Environmental Science and Engineering

Navneeta Bharadvaja · Lakhan Kumar ·
Soumya Pandit · Srijoni Banerjee ·
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Recent Trends and Developments in Algal Biofuels and Biorefinery

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Contents

Chapter 1
Biorefinery for Microalgal Biomass at an Industrial Production Scale

Neha Singh and Vijayanand S. Moholkar

Abstract Latest trend influences consumers to opt for healthy and natural products, which forces the industry to research and develop natural products that have functional ingredients. A natural source of functional ingredients is microalgae which has positive health impacts as these microorganisms are producer of bioactive peptides, essential minerals, enzymes, natural pigments, polysaccharides, polyunsaturated fatty acids, and vitamins. Microalgae are commercially utilized for producing chemical reagents, cosmeceuticals, functional food, nutraceuticals, and living feed and feed additives. Microalgae are also crucial for biofuel development along with environmental protection. The major microalgal products are derived from its biomass; though, mass cultivation produces large quantity of spent cell free media which is still unexploited. Certain extracellular metabolites can possibly be used as antioxidants, drugs, growth regulators or metal chelators. It is important to understand these microalgal extracellular metabolites that may be commercially used. The production process for these microalgal products is moderately economically viable; however, inflated downstream processing cost has impeded commercialization of microalgal biofuels. Hence, a promising solution for development of relevant agricultural, industrial, and pharmaceutical value-added products is the simultaneous extraction of these products during bioenergy production in a biorefinery approach. Thus, microalgal biorefinery helps meet the increasing demands of energy, food and pharmaceutical industries.

Keywords Bioenergy · Biofuels · Biomass · Biorefinery · Microalgae · Nutraceuticals

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1.1 Introduction

The recent surge in the energy consumption is triggered due to the continual growth in the world population. The rising industrialization and urbanization are leading the world towards a noticeable rise in environmental pollution. Milano et al. (2016) reported that in the past four decades, in world primary energy consumption, the highest growth of 5.6% was observed in a single-year (2010). Henceforth, the mean rate of growth in world primary energy consumption remained greater than 2.5%. Fossil fuels have been the main source of primary energy, namely coal, petroleum, and natural gas. Figure 1.1 illustrates the global primary energy consumption in the year 2021. It is easily deducible that the major contribution of about 85% comes from fossil fuels while the contribution of the renewable sources of energy, such as biomass, wind, hydropower, etc., is only 14% of global energy consumption. Wide depletion of fossil fuels has now escalated key concerns over energy security and the risk involved with the change in the climate of the world. Primary concern is the energy crisis as fossil fuels being unsustainable, are depleting at a steep rate, thus shifting the world's focus towards other sources of fuel. Another issue related with fossil fuels consumption is environmental pollution that also raises an alarm concerning the global warming caused by the high CO_2 emissions in the air.

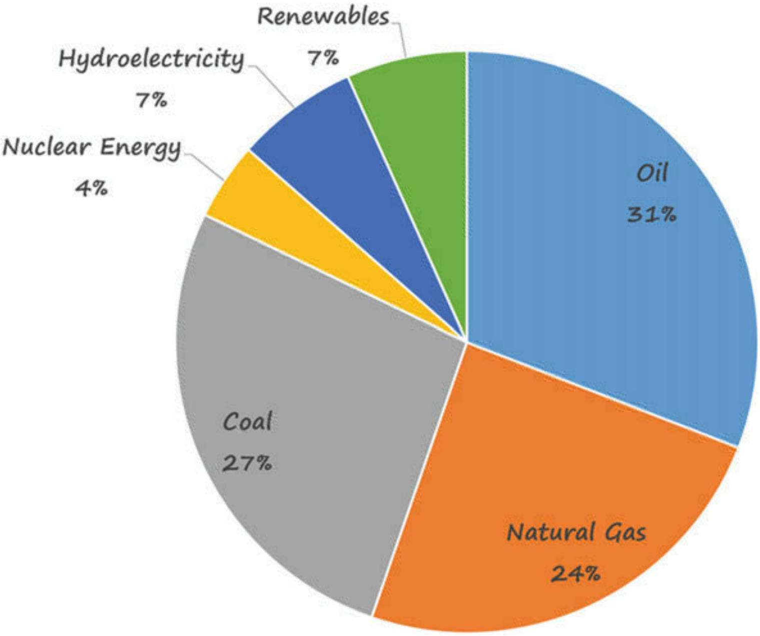

Fig. 1.1 Global primary energy consumption 2021 (bp Statistical Review of World Energy, 71st Edition, 2022)

In recent years, nations are significantly attempting to lower the utilization of fossil fuels, as they are of non-renewable nature, in order to alleviate carbon dioxide emissions and divert the efficiency of energy conversion (Brennan and Owende 2010). The substitutes to fossil fuels may possibly be biofuels that are obtained from biomass sources (renewable). Substrates that are biomass-based are employed in the production of liquid biofuels. Few examples of biomass-based substrates are edible food crops, for instance, sunflower, corn, palm, or lignocellulosic materials such as bamboo, or it could be crops that are non-edible like Jatropha. The capability of plants to capture carbon dioxide by photosynthesis and converting solar energy to chemicals helps in reducing global warming by mitigating CO_2 emission. This, in turn, makes biofuels a renewable option and facilitates in building a green environment. It was demonstrated by Gupta and Tuohy (2013) that lignocellulosic materials could secure approximately 77 Gt of CO_2 yearly, thereby producing 100 Gt of biomass.

Recently, biofuel production has witnessed widespread growth globally. In the USA, Brazil, and Europe, biofuels of the first-generation derived from palatable crops are already commercially consumed. However, competitive consumption of crops as food and fodder has led to the scarcity of such sources. Such a confinement has paved the path for biofuels that are called second generation, which are drawn from non-edible crops (Jatropha, Palm oil, and Castor). These biofuels are essentially the non-edible parts of food crops, such as remains obtained after processing the plants agriculturally or during wood processing. Though these crops preclude the clash with crops from first-generation, however, the requirement of arable lands is still a challenge. Moreover, the conversion rates achieved are also low. In such a scenario, microalgae have the potential to serve as an engaging feedstock to biofuel of third-generation since they have various benefits over first and second generation crops, like, ease of cultivation, the requirement of non-arable land, elevated photosynthesis efficiency, higher content of lipid and faster growth rate. Table 1.1 lists out a few global companies that develop and commercialize microalgae-based products.

Microalgae are unicellular microorganisms having photosynthetic capability that exist independently or in the form of tiny assembles. Microalgae are generally noticeable on the water surface as colored blotches. They indicate the beginning of the marine food chain. Microalgae exist in the form of various species in a varied variety of environmental conditions. They are either eukaryotic (Bacillariophyceae), or prokaryotic (Cyanophyceae), or photosynthetic microorganisms (Chlorophyceae), that use photosynthetic processes for growth and to produce proteins, lipids, and carbohydrates. Furthermore, microalgae are capable of biomass generation, making use of sunlight and water with nutrients (phosphorus and nitrogen) and carbon dioxide.

The size of microalgae varies from μm to mm. They are essentially present on the surface of water under the influence of direct sunlight. The habitat of microalgae is not limited to the aquatic ecosystem. Microalgae live in every ecosystem of the earth, whether it is terrestrial or aquatic, suggesting a widespread and vast species existing underneath diverse environments. Scientists have estimated an occurrence of microalgal species beyond 50,000. Mata et al. (2010) reported that approximately

Table 1.1 List of a few global companies involved in the development and commercialization of microalgae-based products

S. No.	Company name	Location	Products
1	AlgaEnergy, S.A	Spain	Agriculture, Nutrition (both human and animal), Cosmetics and Aquaculture, Pharmaceutical, Biomaterials and Biofuels
2	Algenol	United States of America	Personal Care Ingredients (antioxidants, vitamins, etc.), Food, Green Crude/Biofuels
3	Aliga Microalgae	Denmark	Food ingredients, Supplements, Aquafeed
4	Canadian Pacific Algae	Canada	Marine phytoplankton (Supplements, Exfoliators), Sea salt, Soil enhancer
5	Everflow Global Ventures Pvt Ltd	India	Wastewater reuse, CO_2 Sequestration, Waste management, Biofeed, Biofertilizers, Soluble Algae Products, Algal Super Absorbent Polymer
6	Greenskill Environmental Technology Ltd	United Kingdom	Wastewater treatment, Carbon capture, Biorefinery
7	JUNE Pharmaceuticals Limited	Myanmar	Health Supplements, Functional Foods, Beverages, Skin care, Toiletries
8	Neoalgae	Spain	Cosmetics, Agriculture, Nutrition
9	Provectus Algae Pty Ltd	Australia	Cosmetics, Food and Beverage, Agriculture, Therapeutics
10	Sunchlorella	Japan	Whole Food, Supplements, Cosmetics

30,000 microalgal species have been classified and explored. Microalgae hold the capability of fixing CO_2 by utilizing the sunlight more efficiently in comparison to terrestrial plants. In comparison to conventional food crops, microalgae have a higher growth rate, by almost 10%. Microalgae also consume various harmful pollutants and utilize them for their growth. They have a simple cell structure—uni or multi cells, thus aiding them with rapid growth, and there are high chances of survival even in harsh conditions. Moreover, they have minimal requirements for resources, and they do not compete against food or agricultural crops to obtain various valued resources.

1.1.1 Microalgal Chemical Components

Metabolites are essentially the transitional or the finished products of metabolic pathways. There are two types of metabolites—primary metabolites and secondary metabolites. The metabolites that have a direct association with microalgal growth are the primary metabolites. While secondary metabolites are not straightforwardly connected with microalgal growth, however, these play roles in additional essential ecological functions and are often caused by stress or other environmental conditions.

The structure of the cell wall of the microalgae varies with the type of strain and growth conditions, specifically the thickness and chemical composition of the cell wall. The microalgal cell stores different chemical constituents, which generally depend on the environmental conditions. Some of the key chemical components that exist in microalgal cells are proteins, lipids, and carbohydrates with varying compositions. The major factor that makes microalgae a potential choice for alternate feedstock for biodiesel production is due to its great productivity of lipids. Zhu et al. (2017) stated that the microalgal yield of around 55 tons ha^{-1} $year^{-1}$ is achievable with lipids being up to even 60% in the microalgal cell upon exposure to few stress conditions. Thus, microalgal lipid productivity can rise by approximately 300 times, compared with other normal oil crops. The vital primary metabolites, such as lipids, proteins, and carbohydrates, follow their particular production pathways. Value-added co-products are produced simultaneously that hold numerous applications, such as industrial chemicals, pharmaceutical products, animal feed and human food supplements, and even transportation fuels.

The microalgal structural components vary extensively among species. These structural components vary with the cultivation conditions, hence becoming a non-intrinsic constant factor. Microalgal cells adapt to environmental variations by changing the chemical components since microalgae have the capability to acclimatize to the variations around the surrounding. This is generally carried out by modifying the environmental aspects like temperature, pH, light, salinity, nutrients and usable carbon dioxide quantity. It is possible for microalgae to store a variety of products, as desirable. Microalgal cells also emit chemical signals in order to counter, protect, and for prey selection. Microalgae also serve as an unconventional source of protein since lots of microalgal cells have a protein content. Microalgae species also contain starch, sugars and various polysaccharides, which make them rich in carbohydrate content. It is feasible to utilize the dried microalgal cells as a whole or their components extracted individually as a source of feed since microalgae are effortlessly consumable because of their valuable and distinct composition. One of the major components in microalgae are lipids that range from 1 to 70 wt% in weight among different species and sometimes can be achieved up to nearly 90% of dry cell mass in specific environments. Saturated and unsaturated fatty acids are the major lipid constituents. Fatty acids from the ω3 and ω6 family stand particularly of high significance in comparison to numerous fatty acid families that comprise the microalgal lipid (Blasio and Balzano 2021).

Microalgae typically accumulate two kinds of lipids—polar lipids, such as phospholipids and glycolipids, and neutral lipids, like acyl-glycerides and free fatty acids. The polar lipids facilitate the cell membrane formation, hence also known as structural lipids. The application of neutral lipids is in the delivery of energy. Table 1.2 features the chief chemical components of some microalgae species. Generally, the range of lipids varies from approximately 20–50% of the dry cell weight in microalgal cells. Microalgal cells produce chemical constituents with varying content in various species depending on the growth conditions. Therefore, the content of lipid concentration in microalgae could be modified by cultivating microalgal cells in specific environmental conditions. The surroundings in which microalgae is grown can also be altered by several factors. One such major factor is stress that triggers the production of lipids in the microalgae. These stress could be provided by developing the conditions of nutrient starvation or some other external stimuli. Phosphorous and sulfur depletion leads to modification in the production of neutral lipids, whereas, nitrogen deficiency could lead to enhanced triglyceride formation. Illumination, temperature and salinity are some of the other factors that affect the accumulation of lipids in microalgal cells. Dong et al. (2016) demonstrated that a prolonged cultivation period and nutrient exhaustion leads to a reduction in protein concentration with a simultaneous increase in carbohydrate as well as lipid concentration in microalgae. Yet, the range of deviation depends on the species and strain.

Microalgae comprises of both the minor and the major components. Minor components in microalgae such as pigments have potential application in several industries like food, pharmaceutical and cosmetics. Chlorophylls, carotenoids and phycobiliproteins are some kind of pigments that are present in microalgae. Vitamins are other essential minor components of microalgae. Microalgae act as a warehouse for nearly all the essential vitamins. The growth conditions, the harvesting methods, and the cell drying techniques affect the vitamin quantity within the cells. Vitamins do improve the nutritional value of microalgal cells.

1.1.2 Cultivation of Microalgae

The growth of microalgae depends on several elements such as reactor system, type of reaction, and cultivation condition. The major factors for microalgae cultivation, such as nutrients, light, pH, CO_2, temperature, etc., influence the characteristics as well as constituents of microalgae. Light is the energy source which is mandatory for photosynthesis and growth. The pH and temperature of the microalgal growth medium and surrounding, respectively, needs to be kept at particular values so as to encourage the microalgal cell growth. Carbon dioxide basically serves as the source of carbon, which is required for cell development. Hasnain et al. (2018) described obtaining about 1 kg of biomass of microalgae from 1.8 kg of carbon dioxide. Microalgae typically need phosphorus and nitrogen for growth. The nutrients are obtained from organic as well as inorganic sources, however, utilization of nutrients from inorganic source can lead to pollution, especially water. As a consequence, wastewater

Table 1.2 Microalgal Chemical components suitable for microalgal biorefinery (% dry biomass) (Ravindran et al. 2016; Shuba and Kifle 2018)

S. No.	Microalgae	Carbohydrate (%)	Protein (%)	Lipid (%)
1	*Anabaena cylindrical*	25–35	43–57	4–7
2	*Botryococcus braunii*	20	4	25–75
3	*Chaetoceros calcitrans*	10	58	39
4	*Chaetoceros muellerii*	11–19	44–65	40–57
5	*Chlamydomonas rheinhardii*	17	48	21
6	*Chlorella emersonii*	37.9	9.03	29.3
7	*Chlorella protothecoides*	12–17	15–58	14–56
8	*Chlorella pyrenoidosa*	25	57	2
9	*Chlorella vulgaris*	12–21	51–56	16–40
10	*Chlorella zofingiensis*	11.5	11.2	56.7
11	*Chlorogloeopsis fritschii*	37.8	41.8	8.2
12	*Dunaliella bioculata*	4	49	8
13	*Dunaliella salina*	32	57	6
14	*Dunaliella tertiolecta*	14	29	11
15	*Euglena gracilis*	7–25	30–45	21–38
16	*Haematococcus pluvialis*	15–40	17–45	20–37
17	*Porphyridium cruentum*	40–58	29–40	11–20
18	*Prymnesium parvum*	25–33	28–45	22–39
19	*Pseudochoricystis ellipsoidea*	34	10.2	38
20	*Scenedesmus dimorphus*	17–51	8–18	15–45
21	*Scenedesmus obliquus*	10–16	50–56	20–55
22	*Spirogyra* sp.	33–64	6–21	11–19
23	*Spirulina maxima*	13–16	60–71	6–7
24	*Spirulina platensis*	8–14	42–63	4–11
25	*Synechococcus* sp.	15	63	11
26	*Tetraselmis maculata*	15	52	3

management becomes an essential step in microalgal cultivation. To tackle such an issue, the utilization of wastewater for microalgal growth could be a better approach since the wastewater has abundant phosphorous and nitrogen. This method is not only useful in reducing the cultivation cost of microalgae, additionally, it facilitates in decontamination of the water. Hence, microalgae help in wastewater treatment. Though, for considering wastewater as an alternate source of nutrition, it's important to employ an efficient wastewater evaluation.

The commercial production of biodiesel derived from microalgae is an amalgamation of different processes carried out in a particular sequence. This process includes microalgae cultivation followed by harvesting, and at last, lipids conversion to biodiesel. The cultivation of microalgae can be carried out either in open ponds or in closed photo-bioreactors. Though open systems such as ponds require low capital costs in comparison to photo-bioreactors, they are prone to contamination. Additionally, there is an immense loss of water because of evaporation. Moreover, the utilization of carbon dioxide is not optimal due to the absence of a mixing process. On the contrary, a photo-bioreactor is a sealed and controlled system which needs little area and suffers less contamination. The only disadvantage is the involvement of high production costs.

Microalgal cells are grown either in pure form or mixed cultures. For heterotrophic cultures, the source of carbon and energy are similar, but different sources of carbon and energy are required for phototrophic cultures. Organic carbon, such as glucose, serves as a source of carbon and energy both in the case of heterotrophic cultures. In contrast, for phototrophic cultures, CO_2 and light serve as a source of carbon and energy, respectively. A mixotrophic culture comprises of both phototrophic and heterotrophic cultures together. Such a culture results in enhanced cell production rate and higher productivity of lipids in comparison to individual cultures. Microalgal cells require organic substrate for growth that sums up to approximately 80% of the total cultivation cost. Therefore, to minimize the cost of production of biodiesel, accurate selection of the culture parameters and their proper optimization is highly crucial.

Harvesting, also referred to as dewatering, is a method for the removal of water post cultivating the microalgae. Harvesting is the second step during the microalgal cultivation for production of biodiesel. This process is further split in two steps which includes bulk harvesting and then thickening. The bulk harvesting employs sedimentation, flotation and flocculation methods for collection of solid matter i.e. separation of biomass or microalgal cells from the culture medium. The quantity of biomass is increased by thickening with the help of centrifugation followed by filtration. The cost of harvesting is one of the important factor linked with microalgal biodiesel production, the other major factor is the energy requirement. 20 to 30% of the total cost of production is invested in harvesting. Therefore, it becomes essential to choose a species of microalgae which keeps the process of harvesting easy and simple post growth.

After harvesting, the drying step is a subsequent step that aids in reducing the content of water from the microalgal cells to approximately 10% from 60%. This stage bears high importance as microalgal biomass with elevated water content could have an undesirable effect on lipid conversion. Steriti et al. (2014) demonstrated that lipid yield was higher in the case of dried microalgae in comparison to wet biomass. Takisawa et al. (2013) backed the respective finding with a comparable demonstration. They stated that microalgal biomass with up to 80% of water content, on direct usage, i.e. skipping the drying step, caused various difficulties during the release of lipids from the cells post-harvesting. The process of drying is an energy-demanding procedure, and the exclusion of this vital process can minimize the overall energy expenditure, thus lowering the total production cost. Therefore, it is crucial to create improved technologies that are able to convert lipids into biodiesel even from microalgal biomass with water content without increasing the total cost and at the same time achieving high conversion efficacy.

To summarize, microalgae are potential alternative feedstock for biodiesel production, which are sustainable with distinct advantages. However, a thorough and significant study is required along with optimization of the several governing factors for commercializing microalgal-based production. Also, methods are need to be designed in order to reduce the energy utilization and operation, and total costs associated with microalgal biodiesel production. The other important factors include selection of appropriate microalgae species, finding optimal growth conditions, and adaption of proper protocols for lipids extraction and conversion. Moreover, the recovery of value-added products from microalgae is utmost essential as it generates another source of income for the microalgal biorefinery.

1.2 Microalgae–Derived Products and Their Applications

The products extracted from the biomass of algae or the whole microalgal cells exhibit numerous potential usages. Such purposes span from being a feedstock for bio-alcohol fermentation, feed for the production of biodiesel, animal feed to aquaculture, and a possible supply of nutritional and health products. Additionally, the cultivation of microalgae aids in achieving measured cell quality that avoids infection from herbicides, pesticides, or several additional harmful substances. In a way, each part of microalgae exhibits its individual purpose in various industries. Microalgal cells can also be utilized for various functions, such as CO_2 mitigation and wastewater treatment. Certain uses of microalgal cells and the products derived from them are shown in Fig. 1.2 and are discussed hereafter.

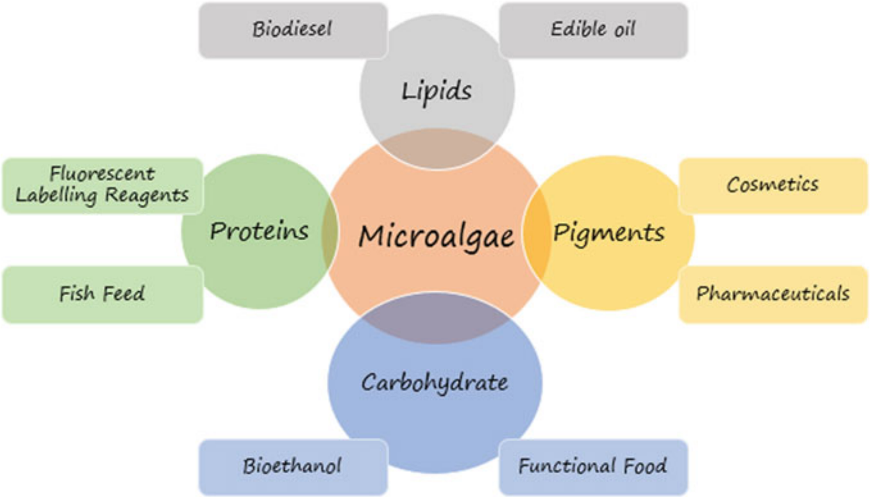

Fig. 1.2 Microalgae-derived products and their potential applications

1.2.1 Lipids as Feedstock for Producing Biodiesel

Microalgal cells exhibit lipids of two different types. Non-polar lipids such as triglycerides play an important role of storing energy. The other are glycerol-phospholipids or polar lipids that perform a vital function in cell structure, hence described as structural lipids. In general, polar lipids contain PUFA's (polyunsaturated fatty acids) long-chains. Few examples of essential PUFAs derived from microalgal cells are Docosapentaenoic acid (DPA), Docosahexaenoic acid (DHA) and Eicosapentaenoic acid (EPA). All the mentioned lipids and some sterols provide a selectively permeable barrier that is beneficial to protect microalgae. Polyunsaturated fatty acids obtained from microalgae also possess several functions, for example, formation of super complex of mitochondria, biodiesel production, and cure for diseases like Atherosclerosis, Alzheimer, and Parkinson. Polar lipids too perform vital functions in different metabolic and biosynthetic procedures by sustaining a standard fluidity of the membrane. PUFAs contribute in various fusion actions occurring in the intracellular membrane. Moreover, cell signaling is one more important function of the structural lipids that promotes changes in the cellular environment thus protecting the cells from unwanted environmental disturbances.

The TAGs or triglycerides, in contrast, are related to the storage of energy within the cells. During photosynthesis, the light energy (solar) gets converted into chemical energy which is then used for growth by microalgae and a few additional metabolic functions. To enable the utilization of this energy, a carbon skeleton (glycerate-3-phosphate) is employed by the microalgal cells that is changed to distinct molecules (such as lipids, pyruvate, amino acid, etc.). Therefore, the growth, maintenance, and various complex metabolism of the microalgal cell is provided by the intense method of energy conversion and storage. Microalgal lipid production with respect to quantity as well as quality differs among microalgal species. Vasudevan and Briggs (2008) stated that the low lipid-producing microalgae strains content grew rapider in comparison with the high lipid-producing microalgae. Later, Francisco et al. (2010) also confirmed that microalgae strains containing elevated content of lipid result in reduced biomass productivity.

Biodiesel is generally an alkyl ester of long-chain fatty acids that possess diesel like-properties. Biodiesel is the chief possible biofuel derived from microalgal biomass, which forms due to the transesterification reaction of lipids from microalgae (Singh et al. 2020). The transesterification reaction for biodiesel production is essentially performed within a closed and controlled system. Once triglycerides are blended with alcohol and a catalyst (acid or alkali), it initiates the transesterification reaction, thus resulting in the formation of glycerol and fatty acid esters. On completion of the process, it is important to segregate the formed end-products in order to get pure biodiesel which can be utilized commercially. This separation of glycerol from esters is achieved through a funnel-type separator. Hence, the separated esters are evaporated so that the remaining alcohol is removed and finally rinsed with water thoroughly to free the products of any impurities. This regular procedure used for producing biodiesel makes it a promising fuel for production at a commercial scale, which can serve as an alternative to fossil-based fuels.

In order to replace the current biodiesel feedstock, such as vegetable oils with oils from microalgae, the lipid-derived biodiesel needs to comply with the set standards of fuel properties. A few examples of such properties are cold filter plugging point, iodine value, viscosity, cetane number, and ignition quality. EN 14214 (Europe) and ASTM D6751 (United States) are two globally accepted standards to define biodiesel properties (Francisco et al. 2010). One of the governing properties, i.e. the fatty acid profile of the biodiesel, is reliant on a few microalgal growth parameters like temperature, light intensity, etc. The biodiesel having greater oleic acid content (C18:1) possesses stable fuel properties, like ignition quality, the heat of combustion, oxidation stability, and viscosity. In colder regions, better oxidation stability and prolonged storage are attained because of the higher oleic acid concentration present within the microalgal FAME (fatty acid methyl ester) profile. Consequently, classes of microalgae having a higher oleic acid concentration in the FAME are preferable for producing biodiesel. Few of the microalgal species, such as *Scenedesmus, Nannochloropsis*, *Botryococcus,* and *Picochlorum*, exhibit high content of oleic acid, thus making essential feedstock for producing biodiesel.

Table 1.3 reviews the previous works on the production of microalgal biodiesel employing diverse catalysts and techniques used with two-step and in situ transesterification processes. Biodiesel production via microalgae is still not commercialized. The main reasons include the high cost of operation and the process being energy intensive. Specifically, biomass drying is a major controlling factor, being cost intensive. Thus, processes employing direct usage of wet biomass possibly will have greater values with respect to energy consumption. Another concern limiting the procedure is catalyst dilution because of the high moisture content in the biomass. Hence, the utilization of catalysts exhibiting higher activity (lower sensitivity to moisture) can provide a way to fix the concern.

On the contrary, subcritical or supercritical conditions can be used to carry out non-catalytic transesterification process. These processes are performed at a higher range of pressure and temperature compared to catalytic routes. Although such routes are energy exhaustive, the discrete benefits of their employment are higher conversion in less reaction time and easier and simpler product separation and purification. Also, biodiesel conversion is comparatively independent of the primary conditions of the feedstock (quantity of free fatty acid and water) under super- or subcritical conditions. This helps in facilitating the use of entire microalgae as a feedstock which contains high content of moisture, approximately up to 90%, along with a higher quantity of FFA content.

Both two-step and in situ transesterification processes are previously reported of being able to be conducted within critical conditions. The first step in the two-step transesterification process is the extraction of lipids with subcritical water or other solvents. The subsequent process involves the transesterification reaction performed at supercritical conditions. As this reaction is sequential, this makes the process energy intensive. Hence, carrying out in situ transesterification reactions in a supercritical environment might support the reduction of consumption of energy.

Table 1.3 Microalgal biodiesel production using different catalyst

Sr. No.	Microalgae	Catalyst	Process	Operating conditions	Yield (%)	References
1	*Botryococcus* sp. (dry)	Immobilized lipase (*Candida antartica* lipase B) 20%	In situ transesteri-fication	T = 50 °C, t = 36 h, reactant: dimethyl carbonate = 5 mL/g, distilled water = 1%	78	Sivaramakrishnan and Incharoensakdi (2017)
2	*Botryococcus* sp. (dry)	Immobilized lipase (*Candida antartica* lipase B) 10%	Ultrasound-assisted in situ transesteri-fication	Ultrasound: 30 kHz, 200 W, T = 50 °C, t = 4 h, reactant: dimethyl carbonate = 5 mL/g, distilled water = 1%	88	Sivaramakrishnan and Incharoensakdi, (2017)
3	*Chlamydomonas* sp. JSC4 (slurry: 31.3% dry biomass)	NaOH (0.5 wt%)	Two-step transesterification	Pretreatment: microwave (350 W, 10 min), solvent extraction: methanol/hexane = 3:1, T = 45 °C, t = 80 min, transesterification: hexane + oil/methanol = 6:1 (v/v), T = 45 °C, t = 15 min, agitation = 600 rpm	95	Chen et al. (2015)
4	*Chlorella vulgaris*	CaO (1.39 wt%)	Direct Transesterification	T = 75 °C, t = 3 h, agitation = 140 rpm, methanol/biomass = 10:1 wt./vol	92.03	Pandit and Fulekar (2019)
5	*Chlorella* sp. (dry powder)	Impregnated pumice (20 wt%)	In situ transesteri-fication	T = 80 °C, t = 3 h, agitation = 500 rpm, methanol/biomass = 12:1 mL/g	42	Daniel et al. (2017)
6	*Chlorella pyrenoidosa*	Graphene oxide (5 wt%)	Microwave-assisted in situ transesterification	Microwave: 600 W (heating) 500 W (holding), T = 90 °C, t = 40 min, methanol/chloroform = 1:1 (v/v)	95.1	Cheng et al. (2016)
7	*Chlorella vulgaris*	CaO.Al_2O_3 as catalyst (1.56 wt%)	Transesterification	T = 50 °C, t = 125 min, methanol/oil volumetric ratio = 3.2:10	88.89	Narula et al. (2017)

(continued)

Table 1.3 (continued)

Sr. No.	Microalgae	Catalyst	Process	Operating conditions	Yield (%)	References
8	*Chlorella vulgaris*	Free Lipase GH2 (150 µL)	Transesterification	T = 30 °C, t = 24 h, agitation = 150 rpm, alcohol/oil molar ratio = 3:1	–	Huang et al. (2015)
9	*Chlorella* sp.	H_2SO_4 (0.6 mL)	In situ transesteri-fication	T = 90 °C, t = 2 h, hexane/ethanol = 1:2 (v/v), solvent = 6 mL	90.02 ± 0.55	Zhang et al. (2015)
10	*Chlorella* sp. FC2 IITG	NaOH and H_2SO_4	Two-step direct transesterification	T = 90 °C, agitation = 150 rpm 1st step: t = 20 min, catalyst/biomass = 0.67 (w/w), methanol/biomass = 49.51 (v/w), 2nd step: t = 10 min, catalyst/biomass = 2.07 (v/w), methanol/biomass = 61.07 (v/w)	98.96	Kumar et al. (2014)
11	Euglena sanguinea	Calcium methoxide (5 wt%)	Transesterification	T = 343 K, t = 75 min, agitation = 500 rpm, methanol/oil molar ratio = 15:1	94.83	Sivagurulingam et al. (2019)
12	Mixed culture	H_2SO_4 (1.5 wt%) and KOH (3 wt%)	Two-step transesterification	1st step: T = 55 °C, t = 90 min, methanol/oil molar ratio = 7:1, 2nd step: T = 60 °C, t = 1 h, methanol/oil molar ratio = 6:1	96	Karmakar et al. (2018)
13	*Nannochloropsis gaditana* B-3 (25% dry biomass)	Novozym 435 (N435)	In situ transesteri-fication	Pre-treatment: high pressure homogenization, T = 40 °C, t = 56 h, methanol/oil = 4.6: 1 (mL/g), t-butanol/oil = 7.1:1 (mL/g), N435/oil = 0.32:1	99.5	López et al. (2016)
14	*Nannochloropsis salina* (moisture: 76.5 ± 1%)	H_2SO_4 (50 µL)	In situ transesteri-fication	T = 100 °C, t = 1 h, wet biomass = 200 mg, methanol = 2 mL	99.7	Kim et al. (2015a)

(continued)

Table 1.3 (continued)

Sr. No.	Microalgae	Catalyst	Process	Operating conditions	Yield (%)	References
15	*Nannochloropsis gaditana* (80% moisture)	HCl (0.3 mL)	In situ transesteri-fication	T = 95 °C, t = 2 h, methanol/chloroform = 1:2 (v/v)	85.7 ± 1.7	Kim et al. (2015b)
16	*Nannochloropsis*	$Mg_2Zr_5O_{12}$	In situ transesteri-fication	T = 65 °C, t = 4 h, methanol/methylene dichloride (10 wt%) = 3:1 (v/v)	60	Li et al. (2011)
17	*Scenedesmus* sp.	CaO (2 wt%)	Transesterification	T = 60 °C, t = 3 h, agitation = 1500 rpm, methanol/oil molar ratio = 11:1	92	Mamo and Mekonnen (2020)
18	*Scenedesmus obliquus* (Trup.) Kutz. (SAG 276-3a)	HCl	Transesterification	T = 65 °C, t = 6.4 h, methanol/HCl/oil = 82:4:1	68.3 ± 1.6	Patnaik and Mallick (2015)
19	*Tetradesmus obliquus* IPPAS S-2023	H_2SO_4 (5 wt%)	Transesterification	t = 1.5 h, 0.01% 2,6-di-tert-butyl-4-methylphenol as antioxidant	–	Ismagulova et al. (2018)

1.2.2 Protein—Utilization in Feed and Food Industry

As illustrated in Table 1.2, microalgal cells are an abundant source of proteins. Thus, they can be utilized in the feed and food industry. Microalgal cells have high quantities of proteins that comprise all essential amino acids of different concentrations (dependent on the type of strain). Microalgae like *Spirulina* can possess proteins higher than 60% of dry cell weight and can be employed for nutrition in humans. Microalgal cells are also usually eaten as tablets, powders or pills in the form of dietary supplements. Few research reported that the majority of bioactive components found in edible microalgal cells become disabled upon treating with heat.

Martínez-Hernández et al. (2018) demonstrated that microalgae in the form of powder are great suppliers of protein and amino acids, reporting their ability to be used as nutrient supplements. Liang et al. (2004) stated that over a hundred industries in China are intent on the usage of microalgal cells as a food supplement. However, substantial studies were conducted earlier to discover the capability of proteins extracted from microalgae to be used as protein supplements for human consumption (Raja et al. 2016). It is reported that the quality of protein from microalgae is comparable to or sometimes even better than the protein extracted from plants. *Spirulina* and *Chlorella* are sometimes blended with the food delivered to poultries and cattle industries to obtain better quality and yield of milk, egg, and meat.

Spirulina is naturally found in lakes, generally in subtropical climates that have high salinity. These species have tolerance to high pH levels, up to 10 pH. The microalgal species *Chlorella* are claimed to be able to be comfortably digested by animals on consumption as paste (5%) upon mixing in animal feed. Harari et al. (2013) reported higher digestibility of protein in pigs by around 56%. *Chlorella* also causes weight gain in sheep. The microalgae companies such as Necton and Allma supply *Spirulina* and *Chlorella* commercially for human consumption in the form of soup, smoothies, millets, juices and dietary supplements, like antioxidants and source of protein (Raja et al. 2018).

1.2.3 Pigments—For Cosmetic and Pharmaceutical Industry

Microalgal cells have shown the presence of various pigments that are associated with light. Chlorophyll is the main pigment in the microalgal cells which is a primary photosynthetic compound besides other essential pigments such as phycobiliproteins (PBPs) and carotenoids. PBPs, for example, phycocyanin and phycoerythrin, are limited to microalgae and vastly utilized in cosmetic industries and for dietary usage. Microalgal carotenoids also have various applications in different industries. For example, astaxanthin and β-carotene are extracted from *Haematococcus* and *Dunaliella*, for application as a red colorant in aquaculture and as supplements for vitamins in pharmaceutical industries, respectively. Additionally, carotenoids such as zeaxanthin, and canthaxanthin are also employed in pharmaceuticals.

Cosmetics: Microalgae such as *Chlorella* and *Arthrospira* are usually utilized in skincare. Some cosmetic companies own the facility of cultivating their own microalgae systems. Pigments extracted from some microalgal cells are utilized in cosmetic products, such as sunscreen, hair care, rejuvenating items, and anti-aging lotions. These microalgal extracts are also used as thickening agents, water-binding agents, and antioxidants. *Spirulina platensis*, *Ascophyllum nodosum*, *Dunaliella salina*, *Chondrus crispus*, *Chlorella vulgaris, Nannochloropsis oculata*, and *Alaria esculenta* are general microalgae used widely in cosmetics. Sharma and Sharma (2017) stated the use of *Spirulina* species in several cosmetic firms, such as in masks and facial scrubs, aimed at cleaning and dead skin removal, improvement of moisture balance as well as immunity.

Pharmaceuticals: Carotenoids are used in several pharmaceutical products because of their biological activities. These microalgal activities hold potential as antibacterials, antifungals, antioxidants, and antiviral agents. Antioxidants exhibit properties like scavenging free radicals, which is useful for an anti-cancer activity. The highly promising raw microalgal antioxidant is astaxanthin. Higuera-Ciapara et al. (2006) detailed the ability of astaxanthin to exhibit about 100X health benefits compared to tocopherol for its higher antioxidant property. Various scientific literatures report the possible usage of astaxanthin for curing different illnesses like diabetes, neurogenerative disorders and cancer. β-carotene is a vitamin A precursor having antioxidant properties, thus, creating a path for its usage in healthy foods. National Cancer Institute declared β-carotene as an anti-carcinogenic compound. Additionally, it assists in avoiding heart diseases and facilitates controlling cholesterol levels. Hu et al. (2008) reported that β-carotene derived from *Dunaliella* exhibited elevated antioxidant properties compared to synthetic β-carotene. This is because synthetic β-carotene is formed with only trans-isomers, while both trans and cis isomers form the microalgal β-carotene.

1.2.4 Carbohydrates—For Producing Bioethanol

Microalgae is a potential bioethanol producer due to its high carbohydrate content. In microalgae, the conversion of carbohydrates to bioethanol is achieved by fermentation. It is a procedure that comprises sugar (cellulose or starch) conversion from the microalgal biomass into alcohol. Firstly, microalgae are exposed to pretreatment prior to fermentation. The process of pretreatment helps in breaking the complex sugars like cellulose into their monomeric state like glucose, thus facilitating simple fermentation using microbes. The produced bioethanol by the microorganisms further needs purification by the distillation process to remove excess water and impurities. Microalgae that have a high quantity of carbohydrates, such as *C. vulgaris,* are a promising source of bioethanol production that helps in attaining bioethanol conversion of up to 65%.

Ueno et al. (1998) described the possibility of employing a green marine microalgae *Chlorococcum littorale* for bioethanol production through dark fermentation. Rojan et al. (2011) also assessed several microalgae (such as *Arthrospira*, *Dunaliella*, *Chlamydomonas, Euglena*, *Chlorella, Spirulina*, *Scenedesmus, and Prymnesium*), which were promising as feedstocks for bioethanol production and further persuaded their use for biofuel from a renewable source, therefore, providing an eco-friendly alternative. In Brazil, bioethanol has been used commercially as fuel or as an additive to gasoline in approximately 86% of cars. However, the usage of bioethanol still has several limitations due to its low vapor pressure and low energy density.

1.2.5 Additional Biofuels Derived from Microalgae

Biohydrogen: Recently, the production of bio-hydrogen is widely being considered because hydrogen remains the purest fuel. Though, commercial production of biohydrogen seems unachievable as the procedure requires high-cost input and produces less amount of biomass. Several works described the introduction of some microalgal cells with stress conditions like scarcity of light to facilitate the production of hydrogen gas in huge quantities. Still, this technique is at the preliminary phase of the study and needs considerable improvisation. The microalgae can generate hydrogen in two ways, i.e., direct generation of hydrogen by microalgae or using microalgae for feedstock by other microbes to produce hydrogen (Ahmed et al. 2021). The direct generation of hydrogen by microalgae can be achieved by three pathways: (1) direct photolysis, (2) indirect photolysis and (3) pathway that is ATP driven. The direct photolysis process is achievable when oxygen and hydrogen produced are separated constantly. This route involves the production of oxygen and hydrogen together that increases the separation cost as well as chances of security risks. Furthermore, in this process, hydrogenase enzyme is used which is oxygen sensitive, thus, indirect process is more feasible compared to direct process. In anaerobic surroundings, starch found in algal cell walls gets transformed to hydrogen to some extent. Several literatures have reported that cyanobacteria use hydrogenase and nitrogenase enzymes as catalysts to mainly produce biohydrogen.

Bio-methane: Biogas which is generated during anaerobic biomass breakdown, is a promising method of obtaining energy. An anaerobic digester is employed for deriving biomass-based energy. It contains a synergy of various microbial populations, which facilitates the conversion of organic compounds from microalgae (proteins, lipids, and carbohydrates) to biogas, that is essentially a carbon dioxide and methane mixture. Methane is vastly exploited as both, a chemical feedstock as well as fuel. Many researchers have explored the capability of various feedstocks like microalgae, solid waste, grass, wood, etc., for producing biomethane and observed microalgae to be a promising candidate. The productivity of microalgal biomass is, in general, greater than plants, although its growth is subjected to the presence of various nutrients. Wang et al. (2016) investigated the thermal pretreatment of microalgae to achieve enhanced biomethane production using *Chlorella* sp. Methane yields obtained from untreated algae were 155 mL/g VS_{add}, whereas thermal pretreatment for 0.5 h at 70 and 90 °C enhanced the methane yield by 37% and 48%, respectively. On increasing the thermal pretreatment temperature to 121 °C for 0.3 h, the highest methane yield (322 mL/g VS_{add}) was obtained, which was 108% more compared to raw microalgae.

Bio-oil: Bio-oils are mainly formed by thermochemical transformation that could be utilized as an alternative to petroleum oils. The bio-oil production process includes two steps—pyrolysis and thermochemical liquefaction. The process of pyrolysis is carried out at a temperature range between 350–550 °C aimed at producing liquid beside gases and solid residues. The liquid portion contains both aqueous and non-aqueous phases, viz. bio-oil/tar, and the remaining microalgal biomass is desiccated. Bio-oil generally comprises of various organic compounds like carbohydrates, proteins, and lipids. Different microalgal categories are investigated for producing bio-oil via pyrolysis or thermochemical liquefaction. The yield of bio-oil varies with the microalgal species, e.g., a bio-oil yield of around 41% was attained in *Spirulina*, it varied from 24 to 45% for *Scenedesmus*, about 37% in *Dunaliella* and approximately 49% for *Desmodesmus.*

1.2.6 Other Microalgal Applications

Aquaculture: The microalgal biomass serves as an important food supply for the larva of marine creatures such as gastropods, mollusks like scallops, oysters, and a variety of fishes, zooplanktons, and shrimps. Hence, after growing the microalgae, they are concentrated in order to make algal pastes and/or solid cubes so as to store at low temperatures with refrigeration by the aquaculture industrialists. Generally, the aquaculture feed formulations are stored in paste form. These formulations may comprise of one or more algal species in order to provide a balanced and complete nutrition. Microalgae feed also serves as an essential source of fatty acids for fish. It is known that Omega-3 fatty acids, for instance, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are extracted and sold as fish oil for human consumption. However, EPA and DHA are not produced by fish, but their accumulation and storage inside fishes are facilitated by the microalgae. Microalgal pastes, too, are desiccated and chilled to form frozen cubes of specific volume for direct use. The process of freezing facilitates in increasing the shelf life of microalgae. These frozen cubes are supplied directly in the tanks of water or indirectly by diluting in the water. Eukaryotic classes of algae which are utilized for producing such aquaculture feeds are Chlorophyceae and Bacillariophyceae. Additionally, *Chaetoceros* sp, *Nannochloropsis oculata* and *Phaeodactylum tricornutum* are some of the marine microalgae which are crucial for the commercial production of paste. These species are blended in the seawater to obtain a microalgal solution of 10% that is subsequently cooled to form frozen cubes for providing homogenous feeds to aquaculture industry.

CO_2 mitigation: The numerous routes investigated for CO_2 capture can be environmentally or economically unfeasible. However, sequestration of CO_2 in microalgal biomass can become beneficial because of value-added product formation like high-grade lipids and pigments, which can be extracted from few microalgae species. In this sense, biological CO_2 mitigation can prove to occur as a breakthrough method of much needed significant reductions in levels of CO_2. Hence, microalgal cells can be advantageous as they could convert carbon dioxide into biomass at higher rates by photosynthesis when compared to typical biofuel crops. Microalgal biomass obtained, by the help of anaerobic bacteria, could be further converted into hydrogen or methane, thus declaring microalgal cells beneficial for the production of hydrothermal methane. Microalgal oil production has acquired recent interest because of the simple production process, wherein the lack of nitrogen source leads to a type of secondary metabolism. Later, the extraction of lipids and subsequent re-esterification is achieved by short-chain alcohols. After extraction, the oils are subjected to hydrolysis and then to re-esterification with moieties of ethyl and methyl alcohol for biodiesel production. To accomplish sustainable biofuel production and microalgae facilitated CO_2 fixation, production of microalgal biomass must be attached with wastewater management set-ups.

Wastewater treatment: The microalgal cells are skilled to utilize water of poor quality, such as municipal and industrial wastewater or agricultural runoffs as the source of growth medium such as phosphorus, nitrogen and other minor nutrients. Phosphorous and nitrogen present in the wastewater serves as nutrients for microalgae which in turn provides dual role by helping in growth of microalgae as well as biologically cleaning the water. With such an approach, the microalgal nutrient requirements are attained by using inorganic compounds like phosphorous and nitrogen available in wastewater. Additionally, microalgae are beneficial in the reduction of harsh consequences of sewage waste and nitrogenous trash coming from fish aquaculture or wastewater from industries which could otherwise impact the biodiversity. Furthermore, the elimination of nitrogen and carbon from wastewater helps in the reduction of eutrophication inside marine ecosystem. Aslan and Kapdan (2006) observed and showed that about 72% and 28% N_2 and P, respectively, were removed from wastewater using the microalgal sp. *C. vulgaris*. The microalgal species of *Spirulina* and *Chlorella* are commonly exploited for removal of nutrients. *Botryococcus brauinii* and *Phormidium bohneri* are also investigated for their ability of removing the nutrients. Thus, merging the microalgal capability of producing biofuel or various value-added bio-products and the procedures like treatment of wastewater might prove to be more sustainable and economical. Hence, several investigations are now focusing on the evaluation of microalgae potential for simultaneously synthesizing value-added products and environmental applications.

1.3 Microalgal Extracellular Metabolites

Metabolites like glycerol and acetate are produced and secreted into the medium by microalgae during their growth. These microalgal extracellular metabolites hold crucial ecological significances. For example, huge quantities of dissolved organic substances are released by marine microalgae, which act as the source of energy for heterotrophs during the symbiotic interactions of algae and bacteria. Considerably, the sequence of allelopathic interactions amongst other microorganisms and the microalgae is determined by the excretions done in pericellular space. Microalgae have certain allelopathic compounds that are considered to be biocontrol agents or environment-friendly herbicides and have straight prospects for use in the biotechnology sector. A forthcoming field for application of microalgal extracellular metabolites may be the use of microalgal extracellular biopolymers for improved oil recovery. Additionally, various microalgal extracellular polysaccharides possess several bioactivities, such as antiviral, anti-inflammatory and antitumor, offering encouraging prospects for application in the pharmaceutical sector. Consequently, the microalgal extracellular metabolites may also be possibly applied in various industries like cosmetics, feed, food and oil. Thus, they have the potential for development and commercialization. Few extracellular bioactive substances having possible biotechnological applications, from industrial microalgae holding commercial interest are described below.

1.3.1 Allelopathic Chemicals

Interactions amongst organisms due to release of chemicals is common within all the microalgal species. The interactions among microalgal species facilitated by the discharge of compounds that are biologically-active, such as allelochemicals or phytotoxins, in the environment is termed as allelopathy and the compounds are called allelopathic chemicals. Numerous microalgal species are capable of excreting significant amount of biologically active compounds within the medium. Some of these compounds are described in brief.

Few polyunsaturated aldehydes are known for showing allelopathic effect. These polyunsaturated aldehydes are formed by enzymatically breaking the polyunsaturated fatty acids by different lipoxygenase pathways and thus forming halogenated metabolites, hydroxyl acids and polyunsaturated aldehydes. An intact microalgal cell releases these aldehydes into the environment in the late stationary phase but can also be released during growth process by damaging the cells. One such example is Oxylipins, which are known for olfactory properties. This compound is a prospective candidate for industrial scale development as it also regulates the hormones in plants and mammals.

Alkaloids are generally found in higher plants but few microalgae also produce alkaloids. *Calothrix* sp., a nitrogen fixing cyanobacteria releases an allelopathic compound indolophenanthridine calothrixin A which is capable of killing many organisms like fungi and bacteria. In *Bacillus subtilis*, this compound is known to inhibit the replication of DNA, synthesis of RNA and thus synthesis of protein. Volk et al. (2009) reported the isolation of a brominated indole alkaloid called Bromoanaindolone, by culturing cyanobacterium *Anabaena constricta*. Bromoanaindolone possess excellent antibacterial activity.

Berry et al. (2008) summarized that several cyanobacteria like *Anabaena*, *Microcystis*, *Nostoc*, and *Oscillatoria* produce toxins that have potential applications as insecticides, herbicides and also algaecides. For instance, the cyclic heptapeptides called microcystins, having an unusual aromatic amino acid and two variable amino acids, that is produced by *Anabaena flosaquae* is secreted out in the growth medium where it paralyzes the growing of the microalgae *Chlamydomonas reinhardtii*. The study of the wide phylogenetic microalgal species demonstrated that the microalgae are also a potential source of methanol.

1.3.2 Exopolysaccharides

Biopolymers of high-molecular-weight are known as exopolysaccharides that are secreted into the growing medium by the microorganisms like microalgae during the growth phase or the propagation phase. They are emitted in the growing environment or can also be roughly joined with the cell wall. Structurally varied exopolysaccharides are produced by several microalgal species, particularly cyanobacteria or a form of red algae. Exopolysaccharides play various roles for the microorganisms such as biofilm formation, cell-to-cell interactions, adhesion and protection of cells from the adverse stress caused in the natural environment. Exopolysaccharides are commonly consumed in the food industry as gelling agents and thickeners, thus improving the overall food texture and quality. Recently, exopolysaccharides are considered a potential candidate in the pharmaceutical sector as they possess anticancer, anti-oxidation and antibacterial properties. The recovery and purification of these exopolysaccharides is very easy as they are secreted in the culture medium.

1.3.3 Extracellular Phytohormones

Phytohormones (or plant hormones) are chemicals that are produced by plants as they are crucial for regulating their growth, their development, longevity, reproduction, death and stress responses. Higher plants are known for their ability to produce and release phytohormones such as gibberellic acid, abscisic acid and auxin. In microalgae and macroalgae, these phytohormones occur in form of few endogenous substances which have the growth stimulating ability. Moreover, the growth of microalgae can also be affected by certain externally applied synthetic regulators. For instance, the growth of the chlorophytes *D. salina* and *Haematococcus pluvialis* can be significantly increased by kinetin and 2,4-dichlorophenoxyacetic acid. More study on the microalgal phytohormone metabolism and regulatory networks can present different opportunities and views on the cultivation and application of microalgae; as the existence and metabolism of these extracellular hormones in microalgae is yet weakly understood. Different phytohormones produced by the microalgae with their ability are described below.

ABA or Abscisic acid that acts against various stress causing factors are produced by plants. Whereas, under various stress conditions, few cyanobacteria and microalgae produce the extracellular abscisic acid. During the growth of *Stichococcus hacillaris* and *C. vulgaris*, stress caused due to acid, salt, or drought increases the production of extracellular ABA by 5–10 counts. After six days of growing the cyanobacteria *Synecbococcus leopolienssis*, *Trichormus versicolor* and *Nostoc muscorum*, in salt stress, the abscisic acid was found to be more than 1 μg/L in the culture medium. The significant job of these extracellular abscisic acid in the microalgae is still widely unknown but it is hypothesized that the production of extracellular abscisic acid has an ecological significance, for example, elevated resistance of microalgae to stress factors or the regulation of the union of microorganism.

Auxins: This class of plant hormones have been studied widely in the microalgae throughout the last decade. The most biologically active auxin known for promoting elongation and cell division is Indole-3-acetic acid (IAA). Research proves the production of extracellular indole-3-acetic acid by some microalgae. Mazur et al. (2001) reported the presence of IAA in the culture medium during the growth of *Scenedesmus armatus* and *C. pyrenoidosa* at lower concentrations. With addition of high glucose (5 g/L) and continuous dark condition for 48 h, the culture medium of *Chlorella minutissima* has increased release of total indole, that showed the effect of glucose concentration and light–dark cycle on the production of these endogenous hormones.

A tetracyclic diterpenoid compound from plants called GA or Gibberellic acid intensifies the development and growth of the plants. In the extracellular metabolites of the cyanobacterium *Scytonema hofmanni*, gibberellic acid-like plant growth regulators were discovered that showed the capability to lighten the effect of salt stress on the rice seedlings. Still, there are scant research evidences available on the existence of gibberellins in microalgae. Hence, severe research has to be conducted to confirm the presence of gibberellins in the microalgae.

1.3.4 Extracellular Proteins

1.3.4.1 Protease Inhibitors

Ishihara et al. (2006) reported the extraction and purification of ECPI-2, an extracellular cysteine protease inhibitor, from growth medium of *Chlorella* sp. The inhibitory effect of these extracellular inhibitors is towards the proteolytic activity of chymopapain, ficin and papain. Under acidic or neutral conditions, the ECPI-2 enzyme is stable that may be due to the high carbohydrate residue (33.6%). To defend the cells against the herbivores or virus attack, *Chlorella* might synthesise these inhibitor proteins. The peptide drugs over the organic compounds, have comparatively less toxicity to the human being. Thus, in the present medicinal chemistry, developing drugs from the peptide inhibitors is a desirable topic for research. In cases of few diseases, these protease inhibitors can be used in treatments. For example, in the case of lung emphysema, elastase is critically important. Thus, advance study of the protease inhibitors from the microalgae are a treasured asset in the development of pharmaceutical industry.

1.3.4.2 Exoenzymes

Few separate studies have revealed that in the ecology of aquatic microbes, the extracellular enzyme activity is from algal sources. Some examples of these exoenzymes excreted by microalgae are β-D-glucosidases, chitinases, proteases and alkaline phosphatases. Exoenzymes effect the microorganism's growth by biogeochemical cycling within the ecosystems and chemical signalling. In aquaculture, the optimization of the strategy to supplement nutrients may be achieved by understanding these exoenzymes. However, merely some of these exoenzymes are isolated and purified. To establish the bioactivity, the structure of these enzymes and their potential applications, additional studies are needed. A few chosen promising exoenzyme classes are described briefly.

A few reports suggest that extracellular proteases are produced by green microalgae *Dunaliella* and *Chlamydomonas coccoides.* Another marine unicellular chlorophyte capable of producing these extracellular proteases is *Chlorella sphaerkii*, and it has the ability to cleave the substrate with very high specificity. Large quantities of proteases are also released by the diatom *Chaetoceros didymus* into the medium and *Kordia algicida*, the lytic bacterium induces the production of proteases. The produced proteases thus, offers resistance to the microalgae towards the negative impacts of the bacterium. These extracellular proteases are attractive objects in drug development as they efficient play role on the life cycle of the viruses.

In seawater, and bicarbonate and carbonate ion are present in concentrations greater than CO_2 in the form of inorganic carbon. The microalgae are capable of converting bicarbonate to CO_2 or directly use bicarbonate for utilization and later photosynthetic fixation. Carbonic anhydrase plays a vital role in the process of CO_2 concentration. Extracellular carbonic anhydrase is produced by various microalgae such as diatoms, haptophytes, dinoflagellates, and green algae. Within few microalgae, the conversion of external bicarbonate into CO_2 is also enhanced by extracellular carbonic anhydrase. The promising applications of these extracellular enzymes comprises of pH regulation, CO_2 recovery and CO_2 supply. These activities of extracellular carbonic anhydrases are obstructed by concentration of CO_2. In *C. pyrenoidosa*, a decline in the activity of extracellular and intracellular carbonic anhydrase occurred due to the enhancement of CO_2. Hypo-osmotic stress also affects the activity of extracellular carbonic anhydrase in *D. salina.* Liu et al.