydrogen, and biodiesel and are derived from biological sources such as wastes, plants, animals, and microorganisms (Godbole et al. 2021). Given the advancement of biofuel technology over the last few decades, biofuels are classified into four generations (Gs) based on the feedstock from which they are derived (Fig. 8.1) (Paul et al. 2021). The first-generation (1G) biofuels, which used food-based feedstocks such as vegetable oils, animal fats, and energy crops, were associated with the disadvantages of using food crops and emitting greenhouse gases. For this purpose, second-generation (2G) biofuels such as biomethane, biohydrogen, and bioethanol were introduced, which were produced from inedible crops and agricultural wastes (Bhardwaj et al. 2020). However, major constraints for the production of 2G biofuels include the

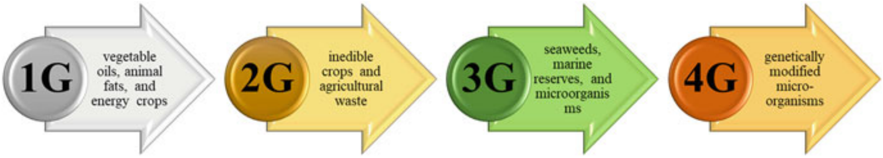

Fig. 8.1 The four generations (Gs) of biofuel with their feedstocks

need for arable land, the use of pesticides, and costly pretreatment workflows that must be supported by an effluent treatment strategy. To further reduce the cost of biofuel production, third-generation (3G) biofuels based on microbes, algae, and marine micro/macroalgae were developed, including bioethanol and biobutanol derived from seaweeds, marine reserves, and microorganisms. Fourth-generation biofuels (4G) emerged as a result of advances in molecular biology and genetic engineering, harnessing genetically modified organisms for increased carbon sequestration and fuel production. These biofuels include solar and electrical fuels generated from genetically engineered photosynthetic bacteria (Paul et al. 2021; Moodley 2021).

Biofuel production (BP) from microorganisms is a very promising potential, with research focusing on bacteria and microalgae containing high quantities of proteins, carbohydrates, or triglycerides to produce biofuels such as biohydrogen, biomethane, biodiesel, and crude bio-oil (Raven et al. 2021; Lin and Lu 2021; Goswami et al. 2020a; Anwar et al. 2019). Various research has also reported the generation of biodiesel via the transesterification process from lipid-rich cyanobacteria, bacteria, microalgae, yeast, and micro-fungi (Sadvakasova et al. 2021; Raven et al. 2021; Kadir et al. 2018). Microalgae are regarded as a promising crude material for BP due to their high carbohydrate and lipid content and decreased lignin content when compared to lignocellulosic feedstocks (Dalena et al. 2019). According to reports, the production of carbohydrates, lipids, and hydrogen by microalgae rises with stress (Nagappan et al. 2019). Furthermore, the cultivation of microalgae with minimal energy inputs and its contribution to reducing competition with agricultural plants as feedstocks increase its utilization as a feedstock for BP (Dalena et al. 2019; Mehariya et al. 2021a). The presence of microalgal oil accounts for nearly half of its biomass, and this high proportion of oil in microalgae contributes to a significant quantity of carbohydrates, proteins, and lipids essential for biofuel production (Raven et al. 2021). Carbohydrate or protein-rich microalgae are utilized in the manufacturing of 3G biofuels, while lipid-rich microalgae are used in the creation of biodiesel (Lin and Lu 2021). Figure 8.2 depicts a brief overview of biofuel production from microalgae. Although microalgae as a source of BP are conferred with numerous advantages, they are not bereft of limitations. Currently, 4G biofuel production is constrained by intensive production costs, low yields of biofuel, and complex upstream and downstream processes (Debnath et al. 2021; Nagappan et al. 2019).

Pretreatment of microalgae whose primary goal is to reduce the energy costs for converting microalgal biomass to biofuel is a critical step in the production of biofuel because it decomposes the complex cell wall resulting in the release of intracellular components and depolymerization of complex sugars into monomers. The limitation of biofuel recovery can be overcome by optimizing the pretreatment procedure for optimal digestion of algal biomass (Costa et al. 2020; Kannah et al. 2021; Cavinato et al. 2017). Although many conventional pretreatment processes are being employed, utilization of an efficient and environment-friendly process is encouraged. As a result, biological pretreatment approaches based on enzymatic and

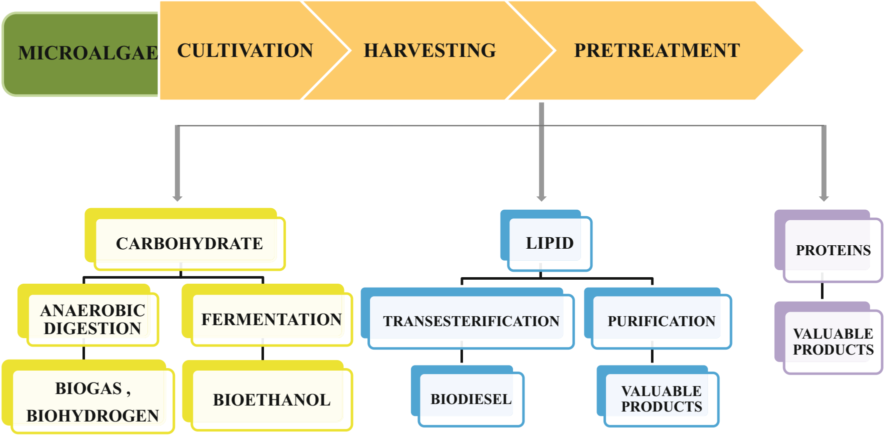

Fig. 8.2 A brief overview of biofuel production from microalgae

biocatalytic processes have piqued the interest of researchers as an ideal pretreatment process since they reduce the need for costly processes for biofuel recovery while also providing other benefits (Barati et al. 2021).

This chapter summarizes the recent advancements in the enzymatic and biocatalytic pretreatment methods of microalgae for enhancing microalgal BP and also discusses the different factors affecting the pretreatment process.

8.2 Pretreatment of Microalgae

Microalgae qualify as a promising feedstock for BP owing to their intracellular contents, which include carbohydrates, proteins, and lipids (Mehariya et al. 2021b). Although various techniques and processes are employed to convert these intracellular contents into useful products, the complex, diverse, and recalcitrant properties of microalgal cell walls present numerous limitations for the extraction of these contents of microalgae. This intricacy in algal biomass structure is owing to the presence of pectin and cellulose, the principal cell wall components, which make hydrolytic enzymes inaccessible (Zabed et al. 2020). The extraction techniques of these contents are expensive due to the requirement of high energy and huge amounts of chemicals required, and so rupturing of the microalgal cell wall is an essential step in BP (Karim et al. 2020). Incorporations of various cell wall disruption approaches such as direct transesterification, utilization of green solvents, pretreatment of microalgae, etc., are important along with the extraction procedures for producing these valuable products from microalgae, and among these, the pretreatment approach for disruption of microalgal cell walls has been preferred because of their efficiency (Karim et al. 2020; Onumaegbua et al. 2018).

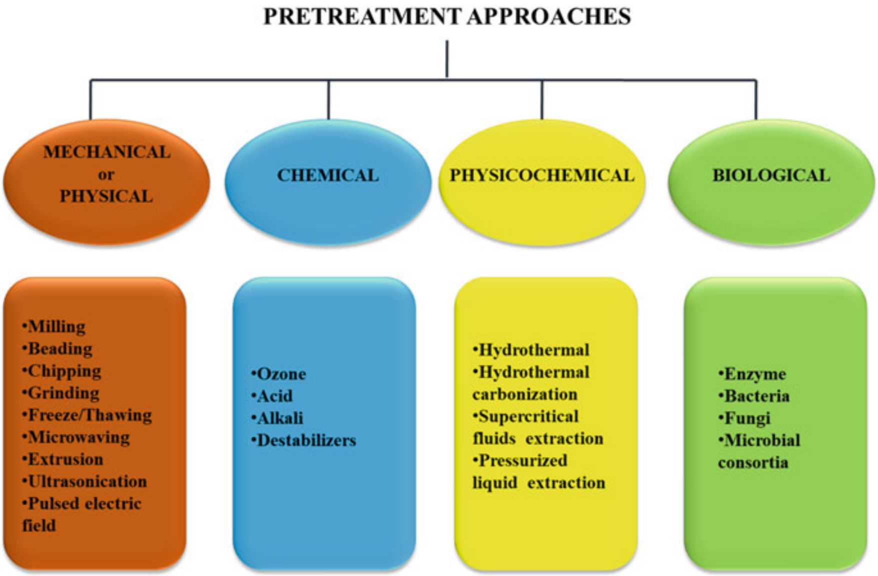

Fig. 8.3 Pretreatment approaches for microalgal biomass

Pretreatment of microalgae, whose primary goal is to make the microalgal biomass readily available for extraction and transformation of their contents into valuable products, is a critical step that primarily contributes to broadening the applications of microalgal biomass by converting them into valuable products such as biopolymers, biochemicals, fermented sugars, and biofuels (Sirohiet. al, 2021; Antoet. al, 2019). Depending on the structure and composition of microalgal biomass, many pretreatment methods have been used to produce biofuel. These methods can be classified as mechanical or physical, chemical, physicochemical, or biological processes (Sirohi et al. 2021) (Fig. 8.3). The selection of these pretreatment techniques is dependent on the morphology and cell wall composition of microalgae and can be used separately or in combination with other techniques (Nagarajan et al. 2020; Alavijeh et al. 2020; Ananthi et al. 2021).

Mechanical or physical pretreatment methods which break down the ultrastructure of lignocelluloses, depolymerize hemicellulose, and also increase the effective surface area of the biomass comprise mechanical and irradiation methods which are based on physical forces. Milling, beading, chipping, grinding, freezing-thawing, microwaving, extrusion, ultrasonication, and pulsed electric field are some of the mechanical or physical pretreatment methods (Sirohi et al. 2021; Kendir and Ugurlu 2018; Goswami et al. 2020b). The chemical pretreatment strategy, which uses ozone, acid, alkali, and various other destabilizers such as ionic liquids, surfactants, and nanoparticles resulting in depolymerization and cell degradation, is based on the defibrillation capacity of chemicals (Kendir and Ugurlu 2018; Ananthi et al. 2020). Physicochemical pretreatment involves the employment of both physical and chemical pretreatment methods for breaking down the cell membrane of microalgae such as combining both physical properties like pressure, temperature, and ultrasound along with chemicals like acids and solvents for the disintegration of microalgae (Sun et al. 2018; Sirohi et al. 2021). Some commonly used physicochemical methods for the pretreatment of microalgae are hydrothermal, hydrothermal carbonization, supercritical fluids extraction, and pressurized liquid extraction (Sirohi et al. 2021).

Although mechanical or physical, chemical, and physicochemical approaches are most commonly applied for the pretreatment of microalgae, these conventional approaches have specific environmental, economic, and technological restrictions (Zabed et al. 2019). The large energy inputs, the toxicity of by-products, and the need for expensive equipment during the pretreatment process are all unintended consequences of traditional pretreatment procedures (Barati et al. 2021; Kendir and Ugurlu 2018). Thus, considering the limitations of the conventional pretreatment approaches, the researchers realized the necessity of a more sustainable pretreatment technology resulting in the development of biological pretreatment methods owing to their advantages of being non-toxic, environment friendly, and cost-effective. Moreover, the biological pretreatment process offers the scope for developing various high-quality products from the products that are produced during the pretreatment process (Zabed et al. 2019; Ferdeș et al. 2020).

The biological pretreatment approach utilizes the hydrolytic ability of various microbial organisms to degenerate the components of the microalgal cell wall and depolymerize the intracellular polymers. The hydrolysis of molecules can be full or incomplete, depending on the efficacy of the microbe, the quantity of enzymes released, and the process condition. This pretreatment strategy is categorized into microbial and enzymatic pretreatment (EP) based on the inclusion of microorganisms or enzymes in the pretreatment procedures, and the time period of the pretreatment also varies (Zabed et al. 2019). Combining biological pretreatment with other traditional pretreatment procedures, such as physical, chemical, physicochemical, or even a distinct biological process, is a new technological innovation in the pretreatment process (Zabed et al. 2019; Alexandropoulou et al. 2017; Mustafa et al. 2017; Thomsen et al. 2016). Each of the biological pretreatment approaches will be discussed in detail in the next sections of this chapter.

8.3 Pretreatment by Enzymes

During the 1970s the hydrolysis of microalgae by the administration of enzymes was developed. Enzymes being biocatalysts catalysing the selective breakdown of compounds, the release of inhibitory compounds can be significantly reduced (Barati et al. 2021). In addition to the disruption of the cell wall, enzymes can be used for the selective hydrolysis of the macromolecules (Magdalena et al. 2018). The pretreatment of microalgal biomass by the application of enzymes includes the exploitation of hydrolytic enzymes such as cellulases, hemicellulases, proteases, lipases, amylases, xylanases, chitinases, pectinases, papain, and lysozymes (Ananthi et al. 2020; Zabed et al. 2019; Zuorro et al. 2016; Carrillo-Reyes et al. 2016). Extraction of these hydrolytic enzymes in pure, unpurified, or semi-purified form is accomplished in order to incorporate them in the pretreatment process, though unpurified enzymes are generally chosen due to their techno-economic advantages. Furthermore, these enzymes are used solo or in combinations in these pretreatment processes, although the employment of enzyme cocktails for the pretreatment process produces a more desirable result than the use of single enzymes (Adsul et al. 2020; Maffei et al. 2018; Carmen et al. 2019). Reports also suggest that crude enzyme mixtures are more effective than commercial enzyme mixtures (Hom-Diaz et al. 2016).

EP includes two groups of enzymes based on their origin: endogenous enzymes and commercial exogenous enzymes. EP with endogenous enzymes is made out of crude enzymatic extracts to degrade cell walls, whereas EP with commercial exogenous enzymes is made up of enzymes with known composition (Córdova et al. 2018). EP is reported from several beneficial product manufacturing processes such as lipid extraction for biodiesel production, biogas, bioethanol, and biohydrogen generation (Zabed et al. 2019). Table 8.1 lists various enzymes used for EP of microalgae for BP.

The enzymatic hydrolysis strategy for microalgae pretreatment appears promising due to several advantages, including a decreased energy need, low cost, the absence of end products that hinder the process, and the selective character of enzymes (Kendir and Ugurlu 2018). Recent advancement in the EP strategy is the identification of the genes responsible for the synthesis of hydrolytic enzymes in organisms and the introduction of these genes into fast-growing strains of bacteria using modern molecular techniques. This results in the synthesis of enzymes that are effective for pretreatment operations, which is particularly advantageous for industrial applications (Barati et al. 2021).

Table 8.1 Enzymatic pretreatment of microalgal biomass for biofuel production

Microalgal biomass	Enzyme	Final product	Reference
Spirulina subsalsa	Mixture of cellulase, protease, and hemicellulase	Biogas	Dar and Phutela (2020)
Porphyridium cruentum	Enzyme mix	Biogas	Çakmak and Ugurlu (2020)
Chlorella sp.	Mixture of cellulase, pectinase and xylanase	Biodiesel	Zhang et al. (2018)
Chlorella sorokiniana	Mixture of enzymes (cellulase, hemicellulase, β-glucosidase), a mixture of enzymes (cellulase, β-glucosidase, xylanase, exoglucanase endoglucanase) and cellulase	Biomethane	Córdova et al. (2019)
Chlorella sp.	Cellulase, hemicellulase, pectinase, papain, and lysozyme	Biodiesel	He et al. (2018)

8.4 Pretreatment by Bacteria

Despite the availability of commercial enzymes, their high cost and the demand for specific conditions for enzyme activation favoured the use of hydrolytic bacteria for pretreatment (Wei 2016). Furthermore, unlike enzymes, bacteria perform the same function as enzymes or enzyme mixes without the requirement for constant addition or a longer incubation time during the pretreatment process. Cellulases, proteases, xylanases, chitinases, pectinases, amylases, lipases, and lysozymes are released, either singly or in combination, to break the cell wall and depolymerize internal biopolymers (Zabed et al. 2019; Barati et al. 2021). Figure 8.4 depicts the breakdown of microalgal biopolymers by hydrolytic enzymes produced by hydrolytic bacteria. These hydrolytic bacteria have been discovered in agricultural waste, soil, organic

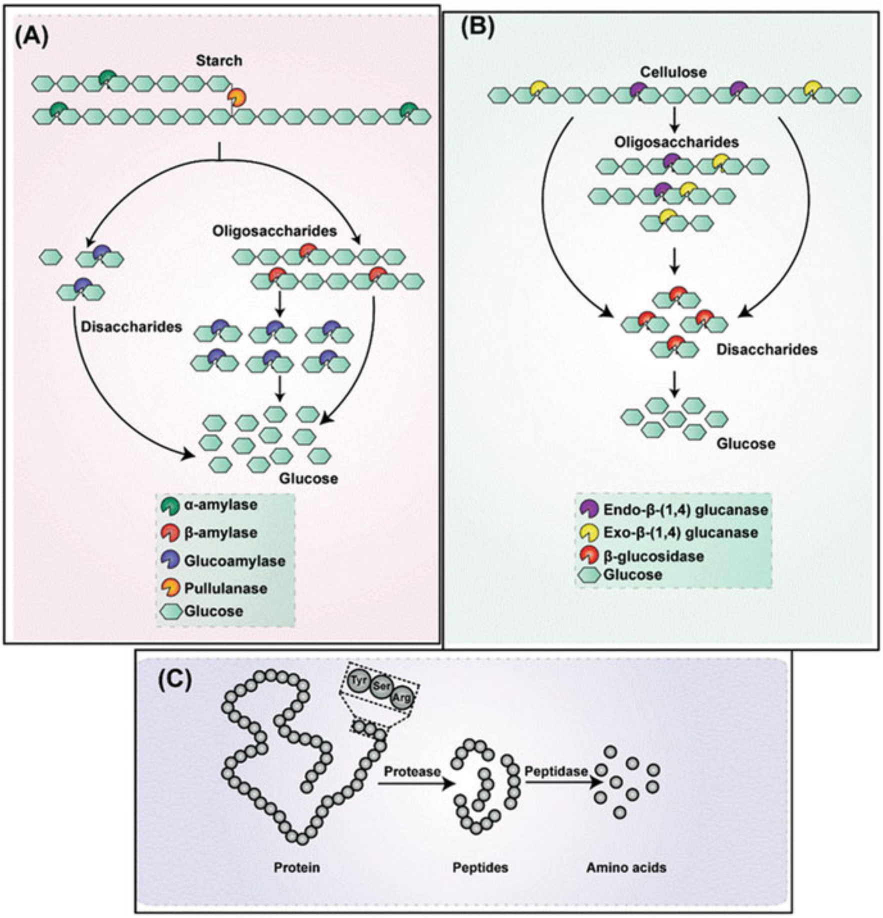

Fig. 8.4 Mechanism of degradation of microalgal biopolymers by the action of hydrolytic enzymes. (**a**) Starch, (**b**) cellulose, and (**c**) protein (Adapted with permission from Zabed et al. 2019)

debris, and even extreme conditions such as hot springs. Recently, ruminant bacteria from the intestines of xylophagous insects like termites and wood louses, as well as ruminants, snails, sea urchins, and fishes, have been included in the microalgal pretreatment process (Barati et al. 2021; Barragán-Trinidad et al. 2017).

Algicidal bacteria, the bacterial group responsible for the lysis of algal cells, have a promising future in the microalgal pretreatment for BP. The bacterial groups recognized as algicidal are *Bacteroides*, *Flavobacterium*, and *Cytophaga* and the common genera that comprise these algicidal bacteria are *Pseudomonas*, *Micrococcus*, *Bacillus*, *Pseudoalteromonas*, *Vibrio*, *Alteromonas*, *Cytophaga*, *Cellulophaga*, *Flavobacterium*, *Saprospira*, *Planomicrobium*, *Raoultella*, *Chryseobacterium*, and *Zobellia* (Wang et al. 2020; Sangwijit et al. 2016; Zabed et al. 2019). Earlier studies suggested the degradation of *Chlorella* sp. by *Bacillus thuringiensis* and *Bacillus licheniformis* (He et al. 2016; Bai et al. 2015). Table 8.2 lists the hydrolytic bacteria responsible for the hydrolysis of microalgae.

Table 8.2 Pretreatment of microalgae by bacteria

Bacteria	Target microalgae	Bioenergy	Reference
Bacillus jerish 03 *Bacillus jerish 04 bacillus* sp.	*Merismopedia ferrophila* *Scenedesmus quadrispina* *Merismopedia tenuissima* *Chlorella vulgaris* *Nodularia spumigena* *Coelomoron tropicalis* *Aphanocapsa koordersii* *Chroococcus lithophilus* *Tetradesmus obliquus* *Arthrospira platensis* *Chlamydocapsa planctonica* *Micractinium quadrisetum* *Tetradesmus largerhemii* *Coelastrum microporum*	Biomethane	Kavitha et al. (2017)
Pseudobutyrivibrio xylanivorans Mz5	–	Biomethane	Vidmar et al. (2017)
Bacillus licheniformis	*Chlorella* sp.	Biomethane	He et al. (2016)
Raoultella ornithinolytica	*Nannochloropsis gaditana*	Biogas	Muñoz et al. (2014)
Bacillus thuringiensis	*Chlorella* sp.	Oil extraction	Bai et al. (2015)
Clostridium sp. *Proteocatella* sp. *Pseudomonas* sp.	*Scenedesmus* sp. *Keratococcus* sp.	Methane	Barragán-Trinidad et al. (2017)

Bacterial pretreatment (BAP) also involves the introduction of organic acid-producing bacteria to biomass in a process known as "ensiling" and generally lactic acid bacteria (*Lactobacillus*) are preferred. The acid production by these bacteria lowers the pH and thus enhances the degradation of polysaccharides, provides suitable conditions for pretreatment, and also keeps a check on the growth of undesirable microorganisms (Zhang et al. 2019). Although this technique of pretreatment involves the inclusion of acids that are weak in nature, it is commonly used because of its efficacy in cellulose and hemicellulose degradation (Rodriguez et al. 2017). Because the presence of sugar is necessary for the formation of organic acids, adequate sugar levels must be regulated, implying that ensiling is more successful in biomasses containing free sugars (Zabed et al. 2019; Barati et al. 2021).

8.5 Pretreatment by Fungi

Similar to BAP, fungal pretreatment (FP) of biomass also possesses several benefits. Low energy inputs and less maintenance of conditions have attracted the use of fungi for pretreatment (Sankaran et al. 2020; Agrawal and Verma 2020).

Various fungal groups, including soft-rot fungi (SRF), white-rot fungi (WRF), and brown-rot fungi (BRF), have been utilized in the pretreatment process to break down complicated chemicals into simpler ones. SRF mainly comprises of *Ascomycetes*, while BRF and WRF consist of *Basidiomycetes* (Zabed et al. 2019). *Basidiomycetes*, a BRF class, act on celluloses and hemicelluloses by modifying lignin (Rouches et al. 2016). However, FP is slower than BAP and more appropriate for multicellular biomass with high content of hemicellulose and lignin (Barati et al. 2021; Zabed et al. 2019).

Though there are numerous reports on FP of lignocellulosic biomass, very limited studies have been reported on algal pretreatments. A study on macroalgal strains *Gelidium amansii* and *Kappaphycus alvarezii* pretreated by fungi *Trichoderma harzianum* reported an increase in the yield of ethanol (Sulfahri et al. 2020). Most studies on microalgae involve the utilization of enzymes extracted from fungi (Barati et al. 2021). Reports suggested the usage of *Trametes versicolor* broth that contains mixtures of enzymes of fungi rather than live fungal cells to increase biogas production (Hom-Diaz et al. 2016). Studies on microalgal pretreatment using live fungi are by far limited. Pretreatment of microalgae with fungi *Orpinomyces*, *Piromyces, Neocallimastix*, and *Anaeromyces* isolated from rumen enhanced the production of methane suggesting that direct utilization of fungi for pretreatment of microalgae can be considered for further studies (Barati et al. 2021; Aydin et al. 2017).

8.6 Pretreatment by Microbial Consortia

Commonly pretreatment by microorganisms is accomplished by the utilization of pure cultures of bacteria and fungi. However, the limitations of lengthy pretreatment time and difficulty in maintaining the pretreatment conditions are associated with the employment of these pure cultures (Zabed et al. 2019). Therefore, considering that in the living world groups of microorganisms synergistically perform the degradation activities of complex substances, the employment of microbial consortia was considered for the pretreatment processes (Padmaperuma et al. 2018). The microbial consortia generally including mixtures of fungi, bacteria, and actinomycetes utilized for the pretreatment of biomass suggest increased BP (Ali et al. 2017; Fang et al. 2018; Muñoz et al. 2014; Hu et al. 2017).

8.7 Factors Affecting the Enzymatic and Biocatalytic Pretreatment of Microalgae

The knowledge of the various factors reported to affect the enzymatic and biocatalytic pretreatment of microalgae is extremely important for maximizing the efficiency of the pretreatment process. These different factors can be associated with different categories such as biomass, bioagents, and the process conditions (Zabed et al. 2019) (Fig. 8.5).

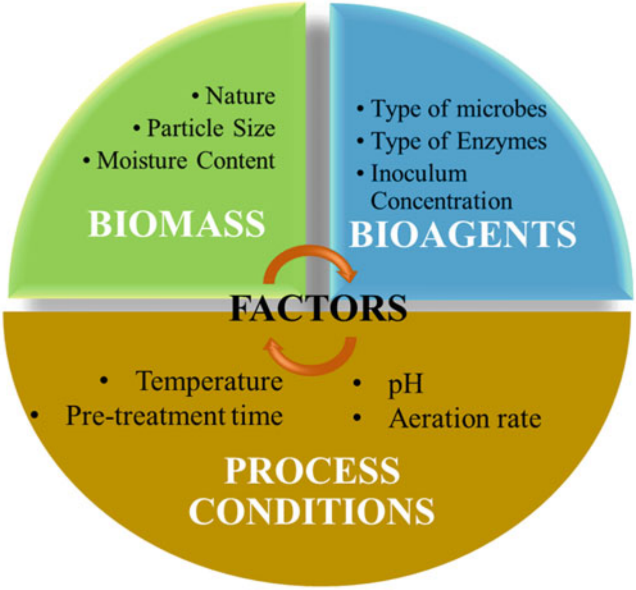

Fig. 8.5 Factors affecting enzymatic and biocatalytic pretreatment of microalgae

8.7.1 Biomass

The nature of biomass, its particle size, and moisture content are the key factors associated with biomass. The type of biomass is crucial since it varies with the chemical composition of the microalgae. Microalgae are generally composed of carbohydrates ranging from 4% to 57%, proteins ranging from 8% to 71%, and lipids ranging from 8% to 71%, although their composition varies depending on the conditions under which they are grown (Zabed et al. 2019). The knowledge of the composition of microalgae is a prerequisite for the efficiency of the pretreatment process as the selection of the enzymes and the microorganisms for the pretreatment processes is based on the composition of the microalgae (Passos et al. 2016).

The size of the biomass particles is another important criterion for enzymatic and biocatalytic pretreatment wherein the surface of biomass includes internal surface area and external surface area that is dependent on the shape and size of the particles (Maurya et al. 2015). Reduction in the size of the particles is necessary to increase the surface area of the biomass for enhancing degradation by microorganisms and enzymes as large size restricts the penetration of the microorganisms. However, very small particle sizes of the biomass also have a negative influence on the circulation of gases between particles suggesting the essentiality for adequate particle size for effective pretreatment (Sindhu et al. 2016).

The amount of moisture in the biomass is another important factor for the pretreatment process. The optimum amount of moisture which may differ with the biomass and the microorganism is essential for the growth and the biodegrading action of the microbes as excessive or very low amounts of moisture inhibit the growth of microbes (Mustafa et al. 2016; Zabed et al. 2019). Although the optimum range of moisture content for bacterial and fungal growth is 40–70%, some fungi are known to have better degradation activity at the high moisture content (Sharma et al. 2019; Saha et al. 2017; Meehnian et al. 2016).

8.7.2 Bioagents

The effectiveness of the pretreatment process utilizing microorganisms depends on the type of microbe employed for the process. Microorganisms with excellent hydrolytic capabilities will deliver better results than the ones with comparatively less hydrolytic capabilities (Tsegaye et al. 2019). However, it is difficult for a single strain of any microorganism to hydrolyse all the components of a biomass as all the enzymes may not be secreted by the single microbe. Therefore microbial consortium is often preferred for the pretreatment processes (Sharma et al. 2019). Similarly, the type of enzyme also influences the pretreatment process, and thus the selection of the enzyme or the enzyme mix is carefully achieved with the knowledge of the substrate that the enzyme needs to hydrolyse. The efficiency of EP is thus largely dependent on the suitable interaction of the enzyme and the substrate (Bhushan et al. 2021). In addition, the concentration of the enzyme employed for the pretreatment is also an important aspect that needs to be taken into consideration. The concentration of the inoculum is also a crucial factor for the efficiency of the pretreatment of biomass. Substrate colonization is entirely dependent on the concentration of inoculums as a lesser amount of inoculum results in the requirement of longer time for substrate colonization (Sindhu et al. 2016).

8.7.3 Process Conditions

The parameters such as temperature, pretreatment time, pH, and aeration rate of the pretreatment also influence the effectiveness of the process. The amount of time required for pretreatment, which can range from a few hours to days, depends on the type of microorganisms or enzymes used, the nature of the microalgal biomass, the process conditions, and the recalcitrance of the biomass (Zabed et al. 2019; Barati et al. 2021). Pretreatment time may be extended for processes involving a single kind of enzyme or microorganism since a single microbe may not be able to meet all of the enzyme needs. Thus utilization of enzyme cocktails and the microbial consortium is preferred over the use of single enzymes and microbes (Barati et al. 2021). During biomass pretreatment, the temperature is an important characteristic that must be kept within an optimal range depending on the type of enzyme and microbe used (Zabed et al. 2019; Sindhu et al. 2016). Maintaining an optimal temperature during the pretreatment is essential for the growth of the microorganisms and the activity of the enzymes. Bacteria and fungus have extremely broad temperature ranges. Bacterial growth has been seen at temperatures ranging from 4 °C to 60 °C (Sharma et al. 2019). Also, the maintenance of optimum pH during the pretreatment process is important for the enzyme production of microorganisms as the activities of the enzymes are inhibited in acidic and alkaline pH (Sindhu et al. 2016; Sharma et al. 2019). Aeration during the pretreatment of biomass is noted to perform some of the important functions, namely, oxygenation, removal of carbon dioxide, maintenance of humidity, dissipation of heat, and distribution of volatile substances produced as a result of metabolic activities of the microorganisms. In addition, high amounts of aeration are also crucial for enhanced production and activity of enzymes (Sindhu et al. 2016; Sharma et al. 2019).

8.8 Conclusion and Future Perspectives

Microalgal biofuels being environmentally sustainable and energy-efficient are considered to be a promising alternative to conventional fossil fuels. Researchers are interested in using microalgae as a feedstock for BP because of the low energy inputs required for microalgae production and the reduction in the usage of agricultural crops as BP feedstocks. Moreover, the intracellular contents of microalgae, namely, proteins, carbohydrates, and lipids, can be transformed into valuable products such as biofuels. Thus, more studies are required in the selection of microalgae with quicker growth rates, high biomass productivity, and increased stability in the extreme environment. Although microalgae have several benefits, they also contain certain drawbacks. The difficulty in digesting the complex microalgal cell wall, which limits the bioavailability of its internal contents, must be addressed. Therefore, pretreatment of microalgae is a prerequisite for BP as it degrades the cell wall resulting in the release of intracellular components. Among the several physical or mechanical, chemical, and physicochemical pretreatment techniques reported so far, the biological approach for pretreatment, which involves the use of enzymes or direct microorganisms, is favoured because of its environmental friendliness, nontoxicity, and cost-effectiveness. However, technological advancements must be researched before biological pretreatment may be implemented on large scale. Despite substantial research on BAP of microalgae, the amount of research on FP of microalgae is minimal. Given the benefits of pretreatment of microalgae with fungi, the FP strategy should be investigated for microalgal BP, and more detailed research is needed in this area. Furthermore, when a microbial consortium was used instead of pure cultures of microorganisms, greater results of pretreatment of microalgae were obtained, owing to the fact that in nature, groups of microorganisms synergistically perform degrading activities of complex chemicals. Future research should be focused on screening and the engineered enhancement of microbial strains capable of producing high amounts of catalytic enzymes that can efficiently break down microalgae while being resilient to variables like temperature, pH, and inhibitors. Additionally, advanced biotechnology strategies for the manufacture of effective enzymes that can contribute to the pretreatment of microalgae should be prioritized.

Competing Interests All the authors declare that they have no competing interests.

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Chapter 9
Innovative and Strategic Upgrades in Large-Scale Microalgal Culture Techniques

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Abstract Microalgae are a diverse group of microscopic, unicellular, photosynthetic algae that have colonized almost every habitat on Earth and exhibit a wide range of ecological adaptations. They have high photosynthetic efficiency and therefore generate enormous biomass under favourable conditions. Currently, the most influential factor is the modern and scientific commercialization of microalgae for its biofuel and biomass production. Large-scale cultivation of microalgae is carried out in open ponds and photobioreactors to meet the marketing demands. Latest technological approaches like design specification, model evaluation, and dynamic optimization of open ponds and photobioreactors are being considered globally. Since algae have come under the limelight as a better biofuel feedstock, it has brought attention towards many raceway pond systems and novel photobioreactors designs. In the near future it will bring fossil fuel usage down to zero. Considering the importance of environmental safety, photobioreactors are good alternatives in order to achieve good economic output from biofuels. Also, compared to conventional bioreactors, open pond systems use less energy. In this chapter, we will look at some of the improvements and adjustments that these systems have included in order to achieve concerned industrial goals.

Keywords Microalgae · Photobioreactors · Open pond system · Algal feed · Biofuels

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Abbreviations

WFCC	World Federation for Culture Collections
CRISPR/Cas9	clustered regularly interspaced palindromic sequences - associated protein 9
TALEN	transcription activator-like effector nucleases
TALEs	transcription activator-like effectors
ZFN	zinc finger nucleases
BHL	high-intensity blue light
WT	wild type
WL	white light
DSB	double-stranded break
EMR	electromagnetic rays
UVR	ultraviolet rays
EMS	ethyl methane sulfonate
FDA	Food and Drug Administration
GRAS	generally recognized as safe
PUFAs	poly unsaturated fatty acids
ALA	alpha-linolenic acid
EPA	eicosapentaenoic acid
DPA	docosapentaenoic acid
DHA	docosahexaenoic acid
ω-3-FA	omega-3 fatty acid
CNS	central nervous system
RWP	raceway pond
PBR	photobioreactor

9.1 Introduction

In recent times we are witnessing a rapid change in our lifestyle compared to the last few decades. The continuous development in science and technology has taught us to keep looking for innovations. These innovations are made to address serious environmental issues, food demand, etc. (Fabris et al. 2020). Most of these problems can be handled by utilizing microorganisms as alternate sources such as microalgae. These are a group of microscopic, unicellular, photosynthetic organisms present in almost every ecological zone. Unlike terrestrial plants, microalgae can be cultivated on non-arable land without fertilizers. Also, the requirement of freshwater is not a necessity for microalgae (Jerney and Spilling 2020; Goswami et al. 2020; Bhardwaj et al. 2020). Thus, it is a better alternative over food crops to meet growing food, feed, and fuel demands.

Over the last decade, industrial models for algal cultivation have been designed based on significant commercial and academic observations. The primary focus of these models includes a reduction in energy consumption and production cost. Microalgae can be cultivated on large scale in two major ways—open and closed systems. Traditionally, they have been grown in simple open ponds, and till now open systems are the most used technology for outdoor solar cultivation (Jerney and Spilling 2020; Mehariya et al. 2021a). However, the contamination of the culture and the requirement for more space are major limitations for open cultivation. In order to resolve the limitations of open pond systems, many innovative photobioreactor (PBR) designs have been designed in recent years.

Owing to their taxonomic and biochemical diversity, many different microalgal species are being continuously exploited for various industrial applications, which include biomolecules production—lipids, proteins, and carbohydrates (Kumar et al. 2020; Agrawal et al. 2020)—for phytoremediation of environmental pollution and to increase biomass production to satisfy commercial demands. Nowadays, microalgae are gaining wide acceptance in the pharmacological industry for their anticancer, antibacterial, and antiviral bioactive components. However, in order to explore them at the industrial level, we have to understand their genetic makeup and take the help of genetic engineering to make them produce a higher amount of desired product (Kumar et al. 2020; Chaturvedi et al. 2020).

Currently, all these cultural advancements are focusing on minimizing expenditure and maximizing the productivity of microalgae. This chapter discusses the maintenance of microalgal culture in labs, collection centres, and further the technologies used for cultivating microalgae at a large scale such as open and closed ponds systems. The factors such as physical, chemical, and biologicals, which affected the large-scale algal cultures, will also be discussed along with the applications of cutting edge gene-editing tools such as CRISPR/Cas9 for microalgae.

9.2 Maintenance of Microalgal Culture

At present, bioprospecting of microbes is a leading research work that examines the potential applications of microorganisms. Microalgae, being one among them, are studied and exploited for their properties. The increase in the commercial value of microalgae has resulted in huge cultivation demand of various microalgal species for various necessary applications. To satisfy the demand, large-scale cultivation of microalgae using advanced technologies, viz. open pond system, photobioreactors, hybrid system, etc., is very much important. In order to preserve a particular microalgal species for a longer period of time, it is necessary to maintain these cultures perpetually under controlled environmental conditions. For sustaining axenic cultures of microalgae, gelatinized agar is preferred (Brand et al. 2013).

Once microalgal species are isolated, the researchers are not always fond of maintaining the culture, maybe due to lack of resources or time. At this point, culture collection centres play an important role in ensuring the consistent existence of microalgae in an available form for further research or large-scale production purpose (Lourenço 2020).

The World Federation for Culture Collections (WFCC) is an international body that responsibly collects all types of living biological specimens, and ideally, all the biological collection centres must be affiliated to it (Lourenço 2020). In most of the large collections, both actively growing and cryopreserved cultures of microalgae are maintained. Curators ensure that minimal transfer of active cultures happens and thus adopt maintenance techniques accordingly (Brand et al. 2013). Actively growing microalgal cultures are maintained usually for one of the following objectives:

- Conservation of stock cultures
- To achieve a specific morphological and physiological status
- For mass culture (>200 ml liquid)

Important factors to be monitored for growing microalgae include light and temperature, containers used for growing, and static or agitated growth (Brand et al. 2013).

9.2.1 Serial Transfer Technique

The serial transfer technique is preferred to maintain active cultures in the laboratory. In this method, small aliquots of older cultures are inoculated into the fresh medium on a perpetual basis. Curators, who maintain the culture, will ensure that the culturing conditions chosen retard the growth rate of active cultures, thereby minimizing transfer frequency. Microalgae can be grown in either liquid or solid substrates. Those that occur naturally on a firm surface are grown on a solid substrate, while those that swim (flagellates) are grown in a liquid culture medium. For an instance, natural seawater is an effective base substrate for marine microalgae, as it constitutes various nutrients in dissolved form. Usually, in a culture collection, strains are maintained on a solid support. However, maintaining actively growing cultures on a regular basis has its own limitations: high cost, time-consuming, labour-intensive, and prone to human error. Apart from that, continuous culturing can lead to genetic mutations and alter the character of the strain (Brand et al. 2013). Thus, the cryopreservation technique is adopted to maintain cultures for a prolonged period.

9.2.2 Cryopreservation

Cryopreservation is an advanced technique used for preserving biotic samples. The sample can be revived by thawing. This technique uses liquid nitrogen at −196 °C to maintain or preserve cultures in a metabolically arrested state for a prolonged period of time. By using this technique, we can store organisms without any changes in their morphological, physiological, biochemical, and genetic properties (Brand et al. 2013). Some microalgal species like *Chlorella protothecoides* can tolerate a cooling rate to an extent of $-200\ ^\circ\mathrm{C\ min}^{-1}$, while many species lack this capacity. Therefore, cryoprotective agents are used to preventing any excessive damage to cells during the freezing or thawing process (Lourenço 2020). Benefits of cryopreserving microalgal strains include:

- Less maintenance cost.
- Requires very little storage space.
- At cold temperatures, axenic cultures will be free of contamination.
- Cells are not subjected to gene modification at a lower temperature (Brand et al. 2013).

9.3 Methods of Cultivation

In the aspect of commercialization, the basic cultivation methods of microalgae need to be improved. That is why a lot of research is also going on for further modification of the already known process as well as for discovering new advantageous processes. All the modified systems should possess the characteristics, including (1) adequate light source, (2) effective transfer of material across the liquid-gas barrier, (3) simple operation procedure, (4) minimal contamination rate, (5) cheap overall building and production cost, and (6) high land efficiency (Energy 2021).

In general, there are very few promising strategies that are being used tremendously for the cultivation of microalgae that include:

- Open cultivation systems
- Open ponds
- Circular ponds
- Raceway pond
- Inclined (cascade) systems
- Closed cultivation systems
- Bioreactors
- Hybrid systems

Below we have discussed these cultivation systems in detail.

9.3.1 Open Cultivation System

Open cultivation system has so far been the method of choice for commercial biomass production. Currently, 95% of microalgae productions are performed in open cultivation systems. The two important species cultivations—*Arthrosporic (Spirulina)* and *Dunaliella* sp. cultivations—have been relying on this method for more than 30 years (Richmond and Hu 2013; Goswami et al. 2021a). Open cultivation systems generally include open ponds, raceway ponds, and inclined systems which are described below.

9.3.1.1 Open Ponds

This open pond cultivation system is one of the oldest and simplest ways to cultivate microalgae on a large scale. This system is so widely used in industries due to its relatively cheaper construction, operation, and maintenance costs. Open pond systems are promising due to their simpler operation mechanism, maintenance, low energy demand, and ability to scale up easily (Tan et al. 2020; Goswami et al. 2021b). There are several variations of open pond systems, which mainly include natural water bodies such as lakes, ponds, as well as man-made water bodies such as circular and raceway ponds. Sometimes, large containers like tanks can also be used to culture microalgae (Richmond and Hu 2013; Goswami et al. 2021c). Cultivation of microalgae from natural water sources via open ponds has few drawbacks like relatively lower cell concentration, and that is why a highly efficient harvesting method is required nowadays (Shen et al. 2009; Bhardwaj et al. 2020). Also, various factors such as rainwater runoff badly affect the growth condition of microalgae such as salinity, pH, erosion, etc. that in turn results in leakage, and also, increasing water turbidity might also affect the productivity of microalgae in open ponds. As of now, one of the biggest commercial cultivations of microalgae in natural water is located at Hutt Lagoon, Australia, which is capable of producing approximately 6 tonnes of β-carotene every year form *Dunaliella* sp. in its 700-ha ponds (Borowitzka and Borowitzka 1990).

9.3.1.2 Circular Ponds

These circular pond systems are the first artificial open pond system to be used in the large-scale cultivation of microalgae. This is so named because of its circular-shaped culture tank, and it usually has a depth of 30–70 cm and a width of 45 m along with a rotating agitator that is located at the centre of the pond (Shen et al. 2009). The function of the rotating agitator is to ensure efficient mixing and to prevent sedimentation (Du et al. 2016). Several drawbacks also come with this method, as this does not allow a larger size, because the size might introduce stronger water resistance, which in turn might cause strain on the mechanical parts of the agitator (Doucha et al. 2005). On the other hand, this design also has disadvantages for using more energy in the agitation process as well as high construction costs (Hamed 2016). And for now, this cultivation system is being used in Japan and Taiwan to culture *Chlorella* for consumption (Shen et al. 2009).

9.3.1.3 Raceway Ponds (RWP)

Raceway pond system is one of the most common commercial modes of cultivation in the current scenario.

They are basically shallow ponds having several cultural units. A single RWP shares an area approximately between several 100 and a few 1000 sq. m. Being composed of two or more tracks levelled at 2–10 m wide, it is approximately 20–25 m deep, separated by a vertical partition (Rogers et al. 2014). These days, most of the advancements of RWP are relying on enhancing the mixing properties, mass transfer, light penetration, as well as relative time of gas bubbles (Mendoza et al. 2013a, b; Park et al. 2011; Putt et al. 2011).

Depending upon this parameter, RWP has been gone through several advancements. Some of the important modifications of RWP are described below.

9.3.1.3.1 Paddlewheel-Driven Raceway Ponds

Paddlewheel systems have proven to be one of the most preferred methods for propulsion in raceways. Firstly because they are very well suited to the high volume when mixing is done properly. Secondly, there is less possibility of damage to algal biomass due to lower shear stress.

This process is used traditionally especially for wastewater treatment, though temperature control in the cold season is an issue with the paddlewheel-driven RWP systems (Xu et al. 2014).

Different parameters play an important role to maintain the efficiency of the paddlewheel. These may include rotational speed, the radius of the paddlewheel, clearance between blades, pond wall, and pond surface (Chiaramonti et al. 2013).

9.3.1.3.2 Sump-Assisted Raceway Ponds

Sump-assisted RWP is the easiest way to construct as it does not require external energy to fabricate (Mendoza et al. 2013a, b). In order to increase gas-liquid contact time, CO_2 is injected in the countercurrent direction. Gas is introduced at the bottom of the sump that has great depth. In this way, it increases the contact time of gas or liquid which directly affects the enhancement of carbon dioxide utilization efficiency and reduces the carbon dioxide loss into the atmosphere (Craggs et al. 2012).

9.3.1.3.3 Airlift-Driven Raceway Ponds

This is the modification of the sump-assisted raceway pond system. In this system, baffles are designed in such a way that one side of the baffle acts as a downcomer to force the culture to flow downwards and the other side of the baffle acts as a riser. In this airlift system, the sump is generally fabricated using plexiglass, and the inner side of the sump is U-shaped (de Godos et al. 2014). The bottom of the riser contains a sparger by which CO_2-enriched air is introduced to the system. The difference between the density (i.e. higher density in downcomer as compared to gas-liquid in riser) is the pivotal force to induce the axial movement of the liquid culture.

Apart from the above-mentioned systems, the hybrid raceway pond, external carbonation column, and closed raceway ponds are other modifications of the RWP (Richmond and Hu 2013).

9.3.1.4 Inclined (Cascade) System

In this system, the culture suspension flows from the top to the bottom of a sloping surface and ends in a retention tank from where it is pumped back. Thus, it achieves a better or concentrated turbulence. Also, the optical path of the flowing culture suspension is relatively short; as a result, the light utilization is comparatively more efficient, and these modifications in turn help in high biomass production.

This system is still on the experimental level. A pilot cascade to produce *Chlorella* biomass as feedstock for bioethanol has been built in a dairy farm at Dulcie (Richmond and Hu 2013).

9.3.2 Closed Cultivation Systems

The cultivation of microalgae from a natural source in relatively lower concentrations, having a high risk of contamination by protozoa and bacteria, may produce undesirable products. So, in order to achieve large-scale products without contamination, it is mandatory to perform the whole set-up under optimum conditions, because there is ‘n’ number of recipes that affect the cultivation process. So, before starting a cultivation process, one should have prior knowledge of the optimization strategies in order to get a large amount of desirable product (Fig. 9.1).

Under closed systems, the chances of contamination are almost negligible, mostly because closed vessels are used commercially for algal culture to perform photosynthesis using artificial illumination sources or sometimes sunlight as the light source. Under a closed system, several types of photobioreactors (PBR) are used to obtain a higher growth rate (Gupta et al. 2015). Photobioreactors are artificial operating systems used to cultivate phototrophs such as microalgae in an enclosed system that uses photons as the main energy source. Photobioreactors are known to be slightly more efficient than the other methods because of their high efficiency, controlled environment of cultivation, as well as low chances of contamination (Hamed 2016). Various types of photobioreactors are in use nowadays. Some of the important photobioreactors are

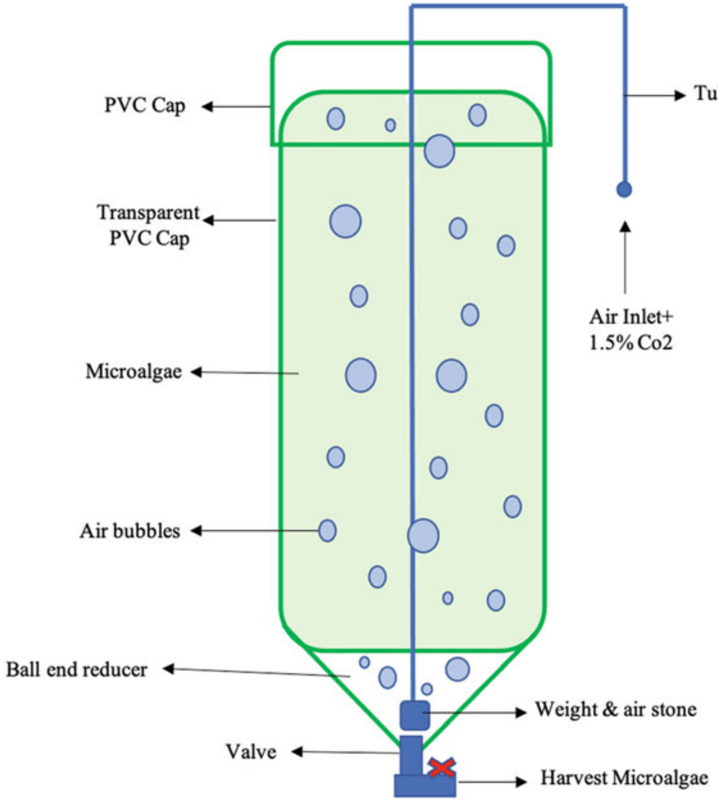

Fig. 9.1 Diagrammatic view of culturing microalgae in transparent PBR made up of PVC. It comprises a specific air inlet that provides adequate CO_2 needed for microalgal photosynthesis. Adapted from Jochum et al. (2018)

- Tubular photobioreactor.
- Vertical column photobioreactor.
- Flat plate bioreactor.

9.3.2.1 Tubular Photobioreactor

Tubular PBRs are the most common design available in the market and also is the most preferred one in commercial algal production. Tubular PBRs are mainly composed of either glass or plastic tubes in which the culture is circulated with the help of a pump by means of an airlift system. Tubular photobioreactors are mainly of three subtypes:

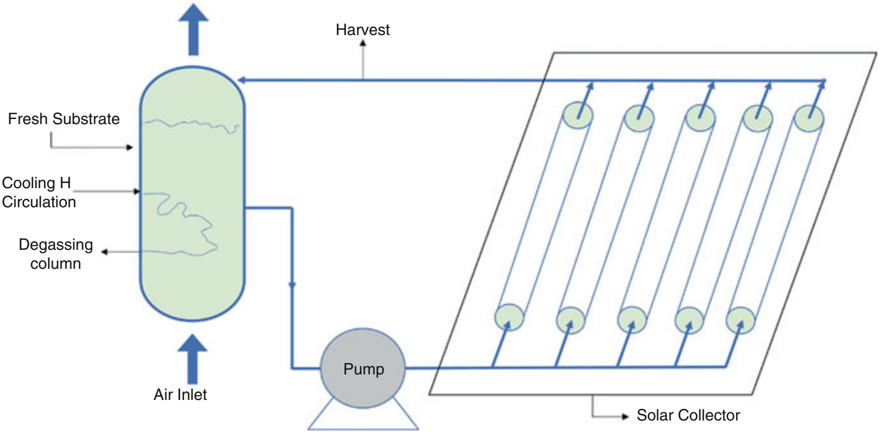

Fig. 9.2 Schematic diagram of tubular PBR comprising of several transparent tube-like components. The tubes of the specific characters are meant for the recirculation of the culture and removal of excess O_2 when needed. Adapted from Tanwar et al. (2011)

- Serpentine tubular PBRs.
- Manifold tubular PBRs.
- Helical tubular PBRs.

Commercial use of some of these large-scale tubular photobioreactor is commonly done in Germany and Israel to grow *Haemapodous* sp. and *Chlorella* sp., respectively (Fig. 9.2).

9.3.2.2 Vertical Column Photobioreactor

Column bioreactors are constructed by transparent vertical cylindrical tubing and sparger which helps in pumping the air bubbles to enable homogenization of the culture. It also allows the transfer of carbon dioxide and oxygen between air and microalgal culture. This system has the best gas-liquid mass transfer ecoefficiency compared to other systems. The reason behind this is the capability of the sparger used for generating smaller bubbles which provide a larger surface area for more efficient transferring of the substrate (Rinanti et al. 2013). Although its design owes to low energy demand, high construction cost and cleaning difficulties make it not so preferable in commercial applications (Huang et al. 2017).

9.3.2.3 Flat Plate Photobioreactor

It is characterized by its rectangular-shaped compartments made out of transparent material having a depth of 1–5 cm. These kinds of bioreactors contain an airlift system that helps in proper mixing utilizing recirculation (Tamburic et al. 2011).

This specific design supports the largest surface area and low oxygen build-up which in turn helps in achieving the highest photosynthetic efficiency out of all the other photobioreactor designs (Yan et al. 2016). The only drawback of this type of PBR is that sometimes the aeration design may cause stress to the microalgae cells. However, research are going on to develop several modifications of flat plate bioreactors to improve the efficiency such as twin layer flat plate and plastic sheet photobioreactor (Vo et al. 2019).

9.3.3 Hybrid System

The hybrid system is the most promising technology apart from the previously mentioned methods. As the name suggests, this system combines the characteristics of both open and closed systems. The efficiency of this system is based on their design that tends to combine photobioreactor and open pond to reduce their cost and increase productivity (Table 9.1).

Most designs of hybrid systems are represented by a covered open pond, in which the cover acts as a separation membrane between the gaseous space above the culture channel from the surrounding environment. This designing approach reduces the chances of contamination by resisting rain, dust, etc. Some other designs can be displayed as it is a partially filled tubular design winded and inflated that also approximately represents a covered open pond (Richmond and Hu 2013).

Table 9.1 Comparison between the open, closed, and hybrid cultivation systems. Adapted from Narala et al. (2016), Cruz et al. (2018)

No.	Character	Open system	Close system	Hybrid system
1.	Contamination	Have high contamination risk	Contamination risk is low	Contamination risk is comparatively lower
2.	Cost efficiency	Open systems are very cost-efficient	Requirement of complex machinery makes it costly as well	Hybrid systems are moderately cost-efficient
3.	Culture maintenance and product yield	Comparatively low productivity due to high maintenance	Due to low contamination risk, the productivity rate becomes high	Productivity rate is comparatively higher
4.	Land requirement	A large water body is required for this system	Large land requirement is not a crucial factor here	A large water body is essential
5.	Energy requirement	Low	High	Moderate

9.4 Factors Influencing the Large-Scale Cultivation

Although cultivation systems such as PBRs are very useful, several factors affect the overall yield of biomass production. These factors can broadly be characterized into physical, chemical, and biological factors/parameters.

9.4.1 Physical Parameters

For algae production, the important physical parameters affecting growth and productivity are light, temperature, and mixing.

9.4.1.1 Light

Light availability is a key component determining photosynthesis and for the development of microalgae. Algae have photosynthetic pigments such as chlorophyll that capture light as a source of energy from the sun. The captured light energy is transformed into chemical energy during photosynthesis (Singh and Singh 2015; Gupta and Choi 2015). Algae use photosynthetically active radiation for photosynthesis to occur (Ren 2014; Mehariya et al. 2021b).

Algal chlorophylls, carotenoids, and phycobilins absorb the visible light range. The optimal range for chlorophyll *a* is 450–475 nm wavelength of visible light, while chlorophyll *b, c1, c2* and *d* have an absorption wavelength of 630–675 nm. Phycocyanin and phycoerythrin use the light range of about 500–650 nm, and the other pigments like α-carotene, β-carotene, and fucoxanthin absorb the range of 400–550 nm for maximum algal growth (Singh and Singh 2015; Ren 2014).

Photoinhibition is a phenomenon that happens when the light intensity exceeds the optimal light and causes a reduction in algal development. As a result, in the absence of photoinhibition, algal growth will be favourable (Chisti 2007; Ren 2014).

Among all the PBRs, flat plate PBRs will have a wider surface area. The wider the surface area of the PBR, the more will be the growth rate (Tredici and Zlttelli 1998; Gupta and Choi 2015). Small diameter tubes increase the light reliability and performance of the PBR. Lowering the light path length is suitable for the effective usage of light and the optimum growth in the PBRs (Gupta and Choi 2015). Vertical column PBR has the advantage of lower photoinhibition among all types of PBRs (Gupta and Choi 2015).

In an open cultivation system, algal growth is affected due to seasonal changes. Shading light in open systems may also suppress algal growth. Algal cells show fast growth if the shading light elements are removed (Singh and Singh 2015).

9.4.1.2 Temperature

Temperature is an environmental factor that can have an impact on the metabolic rate of microalgae. Temperature changes with the intensity of light. Temperature regulates the activity of the enzymes which affect photosynthesis. The algae generally grow at a temperature of 20–30°C. The algae do not show growth at a temperature less than 4°C. When there is an increase in the temperature above 11°C, the interaction between the algal growth and the temperature is exponential (Ren 2014). For the good growth of the algal strain, the selection of the resistant strain is important and beneficial. Each microalgal strain will have a specific optimum temperature for its growth (Slocombe et al. 2016; Agrawal et al. 2022) (Ren 2014). During the early morning, the steady increase in temperature enhances microalgal growth. But if the temperature breaches the optimum level of microalgae, due to photoinhibition, the cell number rapidly decreases (Borowitzka and Vonshak 2017).

9.4.1.3 Mixing

Mixing through the PBRs promotes the microalgae to gain a good light source, nourishment, and preserve algal cells in the suspension (Ren 2014; Ugwu et al. 2008). Gas mixing can be done for the better growth of the desired organism. The efficacy of mixing and oxygen transport is dependent on the impellers and baffles. In stirred bioreactors, the impeller and baffles are primarily responsible for mixing and O_2 transfer. Whereas in air-driven bioreactors, the sparger is primarily responsible for mixing and O_2 transfer (Dunn and Bush 2001; Gupta and Choi 2015). During the daytime, photosynthesis performed by microalgae results in a huge amount of oxygen production in open ponds. The high O_2 concentration inhibits photosynthetic carbon fixation by ribulose bisphosphate carboxylase (Borowitzka and Vonshak 2017).

9.4.2 Chemical Parameters

Apart from physical parameters, there are several chemical parameters, which also play important role in the growth of algal cultures.

9.4.2.1 pH

The pH of the water in which algae grow will exhibit a large variation in their optimal growth. When the growing conditions are alkaline, microalgae may absorb more CO_2 from the atmosphere and produce higher biomass (Zang et al. 2011; Ren 2014). When the pH rises from 8.5 to 9.5, the chlorophyll levels of microalgae fall.

With a rise in pH, CO_2 in water is converted into HCO_3, which is the primary carbon production in weakly alkaline solutions. Hence, microalgae capture more CO_2 under alkaline conditions (Ren 2014).

9.4.2.2 Nutrient Availability

Nutrients have a great impact on microalgal development in large-scale production. One example is the availability of nitrogen. Though some algae do not use urea as the nitrogen source, urea is often utilized in algal cultivations since it is the cheapest source of nitrogen. Ammonia cannot be used as a nitrogen source as its excess concentrations may lead to toxicity of algal cells and also affect the production of certain secondary metabolites such as β-carotene (Azov and Goldman 1982; Borowitzka and Vonshak 2017).

9.4.3 Biological Parameters

9.4.3.1 Contamination

Contamination is common when culturing any kind of organism. The contamination of algae is a major concern in the mass cultivation of microalgae. These can affect the cultivation in two ways—one which affects or destroys the microalgae's cell growth and the others which consume the products produced by microalgae. The entry route of contaminants in microalgae culture systems can occur from water, air, and the blind angles of photobioreactors. The source of contamination is different in different types of cultivation systems (Zhu et al. 2020).

9.4.3.1.1 Closed Systems or Photobioreactors

In closed systems, water acts as a major source of contamination. As microalgae cultivation requires large amounts of water, sterilizing massive quantities is difficult, but disinfection processes like bleaching and filtration are carried out. Even after such preventive measures, there will be still some chances of contamination (Wang et al. 2013; Zhu et al. 2020).

Apart from water, the closed systems are built of various pipe connections and gas distributors, containing many blind places, where the residues of nutrients, salts, and microalgae get accumulated. Such blind angles impart increased risks of contamination and also cause corrosion of the bioreactors.

Another source of contamination is through the air. Culturing of microalgae in closed bioreactors and agitation process requires a continuous supply of air. This gas exchange increases the chances of contamination (Christenson and Sims 2011; Zhu et al. 2020).

9.4.3.1.2 Open Ponds

The mass culturing of microalgae in open ponds provides the advantage of easy gas exchange with the atmosphere. But this mode of culturing also has an increased chance of contamination, as the air directly enters cultivation systems which restricts the growth of microalgae (Zhu et al. 2020).

Another way to limit the possibility of contamination is to grow species with very selective environmental needs such as *Dunaliella salina*, *Arthrospira*, and *Chlorella* (Borowitzka and Vonshak 2017).

9.5 Strategies to Improve Microalgae-Based Products

9.5.1 Genetic Engineering

The present cultivation methods are insufficient for producing microalgae-based products because it is not economically viable, i.e. their production cost is huge and yield is low. In order to overcome this barrier, microalgal genome editing is proposed. Compared to plant and animal genome editing, microalgae genome editing is still in its nascent form (Fayyaz et al. 2020; Goswami et al. 2021d).

In recent years, novel genome editing tools like RNAi, CRISPR/Cas9, TALENs, and ZFN are used to manipulate the genome of microalgae thus procuring desired products (P. K. Sharma et al. 2018; Fayyaz et al. 2020; Jeon et al. 2017). In Table 9.2, some microalgal species manipulated with CRISPR/Cas9 system along with the observed changes are listed.

During the initial period of gene-editing technology, ZFN (protein domains fused to nucleases) attracted researchers and scientists to perform gene editing on organisms (Carroll 2011). The zinc finger domains are involved in creating double-stranded breaks (DSB) on a targeted DNA sequence by directing a pair of proteins to form a protein complex, which functions like molecular scissors. Subsequently, proteins called TALEs were discovered. It created DSB more precisely than ZFN (Joung and Sander 2013). TALEs were fused to nuclease domains to create TALENs, which is again a sequence targeting approach that directs a set of molecular scissors to create DSB at desired gene site. In recent years CRISPR/Cas9 tool came into existence which is a remarkable discovery that has made gene-editing technology readily accessible to the genomic science community (Bolotin et al. 2005). Using CRISPR, a common endonuclease (Cas9, Cas12, etc.) is directed to the desired genomic sequence using a guide RNA (Spicer and Molnar 2018) and thereby either stimulating or repressing the target gene activity (Sharma et al. 2018; Gilbert et al. 2013; Piatek et al. 2015).

Table 9.2 CRISPR/Cas9 tool used on microalgal species and the changes observed

Microalgae (species)	Gene editing tool used	Targeted gene	Result	Source link	Inference
Phaeodactylum tricornutum	CRISPR/Cas9	*CpSRP54* mutation via biolistic transformation	• Exposure to BHL for 1 h: CpSRP54 mutants and WT cultures expressed low photosynthetic efficiency • After 3 h of dim WL exposure: Mutants and WT showed almost complete recovery	Nymark et al. (2016)	Microalgal strains with low photosynthetic ability when exposed to white light for a time period can possibly increase biomass production
Chlamydomonas reinhardtii	DNA-free CRISPR/Cas9	2 gene knockout—*CpFTSY* and *ZEP*	• Constitutive production of zeaxanthin • Improved photosynthetic productivity	Baek et al. (2016)	Increased biomass production
Dunaliella salina	CRISPR/Cas9	β-Carotene hydroxylase gene (*Dschyb*) knockout via salt gradient transformation method	• Mutation of Dschyb gene • High yield of carotene pigment	Hu et al. (2021)	Huge commercial source of carotenoids obtained, like lutein, phytoene, and zeaxanthin

9.5.2 Mutagenesis

One of the main goals behind using mutation techniques on organisms is to make them express our preferred characters in huge amounts. Novel promising strains can be generated by performing traditional mutagenesis such as physical and chemical mutagenesis and genetic manipulation on wild strains (Zhang et al. 2016). Compared to the parental strains, mutants can express certain properties in the required quantity and quality. Thus, mutant strains are preferred for high biomass production, increase in ability to capture CO_2, high contents of lipid or carbohydrate, and high light conversion efficiencies. As the mutation will be random, it is necessary to characterize the mutants for their commercialization potential before going for mass production (Qi et al. 2018). Physical and chemical mutagenesis are commonly used on microalgae to create random mutation, and UV treatment is most common among them (Zhang et al. 2016).

9.5.2.1 Physical Mutagenesis

Physical mutagenesis is performed using methods like UV irradiation and laser mutagenesis. Applying EM rays and UV rays on the organism can result in random mutation, and further, we can characterize the mutated gene to reveal the changes from the wild strain (Xing et al. 2021).

9.5.2.2 Chemical Mutagenesis

In chemical mutagenesis, ethyl methane sulfonate (EMS) is commonly used for inducing mutation in many microorganisms. EMS application resulted in the production of dominant mutants that made the screening of target mutants easy (Zhang et al. 2016) (Table 9.3).

9.6 Importance of Microalgae Cultivation

Microalgae are making way to future products in various fields such as food, feed, fuel, and natural pharmaceuticals. Below we have extensively discussed the various possible utilization of algal biomass and which makes it clear that there is a need for better technologies for the large-scale cultivation of algae.

Table 9.3 Comparison between genetic engineering and mutagenesis

Genetic engineering	Mutagenesis	Reference
• Known genes are directly introduced which will target specific gene expressions	• No genes are targeted here	Zhang et al. (2016)
• This technology generates site-specific mutants	• This technology creates diverse mutants with multiple mutations	Zhang et al. (2016)
• Gene-editing tools like TALEN, ZFN, CRISPR/Cas9 are used for inducing genetic modification	• Physical agents like UV and laser and chemical agents like EMS and MNNG are the mutagens used for inducing mutagenesis	Sharma et al. (2018), Xing et al. (2021), Zhang et al. (2016)
• Difficult to genetically manipulate wild strains with unclear genetic background	• Not necessary to know the complete genetic background of the microalgal strain to perform mutagenesis	Zhang et al. (2016)
• After performing gene editing, we can easily locate the site of modification	• Due to generation of multiple mutation, it is hard to locate the mutagenic effect from the phenotype. But high-throughput screening (HTS) can aid us in identifying the genes responsible for the desired phenotype.	Zhang et al. (2016)

9.6.1 Supplements and Food Nutritive Additives

With the special demand for proteins and food supply, microalgae have been of potential importance for the growing population of the world. In human health, it also plays a key role in the production of bioactive compounds. These bioactive compounds can have anti-oxidative, immunomodulatory, anticoagulant, antihypertensive, anti-carcinogenic, and hepatoprotective properties. Microalgae contain a large amount of proteins in the cells (Prospects and Population 1988). Some algal strains like *Nostoc*, *Arthrospira*, and *Aphanizomenon* are used as human nutritive food suppliers for thousands of years as they produce protein-rich products that are approved by the FDA and GRAS. Microalgae have high importance for the production of protein source, and it is the easiest way of protein production compared to the protein production from plants, beef, pork, and chicken because it requires only low land requirement. *Chlorella and Nannochloropsis* are being used as a good source for protein production (Koller et al. 2014). Lipids are the major metabolites produced by microalgae. Today, lipids are also considered an important component in food supplements (Matos et al. 2016). Different microalgal strains are used to produce lipids and polyunsaturated fatty acids (PUFAs) like ALA, EPA, DPA, and DHA. These strains include *Arthrospira*, *Chlorella*, *Dunaliella*, *Haematococcus*, *Schizochytrium*, *Porphyridium crinum*, *and Crypthecodinium cohnii.* PUFAs are poly chain fatty acids used to treat and prevent cardiovascular diseases (Molino et al. 2018; Saini et al. 2021). Among the strains of microalgae, *Arthrospira and Chlorella* are the commonly consumed food supplements. The microalga *Dunaliella* is used as a source of beta-carotene. Some algae are also crucial in the production of food additive pigments (Caporgno and Mathys 2018). Pigments obtained from microalgae like astaxanthin (red), lutein (yellow), chlorophyll, and phycocyanin are natural dyes. Biscuits and other food products are improved by using microalgae which will increase the iron and the lipid content in them (Abed et al. 2009; Pandey 2015; Manuscript 2012).

9.6.2 Biofuels

Fossil fuels are a source of energy that is not renewable. Usage of fossil fuels has reached above biosafety level. For reducing the usage of fossil fuels and also to reduce greenhouse gas emissions, biofuel production is an alternative. These biofuels include biodiesel, bioethanol, biogas, biohydrogen, and vegetable oils.

9.6.2.1 Biodiesel

Biodiesel is one of the alternatives to fossil fuels. Some of the conventional sources used to produce biodiesel are palm oil, soybean, oilseed rape, cooking oil, sunflower, coconut, peanut, zoetrope, and corn. But a modern and better way for biodiesel production is to use microalgae as an industrial strain. Algal biodiesel will be an alternative to fossil fuel consumption (Axelsson et al. 2012). When compared to bioenergy crops, algal cultivation gives a higher yield of biodiesel. Some of the strains used for biodiesel production are *Schizocytrium*, *Nitzschia*, and *Botryococcus braunii* (Searle 2017; Adeniyi et al. 2018; Axelsson et al. 2012).

There are several advantages of using microalgae over other sources such as microalgae can grow in extreme growth conditions like saltwater, wastewater, liquid aquatic ecosystem, and dormant conditions and give a higher yield compared to other sources. In biodiesel production, the required temperature for algae is 20–30 °C, and they also need optimum pH, moisture, CO_2, light, and water for their growth. Algae can grow on low land compared to food crops. Another technique used in microalgae cultivation is growing them in mixotrophic conditions. In this technique, they use two types of microalgae which are heterotrophic and photosynthetic. They will give more yield and are cost-effective. At the harvesting time, to reduce the cost of downstream processing, algae are combined with fungi, which will form bio-flocculation. It will be easy to separate the biodiesel from the algae, and the most used separation techniques are thickening sedimentation, flocculation, and dewatering of the microalgae. The final steps are filtration, centrifugation, and packing of the product (Adeniyi et al. 2018; Bošnjaković and Sinaga 2020).

9.6.2.2 Bioethanol

In current days, pollution increased by using non-renewable petroleum fuels. Replacing these fossil fuels with renewable bioethanol will decrease pollution. Bioethanol is a sustainable energy fuel obtained by the conversion of biomass (Bušić et al. 2018; Içöz et al. 2009). Bioethanol is grouped into first, second, third, and fourth generations based on the use of raw materials. Among the four generations, the method used nowadays is the third- and fourth-generation method. In this method, they use the industrial strain microalgae (Bušić et al. 2018). In the production process, they developed a catalyst for the transesterification of fatty acids. The ionic liquid is used as a catalyst, and the catalyst works in various situation like low temperature. This will make the algae convert biomass to bioethanol. Some of the pretreatment processes are used within the presence of the ion liquid, and by delignification process bioethanol will be produced. In the third- and fourth-generation bioethanols, they have sustainable advantages and high yield production compared to the first- and second-generation biofuel production. Bioethanol obtained from the algae has great importance because it contains a higher octane ring, and it has higher heat vaporization, compared to the conventional methods (Ahuja and Tatsutani 2009; Axelsson et al. 2012).

9.6.2.3 Hydrogen Gas

Biohydrogen gas is one of the biofuels made by microalgae. The microalgae which are mostly used to produce biohydrogen gas are cyanobacteria. Cyanobacteria, also called blue-green algae, has commercial importance and potential benefits for H2 production. Photosynthetic hydrogen production from microalgae is an interesting and promising clean energy. Hydrogen fuel technology is an eco-friendly way of biofuel production, and electricity is also generated from them. The photobiological production of microalgae will be increased by genetic and metabolic engineering techniques. Some enzymes like hydrogenase nitrogenase will enhance the biohydrogen production in photoautotrophic cells (Sharma and Arya 2017; Azwar et al. 2014; Moreno-Garrido 2008; Dincer 2012; Kumari and Das 2017).

9.6.3 Bioremediation

The process of removing toxic substances and converting them into non-toxic substances is with the help of microorganisms is called bioremediation. This process can be accelerated and made energy efficient by using microorganisms like microalgae. In the wastewater treatment, chlorinated products are dechlorinated by algal strains such as *Chlorella*, *Scenedesmus*, and *Muriellopsis* (Coleman et al. 2001; Abdel-Raouf et al. 2012; Prakash et al. 2021; Goswami et al. 2021e).

Some organic toxic products like dioxins and furans which affect respiratory, reproductive, CNS, skin, liver, and kidney are removed by them. Bioremediation of heavy metals is also done by microalgae. These heavy metals get accumulated in the environment by the rapid industrialization and anthropogenic activities like agrochemicals usage, fossil fuel burning, etc. They persist in the environment because they are not biodegradable. Thus they will get bio-accumulated and bio-magnified along with trophic levels (Ramírez et al. 2018; Singh et al. 2020; Pant 2000).

Some microalgal strains, which are used as the bioremediation agents, are *Chlorella*, *Scenedesmus*, *Phormidium*, *Botryococcus*, *Chlamydomonas*, *Spirulina*, and *Nodularia*. These strains are used to clean the area with a natural, eco-friendly approach. The process of degrading the organic pollutants by the algae is called phytoremediation. Some microalgae produce biosurfactants that bioremediate the hydrocarbon chains. Recently oil degradation was carried out using artificial microalgal bacteria (Perelo 2010; Zoumis et al. 2001; Eljarrat and Barceló 2003).

9.6.4 High-Value Algal Product Development

Microalgae have been known to produce high-value products, such as beta-carotene, astaxanthin, docosahexaenoic acid, etc. Therefore, microalgal biomass is used in various cosmetics, nutraceuticals, pharmaceuticals, and functional foods industries (Suartama and Ardana 2014; Mulders et al. 2014; Kratzer and Murkovic 2021).

9.6.4.1 Lipids

Lipids are one of the major metabolites synthesis by microalgae. Microalgal lipids are reported to have good properties like great moistening nature and viscosity. Therefore they are used as softening agents and also make surface tension stable by the surfactants. They also act as emulsifiers and provide consistency to products, maintaining the integrity of the products (Khan et al. 2018; Cezare-Gomes et al. 2019; De Luca et al. 2021).

9.6.4.2 Medicinal Applications

Microalgae are also used to produce different types of drugs for the treatment of different diseases. Medicinal products which utilize microalgae include antioxidants, vitamins, anticancer, anticoagulant, antithrombotic, anti-inflammatory, antibiotics, and antiviral compounds. These agents are used to treat diseases by inducing the immune system and improving memory, increasing energy metabolism, lowering cholesterol levels, preventing heart diseases, and healing wounds. Treatment of diseases like gout, gallstone, goitre, hypertension, constipation, dysentery, ulcers, lung diseases, and semen discharge is some of the medicinal applications of microalgae (Hoseini et al. 2013; Sotiroudis and Sotiroudis 2013; Tan et al. 2020).

9.7 Conclusion

Microalgal cultivation is nowadays gaining importance as microalgae are successfully used as a sustainable source of various commercial products. In some of the countries, microalgal biomass has helped to bring down the usage of fossil fuels by contributing to their biofuel industries. Slowly the traditional crop techniques are being replaced by modern microalgae cultivation methods as they utilize minimal resources compared to crops. Microalgae have also played important role in the bioremediation of wastewater and environmental pollutants like insecticides and agrochemicals. Products produced from microalgae include proteins, β-carotene, astaxanthin, omega-3 fatty acids, bioactive and functional pigments, and polysaccharides. Further several medical applications of microalgae are also reported with disease-treating drug products like antioxidants, vitamins, anticancer, immune-boosting compounds. Most of the common microalgal strains used in the cultivation are *Chlorella*, *Dunaliella salina*, *Arthrospira*, *Schizochytrium*, *Nitzschia*, and *Botryococcus braunii.* Microalgae can help in diminishing the greenhouse effect. But as their production rate is still not sufficient to fulfil the growing demands, it is necessary that we select robust strains that can contribute significantly to biomass production. This can be achieved with the help of genetic engineering techniques; thus, improving the strain quality will improve the product yield. In the near future, we need to overcome a few challenges like high production costs and slow growth rates in order to achieve complete success in microalgae cultivation. Involving prospective customizations like mathematical methodology for assessing microalgal species growth can help to achieve strategic upgrades in the algal cultivation, and further, the genetic engineering can help in economic product generation.

Competing Interest The Author does not have any conflict of interest to declare.

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Chapter 10
Advances on Harvesting and Extraction Systems in Microalgal Biorefinery

Bobita Ray and Suchitra Rakesh

Abstract Microalgae being the global prominent source for development are proved by its bioproducts that have brought a great change into it. The high cost of production during biomass harvesting and other necessary extraction of valuable compounds is a great challenge. This book chapter aims to brief the various harvesting techniques involved as well as advanced biocomponent recovery for sustainable cost-efficient microalgal biorefinery production.

Keywords Microalgae · Biomass · Biocomponent · Harvesting · Biorefinery

Abbreviations

DAF	dissolved air flotation
DME	dimethyl ether
EPS	extracellular polymeric substance
MNP	magnetic nanoparticles
TAPP	tris-acetate-phosphate-pluronic

10.1 Introduction

With the rise of the global population, the demands have led to more utilization of natural resources. To ease the carbon emissions and uses of fossil fuels, extensive efforts have been taken towards renewable energy. The sustainability is irresponsibly imbalanced prompting to pressurize in high demands of natural resources and high consumer prices. Positively, biorefineries are the core solution to mitigate the

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impacts occurring in environments and help to reduce them. Well, biorefinery is regarded as biomass conversion to any other bioproducts which is self-sustaining and harmless to the environment. It includes various schemes such as biofuels, electricity, cattle feedstock, and high-value chemicals from biomass which benefitted both the environment and commercial progress. Earlier the sources utilized for the production of biorefineries will take edges of time, labour, and money with other sideway loss where microalgae offer the greater potential of success compared to rest.

Microalgae are known to be a predominant, promising source that represents renewable biomass for biorefinery production. Microalgae have potential as feedstocks for biodiesel (Rakesh et al. 2014; Agrawal and Verma 2022) besides their value as a source of bioactive molecules, pigments, nutraceuticals, etc. Microalgae have several unique features like the ability to fix CO_2 and convert it into valuable components via photosynthesis and robust growth with high lipid contents. The microalgae harvesting and qualitative and quantitative estimation of lipid have been reviewed (Rakesh et al. 2020; Arathi et al. 2020). The availability of molecular approaches to increase lipid accumulation and recovery has been extensively discussed (Jothibasu et al. 2021; Chaturvedi et al. 2020). The previous generation sources have failed to reach their goal. As most of the related sources are crops that consume more time, space, and effort to grow, microalgae are completely vice versa in relation to time, space, effort, and currently trying with the expense. It has the advantage of high biomass productivity and short culture periods. Microalgae can easily assimilate CO_2 photoautotrophically for CO_2 sequestration and greenhouse gas reduction. Approximately 50% of its species are responsible for photosynthesis on Earth. Though it seems microscopically minute in nature, it has a vast ability to produce many important products (Suganya et al. 2016; Mehariya et al. 2021).

Microalgae play the principal role in producing multiple products with high-value co-products from the biomasses in a cost-effective and environmentally sustainable manner. Lipid, protein, pigment, carbohydrate, and various other components are used as applications in biofuel, cosmetics, pharmaceutical, and nutritional industries. Among that even microalgal carbohydrate replaces lignocellulosic biomass for the fermentation process in industries (Yen et al. 2012; Kumar et al. 2020; Goswami et al. 2020b). But though the conversion of microalgae into bioproduct might sound easily producible, it is extremely challenging outlining the facts such as expense, time, labour work, and so on, especially while harvesting and extracting the compounds from microalgae. To overcome this constraint, effective cell disruption methods are used, which lead to improved lipid extraction from wet algal biomass using solvents and, ultimately, enhance lipid yield. Autoclaving, ultrasonication, bead beating, high-pressure homogenization, microwave, osmotic shock, and lyophilization (freeze-drying) have been used as cell disruption techniques (Rakesh et al. 2015; Agrawal et al. 2020). Therefore, the main objective of this review will be the study of harvesting and extraction techniques (the outline is shown in Fig. 10.1) researched recently in order to help in the conversion of various biorefinery products. This information will provide a brief idea on every aspect of techniques for future help and commercial development.

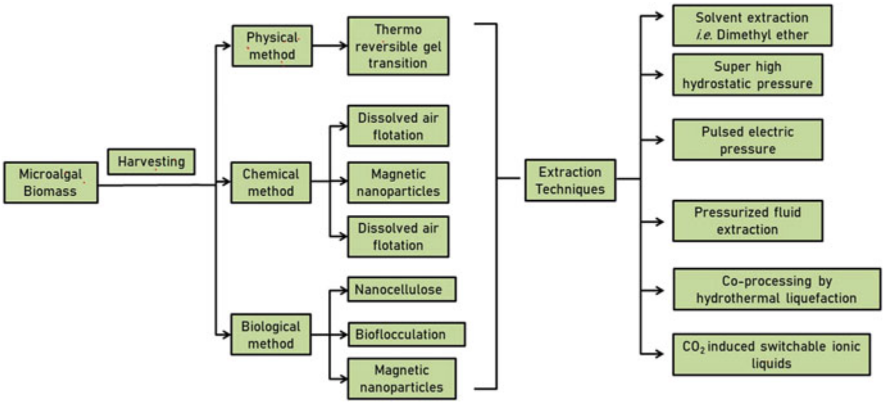

Fig. 10.1 Advanced techniques in harvesting and extraction from microalgae-based biorefinery

10.2 Algal Harvesting Techniques

Harvesting is a process where a gathering of items or yield is acted. It is an ancient method utilized in all aspects of resources such as crops, sawdust residues, aquaculture species, and many more. Surprisingly, few of these methods are continued such as filtration, sedimentation, and centrifugation. The instruments or appliances might be modified or changed from wood to steel, but the techniques and directions the way it is processed are similar. In the case of harvesting microalgae, a few of the earlier techniques have been adapted and still in running point, whereas many others are developed into the more modified and advanced way such as screening, coagulation-flocculation, filtration, gravity sedimentation, flotation, centrifugation, electro-flocculation, electrophoresis, electroflotation, and ultrasonication (Show and Lee 2014; Goswami et al. 2021). Though in these techniques many better consequences have been recorded facing various challenges and circumstances, however, there are even other methods and techniques that need to be observed. The following are the briefly mentioned harvesting techniques, presented in Table 10.1.

10.2.1 Thermoreversible Gel Transition

Thermoreversible gel transition is characterized by either agar gel (Kumar et al. 2017) or sol-gel (Estime et al. 2017) transition. The microalgal cells are generally grown in Tris-Acetate-Phosphate-Pluronic (TAPP) or Bold's basal medium where it is quite reduced to around 10–15 °C for the formation of gelation and incubated along the cultivation time period. After the end of the cultivation period, the gel was heated for transformation into a liquid phase where harvesting takes place as clustered microalgal cells settled at the bottom due to gravimetric sedimentation and biomass is collected by either scraping or centrifuging followed by drying. It is

Table 10.1 Effect of various harvesting techniques related to time (min) on microalgae biomass recovery (%)

Name of microalgae species	Type of harvesting technique	Biomass recovery (%)	Time (min)	Reference
Nannochloropsis maritima	Magnetic nanoparticles	60	10	Fu et al. (2021)
Chlorella vulgaris (with 5% inoculum of bacteria)	Nanocellulose	97	2880 (48 h)	Chen et al. (2018)
Scenedesmus obliquus (with 5% inoculum of bacteria)		91	NA	
Chlamydomonas reinhardtii (with 5% inoculum of bacteria)		97	480 (8 h)	
Nannochloropsis oculata	$FeCl_3$	25	NA	Jafari et al. (2021)
Chlorella sp.	Self-flocculation	97	720	Chen et al. (2021)
Nannochloropsis sp.	Chitosan	97	20	Chua et al. (2018)
Chlorella sorokiniana	Thermoreversible gel transition	80	Depends on the experiment	Kumar et al. (2017)
Chlorella vulgaris	Cationic starch	90	240	Huang et al. (2018)
Chlorella sp.	Bioflocculation	65	150	Luo et al. (2019)
Scenedesmus sp.	Cationic dextrin polymer	98	15	Kumar et al. (2020)
Micractinium sp.		98	20	
Chlorella sp.		97	20	

said that the morphology of algal cells matters more while compared with settling time speed during gravimetric sedimentation (Liyanage et al. 2016). This technique is quite reversible in transition allowing the microalgal cells to grow and settle in controlled confinement with respect to the changes in the system temperature being termed as simple and efficient microalgal harvesting (Randrianarison and Ashraf 2018).

10.2.2 *Flocculation*

The term flocculation refers to the microalgal cells gathering into larger clumps during harvesting. The clumps either form with the help of chemicals, enzymes, alkaline treatment, organic cationic polyelectrolytes, or other sources.

10.2.2.1 Nanocellulose

Cellulose is a type of insoluble substance where polysaccharide is bonded with chains of glucose monomers. It is biocompatible and cost-effective harvesting flocculant for microalgae. Nanocellulose plays a vital role in harvesting which is a cost-effective step for biofuel production. The cellulose nanofibrils flocculate microalgae through the anion surface that is present. It is studied that the more the concentration of cellulose nanofibril, the more the increase of flocculation (Yu et al. 2016). In order to harvest the culture of *Chlorella sorokiniana*, a novel cellulose derivative is used for harvesting called hairy cationic nanocrystalline cellulose. It is characterized by two positively charged amorphous ends bonded with a common crystalline shaft. However, the study was monitored through laser reflectance for better effect of results (Lopez-exposito et al. 2019). Chen et al. (2018) performed a harvesting experiment in the presence of bacterial cellulose in *Gluconacetobacter xylinus* that was inoculated during microalgae cultivation and achieved around 90% of clump formation during harvesting via flocculation.

10.2.2.2 Bioflocculation

Past experiments have shown through bacteria and fungi that it has great potential for microalgae harvesting without spending on expensive techniques or chemicals. But the only problem is contamination and not being reliable for food or feeding production. However, *Pleurotus ostreatus* has solved this situation as it is an edible fungus and also is useful for microalgae harvesting with the consequence of higher harvesting efficiency (Luo et al. 2019; Goswami et al. 2020a).

10.2.2.3 Self-Flocculation

According to previous records we already might have gone through about *Scenedesmus obliquus* containing extracellular polymeric substance (EPS) due to which the microalgal cells bind with each other forming clumps or flocs. Similarly, in a recent experiment it is partially unanswered the reasons behind self-flocculation in *Chlorella* sp., but it might be due to the presence of EPS. It also revealed about the presence of exoproteins in EPS, and also due to the high intensity of light the microalgae might contain higher self-flocculation efficiencies (Chen et al. 2021). On the other hand co-cultivation of microalgae has led to high lipid productivity as well as self-flocculating efficiency too. Here, one of the microalgae acts as adhering capability with charged ions that initially attract the other cells or high pH level properties that react by binding with other microalgal cells forming clumps and later sediments at the bottom. It not only comprises more production but also less time period and cost-effective too (Feng et al. 2021).

10.2.3 pH Modulation Dissolved Air Flotation (DAF)

The knowledge about dissolved air flotation has shown good access to harvesting techniques based on performing air bubble generation to lift the microalgal cells in order to reach the surface of the system efficiently as flocs. The addition of chemical compounds in the solution needs to be proceeded known as coagulation before DAF where it destabilizes the cells to form flocs. In this study, for the coagulation process, NaOH chemical compound is added to maintain the quantity of respective pH instead of using other metal compounds. With the help of the flotation column, the pH modulation was optimized using one factor at a time method for harvesting reciprocated in high biomass (Leite et al. 2020).

10.2.4 Magnetic Nanoparticles (MNP)

Magnetic harvesting of microalgae is another technique whereby the influence of magnetic nanoparticles performs better in biomass gathering, whereas it depends utmost on interactions between microalgal cells and magnetic nanoparticles. As usual, the microalgal cultures are cultivated within its periodic time, while MNP is synthesized by mixing of chemical composition $FeCl_2$ and $FeCl_3$ with the addition of ammonia solution. The composition is kept on a constant vortex and even washed with deionized water until the pH reaches neutral. However, the composition is followed by another addition of $AgNO_3$ solution and mixed thoroughly. Hence, the solution is mixed with the microalgal suspension culture where respective with time it is observed that the microalgal cells will be subsequently harvested (Abo Markeb et al. 2019; Fu et al. 2021).

10.3 Extraction Techniques from Microalgae

Microalgae are boasted as a high growth rate of compounds necessary for biorefinery products. Since it has the capability to grow in non-arable land and prohibits competition from food production, it is presumed to be easily workable with any yield. But the only concern that needed to be focused on is extracting the distinct compound for the production and the techniques specialized on it. The details of those techniques are mentioned in Table 10.2.

Table 10.2 Microalgal extraction technique through various methods

Name of microalgae	Extraction technique applied	Relative Compounds	Reference
Tisochrysis lutea	Pressurized fluid extraction	Fucoxanthin	Gallego et al. (2020)
Nannochloropsis sp.	Microwave-assisted extraction	Lipid	Zghaibi et al. (2020)
Chlorella vulgaris	Pulsed electric field	Protein	Buchmann et al. (2018)
Haematococcus pluvialis	Ultrasound-assisted extraction	Astaxanthin	Chronis et al. (2021)
Phaeodactylum tricornutum	Ultrasound-assisted extraction	Antioxidant	Khawli et al. (2021)
Chlorella vulgaris	CO_2-induced switchable ionic liquids	Lipid	Tang and Row (2019)
Isochrysis galbana	HPLC based on different concentrations of solvents	Carotenoid	Bonfanti et al. (2018)
Nannochloropsis oculata	Supercritical CO_2	Lipid	Jafari et al. (2021)
Tetradesmus obliquus	Supercritical CO_2	Carotenoids, fat-soluble vitamins	Chronopoulou et al. (2019)
Chlorella sorokiniana	Liquid biphasic electric flotation	Protein	Sankaran et al. (2018)
Chlorella sp.	Super high hydrostatic pressure	Lipid	Xu et al. (2021)
Nannochloropsis oculata	Solvent extraction by dimethyl ether	Lipid	Wang et al. (2021a)

10.3.1 Solvent Extraction

10.3.1.1 Dimethyl Ether (DME)

Dimethyl ether is a kind of a gas that represents mainly in saving energy, but it can be easily liquefied at a temperature between 20°and 25 °C and 0.51–0.59 MPa. It is both simultaneously suitable for extracting lipid and dewatering biomass too (Wang et al. 2021a). The working vessel system is basically made of five parts: liquefied DME storage vessel, DME measuring vessel, lipid extraction vessel, liquid from solvent separating vessel, and lastly the moisture trap column of $CaCl_2$. In here, the microalgae sample contained in a cellulose extraction thimble is loaded in the lipid extraction vessel with an entrainer to draw along itself. The liquefied DME from the storage vessel is passed through with the help of N_2 gas and with the appropriate measurement passed into the extraction vessel. After finishing with the lipid extraction after several minutes, the liquid phase flows into the fourth vessel where separation takes place, and DME present in raw lipid is evaporated when pressure is relieved depositing the lipid in the upper surface column. Adding the substance with chloroform followed by filtration, the chloroform is removed by N_2 leaving with raw lipid stored at low temperature (Wang et al. 2021b).

10.3.2 Super High Hydrostatic Pressure

Super high hydrostatic pressure was previously utilized in food technology industries, but recently the outcome for lipid extraction was unexpected. The mechanism process of its work leads to economic development and has the capability of shifting into industrial-scale success. The pressure is usually maintained from 100 MPa to 1000 MPa for processing (Abera 2019). Generally, when the super high pressure lets the extracellular solvent such as chloroform or any other non-polar solvents into intracellular space through the cell wall, it leads to the breakdown of cells where usually lipids get mixed with the solvent naming as high quantity of lipid extraction production. This method of extraction from wet microalgae is considered a beneficial effort technique; firstly taking into wet biomass, time and expense is saved from sterilization and dewatering, secondly, high quantity of lipid yield following with cell disruption, thirdly, presence of non-thermal treatment will guarantee other extraction bio-compounds without destructing, and lastly, these techniques can also be utilized in edible protein or animal feed commercially (Xu et al. 2021).

10.3.3 Pulsed Electric Field

Extraction of protein has been demonstrated in various methods and techniques, but figuring with the history of the pulsed electric field has innovative non-thermal technique to increase the quantity of mass across cellular membranes where the processing can be applied both reversibly and irreversibly based on parameters. This technique consists of a broad side of the image from the insertion of molecules into cells to microbial inactivation and cancer treatment (Campana et al. 2016). Buchmann et al. (2018) reported that maintaining an electric field around 20 kV cm^{-1} during pulsed electric batch by batch in plate electroporation can harvest high microalgal biomass. The protein extraction came into quite easily accessible by high-pressure homogenization through pulsed electric technique.

10.3.4 Pressurized Fluid Extraction

Fucoxanthin is a significant carotenoid that has a great advantage in pharmaceutical properties. Similarly, microalga named *Tisochrysis lutea* has great benefit in carrying fucoxanthin compound. In order to extract the required compound, the pressurized fluid method is applied where various concentrations of 0–100% of ethyl acetate are analysed maintaining the temperature from 40 °C to 150 °C continuing with static extraction cycles. Lastly, the expected outcome of extraction of fucoxanthin from the microalgae experiment is obtained with pure ethyl acetate at around 40 °C with one extraction cycle where the quantity achieved is 132.8 mg of carotenoids (Gallego et al. 2020).

10.3.5 Co-Processing by Hydrothermal Liquefaction

Observing the experiments and results from the past researches, hydrothermal liquefaction is quite familiar with processing with the conversion from whole microalgae biomass into biocrude oil. But Guo and other experts have presented this concept by selecting biofuel microalgae cultures that can produce a high yield of crude with the help of the technique co-processing hydrothermal liquefaction together with a variety of plastic wastes and microalgae at around 300 °C for 15–20 min. The crude oil produced can be utilized in aromatic chemical and biofuel applications (Guo et al. 2021).

10.3.6 CO_2-Induced Switchable Ionic Liquids

Ionic liquids are comprised of various ion solvents perceived as a non-volatile substance with the presence of salt derived from 1-methylimidazolium. With the preference of switchable transition from hydrophobic to hydrophilic transition test including bubbling, CO_2 gas can be expected for extraction of lipids from microalgae for biofuels. The experiment is continued with a cell lysis process stirred magnetically at 500 rpm conducted at room temperature for 12 h. For a better examination of high lipid extraction, different concentrations of switchable ionic liquids are preferred (Tang and Row 2019).

10.4 Conclusion

Microalgae are acknowledged as leading green technology for the future and are thereby needed to be recognized worldwide. But the only hurdles faced while commercializing are harvesting and extracting the yield of biorefinery products that require more time and expense. Therefore the researchers need to act as lynchpin by co-operating with each other about the challenges and knowledge in various fields and disciplines to strengthen the development.

Conflict of Interest The author declares there is no conflict of interest to declare.

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