Clean Energy Production Technologies

Series Editors: Neha Srivastava · P. K. Mishra

Neha Srivastava
P. K. Mishra *Editors*

Basic Research Advancement for Algal Biofuels Production

Springer

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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.

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Preface

The global green concept is spreading very rapidly to achieve long-term sustainability in environment and solve energy crises. Renewable energy and biofuels are potential energy sources being developed at a very faster pace worldwide to replace fossil fuels. Biofuel production from renewable resources-based feedstock is a potential and sustainable option. Among different available biofuels, algal biomass is emerging as a potential alternative as feedstock as well as a direct source of biofuels production. Renewable nature, huge availability, and rich in lipids and carbohydrates are the unique properties of algae which make them potential ideal candidates for biofuels production. However, a basic and key understanding of this microbial group is needed to explore the scientific ground for successful batch as well as mass scale biofuels production. Therefore, this book provides details about basic research for potential and practical algal biofuels production. Chapters 1 and 2 present latest trends in algal biofuels production while Chapters 3 and 4 explore algal biomass mats and algal biofuels production as potential and ideal biofuels options. Further, Chapter 5 and 6 discuss algal biomass as an efficient key feedstock for algal biohydrogen and other biofuels production. Chapters 7 and 8 give new insight on algal biofuels and their property as potential biofuels among other existing biofuels whereas Chapter 9 and 10 discuss the versatility of algae for various clean biofuels. The basic research concept and application utility may be useful to understand and set the mass scale goal for practical application of algal biofuels. Therefore, the aim of the current review is to explore the scope of algae for the production of different biofuels and evaluation of its potential as an alternative feedstock.

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Chapter 1
Recent Advancements in Municipal Wastewater as Source of Biofuels from Algae

Spriha Raven, Arpit Andrew Noel, Jane Florina Tirkey, and Archana Tiwari

Abstract The normal aquatic microflora comprising of bacteria and algae perform a vital role in the maintenance of an ecological balance of water by consuming excess nutrients. Exploring wastewater as a reservoir for nutrients and concomitant generation of value-added products from algae and bacteria is indeed an innovative approach towards sustainability. For the optimum exploitation of current wastewater treatment infrastructure, eutrophic water bodies like lakes, ponds, and water canals can be used for the growth of bacteria and algae, thereby resolving the scalability and economic issues. In this chapter we are elaborating the municipal wastewater remediation potential of bacteria and algae and valorizing the resulting biomass for diverse applications. The microbial enrichment in wastewater can be envisaged as a rapid, economical, and environment-friendly approach for the wastewater remediation coupled with the generation of bioactive compounds. The crucial challenges include the standardization of the culture conditions to grow bacteria and algae in wastewater for nutrient elimination concomitant with the generation of biofuels and valuable products. The biorefinery approach is an efficient tool to combat environmental pollution coupled with the generation of value-added products like biofuels.

Keywords Algae · Bacteria · Biofuels · Remediation · High value products · Wastewater

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1.1 Introduction

With time the global demand for energy is rising, so is the need and usage of fossil fuel. There is still abundant availability of fossil fuel at practicable cost, but most likely to change soon in future. The major concern is the excessive usage of fossil fuels is not so sustainable for longer terms mainly as it contributes to increase in the greenhouse gas release and put-up impact on global warming (Hill et al. 2006). At the same time energy requirement worldwide is increasing constantly. At present fossil fuels are eminent source of conveyance fuel and energy. The demand for fossil oil is anticipated to increase 60% from the present level by 2025 (Khan et al. 2009). Hence, it is very important to identify other renewable sources of fuel which have the potency of carbon neutral (Demirbas 2009; Hill et al. 2006; Rittmann 2008). Biomass, either terrestrial or marine, is one of the renewable sources of energy. Amongst all, algae have the adaptability to develop and cultivate in diverse habitats. It can grow in marine as well as freshwater (IEA Report 1994). The currently available biofuel in majority is mainly bioethanol which is derived from sugarcane or corn starch or biodiesel which is derived from oil crops such as soyabean or rape oilseed. In spite of the fact that biofuels are potentially more beneficial than fossil fuels, there is a dilemma on using these crops for the production of biofuels, whether they are as economical as compared to fossil fuels. Also, this brings more concern over the fact that using these crops as biofuel may impact the availability of food (Demirbas 2009; Hill et al. 2006). Therefore, algae cultivation on wastewater is much more appropriate and feasible which would not affect agriculture. It is sustainable, ecofriendly and cost-effective. Also, algae are capable of using land more efficiently as compared to other traditional biofuel crops (Clarens et al. 2011).

1.2 Advantage of Biofuel Over Fossil Fuels

Biofuel is an effective fuel for today’s generation. Fossil fuel is produced by organic materials over the course of millions of years and it is produced by decomposing plants and animals. Coal, oil and natural gas are examples of fossil fuels. These fuels emit CO_2, and it is harmful to the environment. However, biofuels are very effective and ecofriendly as it is being produced by vegetable wastes, algae, fungi, etc. It is helpful in maintaining the ecological balance and free from carbon dioxide emission. CO_2 emission is bit toxic and it causes global warming. Also, biofuel is a renewable and biodegradable source of energy as it is being produced by organic wastes and materials and practically, we can get enormous amount of fuels. It is safe to be used as it does not involve drilling, mining and other activities for the generation of biofuel. Hence, it is not that dangerous as we need to grow the fuel on farm (Ramos et al. 2016).

Advanced biofuel is being produced by the usage of wastes and residues, such as organic fraction of municipal solid waste, vegetable oil, biological sludge from urban water purification plants. Biofuels such as bioethanol, biodiesel, biohydrogen and other biofuels are produced by the usage of algae, fungi, etc., and it has multiple benefits, such as it reduces greenhouse gas. It is not 100% safe but it is safer than fossil fuels.

1.3 Biofuel Feedstock and Utilization of Wastewater

Microalgae have the ability to produce lipids at a notable concentration. The generated lipids produced, goes up to 80% of the dry weight of its biomass and the type of lipid, i.e., polyunsaturated fatty acids, saturated fatty acids, glycolipid or triacylglycerols depends on the species and its growth habitat (Chisti 2007; Griffiths and Harrison 2009; Hu et al. 2008). Mostly the algae cultured in photoreactor grown cells or batch culture grown cells in laboratory often tend to have maximum lipid production as compared to the algae grown in open pond (Griffiths and Harrison 2009). Recent studies confirm that microalgae which are grown in small batches, bioreactors or small semi-continuous culture showed practicable lipid concentration in microalgae grown in wastewater ranging from more than 10% up to 30% of the dry weight of biomass and the production of lipid could be increased if biomass is cultivated at large scale (Chinnasamy et al. 2010a, b). Recently a study on municipal wastewater along with *Chlamydomonas reinhardtii* was conducted and it was observed that it was very effective in wastewater treatment and total lipid content was 16.6% of the dry weight (Kong et al. 2010). When the microalgae were transferred to biocoil, it continued to grow for 1 month in wastewater, the lipid content increased to 25.5% of its dry weight, lipid productivity to 2505 mg/l/day and biomass productivity to 2000 mg/l/day. Also, there was effective removal of nitrogen and phosphorus (Kong et al. 2010). Likewise, in *B. braunii* level of total lipid content was observed similar in secondary treated municipal wastewater.

The lipid content and biomass were higher as compared to microalgae grown in synthetic grown medium (Órpez et al. 2009). For wastewater treatment microorganism requires large amount of oxygen for degrading organic material biochemically. And for this purpose, aeration devices are used which require electrical power up to 45–75% of plant energy cost (Rosso et al. 2008) which was not economical. Algae when used for wastewater treatment supply oxygen using a little energy required as compared to mechanical aeration. Algae also remove nitrogen and phosphorus which prevent eutrophication with the means of nitrification/denitrification bacteria (Richards and Mullins 2013). Most of the algae species are efficient in sorbing metals and are helpful to treat wastewater containing heavy metals (Li et al. 2009).

1.4 Algal Biofuel Production

Microalgae are a diverse group that helps in providing potential offer to a variety of solution for liquid transportation fuel requirements for a number of avenues. Microalgae are widely used in a variety of purposes. It uses CO_2 efficiently and more than 40% of global carbon fixation occurs and the productivity usually occurs through marine algae (Benemann 2008).

There are various fuels that are produced through algae such as ethanol, biodiesel, biohydrogen. There are many researches going on, but no proper mechanism has been scaled up for biohydrogen. The main algae genera currently cultivated photosynthetically for various nutritional products are *Spirulina, Chlorella, Dunaliella and Haematococcus.* Microalgae are cultivated using sunlight energy and it is produced in open ponds or closed bioreactors. Generation of microalgae for the commercial scale can be done in an open pond. It is much cheaper than closed bioreactors.

Methane is the first source of fuel from microalgae that are considered for their applications in wastewater treatment. Anaerobic digestion of algae is still uncommon but producing biofuel from algae especially biodiesel is the most common attention of algae fuels. Algae-based biofuel production has gained considerable attention in recent years leading to its biodegradability and sustainability (Benemann 2008) (Fig. 1.1).

1.5 Municipal Wastewater as Source of Biofuels

Due to the growth of human population and modern living of people, water pollution is inflated worldwide. Wastewater is primarily by-product produced by various resources such as domestic, municipal, agricultural and industrial sources. The constitution of wastewater is a mirroring of the way of life and various approaches practised. Wastewater compromises organic mass like proteins, lipids, volatile acids, carbohydrates and inorganic content containing sodium, calcium, magnesium, sulphur, bicarbonate, chlorine, ammonium salts, phosphate and heavy metals. Presence of excessive nutrients in the water bodies leads to eutrophication or algal blooms because of anthropogenic waste production.

Municipal wastewater has also been considered domestic wastewater and is generated by domestic sites. In the last few decades, municipal wastewater has increased due to the development of city. It compromises various wastes from human, food and chemical wastes from day today life. Nitrogen and phosphorus are present in less quantity in municipal wastewater as compared to other types of wastewater. Microalgae are expanded in raw concentrate and various algae can convert municipal wastewater into biomass efficiently (Akhtar et al. 2019).

Algae can be grown in various water such as fresh, brackish and marine water, municipal wastewater, industrial wastewater, aquaculture wastewater, animal

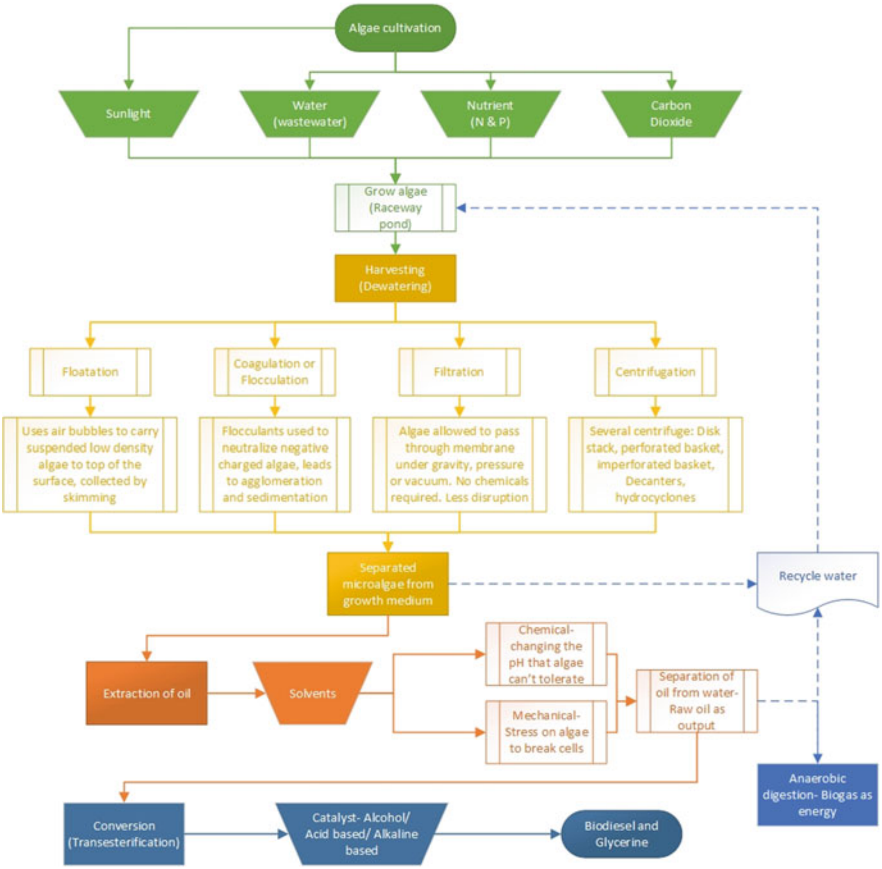

Fig. 1.1 Algal biofuel production

wastewater, domestic wastewater and adequate amount of nutrients such as nitrogen, phosphorus and various trace elements. Wastewater has chemical and physical properties (Mobin and Alam 2014).

1.5.1 Potential of Algae to Grow in Municipal Wastewater

The raising of microalgae for the purpose of biofuel in established wetlands fulfils all the essentials of ecofriendly purification process required for wastewater treatment as it is better to substitute in terms of budget and energy for the process of water treatment (Kadlec and Wallace 2009). Cultivation of microalgae is very advantageous for developing countries as aquatic plants in wastewater require warm climate. Hence it is a boon for developing nation (Zhang et al. 2014). There have been several researches going on about the nutrient and habitat requirement on different configurations of wastewater streams like agro-industrial wastewater, municipal wastewater, etc. (Van Den Hende et al. 2016; Abinandan and Shanthakumar 2015). It was found that the required quantity of water, additives required for green growth development, life cycle are well provided by these wastewater and hence it is beneficial for the growth of microalgae in wastewater as it is economical, ecofriendly (Zhou et al. 2012). Later it was found that the available phosphorus and nitrogen in wastewater sources are used for the green growth for bioremediation processes (Chinnasamy et al. 2010a, b).

Microalgae's chemical and nutritional requirement offers chance for biofuel production and bioremediation which can be achieved with the integration of municipal and industrial utilities with algae system for maximum use of urban resource. This integration is the best example of holistic use of available resource as algae being cultivated on the wastewater treatment system captures carbon dioxide, removes nitrogen, phosphorus from wastewater as it utilizes it for its growth which is being cultivated for biofuel production. These are several benefits that can be achieved from the integration of algae and wastewater treatment. Algae utilize carbon dioxide for its growth, hence, filters it efficiently. Algae uses the available nitrogen phosphorus present in wastewater and helps in wastewater treatment. Algae contain 20–70% lipids of their biomass for biofuel production. It also gives several other by-products such as protein, vitamins, carbohydrate, pigments that are used by fertilizer and pharmaceutical industry. Hence, the integration of algae cultivation with wastewater becomes more cost-effective (Hwang et al. 2016).

1.5.2 Types of Algae for Biofuel Generation

Through pyrolysis algal biomass can be used for the production of biofuel. Macro and microalgae should fulfil certain criteria as listed (Carlsson et al. 2007): it must be high yielding, easy to harvest, capable enough to resist water current in ocean and it should be cost-effective.

1.5.2.1 Macroalgae

Listed below are the strains of microalgae for biofuel production:

For the production of methane, the feedstock that could be used are *Laminaria* sp. (Chynoweth et al. 1993), *Sargassum* sp. (Bird et al. 1990), *Macrocystis* sp. (Chynoweth et al. 1993), *Ulva* sp. (Adams et al. 2009). For the production of ethanol *Ulva* sp. (Morand 1991), *Kappaphycus alvarezii* (Khambhaty et al. 2012). For the production of hydrogen feedstock that can be used as *Gelidium amansii* (Park et al. 2011), *Laminaria japonica* (Shi et al. 2011).

Using macroalgae for the production of biofuel is not economical with the present available technology, but if the process of production is amalgamated with other activities such as removal of pollutant and manufacture of several biobased products then it can be cost-effective (Savage 2011; Pittman et al. 2011).

1.5.2.2 Microalgae

Microalgae are mainly photosynthetic organisms found in fresh as well as marine habitat. At hand research enterprises have confirmed that microalgal biomass is one of the most hopeful sources of renewable biodiesel and it is efficient to fulfil the global demand.

Microalgal biomass has a huge percentage of oil content that can exceed more than 80% of its dry weight (Rodolfi et al. 2009).

Some of the microalgae with its oil range of 20–50% are listed below (Chisti 2007; Rodolfi et al. 2009) (Table 1.1):

Various strains of microalgae that are used for bioethanol production are *Chlorococcum humicola* (Harun and Danquah 2011), *Chlorococcum infusionum* (Harun et al. 2011), *Chlamydomonas reinhardtii* (Choi et al. 2010), *Spirogyra* sp. (Eshaq et al. 2011) (Table 1.2).

Table 1.1 Microalgae with its oil range

Feedstock	Oil content (% dry weight)
Botryococcus braunii	25–75
Chlorella sp.	28–32
Chlorella vulgaris	19.2
Chlorococcum sp.	19.3
Chaetoceros muelleri	33.6
Chaetoceros calcitrans	39.8
Crypthecodinium cohnii	20
Cylindrotheca sp.	16–37
Dunaliella primolecta	23
Isochrysis sp.	25–33
Monallanthus salina	>20
Nannochloris sp.	20–35
Nannochloropsis sp.	31–68
Neochloris oleoabundans	35–54
Nitzschia sp.	45–47
Phaeodactylum tricornutum	20–30
Pavlova lutheri CS182	30.9
Schizochytrium sp.	50–77
Scenedesmus sp. *F & M-M19*	19.6
Scenedesmus sp. *DM*	21.1
Skeletonema sp.	31.81
Tetraselmis suecica	15–23

Table 1.2 Types of algae and phylum

Phylum	Name	Type of algae	Reference
Cyanobacteria	*Cyanobacterium aponinum Cyanobium sp. Phormidium sp. Pseudoanabaena sp*	Freshwater/marine Freshwater Freshwater Freshwater	Hopkins et al. (2019) Mendes et al. (2011) Mendes et al. (2011) Mendes et al. (2011)
Haptophyta	*Isochrysis galbana*	Marine	Ammar et al. (2018)
Chlorophyta	*Chlamydomonas reinhardtii* *Dictyosphaerium sp.* *Dunaliella tertiolecta* *Monoraphidium sp.* *Scenedesmus sp.* *Amphora coffeaeformis*	Freshwater Freshwater/marine Marine Freshwater Freshwater Freshwater/marine	Badrinarayanan et al. (2017) Al-Ghouti et al. (2019) Ranjbar et al. (2015) Das et al. (2019) Das et al. (2019) Godfrey (2012)
Ochrophyta	*Chaetoceros gracilis* *Chaetoceros muelleri* *Nannochloropsis oculata*	Marine Marine Marine	Godfrey (2012) Godfrey (2012) Ammar et al. (2018)

1.5.3 Innovative Algae Cultivation System

The cultivation of microalgae depends on several factors such as light, temperature, pH, carbon source. Its main goal is to produce high lipid concentration and biomass with costeffective methods. Cultivation of algae in wastewater treatment is similar to any other biological wastewater treatment. But difference in pH and temperature can affect the growth adversely. Wastewater comprises of various nutrients which are favourable for the growth of algae, however for increased lipid concentration components such as metals, nutrients, etc. has to be monitored. Also, the salinity has to be controlled as it affects algal cultivation. Most commonly used reactors for algal cultivation are open air, closed air and biofilm system (Hwang et al. 2016).

1.5.3.1 Open Air System

It is the oldest form of algae cultivation system practised in open ponds. It requires less investments, uses solar energy and less energy as compared to others. The temperature and light cannot be controlled. In this method it can be easily contaminated and is preferred for mixed culture cultivation. There is a limitation of time and place in open cultivation (Kumar et al. 2015a, b). There are four basic types of open system: circular pond, shallow big pond, tank and high-rate algal pond (Kumar et al. 2015a, b).

1.5.3.2 Closed System

In closed system (PBR) factors such as light, gas exchange and contamination can be controlled. Hence, monoculture of certain algae is possible. But the limiting actor is the cost of establishment. Also, in closed system temperature rises and overheat is enough to cause damage to algae growth (Pruvost et al. 2016). There have been constant research going on to work on the limitations, it also has difficulty in dissolving oxygen at large scale. Air sparging is a solution to it but it adds to the cost (Hwang et al. 2016). There have been several structures of PBRs designed to make it more economical and effective, most popular once are ultrathin immobilized configurations, flattened plate-type systems and tubular system (Pulz 2001).

1.5.3.3 Biofilm System

It is least common method of cultivating algae but provides good biomass (Hwang et al. 2016). There is still a lot of information required to practice algae cultivation using biofilm for wastewater treatment regarding removal of nutrients, being sustainable and economical (Kesaano and Sims 2014).

1.5.4 Algal Biomass Harvesting Techniques

Algae harvesting means separating or detaching algae from its growth medium (Gulab and Patidar 2018). Harvesting of algal biomass leads to huge operational cost due to dilute nature of the harvest microalgal cultures. The choice of which harvesting technique to use depends on the type of algae species and required final product (Uduman et al. 2010). At present, algae biomass harvesting techniques include mechanical, chemical, biological and electrical methods (Gulab and Patidar 2018). When mechanical method is combined with coagulation and flocculation, then overall efficiency increases with reduced operational and maintenance costs (Demirbas 2010). Several techniques of algal biomass production are coagulation/flocculation, floatation, filtration, centrifugation and electrical based processes (Abdelaziz et al. 2013; Barros et al. 2015).

1.5.4.1 Coagulation/Flocculation

Microalgae are negative charged and are in dispersed state. This natural state leads to slow natural sedimentation. To increase the process of harvesting, flocculation can be done (Chen et al. 2011). Flocculation can happen naturally without adding any chemicals by changes in nitrogen, pH and dissolved oxygen. It is called auto-flocculation (Uduman et al. 2010). Microalgae biomass is also produced by electrolytic oxidation where algae are destabilized and floc towards anode. This is called as electrolytic process (Chen et al. 2011). Bio-flocculation is a process where some micro-organisms produce flocculants that flocculates algae in suspension (Shelef et al. 1984).

1.5.4.2 Membrane Process

Algae culture is subjected to move through the filters managing under gravity, pressure to hold back algae in a thick paste form. Such system is available in continuous or discontinuous. The standard of the harvested biomass is fine with respect to other harvesting approaches as the cells are few disrupted and no chemicals are needed in the membrane harvesting (Wicaksana et al. 2012).

1.5.4.3 Coagulation and Flocculation

Microalgae cell is found in dispersed condition. It has negative surface charge density close to the growth medium. Such stable system ends in easy natural sedimentation and microalgae are easily harvested by preflocculation or coagulation (Chen et al. 2011). Chemicals known as flocculants that neutralize the negative charge and permit agglomeration of microalgae are considered. Flocculation is regarded as a superior method for harvesting microalgae as it is considered for large-scale production. This process can be induced by electrostatic patch (or patching), bridging flocculation. Surface charge neutralization are the major process that are involved in microalgae flocculation (Chen et al. 2011). Flocculants are supposed to be sustainable and renewable that lead to no biomass pollution, and it permits reprocess of culture medium, inexpensive, non-poisonous, productive in low doses or withdrawn from renewable resources.

1.5.4.4 Floatation Process

Flotation is a gravity dissociation procedure in which air or gas bubbles are considered to bring the suspended material to the top of a liquid surface and they can be controlled by skimming procedure (Singh et al. 2011). Because of low density and self-float feature of various micro-algal species floatation process can be relatively rapid and more productive compared to sedimentation. The separation process has manifested systematic harvesting of freshwater and marine microalgae.

1.6 Recent Approaches for the Enhanced Production of Algal Biofuels

The process of cultivation is the foremost aspect for optimizing the best productivity of lipid and biomass production. Algae production needs abundant production of CO_2, water and sunlight for the production of algal biofuels.

CO^2 concentration has a strong impact on the production of biofuel and lipid (Sangela et al. 2018).

1.6.1 Harvesting Algal Biomass

Harvesting is the best procedure to recover raw or unprocessed biomass of algae for the production of secondary metabolites. In order to make biodiesel low priced we need to reduce the harvesting cost, and it would be considered an important step for biodiesel generation. Natural sedimentation is not suitable to harvest the microalgal biomass procedure like centrifugation, filtration, bio-flocculation, electro-flocculation, chemical-flocculation, floatation and co-cultivation are currently in use for harvesting purposes. Flocculation is regarded as the best method to obtain algal biofuels (Sangela et al. 2018).

1.6.2 Lipid Extraction

Lipid production from oil yielding algae is not simple; it has some hurdles for large-scale production. In order to make lipid production low priced and systematic, it is essential to choose a fit solvent for extraction. There are numerous solvent mixtures for lipid extraction, for instance, chloroform:methanol, hexane:diethyl ether, nitro hexane, dichloromethane, etc. in use. However, none of these mixtures results in biofuel production. In order to execute extraction within the solvent, numerous thermal and mechanical procedures are used for cell wall disruption like microwave assisted extraction pulse electric fields, etc. (Sangela et al. 2018).

1.6.3 Conversion of Extracted Lipid into Biodiesel

For biodiesel generation, it is essential to know the profile of fatty acid of feedstock. It is important to have $<1\%$ of free fatty acid (FFA) amount. Various characteristics such as amount of catalyst, solvents, co-solvents, temperature and reaction time have notable result on biodiesel yield. Phosphoric acid (H3PO4), hydrochloric acid (HCl) and acid sulfonates are to execute esterification and transesterification as acid catalysts (Sangela et al. 2018).

1.7 Biofuel Industry Analysis

1.7.1 Market Size

Biofuel industry is gaining substantial growth, as it is an alternative fuel for petroleum derived fuels which is the major reason for global warming. The global market size of biofuel has been US$ 141.32 billion in 2020 which is expected to grow by 8.3% CAGR by 2030 to US$ 307.01 billion (Precedence Research 2021) (Fig. 1.2).

Talking in terms of Petajoules, total world fuel production from biofuel is 4113 PJ, which is just 0.8% of the total world fuel production (Bp plc 2020).

Out of 4113 PJ of global energy production by biofuels, 2552 PJ is from bio-gasoline and 1561 PJ is from biodiesel (Bp plc 2020) (Fig. 1.3).

Global ethanol production is expected to increase from 116 billion litres in 2015 to 128 billion litres by 2025, half of which will originate from Brazil. Global biodiesel production is expected to grow from 31 billion litres in 2015 to 41 billion litres by 2025 (OECD-FAO Agricultural Outlook 2016) (Fig. 1.4).

Usage of maize and sugar crops as feedstock is very common for ethanol production. Lot of research is going on for utilizing cellulose-based biomass as feedstock and it is forecasted that its share will increase to 0.7% by 2025. In case of biodiesel, vegetable oil holds the highest market share of 82% which is expected to

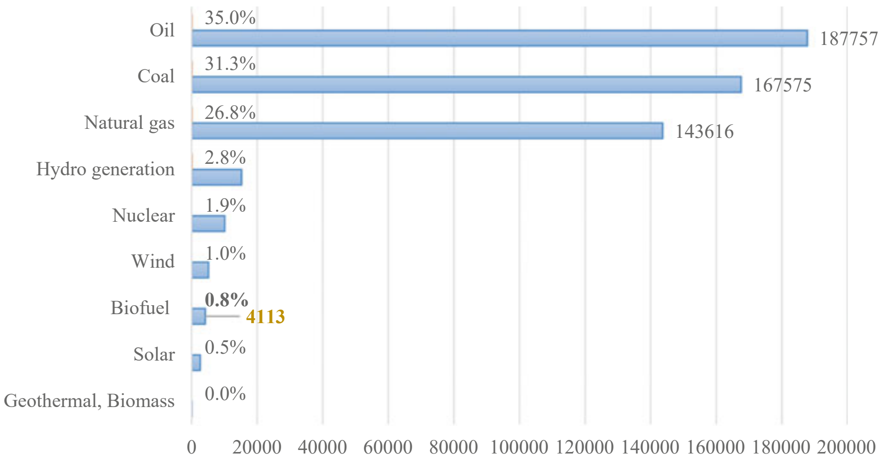

Fig. 1.2 Total world energy production (petajoules) 2019–2020 (Source: Bp plc 2020)

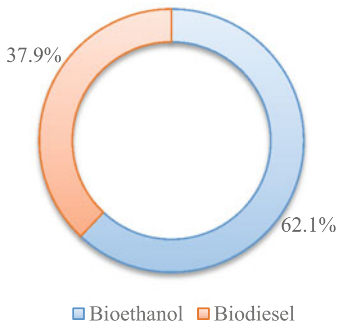

Fig. 1.3 Types of biofuels' market share % (Source: Bp plc 2020)

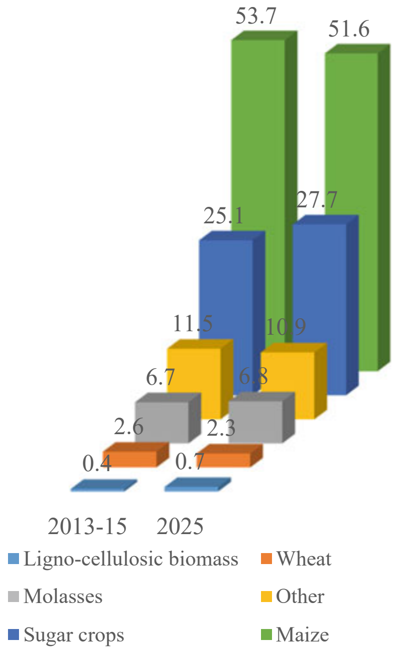

Fig. 1.4 Share of feedstock used for ethanol production % (Source: OECD-FAO Agricultural Outlook 2016)

come down to 72.7%, as companies are exploring opportunities to utilize waste oil from Horeca segments (OECD-FAO Agricultural Outlook 2016) (Fig. 1.5).

North America has captured most of the biofuel market (39%) owning to the government policies favouring biofuel industry over petroleum industry; also feedstocks such as coarse grain, sugar crop, vegetable oil, jatropha and molasses are easily available in abundant. There has been increase in research and development investment in biofuel industry in various regions of Asia-Pacific. Also, there is increase in awareness regarding use of renewable energy source. We can expect a rapid growth of biofuel industry in Asia-Pacific regions as well (Bp plc 2020) (Fig. 1.6).

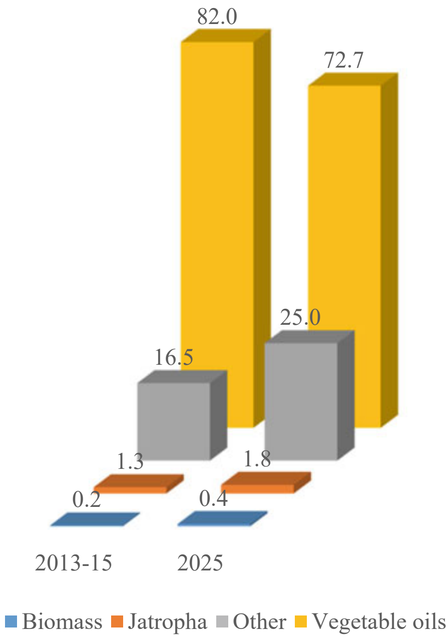

Fig. 1.5 Share of feedstock used for biodiesel production % (Source: OECD-FAO Agricultural Outlook 2016)

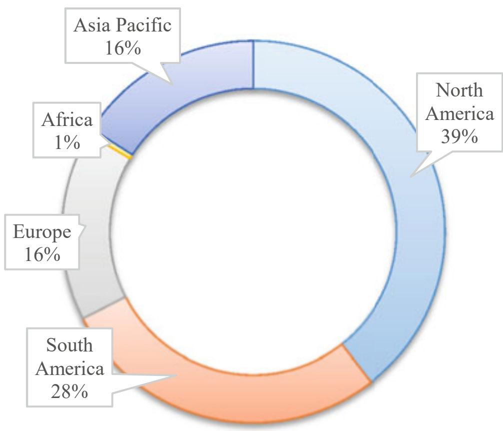

Fig. 1.6 Market share based on production, sub-continent wise (2019–2020) (Source: Bp plc 2020)

1.7.2 Major Companies

Some leading companies competing in global biofuel market are (Table 1.3):

Some other major companies are:

- BTG International Ltd
- Cargill Incorporated
- DowDuPont, Inc.

Table 1.3 Some major companies manufacturing biofuel

Companies	Production (Gallons)	Fuel produced	Raw material used
Wilmar International Ltd	1,20,80,57,859	Biodiesel	Palm oil
Royal Dutch Shell PLC	66,04,30,000	Bioethanol	Corn, sugar
Renewable Energy Group (2020)	49,50,00,000	Biodiesel	Used cooking oil, waste oil, animal fats, plant oil
VERBIO Vereinigte BioEnergie AG	24,05,27,641	Biodiesel, Bioethanol, Biomethane	Rapeseed oil, palm oil, wheat straw, sugarcane and beet
Abengoa Bioenergy S.A.	19,43,46,537	Bioethanol, biodiesel, jet fuel	MSW (Municipal Solid Waste), fats and waste oil
POET, LLC	6,00,00,000	Bioethanol	Corn and cellulose

- Archer Daniels Midland Company
- My Eco Energy
- China Clean Energy Inc.

1.7.3 Their Core Competency

1. *Renewable Energy Group, Inc.:* REG believes in providing the society with right product at right time and place. They are providing cleaner fuel solutions for more than 20 years. They have 12 biobased diesel plants. The total production of 495 million gallons of biofuels in 2019 resulted in 4.2 million metric tonnes of carbon reduction which is equivalent to carbon dioxide removed by 5.5 million acres of forest in one year. The feedstocks they generally use are low carbon emitting like waste and by-products from fats and oils. The energy returned from the biofuels produced is 5.5 times the fossil used for production. The carbon emission is 50–90% lower (Renewable Energy Group 2020).
2. *Abengoa Bioenergy S.A.:* Abengoa is at the top of industry in energy sector, in terms of hybridization of technologies, construction of complex and value-added project. They have more than 280 patents under them. They convert municipal solid waste into jet fuel by gasification and synthesis process. They have presence in all the sub-continents. They produce more than 194 million gallons of biofuels per year that include biodiesel, bioethanol and Jet fuel. A biorefinery that will produce ten million gallons per year of jet fuel from solid urban waste is under construction (Abengoa 2020).
3. *Wilmar International Ltd.:* They have 14 biodiesel plants. 12 are located in Indonesia and 2 are in Malaysia. In 2020, Indonesia has expanded it biodiesel blending mandate from B20 to B30, that is 30% of diesel is biodiesel and 70% is from crude. Although due to low crude oil prices and reduced diesel consumption

due to COVID-19, Indonesia biodiesel consumption came down from 7.5 million MT to 7.2 million MT which is a 4% decline, affecting Wilmar's production and consumption. They produce Palm oil methyl ester which is used in biodiesel. Wilmar's biodiesel contains virtually zero sulphur, there they burn cleaner than traditional petroleum-based fuel (Wilmar International Limited 2020).

4. *POET LLC:* POET is a major producer of bioethanol in the USA. They produce bioethanol in 27 locations. They use dry mill process to produce bioethanol. The raw material used to produce bioethanol is either corn or cellulose. Corn is used in making starch-based bioethanol. Non-grain material/feedstock that provides the cellular structure for all plants is used in making cellular bioethanol. Their estimated annual revenue is 7.2 billion dollars (POET, LLC 2021a, b).
5. *Archer Daniels Midland Company:* ADM produces both biodiesel and ethanol. They have patented processes for production of biodiesel. The process is known as transesterification where vegetable oil is heated with alcohol in presence of catalyst to form mono-alkyl esters which are biodiesel. ADM has biodiesel production plant across the world in EU, Brazil, Canada and the USA. ADM also produces ethanol from corn through an efficient process which also produces animal feed and is partnering with institutes to produce biofuel sourced from cellulose (Stiefel and Dassori 2009).
6. *VERBIO Vereinigte BioEnergie AG:* Verbio is an independent manufacturer of biofuels including biodiesel, bioethanol and biomethane. Verbio is a German based company. They rely on raw materials which are not used for food production. They have a patent pending for a state-of-the-art biorefinery. They had the capacity utilization of 84.8% for biodiesel plant in 2019–2020 due to the increased demand from North America and increased greenhouse gas reduction quota from 4% to 6%. For ethanol, they had capacity utilization of 91% and the sales revenue also increased from 255 million euros (2018–2019) to 275 million euros (2019–2020). This increase in revenue, even when the sales volume almost remain unchanged is because of the high sales price in the pre-COVID period (Verbio 2020).
7. *My Eco Energy (MEE):* MEE is based in India which produced biodiesel marketed as Indizel. Indizel meets Indian and European standards. Indizel is an alternative to diesel having low emission and is compatible with all diesel engines. MEE has 8 fuel stations currently across India (My Eco Energy 2021).
8. *Royal Dutch Shell PLC:* Shell is a global group of companies with 87,000 employees in around 70 countries. They use advanced technology and innovative approach to build sustainable energy for future and developing fuels for transport such as bioethanol. In 2020, 2.5 billion litres of biofuel were formed by Shell. In February 2021, Raízen (JV in brazil) acquired Biosev, which will add to 50% of production capacity in low-carbon fuels. This will increase the production capacity to 3.75 billion litres (Shell 2020).

1.7.4 Supply Demand Gap

In 1990s, when the biofuel industry was at the very nascent stage, growth was stagnant. Consumption of biofuel was more than production. Major reasons of less production were no active government policies and high capital-intensive industry. Research and development in biofuel production from agricultural product was not significant. Large-scale production of biofuel was not considered an alternative source of oil and was not even advised to cover significant fraction of it (Giampietro et al. 1997).

But in the twenty-first century, industry started blooming exponentially. Both production and consumption increased from 200 KBoed to 1800 KBoed. Also, this time, production was more than consumption. This was due to the partial response to climate change; provision of government subsidy and minimal changes in retail distribution was required (Rajagopal et al. 2007).

Increasing the production of biofuel in the USA also affects the crude oil price globally. As USA is the major importer of crude oil, the shift in dependency on crude oil to biofuel will increase the supply of crude oil in the global market, thereby decreasing the price (Osborne 2007) (Figs. 1.7 and 1.8).

1.7.5 Porter's 5 Forces

1.7.5.1 Threat of New Entrant (Low)

As per Jones et al. (1978), The major barriers to biofuel industry are:

- Patent
- Large capital requirements
- Economies of scale
- Government's regulations
- Product differentiation
- Predatory behaviour by cartels
- Ownership of resources

Biofuel industry is at a very nascent stage. Still a lot of research and development is going on. All the major companies are having some patents regarding formula, processes and manufacturing. Patents drive the cost reduction and differentiation (Dos Santos et al. 1999).

1.7.5.2 Threat of Substitutes (High)

Crude oil is the major substitute for biofuel. Due to low price and easy availability, consumers generally prefer crude oil. As gasoline is a product of crude oil, the price

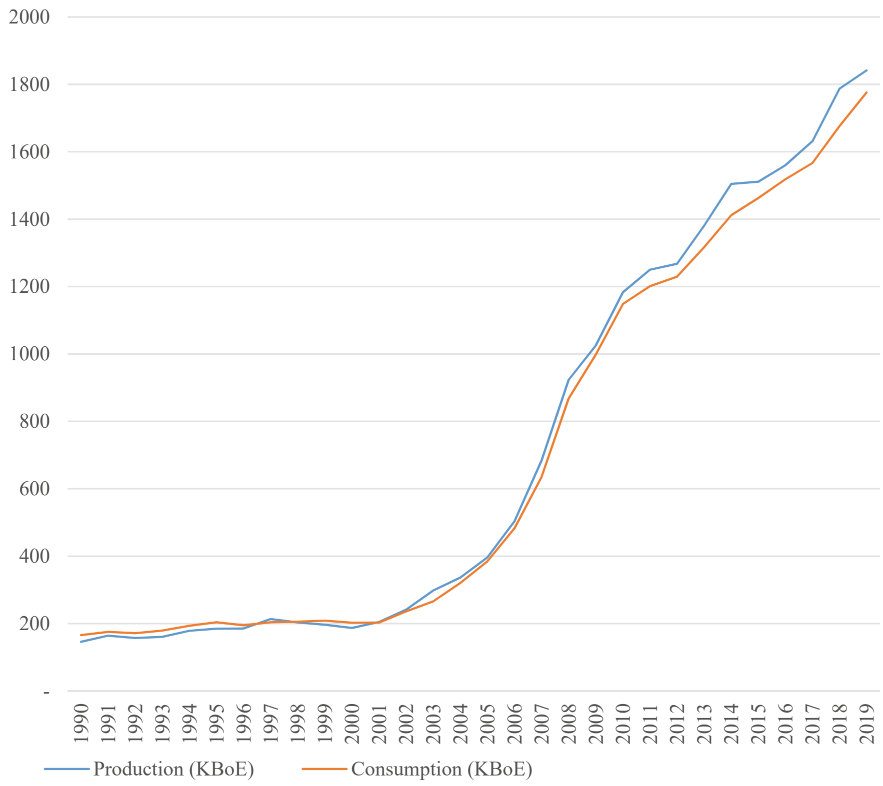

Fig. 1.7 Production vs. consumption of biofuels (Kboed) (Source: Bp plc 2020)

Fig. 1.8 Porter's 5 forces analysis—biofuel industry

of gasoline is driven by the price of crude oil. In 2005, ethanol prices fell below gasoline price. Major reason suggested was expansion in production of ethanol (Pokrivčák and Rajčaniová 2011).

1.7.5.3 Bargaining Power of Suppliers (Medium)

Providing residue to the biofuel manufacturer is an extra income for the farmers. However, excess residue from the agriculture field is made available for ethanol production only after soil conservation and local animal feed needs are fulfilled. Farmers only provide feedstock like corn only if price offered is high enough to compensate for the harvesting, storage and transport of the feedstock (Kumarappan et al. 2009).

1.7.5.4 Bargaining Power of Buyer (High)

Customers will easily switch to the non-renewable and traditional sources of energy as the cost of switching is really low (Indian Brand Equity Foundation 2017). In markets like Sweden where 70% of the biofuel is imported and rest is produced, buyers tend to compare quality and price with the imported one. Here, the buyers are distribution companies who sell to the end consumers. They buy large volumes, therefore for them quality and price are really important. Hence, bargaining power of buyer is high (Folea et al. 2010).

1.7.5.5 Competitors Rivalry

Industry itself is at its nascent stage, players are still establishing themselves in the market, the sector has still not reached the stage of competition. Therefore, competitive rivalry is low (Indian brand Equity Foundation. Renewable energy 2017). Bioethanol and biodiesel are highly standardized products and that is why it is very difficult for any company to introduce a new product and increase its portfolio. Any degree of rivalry can only happen if any company comes up with new producing technology such as cellulose-based bioethanol (Folea et al. 2010).

1.8 Major Challenges in Algal Biofuels Production from Wastewater

Algal biofuel is blooming due to high growth rates, high oil contents that have been a significant part in the capital to turn algae into biofuels. But to generate biofuels from algae, there are multiple procedures and it requires various steps such as where and how to grow algae, to improve the oil extraction and fuel processing. The major challenges include strain isolation, nutrient sourcing and utilization, production management, harvesting, coproduct development, fuel extraction, refining and residual biomass utilization (Saad et al. 2019).

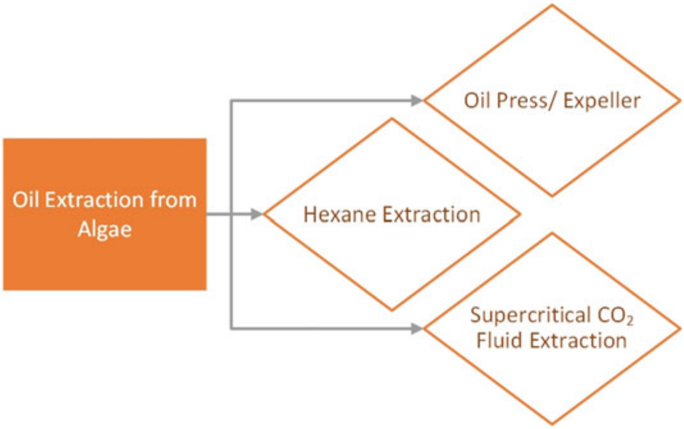

Fig. 1.9 Oil extraction from algae

- Improved engineering might contribute and impact algal biofuel production.
- Oil extraction and downstream processing are other challenges that are easily addressed from engineering side.
- Nutrient challenge: Algae require nutrients, light, water, carbon source for efficient growth.
- Land use
- Water use (Hannon et al. 2010) (Fig. 1.9)

1.9 Future Prospects

1.9.1 Aviation

In 2018, 42 million commercial flight was scheduled, and the industry has grown by 4%. Roughly 2.5% of global man-made carbon emissions is made by aviation industry. But the use of biofuel in aviation industry can reduce carbon emission. Biofuel has already been used by 100,000 flights and is expected to grow to one million by 2020. IAE expects the demand of biofuel in aviation industry to reach 10% by 2030. Currently, several agreements are being made between airline companies and biofuel producers which would cover approximately 6 billion litres of aviation biofuel (Abengoa 2020).

1.9.2 Food Processing Waste Feedstock

Waste from food processing is tough to dispose and treat. Food processing waste contains cellulose, hemicellulose, lipids, protein, starch, etc. which can be used to produce biofuels as it is a good source of carbon and other nutrients (Zhang et al. 2016). The use of waste cooking oil from Horeca industry for biofuel production is also a good method to reduce food safety problems and increase renewable energy sources. To encourage restaurants to give waste oil to the biofuel companies, government should intervene in the whole system, provide subsidy to the Horeca segment for good practices (Yang and Shan 2021).

1.9.3 Algae-Based Feedstock

Trade-off between food crops or crops for fuel is very crucial as choosing either one will affect the environment, food price and fuel price. Here, algal biofuel comes into picture, which holds promising factors to overcome global energy crisis. Algae is high yielding, relatively less capital intensive and does not have to compete with food crops for land and freshwater resource (Kour et al. 2019).

1.9.4 Cellulose-Based Ethanol

It is the hope for future as industry would not have to be dependent on just corn to produce ethanol. Cellulose feedstocks include corn stover, switchgrass, poplar trees, etc. (Tyner 2008). The USA is dependent on livestock producers both domestic and international for corn supplies, which limits corn-based ethanol production. If the cellulose-based feedstock availability increases, then the overall ethanol production can be increased (Osborne 2007). Cellulosic biofuel production is far below the target set in 2007 by EISA. Level of commercial production was quite low. Research and development on the same has to be ramped up to reduce the feedstock cost and increase the operational efficiency to make the production feasible (U.S. Department of Energy, Statistical and Analytical Agency 2012).

1.10 Conclusion

The rapid depletion of fossil fuels and exponential enhancement in pollution call for urgent search for alternative to resolve both issues concomitantly. Algae are potential bio-factories that have acumen to grow on municipal wastewater utilizing the nutrients for their growth and simultaneously generate treasured biomass, which can yield high value products including biofuels. A plethora of biofuels can be produced by algae including bioethanol, biodiesel, biohydrogen to name a few. Investigations are in great progress to envisage sustainable algal biorefineries churning wastewater into biofuels for a better tomorrow.

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Chapter 2
Recent Trends for Production of Biofuels Using Algal Biomass

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Abstract Industrial revolution not only brings comforts to life but also leads to many problems. One of them is limited supply of energy resources; the other is global warming and environmental pollution by burning fossil fuels. These problems lead scientists towards the idea of biofuels. But the production of first and second generation biofuels has many challenges including food vs. fuel war. Recently the production of biofuels by algal biomass also called as third generation biofuels has gained attention. Algal biomass can not only be converted to all forms of energy resources like biodiesel and biogas, but also are ecofriendly as they recycle the CO_2 in the environment and reduce the emission of greenhouse gases as in the case of fossil fuel. But there is need for modern methodology and instrumentation to obtain biofuels from algae. This chapter is about cultivation and harvesting of different algal strains, production of different types of fuels from algal biomass, genetic engineering of algal strains to obtain maximum lipid content, etc.

Keywords Biofuels · GHG · Biodiesels · Biogas · Bioethanol · Genetic engineering

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2.1 Introduction

Fuel is the backbone of today's life. In 1947, usage of fossil fuels started on commercial scale, yet they have been basic need of contemporary civilization since the beginning of modernization. Although our growth has been significantly reliant on fossil fuels obtained from natural resources under the Earth's surface in "solid (coal), liquid (petroleum oil) and gaseous states (natural gas)". However, continuing to consume these finite naturally occurring fossil fuels has a negative effect on the environment and is therefore not very manageable. Concerns about overuse of petroleum products and high "non-renewable energy" consumption lead to increase in the cost of raw petroleum. This causes changes in the environment, extreme weather patterns and distortion of ecosystems have prompted. Policymakers, researchers, governments and scientists are looking for an alternatives to conventional energy sources such as solar, wind and biofuels (Alam et al. 2012). Growing energy and transportation sectors have been connected to total greenhouse gas (GHG) emissions of up to 60 and 70 by percentage, respectively, while agrarian countries such as India and China account for only approximately 9% of global greenhouse gas emissions (Mata et al. 2010).

The continuous and gradual increase in the utilization of fossil fuels in transportation and to fulfil other energy demands has led to enhancement in the emission of greenhouse gases that is badly effecting our environment and ultimately biosphere (Vologni et al. 2013; Chandrasekhar et al. 2015). Additionally, the increased emission of greenhouse gases is the leading cause of global warming. Excess soluble bicarbonates specifically CO_2 in the aquatic ecosystems can alter the pH and leads to destruction of both the sea and land habitats which ultimately result in destruction of sea food chain and also the food supply to human. Furthermore, fossil fuels are a finite source for energy production and are not a continuing process as they are not renewable source of energy and take hundreds of years to form. So in order to get a continuous and long lasting source of energy and also to reduce the emission of greenhouse gases, we have to find out alternative sources of energy. For this purpose we are in need of an eco-friendly and renewable source of energy. These problems lead scientist towards the production of biofuels. In the beginning food crops like corn and soya-bean had been used to produce biofuels (also called as first generation biofuels). But its production was limited because their use for fuel production may lead to food shortage, especially in those areas where they are used as stable food. Then cultivation of special crops like Jatropha (second generation biofuels) for producing biodiesel on the land previously used for food crops cultivation was also an issue. Because it also leads to food shortage and ultimately increase in food price (Mata et al. 2010).

Direct combustion of biomass is currently 95% to 97% source of energy. The driving force behind the production of biofuels was complete combustion of natural biomass along with the combustion of semi-natural biomass (industrial waste, municipal solid waste, agro-industrial waste and also the solid fossil fuels like coal, peat, lignite, etc.) in many countries worldwide (Vassilev et al. 2013).

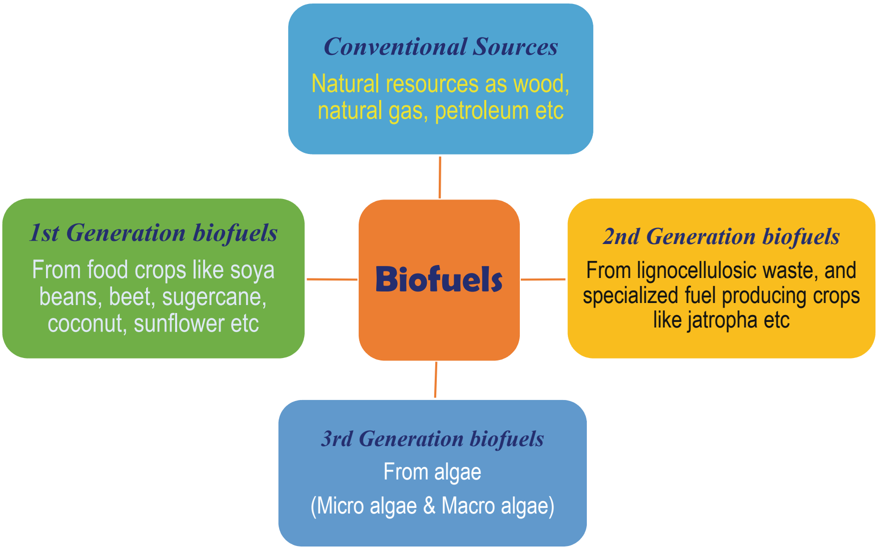

Fig. 2.1 Different types of biofuels

Ecofriendly fuels are actually biofuels, as the name represents are biodegradable and non-toxic fuels that are obtained from natural sources like plants, animals and microbes (Vogel et al. 2019; Xu et al. 2006). Figure 2.1 indicates the different generations of biofuels. In comparison to geochemically produced fuels, these modern biological sources have acquired great attention due to presence of their sustainable feedstock and also with the minimum emission of greenhouse gases. The type of biofuels produced from these sources depends upon the production and accumulation of intracellular component of these biological sources such as lipid and carbohydrate content. Important debates for the global production of these biofuels comprise criteria for production of biofuels, energy balance between fossil and biofuels and emission of greenhouse gases by biofuels (Ullah et al. 2015). For example, first generation biofuels have issue of food shortage, indirect increase in CO_2 and use of land so the production of biofuels from plants was not further extended (Ullah et al. 2015; Noraini et al. 2014; Trivedi et al. 2015). That is why we are in need of sustainable feedstock for the production of biofuels that reduce emission of greenhouse gases, ecofriendly and must be non-agricultural non-food feedstock, including microbes, algae as well as plants that can grow on low quality land (Farrell and Gopal 2008).

Due to simple cultivation and other production parameters, microbial feedstocks have gained attention for the production of biofuels, because of certain restriction for the plant feedstock like water usage, land, long growth period and food vs. fuel issues. Additionally we can manipulate microbial sources on gene level with the help of genetic engineering, to produce recombinant strains having more lipid content. Different algal strains are the best known feedstock as microbial source for production of biofuels including biodiesel and bioethanol. Algae can be easily cultivated in natural (open/covered ponds) or artificial systems on large scale or even in simple continuous stirred tank reactors, (CSTR). Biofuels from algae are also called as third generation biofuels. Requirement of water for algal biomass production can be fulfilled by wastewater sludge, already containing sufficient amount of some nutrients and carbon (Mathiyazhagan and Ganapathi 2011). Microalgae and macroalgae both have potential to produce biofuels. Algae seem to be best feedstock for biofuels due to a number of reasons which are listed below (Anto et al. 2020).

1. Algae is not involve in food fuel and is not used as food in most of world.
2. Small period required for full growth.
3. Low nutrient requirement.
4. Low quality land can be used for its cultivation.
5. Artificial photobioreactors (as discussed in the next part).

2.2 Classification of Algae

Research has been begun on the use of several algal strains to convert them into biofuels through numerous techniques starting from the esterification to anaerobic digestion. Algae are mainly classified into two major groups: microalgae and macroalgae. The macro- and micro algae of bioenergy production must meet the following criteria (Carlsson et al. 2007):

(1) Both types must be very high yielding; (2) they must be easy to harvest; (3) they must be capable of resisting water currents from high sea level; (4) their production must be equal to or lower than those from other available sources (Kraan 2013).

2.2.1 Microalgae

Generally, microalgae are photosynthetic microorganisms found in both freshwater and marine habitats. Microalgae have been classified according to various features such as pigmentation, photosynthetic storage product, the arrangements of photosynthetic membranes and other morphological characteristics. Currently, there are four types of microalgae, namely “Diatoms (*Bacillariophyceae*), Green algae (*Chlorophyceae*), Blue green algae (*Cyanophyceae)* and Golden algae (*Chrysophyceae*)” (Khan et al. 2009). The dominant types of Microalgae for commercial scale production comprise *Chaetoceros*, *Arthrospira* (*Spirulina*), *Dunaliella* and *Chlorella* (Lee 1997). Several strains of Chlorella, which is a microalgae, can switch from phototrophic mode to heterotrophic mode of nutrition (Xiong et al. 2008; Xu et al. 2006). Just like heterotrophic organisms, the algae are also dependent upon carbon sources like glucose for obtaining energy and carbon metabolism. Few algal species have the ability to grow in the presence of mix nutrients. Bio-molecules like proteins, carbohydrates, nucleic acid and lipids are ordinary components of microalgae.

Recent research companies have shown that biomass of microalgae seems to be the one of favourable sources which can be used as renewable biodiesel and can meet global demand. The oil content of microalgal biomass can be as high as 80% by dry weight, depending on the species (Rodolfi et al. 2009). It is common for oil to range from 20% to 50% (Chisti 2007). It is possible to define oil production yield as the volume of oil produced per unit volume of microalgal culture each day as a function of microalgae growth rate and biomass. Various algal strains manufacture high levels of lipids about 50% to 60% of dry weight which acts as storage material. This stored lipid is chemically similar to oil-seed lipids which are obtained from other crops, making an encouraging source for production of biodiesel (Griffiths and Harrison 2009).

For more efficient lipid extraction from microalgae, many approaches have been used like expellers or oil pressing, ultrasonic procedures, extraction of super critical fluid and solvent extraction are the most popular methods. These methods of extraction should have the following characteristics: they must be quick, non-destructive and effective for the lipids removed and they should be easily scaled up (Medina et al. 1998). The modified Bligh and Dyer method for lipid extraction is frequently utilized (Mutanda et al. 2011). Different methods like simultaneous extraction, direct esterification and transesterification could be used to extract microalgal fatty acids from diverse biomass types and making it flexible process for production of biofuels. This process involved several steps that require a combination of solvent extraction, ultrasonication, heating at high-pressure, filtration, separation on the basis of solvent density, oil and liquids recovery via evaporation to dryness. Mondal et al. 2017 described that along with the production of biofuels some species of microalgae can also produce valuable chemicals that could be used in medicines as well as in other applications as described in Table 2.1.

Table 2.1 Different chemicals produced by microalgae and their uses

Algal species	Chemical produced and its use
Arthrospira	Phycocyanin, can be used as antioxidant
Haematococcus	Astaxanthin, used as anti-ageing agent
Phaeodactylum	Eicosapentaenoic acid, used as nutraceutical
Dunaliella	Carotene, used in the medicines to treat cancer
Tetraselmis	Tocopherols, used as anti-inflammatory agent

2.2.2 Macroalgae

Macroalgae account for the most integral part of the marine ecosystem, because they protect marine resources by stopping pollution and eutrophication (Notoya 2010). Macroalgae are inferior plants because they do not have roots, stems or leaves. Instead, they are made up of thallus (leaf-like) and sometime stems and foot. Some species are filled with gas in enclosed structures to aid buoyancy. They can grow very fast, up to tens of metres in size (Lüning and Pang 2003). Macroalgae are different in several aspects, such as morphology, life span and physiology. According to their pigmentation, they are divided into Phaeophyta (brown), Rhodophyta (red) and Chlorophyta (green) algae (Chan et al. 2006). The growth of macroalgae on rocky substrates results in a stable multi-layered perennial vegetation that is capable of capturing nearly all of the available photons in the natural environment. Almost 200 species of macroalgae are utilized all over the world, in which about ten are more commonly cultivated like *Laminaria japonica, Phaeophyta*, *Rhodophyta*, *Undaria pinnatifida*, *Gracilaria*, *Eucheuma*, *Porphyra*, *Kappaphycus Monostroma*, *Kappaphycus and Chlorophyta Enteromorpha* (Lüning and Pang 2003).

Some macroalgae species collect a large number of carbohydrates that can be used as a substrate in microbial conversion activities, such as the manufacture of biofuels with a high product cost (Kraan 2013). Table 2.2 indicates different algal strains that can produce different fuels. Maceiras et al. (2011) recently discovered that a transesterification process could be used to produce biodiesel from triglycerides by using a variety of macroalgae, including *Codium tomentosum*, *Ascophyllum nodosum*, *Fucus spiralis*, *Enteromorpha intestinalis*, *Sargassum muticum*, *Pelvetia canaliculata*, *Saccorhiza polyschides and Ulva rigida.* It has been observed that macroalgae have a higher water content (80–85%) than terrestrial plants, making them better suitable for the thermochemical conversion of microbial conversion than the direct combustion process. Macroalgae like *Gracilaria* spp., *Sargassum* spp., *Laminaria* spp., *Prymnesium parvum* and *Gelidium amansii* are best targets for production of bioethanol (Adams et al. 2009; Wi et al. 2009).

Table 2.2 Different algal strains from the production of biofuels

Strain of algae	Type of biofuel	Reference
Laminaria sp.	Methane	Chynoweth et al. (1993)
Sargassum sp.	Methane	Bird et al. (1990)
Ulva sp.	Ethanol	
Macrocystis sp.	Methane	Chynoweth et al. (1993)
Gelidium sp.	Ethanol	Yung-bum et al. (2010)
Ulva sp. *Gelidium* sp.	Methane	Adams et al. (2009)
Gracilaria sp.	Methane	Bird et al. (1990)
Gelidium amansii	Hydrogen	Park et al. (2011)
Kappaphycus alvarezii	Ethanol	Khambhaty et al. (2012)
Laminaria japonica	Hydrogen	Shi et al. (2011)

2.3 Cultivation of Algal Biomass

The cultivation of algae raw materials is comparatively an easy process and may evolve with limited or no supervision. Algal biomass can be cultivated by using wastewater which has harmful effect if consumed by human being and at the same time absorbing CO_2 from the atmosphere (Bharathiraja et al. 2015). Algae absorb sunlight for the process of photosynthesis, which is biochemical process and used for growth of plant, then they produce chemical energy from sunlight (Brennan and Owende 2010). The CO_2 formed is converted into distinct configuration of chemical energy through photosynthesis, like lipids, carbohydrates and protein. That is why the growth and reproduction of algae require only the supply of basic nutrients like sunlight, CO_2 and water required for regular photosynthesis of plants, and the conversion of solar energy by fixing CO_2 (Hallenbeck et al. 2016). Algae biomass cultivation basically has the following two types, as illustrated in Fig. 2.2.

2.3.1 Natural System for Cultivation of Algae

The natural system for cultivation of algae includes natural habitat of algae like lakes, ponds, lagon, etc. These are classified on the basis of structure and depth of water bodies such as circular pond, shallow ponds, open ponds, covered ponds, etc. These specialized ponds for algal production are constructed in such a way that they are situated above level of ground (Suganya et al. 2016).

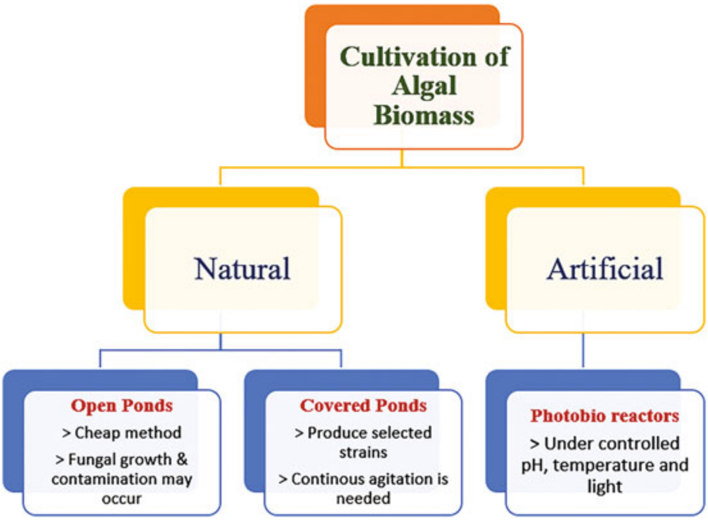

Fig. 2.2 Different types of cultivation systems

2.3.1.1 Open Ponds

These are the best suited places for the cultivation of algae from economical point of view, as they are simply natural ponds containing fresh or salted water according to the selected strain of algae to produce biofuels. These ponds can be scaled up easily up to many hectors. But the main limiting factor for scaling up of these ponds are grazers, fungal growth or contamination of selected strains with unwanted algal strains. Although about 98% of the algal biomass production is obtained from open pond system. Algae can grow really fast so it can produce about 15–20 tons of biomass after drying from each hector per annum. All high yielding strains of algae contain about 50–60% of lipid content by weight that is further processed to obtain biofuels (Singh et al. 2015). There are a number of microalgae strains that could be cultivated by using open raceway ponds. One of these species is "*Chlorella pyrenoidosa*" in the pond containing wastewater and can generate about 1.7 g of biomass per litter of water (Bell and Strang 2020). Reports show that the dried biomass productivity of Dunaliella salina in open pond was 0.097 g/l per day and that of Nannochloropsis sp. is 0.207 g/l per day (Ghorbani et al. 2018).

2.3.1.2 Ponds

The problems associated to open ponds can be sorted out by covered ponds such as production of unwanted strains, fungal growth and protection from grazers. A huge water loss by evaporation in open pond is solved in covered ponds also. But one of the problems with these closed systems is the rise in temperature since the ponds are covered. This issue can be sorted out providing continuous agitation to the system that may increase cultivation cost (Carvalho et al. 2006). Many manipulations in the set-up of both open covered system are being made for significant increase in the yield of biomass of selected strains of algae. In a study (Marchin et al. 2015), a cascade system for the production of algae was made on the roof top, while the force of gravity was used on inclined surface to keep the system in motion simply to provide thorough agitation. The culture of algae was collected at the end of inclined surface in a tank and then the water is pumped back to the roof. The variation in the volume of system due to evaporation in very hot sunny days and in rainy day was managed in the tank that acts as buffering agents. Several different systems were also used by researchers to grow algae in their natural environment (Devi and Mohan 2012).

2.3.2 Artificial System for Algae Cultivation

Artificial cultivation of algae has been achieved by the use of photobioreactors (Lee and Lee 2016). These closed cultivation systems of algae have controlled environmental condition that improves overall yield (Kandiyoti et al. 2016). Water, CO_2 and other nutritional requirements for growth of algae are added in the photobioreactors, while light, pH, temperature and density of the system are maintained at optimum level required for the growth of a particular algal strain (Trivedi et al. 2015). All the limitations that can affect the overall yield of the algal biomass in the natural system could be sorted out by using photobioreactors but limiting factor in the systems is capital cost (Brennan and Owende 2010). The main reason behind the invention of this artificial technology was to overcome the challenges as in the natural systems such as contamination and pollution, fungal attacks that hinder the production of good quality yield of algal biomass at large scale (Kandiyoti et al. 2016).

Photobioreactors are actually manmade cultivation system that enhances growth of specific algal species in the presence of ideal growth parameters including pH, light and temperature (Ullah et al. 2015). They are several forms of photobioreactors on the basis of their shape (Anto et al. 2020). Some types are listed below:

1. Tubular photobioreactors
2. Plate photobioreactors
3. Helical photobioreactors
4. Horizontal photobioreactors
5. Foil type photobioreactors

The algal culture is continuously pumped and recirculated in the photobioreactor for proper growth (Carvalho et al. 2006). The photobioreactors are made up of glass or acrylic (as represented in Fig. 2.3) but are translucent so do not allow natural sunlight to reach the algal biomass. So in order to minimize the need of natural

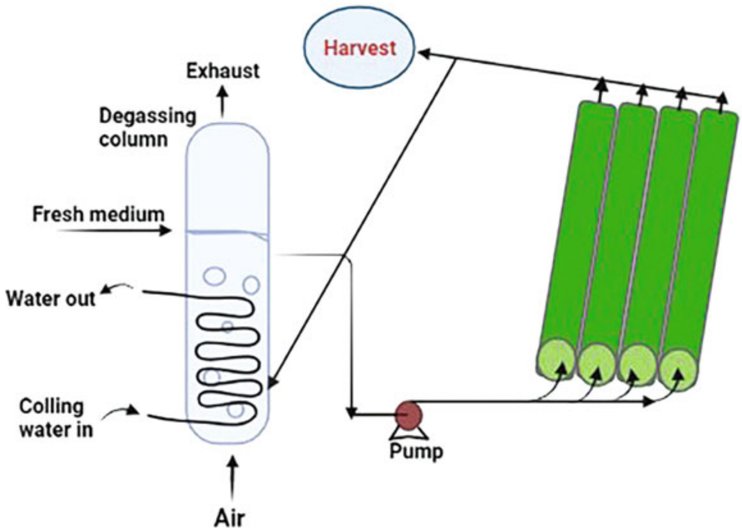

Fig. 2.3 Schematic representation of photobioreactor

sunlight, light emitting diodes are fitted inside the reactors and a yield of 100 g/per hour can be achieved. Increase or decrease in light intensity affects the rate of photosynthesis (Ullah et al. 2015). These manmade reactors have many advantages over natural system, a few are listed below (Anto et al. 2020):

1. As the cultivation systems are fully closed it eliminates the chances of contamination by unwanted algal strains or fungal attacks.
2. Water loss by evaporation is minimum due to no direct contact with sun that reduces the water need as compared to open ponds.
3. More efficient dissipation of heat and nutrients that leads to uniform and maximum growth.
4. Controlled parameters like CO_2, nutrient, pH, etc. maintain the uniform environment.
5. Can also produce biomass at night by photosynthesis due to availability of light by artificial diode system which reduces the cultivation period.

2.4 Harvesting

The next step right after the production of algae is to harvest its biomass present in aqueous environment. Usually cultivation of algal biomass in the aqueous environment faces many hurdles like its down-streaming and to make it water free. The cost of harvesting of "algal biomass is about 20–30%" of the total operating cost of the algal biomass production. This cost could be increased or decreased by different factors such as type of harvesting method used, type/species of algae and its density (Karemore and Sen 2016). That is why the increment in algal biomass and reduction in the overall volume of culture medium will automatically lead to decrease in the reduction of expenditure on the down-streaming. Some of the mostly used techniques for harvesting algal cell biomass are electrophoresis, centrifugation, flocculation, filtration and flotation, etc. (Karemore et al. 2016). Comparison of different types of harvesting techniques is shown in Table 2.3.

Nichols and Scott (2012) opened a gateway for the growth and easy down-streaming of algal biomass by using "polycationic flocculants". The process involves use of optimum amount of chitosan or chitin in the aqueous environment for the production and harvesting of algal biomass. It is used to

1. increase algal biomass
2. more aggregated algae
3. enhance over-all lipid concentration in the algal cell to produce biodiesel
4. downstream the biomass after removing extra water

That lipid containing algal biomass can be further processed to produce chemicals, biofuels like biodiesel, biogas, bioethanol or biohydrogen, for animal and fish feed and in some cases for human dietary purposes. Some anionic polymers

Table 2.3 Comparison of different methods of harvesting algal biomass

Techniques	Advantages	Disadvantages	Dry solids output conc' (%)
Ultrafiltration	Used to handle with intricate cells	High costs	1.5–4
Centrifugation	Efficient method of algal biomass harvesting and can handle almost all algal types	But have high costs	10–20%
Chemical flocculation	Can be low cost method, vast range of flocculants are available	Removal of flocculants, chemical contamination	3–8
Filtration	Vast range of membranes and filter papers are available	Dependent on algal strains but best suitable for large cells of algae but fouling and clogging are main issues with filter paper	2–27
Flotation	Rapid process and can combine with gaseous transfer	Specific for specific algal species and have high costs	7

could also be used like pectin, xanthan gum and alginate for harvesting (Karemore et al. 2016).

Another method to increase concentration of biomass after harvesting is the use of organic flocculant in their low concentration for the production of freely moving single cell algal biomass, which has relatively less diameter of about 5 μm, for example, Nannochloropsis. This process is economically very suitable to produce algae for biofuel production at large scale (Bazarnova et al. 2018). One of the other invented methods to obtain higher concentration of algae is filtration that is performed without addition of any supporting material, and algae have to bear no thermal or any other shock, that is why biomass remains protected. Additionally this technique is used to do harvesting in continuous manner and harvested biomass can be directly used for the production of biofuels or other products. To carry out the algal biomass will have to pass through a series of concentrators that work on the principle of passive filtration. The filtrating device can also be processed by using gravitational force (Kabakian 2014). Centrifugation can also be used to harvest algae in a "mechanical based system" and the biomass is collected with higher concentration. In recent experimentation the combination of all these methods is performed to obtain more concentrated product (Chowdhury et al. 2019).

2.5 Production of Different Types of Biofuels from Algae

There are different kinds of biofuels that can be obtained from algae by using different processing as shown in Fig. 2.4.

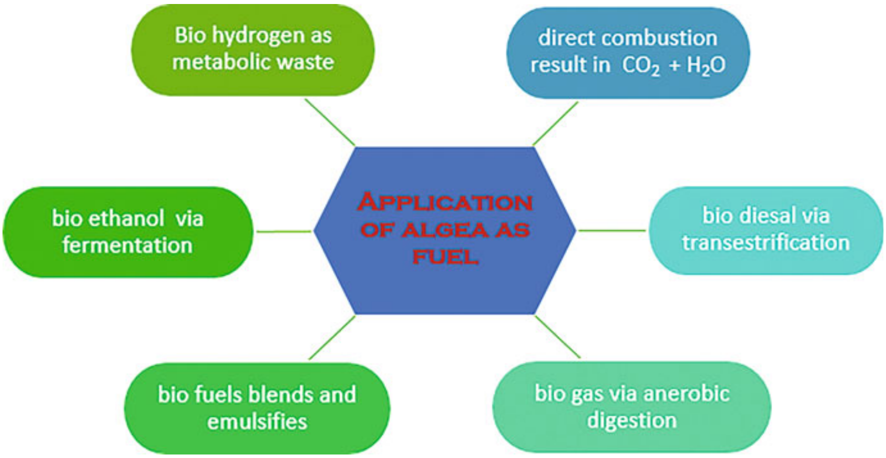

Fig. 2.4 Different types of biofuels that can be produced from processing of algae

2.5.1 Production of Biodiesel

Biodiesel is simply methyl or ethyl ester of fatty acid that is produced by the process of trans-esterification (in the presence of alcohols) of triglycerides. So we can say that biodiesel is alkyl fatty acids (having chain length of C14 to C25), esterified in the presence of alcohols mostly CH_3OH or C_2H_5OH. Biodiesel is most comparable with the diesel from fossil fuels in characters like flash point, "cetane number", viscosity and heating value (Demirbas 2009). Due to these characters biodiesel seems to be the most demanding fuel in future because of limited resources fossil fuels. The other most demanding feature of biodiesel is its eco-friendly nature as it reduces emission of CO_2 by 77% as compared to conventional fuels (Atadashi et al. 2010).

It is the type of fuel that can be degraded by microbes (biodegradable) and non-toxic that is derived from renewable natural resources (Hossain et al. 2008). It also enhances engine performance as well as reduces the emission of sulfur and other particulate matter during combustion (Miao and Wu 2006; Scragg et al. 2002). For the production of biodiesel, triglycerides are transesterified by acids or any catalyst like metals to produce biodiesel and glycerol as by-product (Chisti 2007; Johnson and Wen 2009). By keeping in view all the above mentioned points biodiesels are acceptable in order to fulfil demand of fuel, but its current production by terrestrial biomass is not enough to meet energy demands. In 2007 Chisti noted that about three times of the area currently available for cropping in the USA is needed to fulfil the only half of the demand of fuel for transportation per annum. However algal biodiesel can fulfil this demand with the low quality cultivating land and less efforts (Chisti 2007). But up till now there is no report on the large scale production of biodiesel from algae (Lardon et al. 2009). Different algal species produce biodiesel in different quantities as described in Table. 2.4.

Table 2.4 Production of biodiesel from different strains of algae and per Kg lipid content

Algal strain	Biodiesel production/kg lipid content	Reference
Spirulina platensis	58.9 g	Nautiyal et al. (2014)
Nemo-chloropsis sp.	98.9 g	Susilaningsih et al. (2009)
Scenedesmus sp.	281.5 g	Kim et al. (2014)
Chlorella marina	99.5 g	
Nannochloropsis salina	181.5 g	Muthukumar et al. (2012)

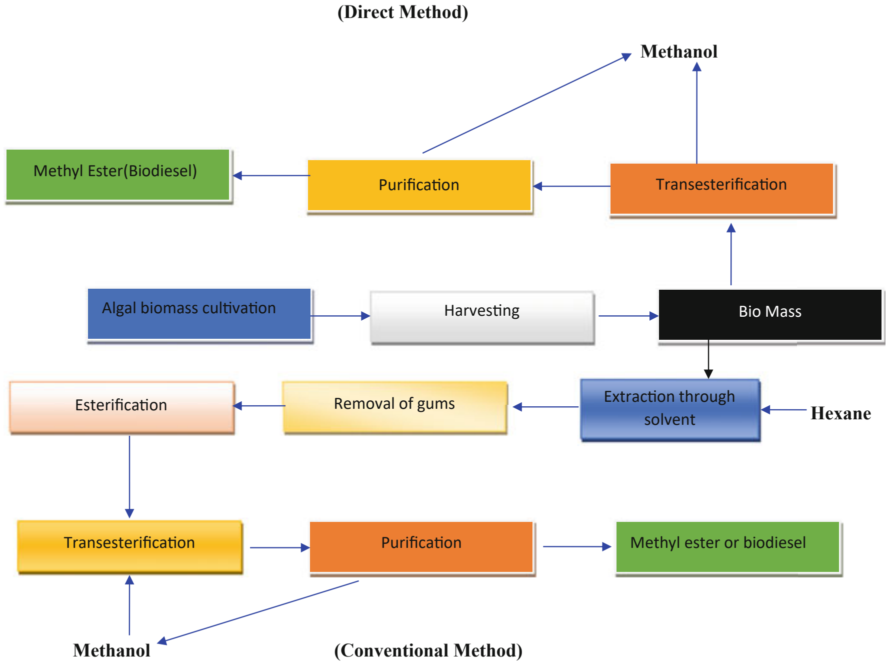

Fig. 2.5 Production of biodiesel through direct and conventional methods of transesterification

The conversion of algal biomass into diesel is a chemical conversion process that needs the reaction of lipids from algal biomass with alcohols (Methanol/ethanol) by adding a suitable catalyst (Suganya et al. 2016; Johnson and Wen 2009; Kandiyoti et al. 2016; Lee and Lee 2016). This process is very compulsory in order to lower the viscosity of algal oils that is greater than the petroleum diesel. That viscosity lowering process automatically increases fluidity of the biodiesel. While glycerol is produced as by-product and used to form different pharmaceutical and cosmetic products. Transesterification can be done by two methods (Johnson and Wen 2009) as illustrated in Fig. 2.5.

1. Conventional method
2. Direct method

2.5.1.1 Conventional or Traditional Method of Trans-esterification

This is a two step process that includes extraction of lipids from algal biomass before the conversion process (Lee and Lee 2016). For this method, there is an essential step of pretreatment that is performed to extract the lipid from algal biomass and removal gums by the action of non-reacting solvents such as hexane (Hossain et al. 2018). These additional methods lead to high yield of extra refined biodiesel that could be directly used for high speed engines (Salam et al. 2016). The addition of these steps increases the over all production cost (Martinez-Guerra and Gude 2018). These processes not only increase the production cost by increasing energy demand but also increase the time of production (Jazzar et al. 2015).

2.5.1.2 Direct Transesterification

This is also called as one step process and involves the extraction of lipid content of algal biomass (Lee and Lee 2016; Ehimen et al. 2010). The wet and unwashed biomass of algae is directly placed in reaction chamber to perform direct transesterification (Jazzar et al. 2015). Excessive methanol is added in the reaction chamber that eliminates the need of processes like pretreatment, removal of gums and extraction of lipids. That is why it gives more yield than the traditional method (Lawayzy et al. 2014).

It was highlighted that yield of biodiesel from the transesterification by direct method of dry biomass of algae is greater than that of wet biomass, pointing about the need of dryness of biomass. In comparison to this use of conventional method for the biodiesel produces equal yield either the biomass is wet or dry (Johnson and Wen 2009). If solar drying techniques are developed, biodiesel synthesis from any biomass became more practical. But the over all yield and the amount of lipid extracted from solar drying of biomass are not currently known (Lardon et al. 2009). To reduce the production cost utilized in drying, pretreatment to reduce the energy consumption for production of algal biodiesel at large scale new and well-established technology is needed (Levine et al. 2009; Sialve et al. 2009). To enhance the over all biodiesel production by increasing lipid content of algal biomass needs to grow algae in nutrient rich medium. It was also described that in order to increase the lipid content of algae genetic engineering is a promising technique. But the previous experimentation in this regard was not very successful (Sheehan et al. 1998a, b). Thus a lot of research work is need in order to manipulate the genes of algal strains for greater yield of biodiesel (Hu et al. 2008).

2.5.2 Biohydrogen Production

Biohydrogen is the gaseous energy source that is considered as the future of energy field stock because of its high energy density and environment friendly nature (Oncel 2013). The traditional method of the production of biohydrogen is by the use of steam that consumes a lot of energy (to produce 1 kJ of biohydrogen 3–3.5 kJ of energy is required). The metabolic processes of microalgae can produce biohydrogen directly (Melis and Happe 2001). Similarly dark fermentative bacteria like (*Clostridium* and *Enterobacter*) can also produce biohydrogen by using macroalgae as substrate. Production of biological hydrogen is relatively simple process that requires less energy supply in mild conditions rather than the high energy consuming traditional method but the biological production of hydrogen is not currently on large scale due to lack of suitable instrumentation. One other main limitation is incomplete knowledge about the metabolic production of biohydrogen from bacteria that hinders the way for genetic engineering to do the process on large scale. Actually, hydrogen is the waste product of metabolic process of algae for the balancing of redox reaction in the absence or presence of oxygen (Dębowski et al. 2013).

But the metabolic machinery for production of biohydrogen in algae and cyanobacteria is different. According to current studies, production of biohydrogen from algae was done only on ice lab scale or pilot-scale, so there are many challenges to upgrade the process for hydrogen production. The metabolic hydrogen enzymes are very sensitive to the presence of oxygen so the process has to be performed in strictly controlled environment without oxygen. But two phase reaction can solve this problem by separating the production of biohydrogen into two phase:

1. Biomass production (aerobic phase)
2. Hydrogen production (anaerobic phase)

To produce biohydrogen on large scale, the aerobic condition can be provided in open ponds to reduce the cost. However, the transfer of biomass after harvesting to the anaerobic environment is still time and cost consuming process. Similarly it is very difficult to prevent inhibition of hydrogen enzymes that is extremely sensitive to O_2 (Márquez-Reyes et al. 2015) even by solving the problem of reduction process, there is still challenge to store hydrogen gas due to its low energy density (Hallenbeck et al. 2016).

2.5.3 Production of Biogas

Biogas can be produced by conversion of organic waste that is obtained from algal biomass. This process involves the breakdown of algal biomass into methane and carbon dioxide that can be used for domestic purpose or as a fuel for automobile engines (Lee and Lee 2016). By this process there is a huge recycling of carbon, in

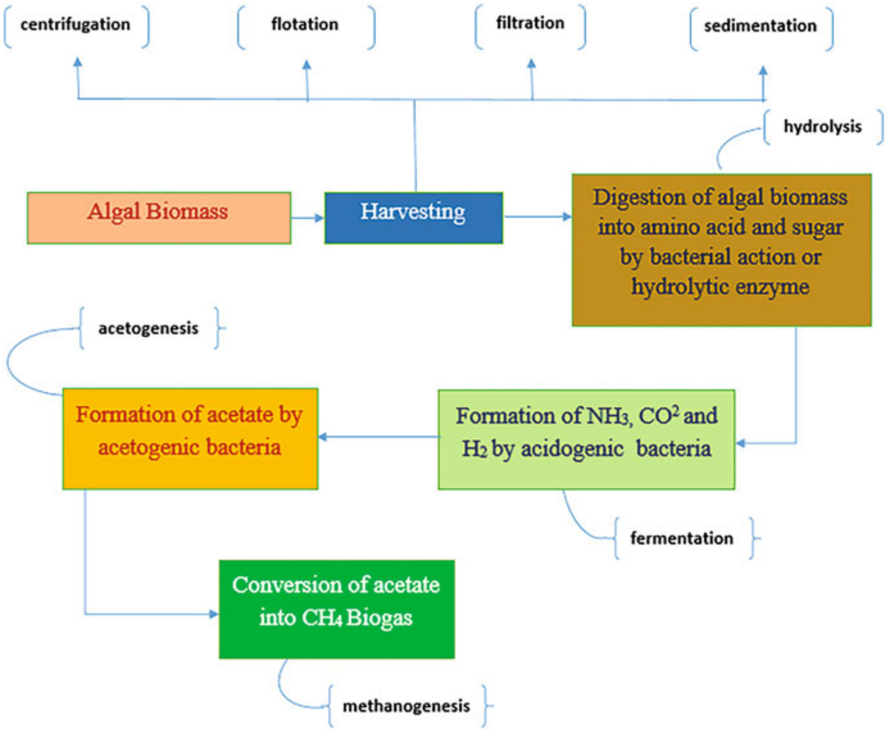

Fig. 2.6 Production of biogas from algal biomass

the form of methane (Adeniyi et al. 2018). This process can also covert biomass having high content of moisture into methane (Trivedi et al. 2015). Production of biogas (methane has the following four steps as illustrated in Fig. 2.6).

1. Hydrolysis of biomass:

   The first step of biogas involves the breakdown of algal cell wall by bacterial or enzymatic action in the digestion tank (Ehimen et al. 2013), which leads to conversion of algal substrate into soluble sugars and amino acids (Trivedi et al. 2015).
2. Fermentation of digested matter:

   This step involves the aerobic digestion of digested organic substrate (Ehimen et al. 2013). Acidogenic bacteria perform their action by converting sugars and amino acids into H_2 (hydrogen), CO_2 (carbon dioxide) and NH_3 (ammonia) (Trivedi et al. 2015).
3. Acetogensis by oxidation:

   This step involves the oxidation of products of fermentation into acetate that has to be utilized further for methane production. In order to oxidize the substrate partial pressure is applied by using hydrogen (Ehimen et al. 2013).
4. Production of methane (biogas):

The final and last step of this process involves the production of CH_4 (methane) also called as methanogenesis (Trivedi et al. 2015). This is achieved by the help of methanogens (such as *Methanosarcina barkeri*). After the production of biogas, there would be enough nutrients as left over that can be utilized for further production of algal biomass (Ehimen et al. 2013).

2.5.4 Bioethanol

Bioethanol/ethyl alcohol is the type of ethanol that is obtained by processing of any natural biological source. It could be utilized as replacement of petrol (Nahak et al. 2013). Demand for bioethanol as a transportation fuel is increased with time. Many countries like Brazil, China and India have started the production of bioethanol and using it as fuel source (Lee and Lee 2016). Over conventional fuels which are obtained from fossil fuels, bioethanol is preferred because of its source and impact over environment. It has lower sulfur content than petrol that is why it emits less greenhouse gases upon combustion. The scope for using bioethanol is high because it is renewable source. Bioethanol is produced after the break down of sugars and starch which are obtained from first or second generation feedstock like lignocellulosic biomass, wheat bran corn, etc. (John et al. 2011). But continuous consumption of second generation feedstock would lead to depletion of food in the world which is already a big issue of recent times. If they continue to use second generation feedstock as a fuel source there would be debate about the water use, use of arable land and food versus fuel.

This pitfall of using second generation feedstock is overcome by algae. Algae are third generation renewable source and are the best substrate to produce Bioethanol. Algae can grow on wastewater also either it is from municipal or industries. Production of bioethanol by using algae would help in bioremediation because algae use CO_2 and other nutrients from wastewater for the process of "photosynthesis" and resulting in treated water (John et al. 2011). Mainly it is obtained through the process of fermentation of cellulose which is the main component of cell wall or by the starch that is a storage material (Ullah et al. 2015). Different species of algae can store starch and other sugars which is stored by *Chlorella vulgaris* is 37% of the dry weight of its biomass. "Blue green algae" like.

Chlorococum sp. and *Spirogyra* species can store high level of polysaccharide. Commonly used algal species (illustrated in Table 2.4) for the production of bioethanol are *Prymnesium parvum*, *Chlorella*, *Scenedesmus*, *Sargassum Gracilaria*, *Spirulina*, *gracilis porphyridium*, *Dunaliella* and *Chlamydomonas* (Chaudhary et al. 2014) (Table 2.5).

Table 2.5 Bioethanol production from different strains of algae

Algal strain	Ethanol yield	Reference
Chlorococcum humicola	0.52	Harun and Danquah (2011)
Spirogyra sp.	–	Eshaq et al. (2011)
Chlorococcum infusionum	0.26	Harun et al. (2010)
Chlamydomonas reinhardtii	0.24	Choi et al. (2010)

2.5.5 Direct Combustion

It is an irreversible chemical change in which algal biomass is converted into gases that are hot and produce energy (Lee and Lee 2016). An interaction among a biomass of algae and O_2 at 100 °C in the steam turbines, furnace or boiler etc., which produces hot mist (steam) that has the power to operate a turbine which drives a "generator" for the production of electricity (Suganya et al. 2016). For the reduction of air pollution direct combustion can be accomplished if the moisture level is lower than 50% (Sheehan et al. 1998a, b). Prior to combustion of biomass pretreatment processes like drying, grinding/milling is necessary (Trivedi et al. 2015). Alkali residues and high ash composition reduces the efficiency of overall conversion process. A new method called fluidized bed method was suggested by Milledge et al. (2013, 2014) which was the best method for the reduction of effect of alkali and high ash content. But for the application of this method algal biomass should be converted into smaller particles, therefore grinded algal biomass required another form of pretreatment (Lee and Lee 2016). Brennan and Owende (Kandiyoti et al. 2017) recommended that the heat which is produced during conversion process is utilized immediately, then the cost of this extra pretreatment process can be lessened (Adeniyi et al. 2018).

2.6 Algal Biofuels Blends and Emulsifiers

Due to the unfavourable effects and depletion of fossil fuels different researches have been done for the improvement of biofuels (Acharya et al. 2017). Due to physiochemical properties biofuels have to encounter several challenges (Rahman et al. 2015; Echim et al. 2012). For producing fuels with desired physiochemical properties blends of biofuels are produced. For this purpose different types of fuels in different proportion are mixed which as a result improve the properties of fuel which helps in the improvement of engine performance (Neto et al. 2013). Diesel produced by using algae can be used as additive or blended with bioethanol or petroleum diesel (Sheehan et al. 1998a, b; Acharya et al. 2017; Barabás et al. 2010), which can be used without any modification to existing engines (Nair et al. 2013). Blending of algal biodiesel and petroleum diesel was begun by Nagane and Choudhari (Patel et al. 2014). They blend about 20% of algal biodiesel which results in good performance of engine. To upgrade the efficiency level of 4-cylinder diesel engine, oxygenating activity of algal biodiesel was combined with addition of butanol. Makarevičienė et al. (2014) found that a blend of 10% algal biodiesel, 60% petroleum and 30% butanol was environmentally benign, with lower NOx and CO emissions (Makarevičienė et al. 2014).

This is due to the fact that direct use of some of these fuels might cause the problems of engine such as incomplete combustion, engine fouling, pollution of lubricating fuel and low fuel atomization (Mofijur et al. 2016). However the blends of these fuels have improved properties as compared to individual fuels (Nair et al. 2013). Because of the oxygenating effect of blends successful depletion in emission of exhaust gases occurs. But when these blended fuels are used in engines there instability causes a big problem, and it might result in failure of engine. Proper biofuel solubility, stability and durability are required, while the temperature, specific gravity and viscosity should be in mind (Khalife et al. 2017).

2.6.1 Blending

There are several methods of blending algal biofuels with petroleum fuels:

- *Splash mixing method*: It is the least precise method of all the blending methods of biofuels that is why it is not used commonly (Mofijur et al. 2016). In this method fuel is pumped into another tank which contains petroleum fuel. The temperature of fuel which is present in tank should be colder than 8 °C and the temperature of pumped fuel should be 18–20 °C.
- *Mixing by in-line method*: for this process an empty vessel called collecting vessel is present which is used for final product collection. In this vessel two different fuels which are used for blending are pumped in exact same ratio through a pipe or hose (Mofijur et al. 2016). For blending of large volumes of fuel this method is perfect but it is preferable to not keep the bended fuel in this tank for long period of time. In this method the blend consistence is much better than splash method but the final product still requires proper adjustment.
- *Mixing by injection method*: Being the most correct and preferable method of blending. It applies control by valves. In this method variable valves are used for the injection of different fuels in a required ratio. This method is used before delivery into final tank at the production point (Mofijur et al. 2016).

2.6.2 Additives

Additives can also be used for the modification of fuel properties like octane number, energy content, lubricity and stability. Few selected additives are tested for better performance of engines (Roy et al. 2016).

2.6.2.1 Types of Additives

Different types of additives are used to increase the performance of algal biofuels as shown in Fig. 2.7.

- *Oxygenated additives*: These additives are used to control compromised combustion which is usually caused by inadequate supply of oxygen (Mata et al. 2010). Oxygenated compounds are added in a specific ratio to fulfil the oxygen need which helps in avoiding incomplete combustion.
- *Additives based upon metals*:

  This type of additives helps to minimize the temperature for oxidation and upgrade the soot oxidation. During soot oxidation they directly react with atoms of carbon and upon reaction with water produce hydroxyl radicals.
- *Water*: In the ignition chamber, upon entry of water the temperature of local combustion is lowered and as a result reduce the emission of NO.
- *Antioxidants*: Antioxidants terminate the chain reaction and prevent the oxidation of other molecules. In this way they increase the shelf life of fuels.
- *Polymeric based additives*:

  This type of additives is used for the improvement of engine parameters and engine performance.

When two different, multifunctional and organic additives are combined it is estimated that upon injection they will exert positive physiochemical effect on engine. Ethanol can also be used as good additive which is used for better emission and combustion performance in engine. Bhale et al. (2009) recommended ethanol

Fig. 2.7 Different types of additives and their function

blended biodiesels which are renewable and can be used as alternative to fossil fuels. These blended also boost the cold flow behaviour and does not affect the performance of engine in cold climate. Sadeghinezhad et al. (2014) examined the factors that influence ethanol solubility in diesel oil and establish a connection between the blend temperature and the water content. When the ambient temperature is warm, dry ethanol can mix with diesel fuel, but when the temperature is lower than 10 °C, both fuels started to isolate. The inclusion of an emulsifier, which holds these blended diesels together by floating microscopic drops of ethanol in diesel, could address this problem. The careful mixing of biofuels like bioethanol or biodiesel with petroleum diesel is a key technique for lowering diesel engine emissions. Although these tidy blends are insufficient for the removal of all pollutants that is why water fuel emulsion approach was developed (Hoseini et al. 2017). For the improvement of thermodynamic stability of fuels micro emulsions are preferable (Karthikeyan and Prathima 2016). These emulsions are evenly diffused in mixtures of liquids comprising polar and non-polar parts that use organic molecules known as emulsifiers or surfactants to lower the interfacial tension among the water and oil phases (Xu et al. 2016). This is the process of creating thermodynamically stable mixes that are transparent, isotropic and nonpolarized (Nair et al. 2013). "Emulsions" are also referring as fuels those are hybrid of diesel since they can combine biodiesel with low-molecular-weight alcohols to lower consistency by using an amphipathic compound referred as a "surfactant" (Dunn 2010).

Several commonly used surfactants are used as cosurfactants with butanol and water such as cetyl trimethyl ammonium bromide (CTAB) and sorbitan monooleate (Span80). These are used for the improvement of emission from the engines that use algal biofuels blends (Xu et al. 2016). Hagos et al. (2017) tried to make a difference between blending and emulsification techniques which are based on boiling point of mixtures. For obtaining neat fuel mixture, blending of those fuels that have similar boiling point should be done. While for emulsion, fuels that have different boiling pints are used. These fuels of different boiling points show phenomenon of small eruption in fuel during mixing of fuel with any oxidizer. Mixture of diesel or biodiesel with water is used to produce emulsions of fuels is an alternative procedure of using microalgae. This emulsified water extends emulsion stability and provides help for interaction between mixtures. This process is described by (Al-lwayzy and Yusaf 2013). Ultrasound treatment is applied to microalgae aggregates which improve the disintegration of these aggregates in dispersed cells and result in fine droplets with wide spray angle in the emulsion. Lei et al. (2012) developed a new emulsifier which is called CLZ. This is based on the firmness of ethanol in blends of biodiesel. It was noticed after testing or using CLZ emulsifiers there was remarkable improvement in engine performance. For the possible reduction of NO and CO_2 emulsion should be used instead of tidy biodiesel blends (Haik et al. 2011).

2.7 Economics of Algae

Petroleum's shifting price continues to establish the economic benchmark that all biofuels must reach in order to be competitive. Lipid production of the microalgae has pronounced potential for renewable fuel production. Cost information of fuel produced by algae is limited to biodiesel but various processes use several forms of microalgae for fuel production. The estimate of cost will be on assumption until unless production plant produces minimum amount of biofuel which is one million gallons per year. Even the best assumptions about the cost of production are most probably may be in precise. For large scale production, economic cost and cost of resources such as water, air and land are unknown. Heterotrophic and autotrophic both processes are used for the production of biodiesel. In heterotrophic process the cost of carbon sources (carbohydrate) or feedstock is reported about 60–75% of total cost of biodiesel production (Brennan and Owende 2010; Borowitzka 1997). The most recent cost of biodiesel which is produced by using Solazyme is about $67 per gallons. That is why it is considered that Solazyme has attained the major milestone in this developing market of biodiesel (Rapier 2010).

Comparison of PBR and PRP was done by Chisti (Chisti 2007). Both techniques are used for the production of 100 s of tons of biomass and used extensively for commercial productions (Spolaore et al. 2006; Tredici 2010; Pulz 2001). First step of obtaining the target product from algal biomass is recovery of algal biomass from the cultural broth. Several techniques such as centrifugation, filtration, etc. are used for the separation of algal biomass (Grima et al. 2003). The techniques which are used for the separation of algal biomass affect the overall cost of process. The recovery costs of PBR technique are somewhat less as compared to the costs of PRP process because in PBR process higher biomass concentration is obtained.

The cost for the recovery of algal biomass from PBR process is somewhat lower as compared to those of PRP process. Because in PBR process there is higher concentration almost 30 times greater of biomass as compared to PRP process. Producing 1KG of microalgal biomass through PBR process would cost almost $2.95 and that same amount of biomass production through PRP process would cost almost $3.80 (Grima et al. 2003; Terry and Raymond 1985). If the amount of production is increased and annual biomass level is almost 10,000tons, then the cost of biomass per KG would decrease which is most probably $0.47 for PBR process and $0.60 for PRP process. For 30% oil extraction efficiency of algal biomass by weight is assumed and the cost for the 1 L of oil which is extracted from biomass would cost almost $1.40 and $5.30/gal via PBR process and $1.81 and $6.85/gal from PRP process (Grima et al. 2003; Chisti 2007).

Algal derived biofuels have standard efficiency about 60%, their conversion would cost about $8.03 via PBR process and $10.38 through PRP process. Improvements, such as growth technique improvements, process efficiency and stability and efficiency of GMOs, also continue to bring the cost of biodiesel made from algal biomass closer to the biodiesel made up of other feedstock such as soybean, canola, palm and petroleum. Recently no such commercial algal plants are established which work consistently for the production of biofuel (Menetrez 2012).

These all theoretical examples about cost which are given above are best putative estimates. Algae-derived biodiesel would be competitive with other biodiesel feedstocks and petroleum-derived diesel at a price of around $4/gal. This hypothetical situation would also have the added benefits of lowering wastewater nutrient loads, lowering plant water demands and CO_2 sequestration. In addition to this importance of nonlipid part of algal biomass cannot be ignored. Ethanol can be produced from high carbohydrate levels and animal feed can be provided from high protein content (Menetrez 2012).

2.8 Genetic Engineering

The illegibility of recombinant algae production to maximize both of the previously mentioned cultivation methods is dependent on its quality of modified algal biomass to modify the standardized attributes of algal biomass in order to generate maximum yields of both the by-products and main products (Snow and Smith 2012). Cultivation process involves purposeful changes in genes of algae in order to make a more efficient substrate for different uses (Tabatabaei et al. 2011). Most of the algal species used a specialized structure called as “antennae” for capturing radiations from sun to perform photosynthesis and is responsible to produce maximum yield. Modification of the DNA which is responsible for these antennae would allow for the expression of genes that are small in sequence, allowing the greater light absorption by the cells of algae. To transfer DNA into algae cells, a variety of transformation methods have been used, including electroporation, particle bombardment, artificial transposons, virus infection, in the presence of glass beads and DNA agitation of cell suspension is done, silicon carbide whiskers, agro-bacterium infection and most recently, agro bacterium-mediated transformation.

Highest transformation rate is obtained in particle bombardment and electroporation methods (Rismani-Yazdi et al. 2011). For reengineering of algal DNA genetic engineering gradually has become the most methodical and time saving method. The reason for DNA restructuring most commonly is that processes like hybridization, sequencing, enhanced evolution and metagenomics can generate or enhance the likelihood of survival in harsh environments (Dana et al. 2012). For achieving the essential metabolism of specific area genomic DNA of algae is modified which also improves the performance in harsh environment. Nontransgenic methods require proper assessment for obtaining new characteristics and it also improves performance and produces best algal strains which can survive under broad scale of abiotic and biotic circumstances (Flynn et al. 2010). But after doing these changes it improvse one characteristic but at the same time it makes other traits harmful, for example, after metabolic changes fuel and synthesis of energy is enhanced but simultaneously it results in the production of such algal strains which are harmful for food and non-food applications (Hall and Benemann 2011). The reason for having such side effects of metabolic changes and genetic improvements is that these changes are made by focusing only on by-products which are produced during process like medical products or cosmetics. By-products are focused because they are used as a compensation for the production cost (Rismani-Yazdi et al. 2011).

This technique of algal engineering was suggested by Snow and Smith (2012) for both organic and inorganic growth since about all of the algal strains can be separated, restructured and hybridized to rapid cultivation even in harsh environments. Snow and Smith (2012) concluded that genetically engineered algae technology can be used to supplement both organic and inorganic cultivation. However, only with the aid of the public and business investors this will become a realistic possibility. As a result of this support, the high price of a working commercialized PBR could be significantly reduced (Davison 2005). Tabatabaei et al. (2011) identified low rates of growth and an impoverished gene as the two most significant problems of recombinant algal production, both of them could have an impact on the global marketing of algae in the future. Organizations like "Sapphire Energy and Monsanto" are trying to develop few new synthetic genes that could enhance the growth as well as other beneficial characteristics.

Few genes and pathways might affect biofuel production by "*Dunaliella tertiolecta*" that is flagellate from marine ecosystem, these are described by Rismani-Yazdi et al. 2011. He described that DNA modification can increase the growth rate and ability to use nitrogen. But there should be a suicidal gene which controls the survival of dangerous algal strains in the environment after an accidental escape (Tabatabaei et al. 2011; Perls 2017). Control of these dangerous algal strain is necessary as they have high risk of polluting environment (Quinn and Davis 2015).

2.9 Effect on Environment

The interaction between the algal fuel industry and the environment is critical to realizing the vision of a continuous and limited industry of fuel based upon algae. By keeping all challenges in consideration all remedies of these problems must be environment friendly and must not confronted with the same issues as the traditional fuel industry. Few techniques like foot printing are used to find out nature of biofuels whether they are green and environment friendly or not (Dassey et al. 2014; Farooq et al. 2015). Therefore emerging techniques should be according to claims and action and focus on clean manufacturing of renewable fuels. Farming of seaweed is the traditional way of harvesting microalgae for many applications. But it has negative effect on environment (Dunningham and Atack 2014; Titlyanov and Titlyanova 2010).

If the farms are not built in accordance with the rules, they could pose a serious issue to aquatic assets and ecosystem balance. As a result of this, the farm should be built in a location where the seaweed planting would not restrain with the natural aquatic environment nor will it pose a threat to the biological clock of the local fish. Aquatic ecosystems like sea possess a synergistic relationship with farming systems for fishes. According to some estimates, the population of fish may act as danger for farming of a few aquatic weed species, however, fluctuations in temperature of water may cause fish populations to migrate in the direction of the seaweed farm, allowing it to be established. In other words, to restrict the fish population in a limited area, it is an even more complex problem issue than the growth of microalgae in aquatic system. Contrastingly cultivation of wild microalgae in beach cast area and eutrophic zones might have positive effect in the cleaning of coastal zones.

Enormous amount of seaweed is accumulated which is used for production of biofuels and aid in balancing the environment and ecology as well as help in biofuels market. Marine algae plays important role in ocean environment. It manages the function and balance of marine habitats and helps in stability of marine environment. If there is no control and cultivation is not used properly then harvesting of macroalgae in an open sea farm might be invasive. Because it results in depletion in biodiversity, changes in environmental structure and can cause economic loss as well (Wang et al. 2013). When water balance is changed, attack on marine environment by wild macroalgae has been reported. This would allow for contaminated seawater to clean up and huge accumulations of algal biomass to accumulate and process it to fuel instead of cultivating macroalgae for fuel algae. Although this method is in favour of production of biofuel from algae but periodic changes and environment of sea may not be stable for providing a consistent amount of algal biomass for industrial scale. Another issue inside the macroalgae farmlands is the impact of human activity on the environment. As a result of the growing demand for macroalgae-derived compounds, the number of manufacturing facilities in developing countries is also increasing. Farmlands with a low level of expertise and untrained workers can be terribly damaging to water ecology. In order to ensure long-term production, the overall process should be modified and comprehensive to account for any potential instrumentation or other problems that may arise, which is difficult given the volume of continuous growth. Regulations in the USA and the European Union, as well as international and local legislation, secure the rights of individuals, the concerns about biofuels, such as algae (Benson et al. 2014; McGraw 2009).

Targets are set by European Commission about greenhouse gas emission, CO_2 reduction, development of continuous technology and usage of algal biofuel, etc. it is estimated that till the year 2020 reduction of 20% was the main goal (Ribeiro et al. 2015). But to secure energy is the main problem of developing countries (Saifullah et al. 2014). In order to talk about energy and management problems, the most important issues to consider are access to electricity, utilization of fuels for utilization of man, and presuming enough power for today fast growing world and in the succeeding era (Rimmer et al. 2015). Political fluctuations, reliance on the few limited petroleum providing organization, high prices of fuel and the occupation of limited countries on trading of energy reservoirs all contribute to issues of energy insecurity. There should be balance between energy which is produced from fossil fuels and any other energy sources which provide reliable, safe, cheap energy for consumption at large scale (Kose and Oncel 2017).

2.10 Conclusion

From above discussion it is concluded that biofuels are the basic need of today's world because they are environment friendly. Fuels obtained from fossil fuels are harmful for environment; result in emission of greenhouse gases and cause global warming. So we need to move towards safe fuels that do not cause any harm to the environment. Algae are best option for producing biofuels because algae not only could be processed into all types of fuels like bioethanol, biohydrogen, etc. algal fuels can be used for making blend and emulsifiers which improve the engine performance but also have easier and simple procedure for cultivation. From future perspective it is important to do more research on genetic engineering of algae so that we produce specifically that species of algae which gives maximum fuel production because fuel production from algae is eco-friendly, cheap and time saving. It might be possible that in future algal biofuels or biofuels may totally replace fossil fuels.

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Chapter 3
Microbial Mats and Its Significance in Biofuel Production

Muhammad Asad Javed and Ashraf Aly Hassan

Abstract Microbial mats are natural ecosystems rich in biodiversity, usually found in shallow hypersaline water and marine ecosystems. These microbial mats comprise mainly microalgae and cyanobacteria along with other aerobic and anaerobic phototrophs and heterotrophs. These communities are considered the primary producers of biofuels such as hydrogen (H_2) and methane (CH_4), as well as some other greenhouse gases such as carbon dioxide (CO_2). The biodiversity in microbial mats makes it capable of fixing some hazardous gases and organic contaminants, i.e., algae and cyanobacteria can produce H_2 and oxygen (O_2) by fixing atmospheric CO_2 and nitrogen (N_2) during the photosynthetic process by suitable electron transfer in photosystem I and II. The H_2 production by microbial mats is catalyzed by hydrogenase and nitrogenase enzymes present in algae and cyanobacteria during biophotolysis. The sulfate-reducing and methanogenic bacteria are able to fix the hazardous hydrogen sulfide (H_2S) gas by maintaining a low concentration of sulfate in the ecosystem resulting in CH_4 production. This chapter highlights the phenomena of biofuel production based on currently available data by using microbial mats, their composition, and structure, along with their metabolic processes that need to be explored to make microbial mats bioenergy production more feasible in the future.

Keywords Microbial mats · Hydrogen production · Methane generation · Biofuel · Microalgae · Cyanobacteria

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3.1 Background

Microbial mats are the oldest microbially induced sedimentary structures that evolved 3480 million years ago in Western Australia (Krumbein et al. 2003; Noffke et al. 2013). Mat structure has continuously evolved from that early stage to one of the currently existing modern mats, which might not contain photosynthetic bacteria. There are possibilities that these non-photosynthetic mats might have evolved 4000 million years ago. The coexistence of carbon (C) metabolic pathways and nitrogen (N_2) fixation archaea also characterizes these mats as 2.1 billion years old (Aubineau et al. 2021; Cerqueda-García and Falcón 2016). The evolution of such mats might have made the hypothesis that the energy source for such microbial mats might be high-pressure hot springs in submerged volcanoes. This might have resulted in the evolution of bacteria and archaea (Nisbet and Fowler 1999). The earliest microbial mats were considered small and single species chemotrophic biofilms supplied with energy and chemicals by hydrothermal reactions (Khimasia et al. 2021). After a period of time, by the development of geological habitat (for example, by the deterioration of microorganisms), an ecological environment (aerobic or anaerobic) was created for uptaking of heterotrophs, possibly methane-emitting and the reduction of sulfates by microorganisms which created new mats or layers in the environment and supplied biologically rich chemicals (Nisbet and Fowler 1999).

Microbial mats can develop in a wide range of environments. This environment includes sea salt ponds and hypersaline lakes, which are known to consist of coastal sediments and coral reefs. Phototrophic and chemotrophic microorganisms virtually form all these microbial mats. Most phototrophic microbial mats consist of oxygenated organisms such as cyanobacteria and microalgae; however, the chemical microbial mats are found in deep seas and specific sulfurous groves containing sulfur-reducing microorganisms. Microbial mats are generally vast communities of microbes found in shallow fresh and saline water interface systems. These microbes are found in microlayers and comprise photosynthetic oxygen (O_2) producing microorganisms (algae and cyanobacteria) and non-O_2 producing green and purple sulfur bacteria.

Due to the presence of microbial aerobic and anaerobic organisms, the mat surface is always assumed to be aerobic because of the presence of photosynthetic microorganisms. Such microorganisms, especially aerobic bacteria, respirate and deplete photosynthetic O_2 establishing anaerobic niches deeper inside the mats. It has been reported in several studies that the anaerobic environment causes the production of sulfides in mat samples that also indicates the presence of sulfide reducers in mats (Nakagawa and Fukui 2002; Kubo et al. 2011). Chloroflexus, a filamentous bacterium, has revealed its sulfide utilization ability as an electron donor under illumination and anaerobic condition, which is further stimulated by carbon dioxide (CO_2) (Kubo et al. 2011; Madigan and Brock 1975). It was also reported by Kubo et al. (2011) that a sulfur cycle was established inside mats by the interspecies interaction between sulfide producers and sulfide consumers. This cycle also runs in parallel with the methane (CH_4) and hydrogen (H_2) production metabolism.

Although photolysis also creates O_2 on Earth and in microorganisms, it is unknown if the process provides the surrounding environment with sufficient O_2. This indicates that carbon dioxide (CO_2) is probably the most prevalent electron acceptor in microorganisms for H_2 oxidation (Chapelle et al. 2002). These microorganisms can acquire energy by combining H_2 oxidation to reduce molecules such as O_2, nitrate (NO_3^-), iron (Fe-III), sulfate (SO_4^{2-}), and CO_2. This microbial community is also capable of producing biofuels through photolysis and fermentation under an anaerobic environment. These biofuels mainly comprise biohydrogen (bioH_2) and biomethane (bioCH_4). However, the production capacity of H_2 and CH_4 is in a low concentration and depends upon the abundancy of microbial community. The production of H_2 and CH_4 by microbial mats is reported in a few studies; however, a comprehensive investigation is warranted.

This chapter will describe the biofuel potential of microbial mats, their parametric, metabolic, and kinetic activity in microbial mat ecosystems. The evidence of such microbial biofuels can address global bioenergy economy, clean and green energy, overview and integrate the recent technologies for future developments.

3.2 Composition and Structure of Microbial Mats

3.2.1 Composition

Microbial mats have a diversity of microorganisms depending upon the environment and abundance of nutrients. Three types of mats are set along with low intertidal region (a) biofilm, (b) endobenthic microbial mats, and (c) epibenthic microbial mats (Noffke et al. 2001). Biofilms may usually develop around individual sedimentary particles in highly flooded regions in the lower intertidal zone; however, microbes cannot grow in constantly flooding areas. Such biofilms do not have much impact on sedimentary surface modification. Conversely, the biofilms in upper intertidal zones where the water exposure time multiplies allow microbe growth, resulting in microbial mats formation (Callefo et al. 2021; Zammit et al. 2021).

Most commonly found mats are algal mats and bacterial mats, which are referred to as microbial mats. Due to the deposition and accumulation of various nutrients as food, microorganisms grow and form a layer that is termed biofilms. These biofilms grow further, form a thick mucus layer, and deposit various nutrients for other microorganisms, mainly algae and bacteria. The type of biofilm that is thick enough up to some centimeters (cms) and forms a mat-shaped layer is microbial mats which can endure and survive through physical and environmental stresses. These colonies and communities of algae and bacteria can grow on any surface with different interface types such as between air/water and sediment rocks inside water, between soil and bedrock, on the water surface through deposition, etc. Such formations have chemical gradients within the communities growing on top of each other, making microorganisms grow at different levels, thus divide the whole microbial community into layers in a single mat, which may sharply or gradually merge into each other.

Each layer induces its own photosynthetic or non-photosynthetic phenomena depending upon the microbes. An abundant and well-known type of microbial mat is laminated and perforated/porous type, laminated by algae or photosynthetic bacteria on the top. The microbes in the mats slowly move upwards as the deposition of sediments carries out on their surfaces.

3.2.2 Structure

A microbial mat is mainly composed of 4–5 layers up to a few millimeters, each of which is made explicitly of microorganisms such as microalgae and cyanobacteria (Fig. 3.1). The composition and structure of each layer vary depending upon the type of microorganism and the byproduct nutrients of one layer provided as the nourishment for the other layer. In lakes, ponds, and wet environments with abundant sunlight as a primary energy source, the uppermost layer comprises photosynthetic aerobic microorganisms, e.g., microalgae and cyanobacteria having green chlorophyll pigment for photosynthesis. In contrast, the lowermost layer is usually dominated by non-photosynthetic anaerobic microorganisms, e.g., sulfate-reducing bacteria. The intermediate layers in between are photosynthetic only during daytime and the light penetration capacity. For example, in hypersaline lake water near Guerrero Negro (Mexico), intermediate layers are usually purple layer composed of photosynthetic purple cyanobacteria (Stal 1995).

Microbial mats are characterized by oxygen concentration variations and primarily due to cyanobacteria/algae physiology (Revsbech et al. 1983). In the light, during

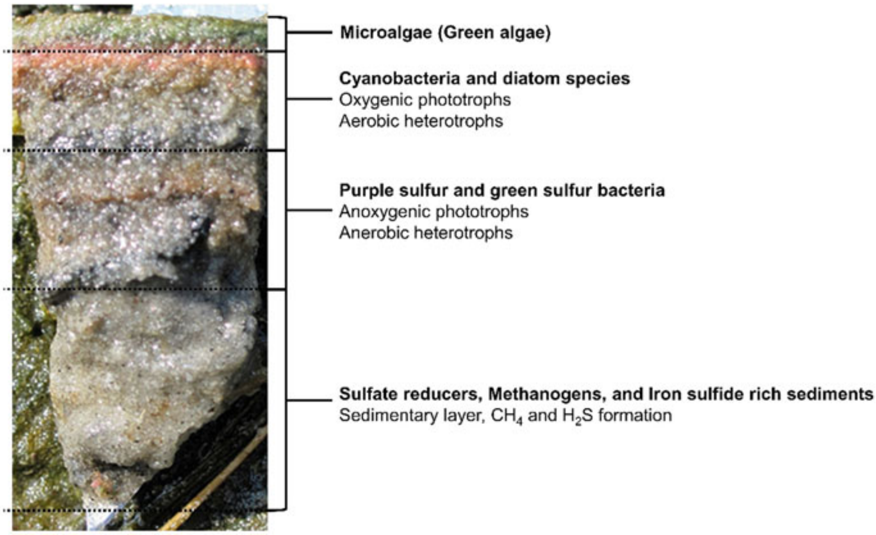

Fig. 3.1 Structure of microbial mats (Armitage et al. 2012)

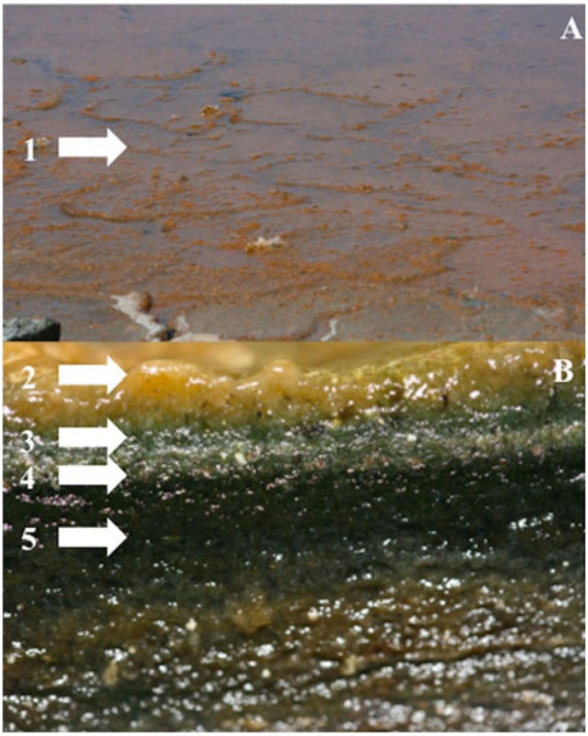

Fig. 3.2 A cross-sectional view of structural composition showing the laminated microbial mat. (1–2) EPS, (3) First layer (0–1 mm), (4) Second layer (1–2 mm), (5) >2 mm (photos by S. A. Cantrell) (Cantrell and Duval-Pérez 2013)

photosynthesis, these organisms carry out oxygen resulting in oversaturation of the mat. The primary mode in the dark is endogenous glycogen respiration (Smith 1983). The diffusion of oxygen in the mats is usually enough in establishing the microbial mats to satisfy the demands and anoxic conditions. Therefore, to survive, cyanobacteria have to move to another way of generating energy. Organisms isolated from those habitats have been investigated and proved capable of fermentation for their mechanisms to produce anaerobic dark energy.

The microbial mats are laminates of bacterium and archaea and often even eukaryotic microorganisms and are strictly microbial communities (Figs. 3.2 and 3.3). The microbial mats start blooming in conditions too harsh to survive for most eukaryotes, such as thermal springs, sulfide, and/or high salinity habitats. These systems provide a three-dimensional environment for metabolic coexistence and interaction between various microbial species. In such a habitat, the micro-environment is described by steep biogeochemical gradients (O_2, pH, redox) and various micro-habitats of different types and populations (Stolz 2000).

The role of microbial mats is of great significance in the composition, alteration of the atmosphere, and the production of O_2, H_2, and CH_4 (Hoehler et al. 2001) throughout Earth's history, as well as constitute the first habitats and stromatolites. Therefore, microbial mats are, without exception, the diverse microbial community

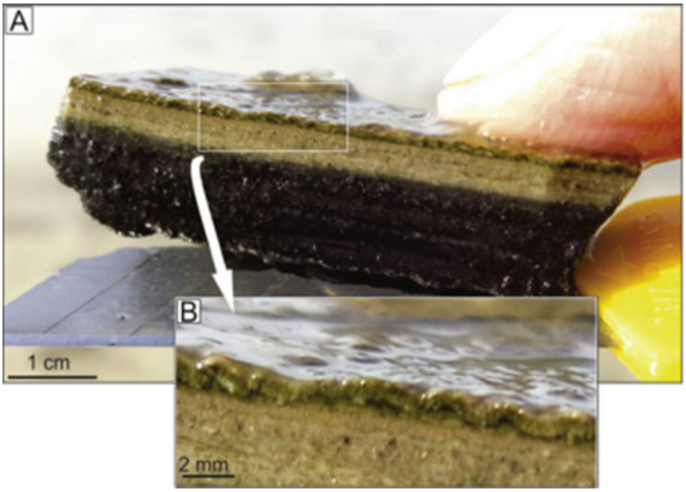

Fig. 3.3 (**a**) Vertical section of microbial mat showing, mainly two layers, anoxic one above and anoxic below. (inset (**b**)) Two uppermost layers present a wavy characteristic (Cuadrado 2017)

(w.r.t patterns and structures) in which evolutionary processes can be analyzed and adjusted to extreme conditions (Inskeep et al. 2013).

3.3 Metabolic Process

A variety of primary biofunctional groups are the microbial mats, such as cyanobacteria and anoxygenic bacteria (non-sulfur green bacteria), green sulfur bacteria and purple bacteria, aerobic heterotrophic and anaerobic bacteria, sulfide reduction bacteria (SRB), and methanogenic archaea (van Gemerden 1993; Klatt et al. 2016). These microorganisms undergo a series of photosynthetic and respiration processes along with natural anaerobic fermentation and biophotolytic reaction. The photosynthetic CO_2 fixation supplements the organic substance to the sediment that forms the foundation of a complex microbial community. Other microorganisms can use organic matter generated by cyanobacteria and algae in several ways. This is because O_2 builds up in the microbial mats during the light phase, and CO_2 depletes. Such conditions encourage photorespiration, during which O_2 is quickly absorbed, and sediment becomes anoxic during the dark. Cyanobacteria and microalgae begin photolytic processes and fermentation of carbohydrates that excrete H_2 hydrogen due to biophotolysis and fermentative products such as methane (Fig. 3.4).

Photosynthesis is the primary source of energy and nutrients for microbial mats (Ley et al. 2006). A mechanism performed by cyanobacteria using light energy to convert the inorganic carbon in the form of CO_2 to organic carbon $(CH_2O)_n$ by releasing the oxygen is the first survival stage of this trophic network in a standard mat (Severin et al. 2010; Baumgartner et al. 2006). Microbial mats are a consortium

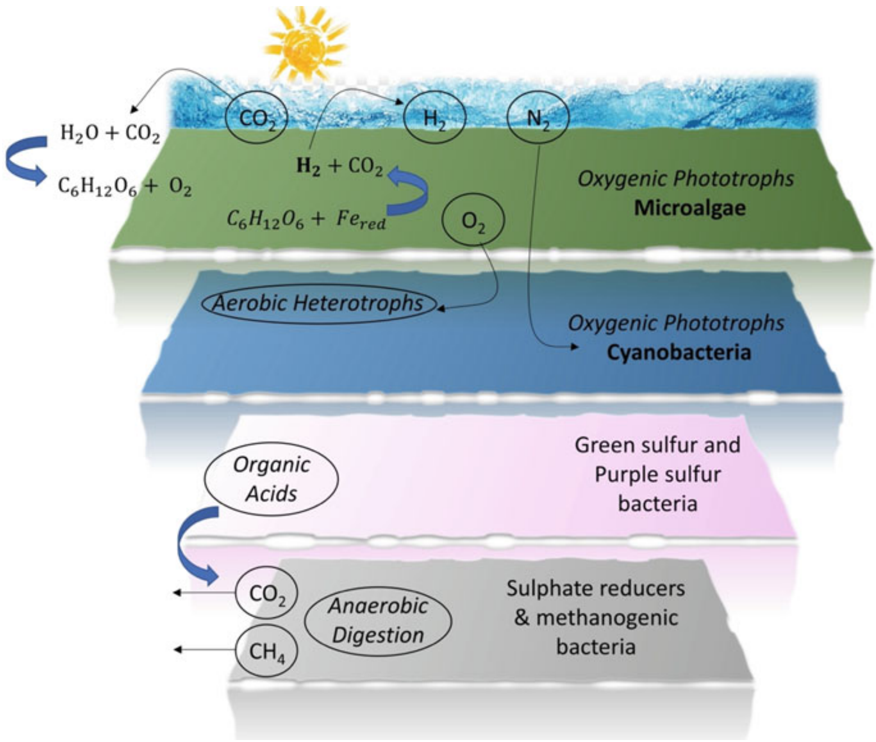

Fig. 3.4 Structure and metabolism overview of photosynthetic mats

of biogeochemical and biochemical cycles, which allows for the availability and use of other microorganism products from the metabolism of one community (Paerl et al. 2000).

Microalgae undergo a light dependent two step photochemical oxidation pathway in direct photolysis (Eqs. 3.1 and 3.2) and indirect photolysis (Eqs. 3.3 and 3.4):

$$2H_2O \xrightarrow{\text{Sunlight}} 4H^+ + 4e_- + O_2 \tag{3.1}$$

$$4H^+ + 4e_- + Fe_{red} \xrightarrow{\text{Hydrogenase}} 2H_2 + Fe_{ox} \tag{3.2}$$

$$12H_2O + 6CO_2 \xrightarrow{\text{Light energy}} C_6H_{12}O_6 + 6O_2 + 6H_2O \tag{3.3}$$

$$C_6H_{12}O_6 + Fe_{red} + 12H_2O \xrightarrow{\text{Hydrogenase}} 12H_2 + 6CO_2 + 6H_2O \tag{3.4}$$

The production of excessive amounts of sulfate (SO_4^{2-}) in the hot-spring or other marine type mats encourages the growth of sulfate-reducing bacteria and thus the production of H_2S (Castenholz 2009). The production of fermentative byproducts such as volatile fatty acids (VFAs), acetate, H_2, and CO_2 also supports the growth of methane-producing archaea.

Microalgae and cyanobacteria are the key producers of biohydrogen (bioH_2) in microbial mats as these microorganisms contain two types of enzymes: hydrogenase and nitrogenase capable of producing H_2. Hydrogenase enzyme is a protein complex that can produce or oxidize H_2 in the presence of suitable electron transfer in photosystem I or photosystem II during direct and indirect photolysis. The H_2 and O_2 produced during biophotolysis are activated by hydrogenase and nitrogenase enzymes in microalgae and cyanobacteria, respectively. However, this bioH_2 production is susceptible to O_2, the primary inhibitor of hydrogen yield in microalgae. The O_2 produced in an algal and cyanobacterial layer is likely consumed by aerobic heterotrophs and oxygenic phototrophs, while the layer below containing purple and green sulfur bacteria can feed on the byproducts by algal and cyanobacterial layer in the absence of O_2. These purple and sulfate-reducing bacteria, along with sulfate reducers, act as sulfate-consuming microorganisms.

3.4 Transitioning to Microbial Mats from Biofilms

A specific biofilm type might be regarded as microbial mats. Interfacial marine ecosystems, in which numerous microbial species are compressed together laterally to form a thin mat, are an extreme example of transitioning to microbial mats. The average thickness of microbial mats from several millimeters to 1 cm segregates it vertically (Lichtenberg et al. 2020). Another defining feature of microbial mats is that the inorganic carbon is fixed into autotrophic biomass whether by photosynthesis or chemosynthesis.

Biofilms are a thin algal formation of cells found in shallow water with an abundant supply of nutrients. These films are typically comprised of microalgae, cyanobacteria, and sediments with the depth size in millimeters and in some cases up to centimeters. Formation of such microbial mats occurs when alternating layers of algae, blue-green bacteria, and sediments keep depositing on layers after layers each other creating a deep layer of microbes up to inches. Mat microbial communities interact in sequential physiological connections in a manner similar to biofilms. Such microbial mats are usually found in the interface of hot springs, hypersaline lakes, and marine ecosystems. These mats have the ability to sustain the whole mat system by majority of the biogeochemical cycles (Rich and Maier 2015; Hörnlein et al. 2018).

The blue-green algae form a sticky layer (extra polymeric substance, or EPS) of microbes, including algae and cyanobacteria (phototrophs and oxygenic heterotrophs) in shallow marine water environments. The nutrients and microbes or sediments present in the water system keep depositing on this layer, and over time, the layer becomes thicker and thicker, forming a microbial met. Such microbes possess symbiotic behavior, which relies on each other and keeps giving the nutrients to each layer while algae and cyanobacteria keep doing the photosynthesis on the upper surface of microbial mats. The lowermost layer in such mats is usually sulfate reducers and methanogenic organisms, reducing or oxidizing various sulfate compounds producing methane gas.

Such mats depend on microalgae and cyanobacteria to feed/nutrient the remaining microbes and control the environment around layers. The pH range also depends upon the algae and cyanobacteria and their photosynthesis or fermentation of various biodegradable substances in sediments.

3.4.1 Kinetics of Mass Transfer Within Microbial Mats

The nutrient and microbes transfer within different layers of microbial mats is related to the mass transfer resistance from the water matrix of one layer to the hydrodynamic boundary layer of surroundings or other layers (de Beer and Kühl 2001). The microbial, nutrient, and solutes transfer between various layers is a diffusional phenomenon through the porous structure of mats. This creates a nutrient and microbes gradient that sweeps through the layers and changes the chemical composition of various layers. As a result, the types and rates of microbial transformations are substantially affected. The mass transfer resistance is typically an interference to most chemical conversion processes that can only occur within the specific layers of microbial mats due to the unique microbial circumstances. Anaerobic conversion process such as H_2 yield, CH_4 production, sulfate reduction, and denitrification can occur in anoxic or oxic environments due to anaerobic and aerobic archaea present in the community. The kinetics of such microbial environment and its interaction with mass transfer resistance is mandatory to understand the H_2 and CH_4 conversion inside microbial mats.

The microbial activity and all nutrients or microbes transport within each layer of the microbial mat are considered to be distributed homogeneously. This distribution is diffusional within the layers, and adjacent to these layers are the boundary layers where this diffusion gradually changes. This variation can be found using zero- and first-order kinetics because microbial community behaves as a mixed-order saturation type that is *Monod kinetics* (de Beer and Kühl 2001). For first-order kinetics, the internal mass transfer can be found using effectiveness factor, η, as:

$$\eta = \frac{\tanh(\Phi)}{\Phi} \tag{3.5}$$

where.

Φ is the first-order Thiele modulus:

$$\Phi = L_b\sqrt{\frac{k}{D_b}} \tag{3.6}$$

where; L_b is the biofilm/mat thickness; k is the first-order reaction-rate constant; D_b is the effective diffusion coefficient.

For most biological conversions, zero-order kinetics is considered where the bulk (c_b) and surface (c_0) concentrations are used to differentiate the penetrations of interlayer mass transfer in microbial mats. Due to the low saturation concentration (K_m) of H_2 and CH_4 production in microbial mats, zero-order kinetics is often taken more realistic. For zero-order kinetics, the zero-order *Thiele modulus* can be found as:

$$\Phi_0 = L_b \sqrt{\frac{k_0}{D_b c_0}} \tag{3.7}$$

3.5 Role of Microbial Mats in Biofuel Potential

3.5.1 Biohydrogen

Hoehler et al. (2001) investigated microbial mat generation of reduced gasses and found the large-scale impact of ancient microbial mats. The development of the modern mats is linked to oxygenic photosynthesis during the diurnal cycle and produces CO_2, H_2, and CH_4 at different levels. Oxygenic photosynthesis increased microbial productivity by carbon fixation and lowered the gasses by 100–1000 times (Marais 2018). Hoehler et al. (2001) proposed that no burial or escape from reducing alternatives may have resulted without oxidation on the outside of the Earth and that escape to outside space may be due to molecular H_2 loss to the outer surface. In modern mats, CH_4 generation is limited even in artificially reduced sulfate conditions—most likely due to the lack of oxygen-sensitive methanogen access to the organic material (Bebout et al. 2004).

The capacity to produce bioH_2 by anoxygenic photosynthesis is found in the microbial mat in cyanobacteria and purple bacteria (Lambert and Smith 1981; Sasikala et al. 1993). The H_2 production in cyanobacterial mats is usually triggered due to nitrogenase (nitrogen-fixing) enzymes which is a less effective process than hydrogenase-operated medium in algal mats concerning the ATP requirements. However, Skyring et al. (1989) observed that hydrogenase enzyme was present in all types of mats, though the H_2 production was only limited to nitrogenase systems. The maximum H_2 production rate in natural mats was reported to be 6 nmol cm^{-2} day^{-1}. Allen and Arnon (1955) enriched the mat medium with an additional substrate such as silage wash (10 g L^{-1}) to supply VFAs, predominantly lactic acid and acetic acid, for the photosystem (PS) II. A nitrogen-based silage that includes nitrogen complex and amino acid compounds also contributes to H_2 production without interfering with the nitrogenase enzyme activity. Such silage based mat systems developed 27 μmol H_2 L^{-1} day^{-1}, which was much higher than

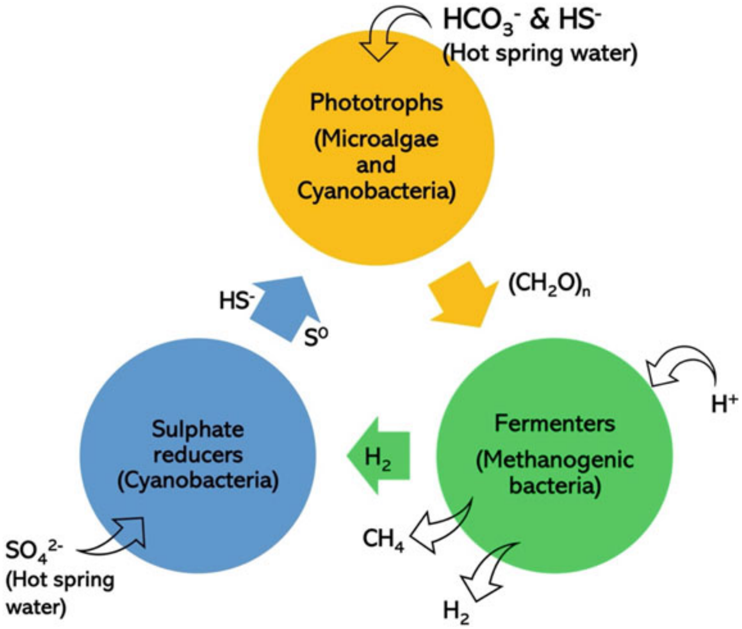

Fig. 3.5 Electron flow model in microbial mats (adopted from Otaki et al. (2012))

natural silage-free mats (Hill 2001). Nielsen et al. (2015) reported 32 cyanobacterial mat species with a maximum H_2 concentration of 40 μmol H_2 L^{-1} day^{-1} having the previous history of light exposure was reduced to almost zero in 7 h. The primary reason for this decline was the consumption by sulfate reducers that lead to a higher concentration of H_2 yield for a more extended period. Revsbech et al. (2016) also found the peak H_2 accumulation of 30 μmol H_2 L^{-1} in the cyanobacterial mat during the dark cycle on the first day and the second highest peak of 46 μmol H_2 L^{-1} on the second day in the mid-dark period.

It is also reported that the electron flow in such microbial ecosystems is an important parameter to understand better the H_2 production phenomena (Otaki et al. 2012). The primary H_2 producers and consumers were also identified w.r.t electron flow for interspecies H_2 transfer as H_2 is considered a key electron donor in an anaerobic environment. The cell dispersal in the mat may affect the intercellular contact between fermenters and consumers of H_2, causing H_2 gas to escape into the gas phase (Fig. 3.5). The H_2 transfer should be supported by the enormous densities of bacterial cells in the mats and close cell proximity of less than 2 μm (Ishii et al. 2005). The maximum H_2 production by microbial mats in artificial hot springs at 65 °C was reported as 2.1 μmol (g wet weight of the mats)$^{-1}$ vial^{-1} after 10 h of incubation in the dark (Otaki et al. 2012). It was also observed that H_2 was produced during the growth phase of microbial strain in mats, while some VFAs such as lactate and acetate were also detected along with CO_2 due to an anaerobic fermentative environment.

Table 3.1 Comparison of hydrogen (H_2) production in different microbial mats

Sr #	Reference	H_2 production
1	Skyring et al. (1989)	6 nmol cm^{-2} day^{-1}
2	Hill (2001)	27 μmol H_2 L^{-1} day^{-1}
3	Otaki et al. (2012)	2.1 μmol (g wet weight of the mats)$^{-1}$ vial^{-1}
4	Burow et al. (2012)	[a]160 nmol cm^{-3} h^{-1}
		[b]200 nmol cm^{-3} h^{-1}
		[c]38 nmol cm^{-3} h^{-1}
5	Nielsen et al. (2015)	40 μmol H_2 L^{-1} day^{-1}

[a]Hydrogen production in 4–15 mm sectioned depth layer of microbial mats
[b]Hydrogen production with DCMU (20 μM) supplementation in microbial mats
[c]Hydrogen production with NH_4Cl addition in microbial mats

Some hypersaline and coastal region cyanobacterial mats are well known to produce H_2 during darkness; however, part of the H_2 production in the light period may also be seen as a result of biophotolysis (Lee et al. 2014; Hoffmann et al. 2015; Nielsen et al. 2015). Burow et al. (2012) demonstrated that the H_2 production in the dark might be attributed to cyanobacterial fermentation in the hypersaline microbial mat, but the nitrogenase enzyme activity responsible for N_2 fixation and H_2 generation was not proven. Moreover, due to the low concentration (Table 3.1), mats are still not considered feasible for a practical energy source at a large scale (Skyring et al. 1989). However, mats may produce a much higher concentration of H_2 by artificially developing a synthetic or lab-scale microbial mat comprising a wide range of required bacteria and microalgal strains or by combining a specially designed microbial strain for H_2 production (Rechenberg 1998).

3.5.2 *Biomethane*

Reduced gas emissions like CH_4 from microbial mats are significant for understanding early biosphere developments (Hoehler et al. 2001; Pilcher 2003; Shuai et al. 2021). The decreased O_2 level in the water environment enhances the H_2 production in microalgae and cyanobacteria during biophotolysis on the upper surfaces of microbial mats. However, other sulfate-reducing bacteria and methanogenic bacteria present in the bottom layer of microbial mats can also significantly enhance the methanogenesis process relative to the marine water environment having an abundant supply of O_2. Sulfate-reducing bacteria maintain the low sulfate concentration in the bottom layers of microbial mats, which can be the primary producers of CH_4 under hypersaline water conditions (Bebout et al. 2004). It has also been suggested by Bebout et al. (2004) that a low concentration of sulfate does not affect primary production. However, the sulfate reduction rates decreased three times, and CH_4 production flux increased ten times to 14 μmol m^{-2} h^{-1}. These variations in H_2 availability in microbial mat surface generate vertical segments of methanogenic archaea in microbial communities. Moreover, the study also suggested that due to the very low conversion efficiency of photosynthetic products into CH_4, such mats are a relatively unworkable source for methane generation at a large scale, even in a strict anaerobic environment and low sulfate concentrations.

The oxidation of CH_4 can uptake 1–90% of gross produced CH_4; however, the interactions of methylotrophs and methanogens can persist CH_4 production in aerobic and anaerobic conditions. Factors may constrain this CH_4 flux in microbial mats, such as availability of nutrients, competition between methylotrophs and methanogens for nutrients, and environmental conditions (Buckley et al. 2008). The entirely different environmental conditions present in the Archean ocean having less concentration of sulfate (<200 μm) have given the assumptions to raise CH_4 production rate in the Archean ocean (Habicht et al. 2002; Hurtgen et al. 2002). Low O_2 concentration in water (dissolved oxygen) and sulfate concentration favor the activities of methanogenic bacteria by yielding an excessive concentration of CH_4 (Pavlov et al. 2003). This CH_4 production potential was higher in microbial mats in the spring season as 17.2 ± 4.5 nmol CH_4 cm^{-2} day^{-1} and relatively low in the fall season as 3 ± 1.1 nmol CH_4 cm^{-2} day^{-1} (Buckley et al. 2008). It was also reported that the CH_4 flux does not vary evidently with the depth of mats but with the presence of H_2 as a substrate for methanogenic archaea. On the mat surface of depth 0–10 mm, H_2 seems to be of high significance as a substrate for methanogenesis, while the proportion of methanogenic archaea increased with the depth. This leads to an unequal and imbalance proportion of H_2 dynamics which indicates the coexistence of methanogens and sulfate-reducing archaea in the microbial mat system.

Due to the strict anaerobic environmental conditions, methanogenesis is excluded from carbon recycling during the photosynthesis period in daylight in microbial mats. These microbes are abundantly present in the lowermost surface of microbial mats where there is minimum penetration of light resulting in minimum photosynthesis rate and O_2 production, therefore, enhance methanogenic activities. Conversely, it was also reported in a previous study that in situ microbial mats production is likely to be an ineffective method of enhancing CH_4 flux 100–300 PPM, which is way less as compared to the proposed 50% of net primary production (Pavlov et al. 2003).

3.6 Other Applications of Microbial Mats

- In the treatment of wastewater by phototrophic or heterotrophic biofilms and microbial mats (Paniagua-Michel 2017).
- For the removal of heavy metals, radionuclides, and hazardous organic pollutants in bioremediation (Bender et al. 2000; O'Niell et al. 1999).
- To enhance the soil fertilization by nitrogen-fixing using cyanobacteria (Fernández Valiente et al. 2000), by fixing greenhouse gas potentially carbon dioxide during photosynthesis using microalgae (Oren et al. 2001).

- Studying the evolution of life on planet Earth in the hypersaline environment as microbial mats are the complete functioning ecosystem in a microscale environment.

3.7 Conclusion

Microbial mats are the primary producers of oxygen and for the existence of life on the Earth. The production of clean energy such as hydrogen and greenhouse gas such as methane can be the final byproducts of hypersaline microbial mats. These gases can be utilized as a potential energy source from naturally generated communities of microbes such as microbial mats. The naturally induced hydrogen and methane from these microbial mats are biofuels that further reduce fossil fuel dependency. The mats with their hydrogen and methane production potential can also be used as nitrogen and carbon dioxide fixers and sulfate reducers. The potential of microbial mats for biofuel production seems to be a promising technique that needs further investigation and study of the existence of life on other planets. The adverse effects of eutrophication can also be compensated by considering the potential of biofuel production through these microbial mats. The economic advantages of using natural systems from energy generation and biofuel production might also be best utilized by integrating it with other modern modes of energy generation.

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Chapter 4
Algal Biohydrogen Production: Opportunities and Challenges

Meenal Jain, Meenakshi Mital, and Puja Gupta

Abstract Biohydrogen is a renewable source of energy which is cleaner and more cost-effective than other biofuels. Hydrogen produced through the action of living organisms is called biohydrogen. Algae have received significant attention as a novel biomass source for the generation of renewable energy. Microalgae are an excellent source for hydrogen production owing to their carbon mitigating properties and the consumption of solar energy as the energy source by microalgae, which is a renewable source of energy. A number of microorganisms can be used in the production of hydrogen; however, the most commonly used and accepted are cyanobacteria and green microalgae. They are considered being more efficient at producing chemical energy from sunlight with a smaller footprint and less requirement for water. The chapter throws light on biohydrogen as an energy source and the various technologies available for algal biohydrogen production. Further, it discusses the opportunities and challenges associated with the production of algal biohydrogen.

Keywords Biohydrogen · Biofuel · Microalgae · Technologies for algal biohydrogen production · Opportunities and challenges of algal biohydrogen

4.1 Introduction

Industrialization, urbanization, population growth, and changing lifestyles are some of the main reasons for soaring energy demand in countries around the world. However this trend is increasingly evident in emerging economies like India due to their huge population base and fast pace of economic growth. The major sources of primary energy are conventional fossil based fuels which have led to an accelerated accumulation of greenhouse gases (GHGs) in the environment exceeding the

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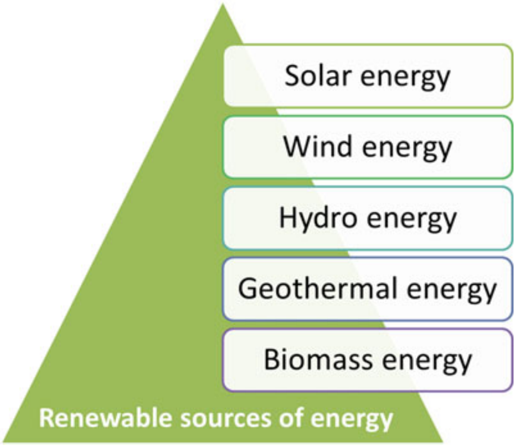

Fig. 4.1 Renewable sources of energy

dangerously high threshold of 450 ppm CO_2 (Singh and Rathore 2017). Global dependence on fossil fuels has led to the release of over 1100 $GtCO_2$ into the atmosphere since the mid-nineteenth century. Currently, energy related GHG emissions, mainly from fossil fuel combustion account for around 70% of total emissions including carbon dioxide, methane, and some traces of nitrous oxide (Sims et al. 2007). Further, the depletion of fossil resources at an alarming rate has stressed that they are an unsustainable source of energy.

The concerns related to energy security, climate change, and sustainability have encouraged research in renewable and cost-effective sources of energy, which have a small carbon footprint (Singh and Olsen 2012). Industrialized and emerging economies alike are thus looking toward new sources of energy which are able to meet their growing demands, are renewable and cost-effective, give them energy security, reduce their carbon emissions, and shape their economy. Renewable energy production in various forms like solar and wind farms, hydropower, and biomass has been initiated. Figure 4.1 summarizes the different sources of renewable energy.

The different fields of technology for use of solar radiation include chemical/physical methods like photovoltaic, concentrating solar power, thermovoltaic, photochemical, thermochemical, and use of biological approaches such as artificial photosynthesis and bio-photolysis. Practical use of solar energy requires conversion of the energy into an energy carrier and one of the promising candidates for alternative energy carriers is hydrogen.

4.2 Hydrogen as an Energy Source

Hydrogen (H_2) offers tremendous potential as a clean, renewable energy source. Hydrogen has a high heating value of 3042 cals/m^3 and is regarded as a clean non-polluting fuel (Suzuki 1982). Hydrogen is used for the manufacture of ammonia, in oil refineries for removal of impurities, in methanol production, and as a fuel in rocket engines (Nath and Das 2004; Das and Veziroĝlu 2001). Ninety-nine percent of the total hydrogen is produced from fossil fuels using the classical methods of production which are both energy intensive and negatively impact the environment. Recent reviews on hydrogen have indicated that worldwide need for hydrogen is increasing with a growth rate of nearly 12% per year and contribution of hydrogen to total energy market is projected to be 8–10% by 2025.

Hydrogen is seen by many as the fuel of the future because it has a very high energy density, three times that of petrol or diesel and because its use produces only water instead of greenhouse gases and other exhaust pollutants. Using petrol and diesel in combustion engines waste at least two thirds of the energy in the fuel, whereas hydrogen can be used in fuel cells, which are about twice as efficient. Hydrogen is one of the most abundant elements in the world that accounts for 75% of the universe mass (Johnston et al. 2005). It is a colorless, odorless, tasteless, and a non-poison gas. Currently, hydrogen is produced using non-renewable technologies such as steam reformation of natural gas (~50% of global H_2 supply), petroleum refining (~30%), or the gasification of coal (~20%). However, the viability of a future H_2 economy depends entirely upon the development of efficient, large scale, and sustainable H_2 production systems. The development of H_2 technologies has been given high priority in the European Union, the USA, Japan, and China.

4.2.1 Biohydrogen

Hydrogen produced through the action of living organisms is called biohydrogen. Biohydrogen holds promise as a potential clean, renewable, and environmentally friendly energy source. Biohydrogen production is particularly useful because it can be done at an ambient temperature and pressure with minimal energy consumption and therefore is more environmentally friendly. Biohydrogen production methods can be broadly categorized into four primary groups, namely photo-fermentation, dark fermentation, direct bio-photolysis, and indirect bio-photolysis.

Biohydrogen is made from bio-fuels which can be classified into first generation, made from food crops; second generation, made from non-food crops or wastes; and third generation, made using microbes. Third generation biofuels have several advantages over first and second generation biofuels. The first generation biofuels have caused increases in food prices unlike the third generation biofuels. In comparison to second generation biofuels, third generation biofuels can capture sunlight energy ten times more efficiently, meaning that smaller land parcels are needed to produce enough fuel (Shaishav et al. 2013). Many types of microbes can convert renewable energy sources into hydrogen. Biohydrogen is particularly attractive because of the excellent properties of hydrogen as a fuel and because biohydrogen being very easy to collect from the bioreactor (Rupprecht et al. 2006).

Microorganisms are able to convert a diverse number of renewable resources into hydrogen (Levin et al. 2004). Microbial hydrogen production through the direct fermentation of organic wastes is one of the potential technologies for producing renewable hydrogen that couples the need for waste reduction and by-product recovery, simultaneously (Show et al. 2012). The biological processes of hydrogen production are fundamentally dependent upon the presence of a hydrogen producing enzyme. These enzymes catalyze the chemical reaction $2H^+ + 2e^- \leftrightarrow H_2$. Three enzymes carrying out this reaction are known: nitrogenase, Fe-hydrogenase, and NiFe-hydrogenase (Hallenbeck and Benemann 2002). Fe-hydrogenase enzyme is used in the bio-photolysis processes, whereas photo-fermentation processes utilize nitrogenase. Among various hydrogen production processes, microbial/algal (biological) methods are known to be less energy intensive, for it can be carried out at ambient temperature and pressure.

Recent reviews on hydrogen indicated that the worldwide need for hydrogen is increasing with a growth rate of nearly 12% per year for the time being and contribution of hydrogen to total energy market will be 8–10% by 2025 (Lemus and Duart 2010).

4.2.2 Classification of Biofuels

Biofuels can broadly be categorized into two groups: primary and secondary. Primary biofuels consist of all those biomass materials which were traditionally used, i.e., firewood, wood chips, animal waste, crop residue, etc. Secondary biofuels consist of other products and processes, which use biomass and provide liquid, solid, or gaseous fuel. This group can further be divided into three categories on the basis of substrate that they use, viz. first, second, and third generation biofuel (Fig. 4.2).

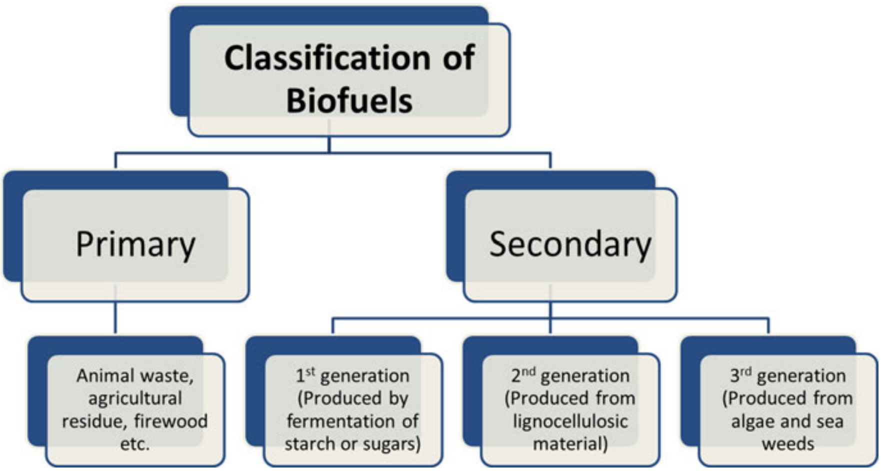

Fig. 4.2 Classification of biofuels

4.3 Algae and Microalgae

The discovery that algae can produce hydrogen on illumination was done 70 years ago by Gaffron and coworkers. Algae are some of the most robust organisms on earth, able to grow in a wide range of conditions (Shaishav et al. 2013). Algae usually grow in damp places or water bodies and hence found both in terrestrial and aquatic environments. Algae have received a great deal of attention as a novel biomass source for the generation of renewable energy. Figure 4.3 illustrates algal biofuel generation.

Algae are both unicellular and multicellular aquatic life form and lack the various structures that characterize land plants, such as phyllids (leaves) and rhizoids in nonvascular plants, or leaves, roots, and other organs that are found in tracheophytes (vascular plants). Its uniqueness that separates them from other microorganisms is the presence of chlorophyll and having photosynthetic ability in a single algal cell, thus allowing biomass generation. Well defined nucleus, a cell wall, chloroplast containing chlorophyll and other pigments, pyrenoid, a dense region containing starch granules on its surface, stigma, and flagella are the major components of green algae.

Algae are generally divided into two groups: macroalgae and microalgae. Both groups of algae do not have roots, stems, and leaves. Macroalgae (or seaweeds) are photoauxotrophic organisms that are able to produce and store organic carbons by utilizing CO_2 and HCO_3 (Chung et al. 2011). Macroalgae are photosynthetic large celled organisms that can be seen without the aid of a microscope. They are classified based on their pigmentations and fall into four basic groups: blue-green algae (Cyanophyta/Cyanobacteria often associated with blooms in rivers); green algae (Chlorophyta) such as sea lettuce; the brown algae (Heterokontophyta); and the red algae (Rhodophyta) most diverse group of all. Macroalgae is low in proteins and lipids but have high content of carbohydrates and water (Sambusiti et al. 2015).

Microalgae are small microscopic aquatic photosynthetic unicellular or simple-multicellular microorganism that cannot be seen by the naked eye. They are small

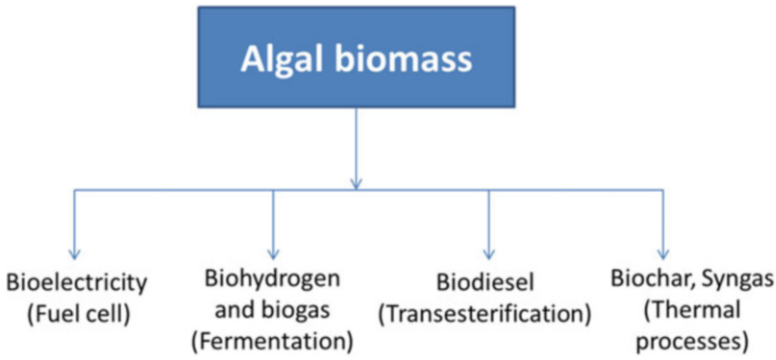

Fig. 4.3 Algal biofuel generation

Table 4.1 Comparison of different algal harvesting methods

Harvesting methods	Details
Filtration	Both large (>70 μm) and small (<30 μm) sized cells can be filtered under pressure or using ultrafilters, respectively. The process is time-saving
Flotation	Algal cells are trapped by air bubbling. It is a comparatively cheaper process
Centrifugation	This process includes sedimentation as per the cell size and velocity. It is a rapid process suitable for large microalgae
Precipitation	Self-precipitation happens in some of the algae wherein they settle at the bottom after stopping circulation. It is a natural process not requiring any toxic chemicals

Source: Giovannoni et al. (1990), Brennan and Owende (2010), Schenk et al. (2008), Gröschl (1998)

free floating organisms and come in different sizes, shapes, and colors. Microalgae can be grouped into prokaryotic microalgae (cyanobacteria Chloroxybacteria), eukaryotic microalgae (green algae Chlorophyta), red algae (Rhodophyta), and diatoms (Bacillariophyta) (Sambusiti et al. 2015). They are able to tolerate and adapt to a wide variety of environmental conditions (pH, temperature, light, etc.) and can be produced all year round (Uggetti et al. 2014). Moreover when cultured at optimal conditions, they are able to double in number within hours, thus permitting a short harvesting cycle. Unlike macroalgae, microalgae are mainly composed of proteins (40–60%), carbohydrates (8–30%), lipids (5–60%), and other valuable components (Uggetti et al. 2014). Microalgae are the principal producers of oxygen in the world and exhibit enormous potential. Microalgae cultivation is an efficient option for the reduction of CO_2 from gaseous effluent and from the atmosphere. The productivity per unit area of microalgae is high compared to conventional processes for the production of raw materials for biofuels and microalgae represent an important reserve of oil, carbohydrates, proteins, and other cellular substances that can be technologically exploited (Chisti 2007; Gressler et al. 2012). There are different algal harvesting methods as summarized in Table 4.1.

The microalgae biomass can produce biodiesel, bioethanol, biogas, biohydrogen, and bio-oils. Microalgae, although having simple structure, have a high photosynthetic efficiency with a growth doubling time as short as 24 h. Moreover, microalgae can be produced all year round. The species abundance and biodiversity of microalgae over a broad spectrum of climates and geographic regions make seasonal and geographical restrictions much less of a concern compared with other lipid feedstocks. The limitations of H_2 production by microalgae are mainly the absence of large scale method, low yield and energy conversion efficiency, and inhibition of hydrogenase by oxygen, a by-product of photolysis. The sulfur deprivation approach, which is common method employed to enhance hydrogen production leads to anaerobic conditions in the culture, thereby providing an environment for efficient hydrogen production (Zhu et al. 2014; Zhang et al. 2014). While promising for the production of clean and sustainable biohydrogen, these processes require improvement to be economically viable.

Biohydrogen production using biological methods is a manifold metabolic process facilitated through microorganisms; effective H_2 generation and steady system performance are necessary to make this method accessible. The use of various substrates impacts hydrogen production due to the structure of the biomass which needs optimum disintegration to enhance hydrogen production. Various biomasses such as waste activated sludge, rice straw, and macroalgae have been used to produce biohydrogen. Macroalgae are an effective bio-feedstock used for renewable fuel production and have attracted increased attention in recent years. To quicken the solubilization of macro-algal biomass, it is essential to disintegrate the biomass with optimal pretreatment methods. Pretreatment methods such as biological, thermal, mechanical, and chemical could abolish cell walls and enhance hydrogen production. Algal cell walls disintegrate with pretreatment resulting in release of inner components such as proteins, carbohydrates, etc. H_2 generation from macroalgae has numerous advantages such as cultivation ease, rapid growth, high CO_2 capture, along with having rich protein and carbohydrate content.

4.4 Benefits and Limitations of Biohydrogen

Biofuels, hydrogen, natural gas, and synthesis gas are some of the important alternative fuel sources. Hydrogen, among these is the most preferred as it is categorized as a renewable energy source and does not emit greenhouse gases. Further it liberates large amount of energy per unit weight and can easily be converted into electricity by fuel cell. Biological hydrogen production uses simple and sustainable technology and holds a lot of potential for fulfilling future energy demands compared to the chemical production of H_2. Further biological hydrogen production can happen through a wide spectrum of substrates, namely organic wastes, industrial by-products, and biomass as feedstock, which are by and large available free or at a very low cost.

The main limitation of biological hydrogen production is lower yield and slow rate of production vis-a-vis other hydrogen production methods. Therefore, there is a need to develop technologies that can increase the yield and rate of production of biohydrogen which shall overcome the main obstacles being faced presently. Partial pressure of hydrogen gas in the produced gas mixture, competing reactions, bioprocess technology, insufficient active hydrogenase enzyme, and efficient hydrogen producing cultures are some of these potential strategies that can go a long way in making biohydrogen a viable fuel source.

4.4.1 Sustainability Assessment of Biohydrogen

Sustainability usually refers to largely three aspects, namely economic, environmental, and social. Developing a biohydrogen economy means improving the biological hydrogen production processes which is being furthered by research in countries around the world. However, development of biofuel industry in an economy is multifaceted and complex encompassing not only scientific and technological aspects but also social, economic, and environmental aspects of the biofuel eco-system in the country. This can be accomplished by feasibility assessment, evaluation of biofuel sustainability, life-cycle, and techno-economic analysis. The energy ratio and GHG emissions of biohydrogen compared favorably with diesel and other hydrogen production pathways, thus making it worthy of consideration in the planning and development of a hydrogen economy, both from energy and an environmental perspective.

The efficiency and sustainability of biohydrogen production are driven by production rate and its purity. Physicochemical methods are highly efficient in both productivity and purity of hydrogen but they are not cost-effective due to high energy demands in the production process. The biological methods for hydrogen production, however, operate in mild conditions, have lower energy demands, are cost-effective, and hence have caught significant attention in the last decades.

4.4.2 Economic Feasibility of Biohydrogen

Biohydrogen is an economically feasible energy source and is being commercialized successfully. In many countries, this is likely to happen before the timelines that had been set for its commercialization. However some economic incentives may be critical in establishing biohydrogen as an alternate fuel. Levelized cost of energy (LCOE) of biohydrogen is less sensitive to the cost of biomass feedstock, but is more sensitive to the capital cost, operating and maintenance cost. Studies have reported that biohydrogen and biobutanol can replace fossil fuels with high economic feasibility.

Several studies have looked into the development of the biohydrogen sector in countries across the world. China has emerged as the largest biohydrogen market followed by the USA, Japan, and India. Further increased investment in the sector will encourage rapid development of the biohydrogen industry in all the four countries. Further an investment of US$1 in the biohydrogen industry will generate a total output of US$3.22, 3.50, 3.09, and 3.00 in the four economies, respectively, from 2011 to 2050. The study also revealed that investing in the development of biohydrogen technology will provide more benefits than investing in hydrogen infrastructure (Lee and Chiu 2012).

4.4.3 Environmental Safety Through Biohydrogen

Stanislaus et al. (2017), in their study on biohydrogen production from Ipomoea aquatica and digested sludge, stated that during the fermentation process, there was lesser energy consumed as compared to the energy produced as a result of the process, showing a positive energy balance. The biohydrogen system is able to have a negative global warming impact and low cumulative non-renewable energy demand. Thus, it is shown that biohydrogen can be yielded with a positive Net Energy Ratio (NER) which would be a sustainable practice. Sekoai and Daramola (2015) and Singh et al. (2016) opined that hydrogen is the safest fuel owing to its advantages like non-toxicity and being dispersive in nature.

Wulf et al. (2017) did a life cycle assessment of the different technologies and processes used for biohydrogen production for their environmental impact. The study was carried out in Germany, wherein biohydrogen production from biomass sources derived from forestry, herbaceous biomass, energy crops, and biowaste was considered. It was reported that the source has significant effect on the environmental impact of biohydrogen production pathways. Further, they stated that gasification and reforming of biomass are potentially climate friendly. Steam methane reforming (SMR) technology was reported to be the most promising technology regarding the environmental impact.

4.5 Technologies for Algal Biohydrogen Production

Hydrogen is the most abundant element found in the universe and all the matter contains about three quarters of hydrogen (Wang and Wan 2009). On Earth, hydrogen is found mainly in the form of water but also in other compounds such as organic matter and fossil fuels. If energy is provided from external sources, it is possible to extract hydrogen from these compounds to produce molecular hydrogen which is an energy carrier and has the ability to combine with oxygen easily. This reaction leads to the production of energy and water. On extracting molecular hydrogen from water, the by-products are nearly entirely recycled. Therefore, molecular hydrogen has a high potential as a clean and sustainable energy carrier (Veziroğlu and Şahin 2008). Hydrogen has several advantages like it can be transported over long distances by tankers and pipelines and be stored for long time periods. It also has the capability to be compressed and used on board vehicles which makes hydrogen a brilliant alternate for fossil fuels (Bayro-Kaiser and Nelson 2017).

It is crucial to explore all available renewable resources for hydrogen production to take care of hydrogen demands arising in future (Rozendala et al. 2006). As the world is dealing with the challenge of carbon emission reduction, hydrogen emerges as a more attractive fuel as its combustion produces only water. Moreover, hydrogen has a 2.7–3.09 higher energy density (~120 (LHV) to 140 (HHV) kJ/g) than other hydrocarbon fuels (Gupta et al. 2013). The technologies for hydrogen production presently in practice primarily are based on fossil fuels. At the same time, they are energy intensive and thus, using fossil fuel-derived hydrogen should be avoided. Biohydrogen production can be done using different methods like microbial methods. Microalgae are an excellent source for hydrogen production owing to their carbon mitigating properties and the consumption of solar energy as the energy source by microalgae, which is a renewable source of energy (Nagarajan et al. 2021).

Biohydrogen production is a sustainable fuel option as it uses renewable carbon sources including wastewater, offsetting carbon dioxide from the environment. Glucose and sucrose are easily degradable and therefore, they are preferred as ideal substrates for the production of hydrogen (Azwar et al. 2014; Behera et al. 2015). A number of microorganisms can be used in the production of hydrogen; however, the most commonly used and accepted are cyanobacteria and green microalgae. They are considered being more efficient at producing chemical energy from sunlight with a smaller footprint and less requirement for water (Kotay and Das 2007; Manish and Banerjee 2008; Moreno-Garrido 2008). As far as biofuel production is concerned, algal biomass is considered to be an important and alluring source. Nowadays, photobioreactors and open-air systems are being increasingly used for algal hydrogen production (Sharma and Arya 2017).

Microalgae, a microscopic type of algae, are such organisms, containing chlorophyll and having efficiency for photosynthesis. Microalgae have the ability to produce and accumulate huge amounts of carbohydrate biomass. Conducive environments for these organisms include fresh water, seawater, and wastewater. There are a number of advantages of microalgae over terrestrial plants, like higher growth rate, better CO_2 fixation capacity, no requirement of arable land for growth, and so on (Wang and Yin 2018).

Traditional methods of hydrogen production include the use of non-renewable sources like fossil fuels which are resource intensive in terms of money as well as energy, and at the same time; they release high levels of carbon dioxide. However, renewable biohydrogen production systems using microalgae and cyanobacteria are potentially carbon negative and consume much lesser energy (Gupta et al. 2013; Karthic and Joseph 2012).

Hydrogen production using oxygenic photosynthesis is the most innovative biological hydrogen production method as it uses solar energy to break down water into protons (H^+), electrons (e^-), and O_2. Further, it recombines the protons and electrons by either hydrogenase or nitrogenase enzymes to produce hydrogen. The consumption of protons and electrons derived from water has only been seen in green algae and cyanobacteria (Blankenship et al. 2011).

4.5.1 Processes for Hydrogen Production

Traditionally, for hydrogen production, the procedures which have been used include steam formation from natural gases, gasification of coal, waster electrolysis, etc. All these methods are not environment friendly, requiring rigorous energy and temperatures as high as 840 °C and more. Out of the technologies of hydrogen production mentioned above, water electrolysis is the cleanest method; however, it can only be used in places with cheap electricity as its operational cost can be 80% higher than other technologies (Saifuddin and Priatharsini 2016).

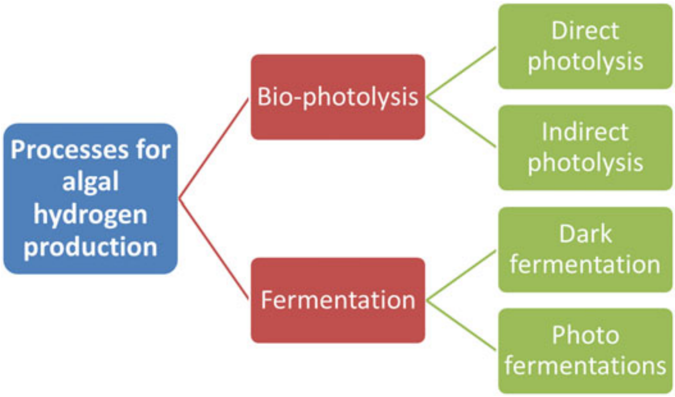

Fig. 4.4 Processes for algal hydrogen production

There are two hydrogenases which catalyze the production of hydrogen, namely [FeFe]-hydrogenase and [NiFe]-hydrogenase. These enzymes are found in many organisms such as members of the genera Clostridium, Desulfovibrio, Ralstonia, and the pathogen *Helicobacter. E. coli* (Cammack et al. 2001). There are several pathways and processes for hydrogen production as illustrated in Fig. 4.4. The pathways for hydrogen production are based on the type of energy, feedstock, and the end-use purity required (Dawood et al. 2020).

4.5.1.1 Direct Photolysis

This method has resemblance with the processes found in plants and algal photosynthesis. Here, photosynthetic reactions are used to convert solar energy directly to hydrogen. In other words, sunlight leads to water being broken down into hydrogen and oxygen (Eq. (4.1)) (Akkerman et al. 2002; Johnston et al. 2005).

$$H_2O \rightarrow H_2 + ½O_2 \tag{4.1}$$

Direct photolysis has been reported to have the highest efficiency in microalgae (Melis and Happe 2001; Melis et al. 2000; Scoma et al. 2012; Volgusheva et al. 2013). Green microalgae have chlorophyll and thus can undergo photosynthesis in the presence of sunlight (Das and Veziroglu 2008; Ghirardi et al. 2000). The process includes channelizing the electrons derived from the breakdown of water (under the action of sunlight) directly to hydrogen producing hydrogenase (Melis and Happe 2001; Melis et al. 2000).

The advantage of this solar energy driven hydrogen production is that it has potentially the highest photon conversion efficiency, as high as tenfold more solar conversion in green microalgae. However, limitation is the practical implementation of this process, which poses technical challenges. The enzyme hydrogenase has high sensitivity to oxygen which hinders its activity and prevents hydrogen production. Furthermore, it also needs high intensity of solar light for the conversion process (Melis et al. 2000; Sharma and Arya 2017).

As the process begins, the light-harvesting complex (LHC) proteins absorb the sunlight. These LHC proteins can be divided into two types, LHCI or LHCII depending on their primary interface with either photosystem I (PSI) or II (PSII), respectively. The LHC proteins hail from a large gene family, which in case of green alga, Chlamydomonas reinhardtii, has more than 20 members (Dittami et al. 2010). The LHCII transfers the energy to PSII, leading to the photosynthetic water splitting reaction, resulting in the conversion of water into H+, e^-, and O_2 (Eq. (4.2)) (Oey et al. 2016).

$$2H_2O \rightarrow 4H^+ + 4e^- + O_2 \tag{4.2}$$

In aerobic environment, photosynthesis generates carbohydrates which lead to mitochondrial respiration and cell growth. However, in anaerobic light environment, mitochondrial oxidative phosphorylation is hindered by the absence of oxygen, which slows down the electron transport chain. In such an environment, the H^+ and e^- extracted from water can be given to hydrogenase (HYDA) via the electron transport chain, which recombines the H^+ and e^- to produce H_2 (Eq. (4.3)).

$$4H^+ + 4e^- \rightarrow 2H_2 \tag{4.3}$$

Microalgae has high hydrogen production efficiency as the efficiency of the algal-hydrogenase is hundred times more than that of other hydrogenases (Lubitz et al. 2014; Volgusheva et al. 2015, 2013). The process takes place at ambient environmental conditions (temperature and pressure) and thus is a sustainable option.

One of the most researched microalgae species for hydrogen production is *Chlamydomonas reinhardtii* (Nield et al. 2004; Posten and Walter 2012; Tokutsu et al. 2012). This species is able to produce hydrogen via a number of processes including direct and indirect photolysis and dark fermentation (Eroglu and Melis 2011; Stripp and Happe 2009; Tolleter et al. 2011; Volgusheva et al. 2013; Wecker and Ghirardi 2014).

4.5.1.2 Indirect Photolysis

The indirect photolysis process can occur in microalgae as well as cyanobacteria (Kruse et al. 2005; Mathews and Wang 2009; Melis and Happe 2001; Melis et al. 2000; Rathore and Singh 2013). This process takes place in two stages. The first step includes breakdown of the water molecules under the action of sunlight, resulting in the formation of protons and oxygen. Subsequently, in the second step, fixation of carbon dioxide takes place leading to hydrogen production by hydrogenase (Eqs. (4.4) and (4.5)) (Prince and Kheshgi 2005).

$$12H_2O + 6CO_2 + \text{light energy} \rightarrow C_6H_{12}O_6 + 6O_2 \quad (4.4)$$

$$C_6H_{12}O_6 + 12H_2O + \text{light energy} \rightarrow 12H_2 + 6CO_2 \quad (4.5)$$

Blue-green algae or cyanobacteria are favorable microbes for this process. The benefits of this process include separation of hydrogen evolution from that of oxygen. Also, hydrogen production is relatively more in this process. However, there are also a few disadvantages such as requirement of continuous light source which is challenging in large scale procedures (Melis and Melnicki 2006; Momirlan and Veziroglu 2005; Mathews and Wang 2009; Das and Veziroglu 2008).

In the process, firstly the energy from sunlight is transformed into chemical energy in the form of carbohydrates. These carbohydrates are subsequently utilized as substrates for hydrogen production. Cyanobacteria use both nitrogenases and hydrogenases for production of hydrogen (Dutta et al. 2005); however, microalgae exclusively use hydrogenases (Oncel et al. 2015). Most commonly, in algal systems, there is a temporal separation of oxygen and hydrogen production phases or the aerobic and anaerobic phases so that oxygenic inactivation of the hydrogenase can be avoided (Kruse et al. 2005; Melis and Happe 2001; Melis et al. 2000).

4.5.1.3 Dark Fermentation

As the name suggests, this process includes production of hydrogen from organic substrate through fermentation in a dark setting and in the absence of sunlight, oxygen, and water. Fermentative microorganisms hydrolyze complex organic polymers to monomers which are further converted to a mixture of lower molecular weight organic acids and alcohols by necessary hydrogen producing bacteria (Das and Veziroglu 2008; Lin and Jo 2003; Schara et al. 2008). The process has several advantages including the use of a variety of carbon sources and hydrogen production in the absence light. Further, valuable by-products are also produced like butyric acid, lactic acid, and acetic acid. However, the yield of hydrogen is relatively lower and carbon dioxide is produced which has to be separated (Saifuddin and Priatharsini 2016).

4.5.1.4 Photofermentation

This process involves fermentation for converting organic substrates into hydrogen and carbon dioxide in the presence of sunlight (Eq. (4.6)).

$$CH_3COOH + 2H_2O + \text{light} \rightarrow 4H_2 + 2CO_2 \quad (4.6)$$

Sunlight is used to oxidize the organic acid substrates using the tricarboxylic acid cycle (TCA). As a result, it produces electrons, protons, and carbon dioxide (Manish and Banerjee 2008; Akkerman et al. 2002). This process is advantageous in removal of environmental pollutants, use of industrial waste, and use of organic acids produced from dark fermentation. However, it requires pretreatment of toxic industrial effluent (Mathews and Wang 2009).

4.5.2 Algal Biohydrogen Production

With the increasing concerns for global warming, renewable energy plays a significant role in mitigating the greenhouse gas emissions from burning of fossil fuels. It also acts as a substitute for meeting increasing world energy needs while preventing depletion of fossil fuel reserves (Kanai et al. 2005). Biomass in the form of several algal species has immense potential for hydrogen production under suitable environment (Li and Fang 2007). Table 4.2 summarizes the comparison of algal biohydrogen production and fossil fuels production.

Algal biomass is a good source of carbohydrates which can be used for dark fermentation. It is even more important when biomass is produced by capturing carbon dioxide from the atmosphere (Roy et al. 2014). Divergence of biomass sources for energy production is an issue requiring attention. Production of algal biohydrogen has lately been receiving much attention. However, production of microalgae biofuels including biohydrogen at commercial scale is still not viable due to low biomass concentration and costly techniques. Exposure of some algal species to environmental stress, like preventing the algae from receiving sulfur from sunlight, can lead to the production of hydrogen gas in substantial quantities. This practice, however, is still in nascent stage with a lot of potential for research and development (Saifuddin and Priatharsini 2016). Further, certain technical barriers have to be dealt with to make biomass a financially feasible biomass option for hydrogen production like having low-energy intensive procedures for harvesting

Table 4.2 Comparison of algal biohydrogen production and fossil fuel production

Parameters	Algal biohydrogen production	Fossil fuels production
Source	Various species of algae	Fossil fuels like coal, natural gas, petroleum, etc.
Characteristics	Renewable in nature, high calorific value of the produced hydrogen	Non-renewable in nature, finite resources
Environmental impact	Non-toxic and pollution free by-products of hydrogen combustion, green and clean source of energy	Leading cause of global warming, climate change, air-pollution, water-pollution, greenhouse gases like carbon dioxide and methane
Production technologies	Different hydrogen production pathways like photofermentation, dark fermentation, direct photolysis, indirect photolysis, genetic engineered pathways, etc.	Energy intensive combustion of fossil fuels for conversion of biomass to gaseous fuels

Source: Karthik et al. (2020), Akkerman et al. (2002), Show et al. (2018); Asongu et al. (2020), Pan et al. (2018), Chufo et al. (2015)

microalgal cells, having large scale and continuous production of biomass, lack of cost-effective bioenergy carrier extraction methods, etc. (Radakovits et al. 2010).

Algae are combination of similar organisms in terms of their morphological and physiological structure. Microalgae have the ability to fix carbon dioxide from the atmosphere and thus, they are suitable for biomass cultivation (Radakovits et al. 2010). There are numerous species of algae available; however, only over 40,000 species of algae have been defined, which is only a small fraction of the total number. The U.S. Department of Energy's Aquatic Species Program analyzed approximately 3000 different microalgae for their potential to produce biofuels (Kars et al. 2006).

Recent years have seen increased use of microalgae in biotechnology. These organisms are associated with numerous industries like food, aquaculture, pharmaceuticals, cosmetics, and several other industries (Chaumont 1993). Algal hydrogen production is a process involving photo-biological breakdown of water, being carried out in a closed photo-bioreactor (Gimpel et al. 2013; Hemschemeier et al. 2009). It has been found that under the process of photosynthesis, if *C. reinhardtii* algae are deprived of sulfur, they will produce hydrogen instead of oxygen (Melis et al. 2000).

Cyanobacteria and green algae break down water into hydrogen ions and electrons under the action of photosynthesis. The electrons are transported over ferredoxins (Peden et al. 2013). Fe-Fe hydrogenases combine them to form hydrogen gas. In case of Chlamydomonas reinhardtii, photosystem II leads to the production of about eighty percent of electrons from direct sunlight. These electrons result in the formation of hydrogen gas (Volgusheva et al. 2013). Photosystem II is a light-harvesting complex system which promotes efficient light energy dissipation (Grewe et al. 2014). The Fe-Fe-hydrogenases are rendered inactive by oxygen and thus require anaerobic surroundings (Langner et al. 2009). Recently, in the year 2020, it was reported that scientists have developed algal-cell based micro-droplets for multicellular spheroid microbial reactors which have the ability to synthesize hydrogen along with either oxygen or carbon dioxide through photosynthesis in sunlight. It has been found that enclosing the microreactors with synergistic bacteria increases the levels of hydrogen production (Xu et al. 2020).

The biophotolysis processes, direct as well as indirect, are inherently related to photosynthesis and the connected electron transport chain. Other than these two photosynthetic pathways, there is also the fermentative metabolism which contributes to hydrogen production as discussed above. In dark surroundings, the enzymatic activity of pyruvate: ferredoxin oxidoreductase (PFR) in *Chlamydomonas reinhardtii* is responsible for the reduction of Fd and the passage of electrons toward hydrogenase. Largely, this process behaves in similar ways as those seen in bacteria (Noth et al. 2013).

As far as early anaerobic stages are concerned, the buildup of complex carbohydrates like starch has been found to be favorably linked with hydrogen production. Further, it has been seen that exogenous carbon-rich media further catalyze its production. Fermentative bacteria make use of the anaerobic processes to convert carbon into several by-products, hydrogen being one of them. Hydrogen production methods like dark fermentation and photofermentation have been regularly used in bacteria species, namely *Escherichia coli*, *Clostridium* spp., *Thermococcales* spp., *Rhodobacter* spp., and *Rhodopseudomonas* spp. (Lee et al. 2010; Jiménez-Llanos et al. 2020).

The photofermentation process converts organic substrates derived from waste into organic acids, alcohols, carbon dioxide, and hydrogen in the presence of light, however, the yield is low. Similarly, in the absence of light, dark fermentation process uses various substrates and waste, releasing hydrogen, among other component mixtures (Anwar et al. 2019; Bolatkhan et al. 2019). It has been reported that the production of hydrogen can be significantly increased (up to 60%) compared to *Chlamydomonas reinhardtii* monoculture systems, by using co-culture systems with *Escherichia coli*. Growth media glucose-rich are exploited by bacteria that produce acetic acid, which can be used in algal metabolism (Fakhimi et al. 2019). Collectively, these photobiological and fermentative microbial processes can be used to increase the production of hydrogen (Fakhimi et al. 2020).

Microalgae are capable of adapting to various extreme environments and surroundings, otherwise hostile to most other living organisms. Thus, their growth conditions can be moderated and controlled to obtain the required composition of biomass (Wang and Yin 2018; Nagarajan et al. 2020). Single-celled green alga, namely *Scenedesmus obliquus* has the ability to biodegrade the phenolic content present in the olive oil mill wastewater, making it possible to solve the Mediterranean problem by creating favorable settings to activate hydrogen production, as the biotransformation carried out utilizes oxygen (Papazi et al. 2019). Another example which has shown significant growth is a group of microalgae cultivated in pig manure, mainly composed of *Scenedesmus* and *Chlorella* species. The algal growth could be achieved without the addition of external nutrients and significant fermentative hydrogen production (Kumar et al. 2016, 2018).

These approaches have a limitation of increased costs in terms of management and purification of the components, including toxic by-products. Further, it has been seen that even though microalgae biomass has a lower level of lignin content as compared to other lignocellulosic feedstock, primary treatments (mechanical, thermal, chemical, or biological) are often essential for extraction of microalgae content (Wang and Yin 2018; Nagarajan et al. 2020).

Algal biohydrogen has gained commercial awareness as it is a reliable and renewable source of energy. Hydrogen as a fuel only produces water as a by-product, thus making it an eco-friendly option. Further research in the genetic and metabolic engineering may increase the photobiological algal hydrogen production considerably. Modulating the key enzymes such as hydrogenase and nitrogenase may alter the metabolic pathways, leading to enhanced hydrogen production. Several photobioreactors have been developed for large scale biomass and hydrogen production (Khetkorn et al. 2017).

4.6 Algal Biohydrogen Production: Opportunities and Challenges

Hydrogen is considered to be a prospective energy for future by virtue of the fact that it is a clean energy source, has high energy content as compared to hydrocarbon fuels, can be easily converted to electricity by fuel cells, and on combustion gives water as the only by-product. Against the projection of global energy crisis and global warming caused due to conventional energy sources, hydrogen holds a promising role as a sustainable fuel for the future. Algal biohydrogen or hydrogen produced from algae is renewable technology as it generates a resourceful fuel through utilization of plentiful natural resources like sunlight and water.

4.6.1 Opportunities and Prospects for Algal Biohydrogen Production

Algal biohydrogen production still remains at the infancy stage of its development. A proficient technology needs to assess and overcome its challenges in order to contribute toward an existing need or demand. Any major breakthrough in process efficiency, reliability, economic viability, social acceptability, and research advancement would act as an opportunity to commodify algal biohydrogen as a fuel. Some of the opportunities that are in accord for algal biohydrogen production have been mentioned below.

4.6.1.1 Interventions of Genetic/Molecular Engineering

Advances and research using molecular engineering approach can prove to be effective to overcome oxygen inhibition of hydrogenase enzyme. This would significantly be a breakthrough to increase the overall yield and productivity of algal biohydrogen production. Advancement in molecular bioengineering also indicates that genetic engineering might offer a feasible approach in developing oxygen-tolerant algal mutant (Show et al. 2018). Genetic manipulation or modification of the hydrogen producing microorganisms may play a vital role in tackling the problem of low yields (Hankamer et al. 2007).

4.6.1.2 Wastewater Integrated Production Process

One of the great opportunities is that microalgae can be cultivated in urban wastewater, which contains sources of carbon and nutrients, helping to reduce the cost of biomass and energy production. The simultaneous treatment of urban wastewater using microalgae and the energetic valorization of the obtained biomass has been tested and proved to produce biohydrogen (bioH_2), a clean energy carrier, through dark fermentation by a strain of the bacteria *Enterobacter aerogenes.* The wastewater was treated using various species of algae; after nutrient depletion the microalgae remained for two more weeks in the photo-bioreactor (PBR) under nutritional stress conditions, to induce sugar accumulation. The stressed biomass was then converted into biohydrogen (bioH_2) (Batista et al. 2015). Through this technology, dual advantage is achieved: energy production and wastewater treatment (Ruiz-Marin et al. 2020).

4.6.1.3 Cost Reduction Through Advancements in Production Process

There have been various research in process efficiency and advancements in production processes that result in reduction in overall cost of the process. This can reinforce industrialization of algal biohydrogen. Various researches like The DISCOVR project, at Pacific Northwest National Laboratory's (PNNL's) Marine Sciences Laboratory in Sequim, Washington, are working to lower the cost of producing biofuels from algae by utilizing an indoor system that mimics the conditions of outdoor ponds. The project utilizes mini-photobioreactors to cultivate algae indoors, in a controlled environment while mimicking the frequently shifting water temperatures and lighting conditions that occur in outdoor ponds. The system is made up of rows of glass column photobioreactors that mimic small outdoor ponds, allowing researchers to grow different strains of algae simultaneously while exposing each row to unique temperature and lighting conditions (Bioenergy Technologies Office 2017). Such advancements would definitely reduce the production cost of algal biohydrogen and would act as an opportunity for this novel technology.

4.6.1.4 Increased Social Acceptability Toward Green Fuel

Algal biohydrogen is a renewable source of fuel that produces only water as an exhaust product and not NO_x. It is very efficient production method in a way that it requires a small land area, produced directly from sunlight in anaerobic conditions, and can outperform traditional hydrogen production from energy crops. It holds the capacity to overcome some of the economic constraints to fulfill energy needs without polluting the environment. Since the current utilization of fossil fuels contributes toward the resource depletion and climate change, there has been an increased acceptability of alternative sustainable fuel production methods by various stakeholders like environmentalists, politicians, technicians, and even industrialists. The idea that hydrogen is the cleanest source of energy and is a promising alternative to conventional fossil fuels is being socially accepted. Social acceptability can support the redesigning of this innovative technology and adaptation of the framework conditions toward the implementation of algae biofuels on a large scale (Villarreal et al. 2020).

However, regardless of being an attractive alternate technology, algal biohydrogen production faces its own set of challenges that inhibit it from scaling up and be utilizable at an industrial scale in the energy sector.

4.6.2 Challenges of Algal Biohydrogen Production

4.6.2.1 Inhibitory Action of Oxygen

One of the major constraints of algal biohydrogen is associated with its production via direct bio-photolysis. During photolysis, along with hydrogen, there is a concurrent production of oxygen which produces an inhibitory effect on the key enzyme, resulting in reduced efficiency of the production process (Show et al. 2018). Hydrogenase enzyme, that catalyzes the hydrogen production process, is extremely sensitive to oxygen (O_2). Exposure to oxygen leads to complete or irreversible inactivation of algal hydrogenase, affecting the conversion of light energy to hydrogen energy (Saifuddin and Priatharsini 2016). Therefore, in order to commercialize hydrogen production at large scale, it is necessary to remove the O_2 as it is produced, which is quite challenging. Some of the promising solutions that can overcome this challenge include: use of O_2 absorbers, use of O_2 tolerant hydrogenase enzymes or to develop O_2 tolerant hydrogenase (Sharma et al. 2013).

4.6.2.2 Maintaining Stable Hydrogen Production

Another major challenge associated with the production process is to maintain continuity in hydrogen production in indirect bio-photolysis. Green algae are able to produce hydrogen in sulfur-free media. Sulfur deprivation causes the inactivation of the photosystem II due to which oxygen consumption is reduced and an anaerobic condition is created in the growth medium, resulting in increased hydrogenase activity (Nagy et al. 2018). However, hydrogen production by sulfur deprivation is time limited. Sulfur limitation substantially induces hydrogen production to last for several days after which the yield begins to gradually level off. After about 100 h of sulfur deprivation, the algae need to go back to normal photosynthesis in order to be rejuvenated by replenishing endogenous substrate affecting the stability and continuity of the hydrogen production process (Show et al. 2018).

4.6.2.3 Purification, Storage, and Transport

Unlike other processes, dark fermentation process used to produce biohydrogen does not produce pure hydrogen. A mixture of gaseous elements is produced that constitute primary hydrogen (generally less than 70%), carbon dioxide, and/or methane, ammonia, moisture, and hydrogen sulfide. Therefore, in such cases, purification of hydrogen gas to be utilized as a fuel becomes a challenge as it is quite energy intensive. Additionally, the storage methods for hydrogen have to be governed by safety regulations and devised in a manner that they can withstand different influxes of pressure without being reactive or affective for a long period of time (Gupta et al. 2013). There is as yet no robust infrastructure developed for hydrogen storage and transportation. This poses a great challenge in its distribution and needs to be addressed in order to improve biohydrogen contribution to the present energy demand.

4.6.2.4 High Cost Requirements

Despite technological advancements, commercial production of algal biohydrogen is still questionable because the cost requirements for the same are quite high. The high cost of biohydrogen production is attributed to photo-bioreactor construction cost, chemical cost of nutrients, strict control of environment (light, climate, land, or space), operational cost, etc. Any intervening technology that might be used to enhance the efficiency and stability of biohydrogen production adds to the cost of production. For example, use of oxygen absorbers, gas separation technologies, pretreatment of enzymes, transportation or handling, research and development, all are energy intensive process and add significant cost to the total production cost. There is also high cost requirement for replacing existing carbon-based fuel infrastructure for proper distribution and storage of hydrogen. Based on the solar energy conversion efficiency of 10% photosynthetic capacity of microalgae used for biohydrogen production, the cost of tubular photo-bioreactor is estimated to be US\$50/m^2 (Wang et al. 2021). This amount is quite high as compared to the production cost of other fuel alternatives.

4.6.2.5 Commercial Viability

The above stated challenges collectively become an obstacle in the path of large scale production of biohydrogen from algae. Reduced hydrogen yield and overall low production rate, lack of infrastructure for storage, transport, high capital and operational cost, all oppose the commercialization of algal biohydrogen production. Currently, only a few pilot scale biohydrogen production facilities are operational; commercial scale production units still face a number of technical and economic barriers. The technology readiness level is low and thus cannot compete economically with fossil fuels. Therefore, immense improvements, research, and interventions are required that can aid in increased commercial viability of such fuels.

4.6.2.6 Other Challenges

Some of the challenges are less talked about, yet have significant role in curbing the growth of algal biohydrogen production. To begin with, lack of knowledge, attitude, and perception plays an important role to bloom an industry. The magnitude of effort, capacity building, and time needed to replace the existing carbon-based fuel utilization is another challenge. Acceptability of various interventions that are employed in order to increase the hydrogen yield, like usage of the genetically modified algae is still low because of the possible risk of horizontal transference of genetic material. Various stakeholders are not convinced about the need to alter natural occurring algae strains to increase productivity, arguing that there is a huge unexplored variety and that the consequences of using genome editing are still unknown. Furthermore, there are no proper governmental initiatives that reinforce the production of algal biohydrogen. Moreover, there are huge research and developmental gaps in the field of algal hydrogen production that must be tackled.

4.7 Conclusion and Future Perspectives

Biohydrogen is a green and renewable source of energy. It is cleaner and more cost-effective than other biofuels. For sustainability of the economy, it is crucial to develop biohydrogen as an alternative fuel owing to its long term advantages like energy security and environmental safety. Genetic engineering can be seen to play a key role in bringing paradigm shift in biohydrogen production in terms of increasing the produce and bringing down the costs.

Renewable sources of energy have multiple advantages. They are sustainable, reduce dependence on fossil fuels, increase energy security, solve environmental problem, and decrease carbon emissions (Singh and Rathore 2017). At the same time, renewable sources of energy are crucial in global and national level decisions regarding climate change and sustainability (Singh et al. 2010a, b, 2011a, b, c).

Biohydrogen production uses simple technology, leading to the formation of clean hydrogen and therefore is a better alternative than chemical hydrogen production which is being used as a predominant hydrogen production technology. Researches on production of biohydrogen have a huge scope in the coming future. Worldwide research data reveals that the US will continue to have the world's largest hydrogen consumption. Talking of Asian nations, biohydrogen production focuses primarily on dark fermentation.

The contemporary production technologies are suitable for decentralized small scale systems, working with waste from different sources like agriculture, industries, etc. Reactors operate with integrated microflora like aerobic, anaerobic, thermophilic, photo non-sulfur producing bacteria, or pure cultures enriched from natural sources. It is anticipated that in the coming times, biological hydrogen production will be determined by a combination of factors including advanced researches, genetic engineering of microorganisms, efficient bioreactors, fuel economics, and wider adoption of these systems. Also, molecular tools can be explored for identifying viable hydrogen producing microorganisms. For sustainable biohydrogen production, the key is to have cost-effective technologies.

Biohydrogen has numerous ecological benefits including efficient waste management. The demand for biofuels is going up, leading to an increased need for researches to explore sustainable systems for biofuel production, including biohydrogen, as per the local conditions of countries. Sustainability of biofuel production must ensure reduction in greenhouse gas emissions and increased social acceptability.

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Chapter 5
Using Algae as a Renewable Source in the Production of Biodiesel

Nesrin Dursun

Abstract Environmental pollution and energy demand have been increasing each passing day. Consequently, the utilization of fossil fuels as the primary resource of energy in the energy and automotive industries has led to a decline in non-renewable fossil fuels. Therefore, renewable, sustainable, and environmentally friendly alternative energy sources have been widely researched. The use of third-generation algae as a basic material in biofuel production research has introduced a new trend in the field of alternative fuels. The production of liquid fuel biodiesel, which is one of the types of biofuels, using algae has recently attracted great interest. Biodiesel has been proposed as a viable alternate to petrol and diesel. It was reported that this fuel could be directly used with diesel engines. The vital factors of nutritional mode, light, and the presence of substrate are known to switch the structural characteristics of algal biomass in the manufacture of biodiesel using algae. The manufacture of biodiesel from algae can be more economical by assembling the wastewater treatment and biofuel manufacture processes. This chapter examines each type of algae used to produce biodiesel under separate headings.

Keywords Algal type · Biodiesel · Liquid biofuel · Production · Renewable fuel

5.1 Introduction

Biofuels have become more practicable in recent years. Its reasons can be listed as follows: (1) the prices of fossil fuels are higher compared to the past, and the cost of biofuel research is affordable compared to that of fossil fuels; (2) consuming biofuels is more attractive due to the use of raw materials that prevent environmental damage since concerns about global warming and potential environmental disasters have become more of an issue; (3) since biofuel is a domestic product, it reduces the

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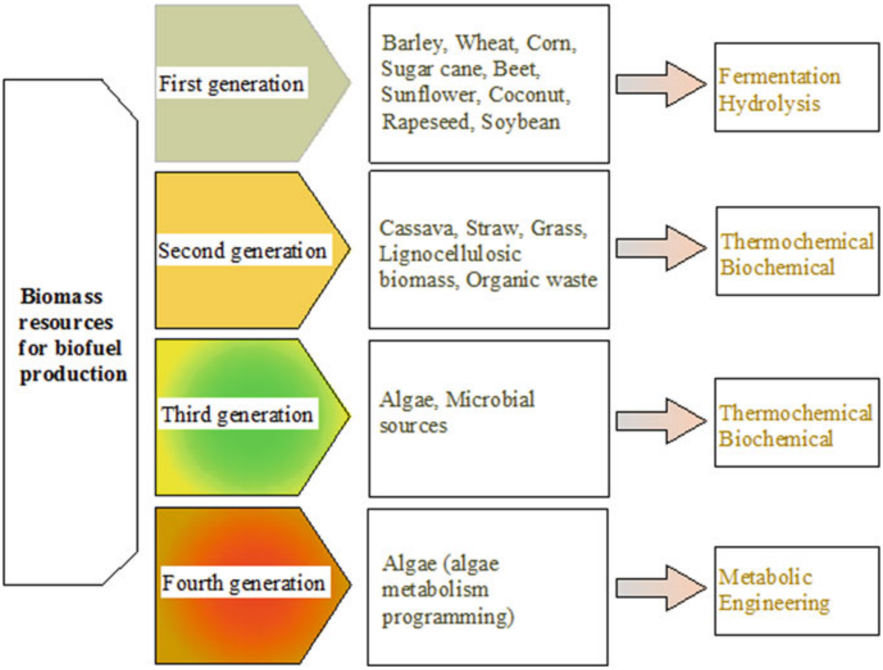

Fig. 5.1 Biofuel generations according to the type of their raw material

outbreak of various wars within the scope of energy independence (Sahay and Braganza 2016).

The continuity, scalability, and efficiency of the biomass source are significant factors in the selection of biofuel raw materials (Chen et al. 2015a). In this context, as seen in Fig. 5.1, biofuels are classified as the first, second, third, and fourth-generation biofuel according to their raw material.

As presented in Fig. 5.2, various raw materials such as algae, palm oil, mustard seed oil, rapeseed, sunflower, peanut, cotton seed, soybean, and jatropha can be employed in the manufacture of biodiesel (Singh et al. 2017). The determinant element contributing to the expense of biodiesel is the selection of basic materials, which accounts for 50–85% of the total expense of the fuel (Fang 2013). Since algae have a rich lipid content, they can be readily processed into biodiesel. Transesterification handling heterogeneous and homogeneous catalysts and in situ transesterification techniques can be used for biodiesel production from algal lipids (Kim 2015). To reduce the biofuel cost to the minimum level, the use of raw material is significant in the way of its quality, yield, and use of by-products. The biochemical compound of the algal biomass can be interchangeable by modifications in the upgrowth circumstances. Biodiesel obtained using microalgae has a fatty acid compound alike the vegetable oils used in the production of biodiesel (Fang 2013).

The manufacture of biodiesel has been fulfilled commercially by transesterification (alcoholysis) of triglycerides using vegetable fat and animal oils

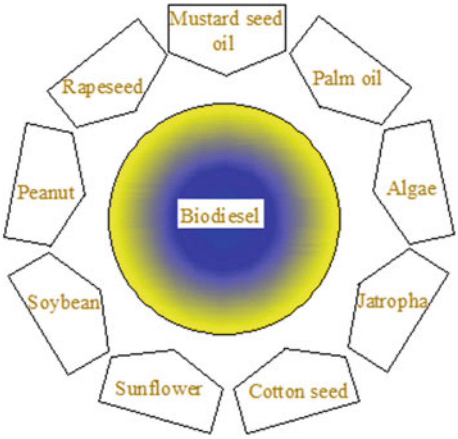

Fig. 5.2 Raw materials used in the biodiesel production

Table 5.1 Data indicate that microalgae almost alone have the potential to replace fossil diesel (Wu et al. 2013; Priyadarshani and Rath 2012)

Crop	Oil yield (L ha^{-1} $year^{-1}$)
Canola	1.190
Jatropha	1.892
Coconut	2.689
Oil palm	5.950
Corn	172
Soybean	446
Microalgae (30% oil in biomass)	58.700
Microalgae (50% oil in biomass)	97.800
Microalgae (70% oil in biomass)	136.900

on the international market or other vegetable oils. Conversely, macroalgae have been mostly used for the production of biohydrogen, biomethane, and bioethanol. It was stated that this is generally due to the almost absence of triglyceride content. It has been reported that a few studies were conducted on the production of biodiesel using macroalgae and the efficiency was very low collated to the efficiency notified from the studies on biodiesel manufacture from microalgae. It has been notified that macroalgae are generally converted to bio-oils such as free fatty acids (FFAs) and lipids, and then the lipids are segregated for biodiesel manufacture. Although FFAs were the pioneer in producing biodiesel, the high content of FFAs in the oil can limit targeted conversion (Chen et al. 2015a).

However, using microalgae as a basic material for biodiesel manufacture offers several advantages. These advantages can be stated as follows: (a) Microalgae, which have a simple structure, show a photosynthetic efficiency twice as high as the current one in a very short time as 24 h. Besides, microalgae can be manufactured throughout the year. Table 5.1 presents the data indicating that microalgae almost alone have the potential to replace fossil diesel (petrodiesel). (b) Microalgae can grow in eutrophicated saltwater lakes, freshwaters, oceans, non-agricultural/barren lands, etc. Moreover, considering a wide geographical region, species abundance, biodiversity, seasonal constraints, it causes less concern compared to other lipid feedstocks (Wu et al. 2013). (c) Microalgae can effectually remove nutrients like heavy metals, phosphorus, and nitrogen from wastewater. They can diminish a big proportion of greenhouse gaseous emissions, which are the reason for global warming. This resulted in the synthesis of proteins, lipids, carbohydrates, pigments, and biomass (Wu et al. 2013; Mohan et al. 2019). (d) The usage of microalgae in the manufacture of biodiesel contributes to the atmosphere by near-zero CO_2 and sulfur emission (Wu et al. 2013). (e) Inducing nutrient stress in microalgae initiates the process of the accumulation of lipids and another precious by-products by increasing biomass (Mohan et al. 2019). Microalgae can produce several valuable products (polysaccharides, pigments, proteins, etc.) (Wu et al. 2013).

It has been stated that technically, biodiesel can compete with petrodiesel. Following features can be listed as the advantages of biodiesel: being a domestic resource; renewability; miscibility with petrodiesel with all mixing ratios; reducing most of the regulated exhaust emission levels, except for nitrogen oxides (NOx); positive energy balance; higher flash point; biodegradability; lack or very little sulfur and aromatic content; inherent greasiness. It was reported that oxidative steadiness and poor chilly flow peculiarities were the technical problems in biodiesel (Knothe 2013; Demirbas 2006).

It has been notified that the transesterification reaction is commonly employed in the manufacture of biodiesel. Moreover, a significant point is that the fatty acid profile of biodiesel corresponds to the main fat. The fatty acid profile determines most of the fuel peculiarities of biodiesel. It has been reported that the above-mentioned technical problems related to biodiesel can be generally traced back to the fatty acid profile. Knowing the peculiarities of the components of biodiesel makes allows us to predict the properties of this fuel relatively accurately (Knothe 2013).

In this section, the algae used for the manufacture of algal biodiesel have been examined considering the up-to-date literature. Mostly, *Scenedesmus* sp. and *Chlorella* sp. were surveyed in the studies on algae-derived biodiesel. Moreover, the number of studies investigating the use of multiple types of algal biomass together has been increasing in recent years. Although the algae diversity is high in nature, the number of studies on using them in biodiesel production was found to be few.

5.2 Some Algae Types Used in Biodiesel Production

The diagrammatic representation of algal biodiesel manufacture is given in Fig. 5.3. The algae given in Fig. 5.4 have been examined under separate headings within the scope of the latest developments in algal biodiesel production.

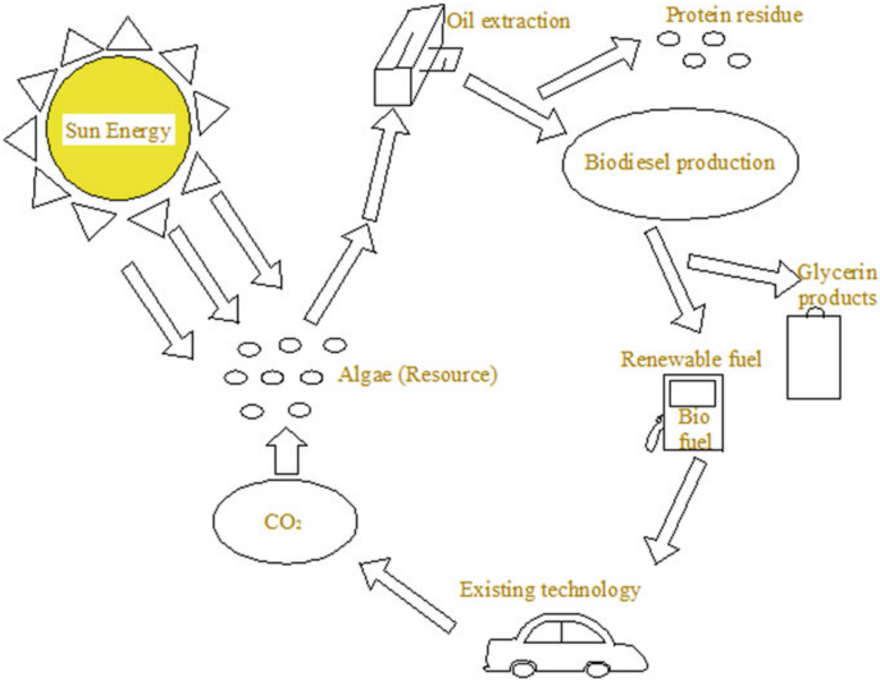

Fig. 5.3 Algal biodiesel production (Bajhaıya et al. 2012)

5.2.1 Ankistrodesmus fusiformis

There are very few studies on the production of biodiesel from *Ankistrodesmus fusiformis*. In this context, the study conducted by He et al. (2015) aimed to optimize the illumination regimens inclusive of first inoculum intensity, light density, and photoperiod on lipid accumulation and upgrowth in *Ankistrodesmus fusiformis* H1 biomass for biodiesel manufacture. Subsequently, 4.47 mM urea with first OD_{680}–0.5, 18:6 h light/darkness period and 200 μmol photon m^{-2} s^{-1} regimens were optimized. Finally, the lipid productiveness was notified to be 116.88 mg L^{-1} d^{-1}, and the neutral lipid was found to be 57.58% in total lipid. In addition, the changes in the photosynthetic activity, biochemical compositions, and pigment contents indicated more carbon inflow to the lipid syntheses. Thus, *Ankistrodesmus fusiformis* H1 was notified to be an ideal resource for biodiesel manufacture when the light is used properly. It was revealed that the 4.47 mM urea strategy contributed to fat accumulation in *Ankistrodesmus fusiformis* H1 biomass and that microalgae had oil potential.

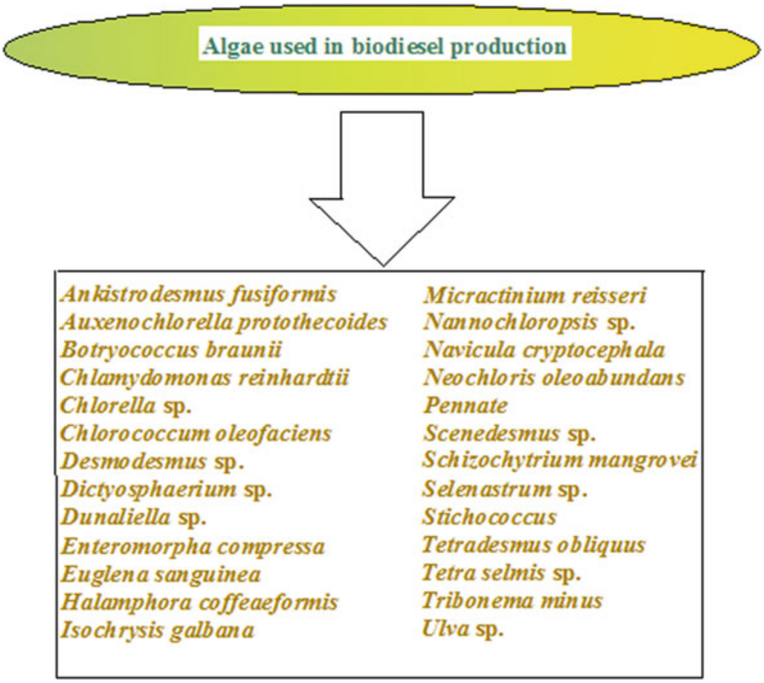

Fig. 5.4 Some algae used in the algal biodiesel production

5.2.2 Auxenochlorella protothecoides

Auxenochlorella protothecoides is one of the algae that has been researched to obtain biodiesel in recent years. Krzemińska and Oleszek (2016) conducted a study to determine the effect of lipid accumulation, algal growth, and fatty acid profile on *Auxenochlorella protothecoides* biomass. They tested the effects of glucose supplementation at various concentrations in their study. With the addition of glucose, the biomass chlorophyll content was observed to decrease, while the growth rate was observed to increase. In their search, the fat content was determined to increase in mixotrophic cells compared to the photoautotrophic cells. The glucose supplementation also led to alterations in the fatty acid profile. The cells grown in a glucose-free medium were found to have higher contents of saturated fatty acids. When annexed with 5 g L^{-1} glucose, oleic acid was determined to be the preponderant component in mixotrophic cells. However, linoleic acids were found to dominate in cultures supplemented with both 1 g L^{-1} glucose and 3 g L^{-1} glucose. The reductions in total polyunsaturated acids (PUFA) and linolenic acid levels were associated with glucose supplementation. The alterations in the fatty acid profile in mixotrophic cells were reported to be convenient for biodiesel production. Moreover, carbon addition was found to provide the requested fatty acid composition in the *Auxenochlorella protothecoides* biomass for biodiesel manufacture.

Iron sources and optimum concentrations of these sources were investigated using *Auxenochlorella protothecoides* microalgae for the manufacture of high-quality biodiesel. The concentration of *Auxenochlorella protothecoides* microalgal biomass with FAME composition was examined in terms of lipid, lipid quality, and specific growth ratio using three ferrous compounds: EDTA (ferric ethylenediaminetetraacetic acid), ferrous sulfate, and ferric chloride. *Auxenochlorella protothecoides* microalgae showed high resistance to high concentrations of iron compounds. Even it was observed to grow at high iron concentrations such as 21.60 mM. The utmost SFA (saturated fatty acid) was observed with the 1.08 mM ferrous sulfate, while the utmost biomass production and 78.5% of FAME were determined with the 1.15 mM ferric chloride. In addition, diesel and biodiesel fuel qualities were reported to be adequate for microalgae grown at 7.19 mM ferric EDTA, 0.07–21.58 mM ferric chloride, and 0.2–14.4 mM ferrous sulfate concentrations. Also, it was stated that the second concentration may be more practical. The reason for this was reported as the usage of this iron compound provided the desirable end in wider ranges. In the overall assessment, it was stated that high-quality biodiesel and lipid manufacture could be achieved by replacement the concentrations of iron resources and compounds (Polat et al. 2020).

5.2.3 Botryococcus braunii

Botryococcus braunii was determined to be one of the algae investigated for biodiesel production in recent years. It was recorded that biodiesel manufactured from microalgae was preferable than fossil fuels in the sense of their life-cycle energy performance. It was stated that the *Botryococcus braunii* green algae may be one of the convenient sources for biodiesel manufacture owing to their relatively higher lipid content. In a study on this alga, the cultivation of *Botryococcus braunii* green alga in a laboratory-scale 4 L continuous stirred tank reactors (CSTRs) with batch feeding was investigated through cheap RNTEM (red Nile tilapia effluent medium), carbohydrate, lipid, protein, hydrocarbon manufacture, biomass growth, and fatty acids profiles. Also, the feasibility of biodiesel production directly using *Botryococcus braunii* green algae obtained by direct transesterification process at laboratory scale was investigated. The maximum biomass yield of *Botryococcus braunii* algae growth was reported to be 8.57 g L^{-1}, and the hydrocarbon context was found to be 35.32%. Moreover, the carbohydrate, lipid, and protein percentages under laboratory conditions were found to be 38.21%, 47.59%, and 16.39%, respectively. The FAME (fatty acid methyl esters) syntheses were carried out by direct transformation of the *Botryococcus braunii* biomass using methanol as a resolvent and sulfuric acid as a catalyst. The conclusions of the research proved that green algae *Botryococcus braunii* can be cultivated in the red Nile tilapia effluent medium (Ramaraj et al. 2016).

A research was fulfilled to thoroughly examine the upstream and downstream processes for obtaining biofuel. In the study, the native strain *Botryococcus braunii*

TN101 was insulated and acclimatized below laboratory conditions. Algae were cultivated in batch mode for 6 days by half-continuous cultivation. Subsequently, 40% of the algal culture was reaped every 3 days. The native strain, which was well cultivated in the semi-continuous system, showed elevated biomass productiveness as 33.8 g m^{-3} day^{-1}. A 2-stage comprehensive reaping process was engineered exercising organic polymer Poly-(D) glucosamine and ferric iron, and the harvested percentage of the biomass was found to be 99.5%. Lipid subtraction was optimized exercising unlike resolvents, methanol, and cyclohexane at an assistance ratio of 3:1 for the highest lipid subtraction up to 26.3% in *Botryococcus braunii* algae. According to the analysis results, the physicochemical properties of the lipid were found as follows: iodine values, 92; ester values, 164; saponification values, 184; average molecular weight of the lipids, 920 g mol^{-1}. The free fatty acids level of the lipid was found to be 9.7%; therefore, contemporaneous esterification and transesterification of triacylglycerides and free fatty acids were optimized to obtain biodiesel. Also, methyl ester yields of 84% have been reported. An optimization work was fulfilled to remove the pigments in biodiesel. It was concluded that 99% of the pigments were dissipated from the biodiesel by exercising activated coal. The biodiesel profile analysis was conducted using GC-MS, ^{1}H, and ^{13}C NMR. Methyl oleate and methyl palmitate were determined to be the chief fatty acids. According to the volumetric and areal biomass productiveness, it was surmised that the native strain could propagate 101 tons ha^{-1} $year^{-1}$ of biomass (Ashokkumar et al. 2014).

5.2.4 Chlamydomonas reinhardtii

One of the algae studied for the purpose of biodiesel production in recent years is *Chlamydomonas reinhardtii*. Due to today's energy needs and environmental pollution, the lipid obtained from microalgae is considered to be one of the sources that can contribute to biodiesel production. The lipid productiveness, lipid content, and biomass productiveness parameters were analyzed in TAP (Tris-Acetate-Phosphate) media, while the nutrient starvation such as glucose (0.2%, 0.15%, 0.1%, and 0.05%), nitrogen, and phosphorus starvations, vitamin B12 supplementation (0.003%, 0.002%, and 0.001%) was investigated in the early constant stage of culture *Chlamydomonas reinhardtii* CC1010 green alga. In the nitrogen starvation medium, the lipid content was found to be 61%, which was noted to be 2.34 times higher than the Tris-Acetate-Phosphate media, which is an adequate nutrient. In the study, the vitamin B12 supplements were found to have no effect on biomass and lipid manufacture; however, glucose supplementation was found to support a proportional increase between the increase in glucose concentration and biomass productivity. Furthermore, the FAME (fatty acid methyl ester) profile of *Chlamydomonas reinhardtii* CC1010 unearthed upwards of 80% of the total saturated fatty acid and monounsaturated fatty acid context. The quality control parameters of biodiesel such as iodine count, cetane count, saponification value, and unsaturation extent were analyzed. Also, the fuel peculiarities of the biodiesel

Table 5.2 Oil content of some algae (Priyadarshani and Rath 2012; Avagyan and Singh 2019)

Algae	Oil content (% dry matter)
Chaetoceros calcitrans	14.6–39.8
Chaetoceros muelleri	33.6
Chlorella emersonii	25–63
Crypthecodinium cohnii	20
Cylindrotheca sp.	16–37
Nannochloris sp.	20–56
Nannochloropsis oculata	22.7–29.7
Nannochloropsis oculata NCTU-3	30.8–50.4
Neochloris oleoabundans	29–65
Nitzschia sp.	45–47
Schizochytrium sp.	50–77
Thalassiosira pseudonana	20.6

were detected to pertain to the international standards of EN 14214 and ASTM D6751. As a general assessment, among all transactions, nitrogen hunger with 0.1% glucose adjunct resulted in tall lipid context in *Chlamydomonas reinhardtii* (Karpagam et al. 2015a).

Chen et al. (2015b) notified that although obtaining biodiesel through microalgae was hopeful, there was still a deficiency of technology for cost-effective and rapid transformation of biodiesel using wet microalgae. In a work conducted in this context, the combination of the open-vessel microwave digestion system, low-speed centrifugation, and methanol treatment was used to develop a new method for microalgal biodiesel manufacture. In this work, *Chlamydomonas* sp. JSC4 algal biomass with 68.7 wt% water context was used as raw material obtained from transesterification with partial dewatering, oil extraction, and without removing co-solvent before hand. Direct transesterification was conducted with degraded wet microalgae. Biomass content increased in wet algae by 56.6 wt% after microwave disruption and 60.5 wt% later partial dewatering processes. Oil extraction of about 96.2% was achieved under the following conditions: hexane/methanol rate, 3:1; extraction temperature, 45 °C; extraction duration, 80 minutes. The transesterification process of the extracted fat resulted in 97.2% transformation in 15 minutes at 45 °C at 6:1 solvent/methanol rate with isochronous removal of chlorophyll along the process. The direct transesterification processing of microalgal biomass with degraded oil content resulted in almost 100% conversion to biodiesel.

Table 5.2 presents the oil content of some algae used in biodiesel production.

5.2.5 Chlorella *sp.*

Most of the works on biodiesel manufacture through algae examined the use of *Chlorella* sp. Malla et al. (2015) reported that the integrated approach based on phycoremediation where wastewater refining and microalgal biodiesel manufacture were carried out together was a hopeful process. The setup of the experiment was prepared to search the phytoremediation potential of *Chlorella minutissima* for removing the fouling burden from the Indian Agricultural Research Institute's (IARI) primary treated wastewater and Common Effluent Treatment Plant's (CETP) tertiary treated wastewater and examining the biomass for biodiesel production later reaping. The study investigated several chemical and physical parameters like potassium, EC, TDS, phosphate, nitrate, BOD, and COD. *Chlorella minutissima* was found to remove about 90–98% TDS, 60–70% P, 70–80% N, and 45–50% K than the wastewater within 12 days. Also, the COD level decreased by 75%, while the BOD level decreased by 60%. Algal biomasses were harvested in order to perform biodiesel extraction. The supreme specific lipid productiveness was found to be 0.171 g-lipids g-cell^{-1} day^{-1} for the wastewater of the Common Effluent Treatment Plant, while it was 0.132 g-lipids g-cell^{-1} day^{-1} for the wastewater of the IARI. The oleic acid was found to increase by 59.6% in the CETP wastewater compared to that of the Indian Agricultural Research Institute's wastewater. The study was determined that the algal strain *Chlorella minutissima* did not solely contribute to the betterment of pollutant burden but could be also expended as a potential material for biodiesel manufacture.

In a study where alga *C. vulgaris* was cultivated, the phytohormone generating green microalga *Chlorella vulgaris* MSU-AGM 14 (C.V) and *Streptomyces rosealbus* MTTC 12,951 (S.R) were also upbrought in a co-culture system for assessing their exogenous hormonal action. The biosynthesis of IAA (indole-3-acetic acid) and its pioneers were inspected quantitatively using the method of HPLC (high-performance liquid chromatography). Because of symbiotic interplay among *Streptomyces rosealbus* and green microalga *Chlorella vulgaris*, the indole-3-acetic acid concentration (0.72 ± 0.02 μg mL^{-1}) was found to increase in the co-cultivation system. In return, a sufficient volume of tryptophan (Trp) was produced by microalgae to initiate the biosynthesis of the IAA. The tryptophan stress in the late exponential stage promoted fat accumulation (175 ± 10 mg g^{-1}). The bio-flocculation feature of microalgae has reduced the energy input by 148% compared to the conventional method, resulting in an economical and potentially viable harvesting process. The result of the research revealed that cultivating *Streptomyces rosealbus* together with microalga *Chlorella vulgaris* exhibited positive behavior. Moreover, the study proved to be a promising cultivation process for green microalga *C. vulgaris* in periods of energy conservation and cost yield (Lakshmikandan et al. 2021).

The use of glyphosate for lipid induction in microalgae for sustainable biodiesel manufacture was investigated by Jaiswal et al. (2020). In the work, the impact of glyphosate herbicide stress on lipid induction and metabolic growth of the freshwater green microalga *Chlorella sorokiniana* was investigated. During the 96 hours of incubation, the glyphosate herbicide concentration of IC_{50} (30.10 ppm) resulted in semi-maximum inhibition. At the end of 24 days of harvest, it was found that *Chlorella sorokiniana* produced 427.73 ± 5.0 mg/L and 442.18 ± 9.1 mg/L dry cell weight (dcw) of biomass in glyphosate IC_{50} and the control medium, respectively. Due to metabolic biosynthesis stress in the glyphosate IC_{50} medium, a weighted reduction of ~3.26% was observed in biomass production. Conversely, unlike its effect on biomass manufacture, the glyphosate IC_{50} stress contributed to the inducing of fat biosynthesis in microalgae cubicles. The progress in the fat syntheses was determined to be ~17% more in glyphosate IC_{50} medium with respect to the control medium. FTIR spectroscopy was used to analyze the chemical structures of the biomass, metabolites, and lipids of *Chlorella sorokiniana*. The lipids extracted from the green microalga *Chlorella sorokiniana* were exercised for methanolic-H_2SO_4 catalyzed transesterification for producing biodiesel. FTIR and ^1H NMR spectroscopy methods were used to analyze the synthesized biodiesel. In the end, the transformation yield of *Chlorella sorokiniana* into biodiesel was calculated as ~77%.

Direct transesterification using cyclopentyl methyl ether or 2-methyltetrahydrofuran co-solvents as an alternate to chloroform was investigated for biodiesel manufacture from the *Chlorella pyrenoidosa*. The acid-catalyzed transesterification method utilizer HCl was suggested for biodiesel manufacture. Biodiesel was purified by the colon chromatography method using someone of 2 verdant resolvents as eluents. The high degree of ecologic biodiesel quality was with respect to the biodiesel obtained exercising chloroform as a co-resolvent. In order to optimize the manufacture of fatty acid methyl esters, a 3^3 factorial design was exercised for the studied independent variables, which were resolvent and catalyst rate (methanol:co-resolvent:HCl), solvent and catalyst volume, and temperature, for a reaction period of 150 min. The biodiesel manufacture was found to be affected mainly by the temperature increment. The achieved efficiencies using chloroform, 2-methyltetrahydrofuran, and cyclopentyl methyl ether were reported as 61–76%, 67–91%, and 71–92%, respectively. The fatty acid methyl esters profile of biodiesel obtained with 2-methyltetrahydrofuran and cyclopentyl methyl ether was found to contain about 45% polyunsaturated components. It was reported that the manufactured biodiesel complies with the ASTM D6751 and EN 14214 standards. The researchers also reported that replacing fossil solvents used in direct transesterification of biomass with a green solvent resulted in a better ecological biodiesel production process for a circular bioeconomy (de Jesus et al. 2020).

Another research examined the heterotrophic cultivation of the alga *Chlorella* sp. TISTR 8990 at dissimilar agitation rates in a medium with various first C/N rates. In the investigated range, only the agitation speed was not affected by the C/N rate. However, total fatty acid coverage, fatty acid composition, and biomass production were affected. The biomass manufacture was found to maximize at a C/N mass rate of 29:1. At this C/N rate, biomass productiveness of 0.68 g $L^{-1}day^{-1}$ was achieved. In other words, it was reported as about 1.6-fold the optimum productivity that can be achieved as a result of photoautotrophic grow-out. The biomass efficiency ratio in glucose was found to be 0.62 g g^{-1} during the exponential grow-out. The maximum total fatty acids in the freeze-seared biomass at a C/N rate of 95:1 were found to be 459 mg g^{-1}. The fatty acid context of the biomass was observed to decrease at the lower values of the C/N rate. The highest productivity of the total fatty acids (TFAs) was found to be (186 mg $L^{-1}day^{-1}$) at the C/N ratio of 63:1, which is a better value.

Mostly, polyunsaturated fatty acids were observed under these conditions. After nitrogen (N) depletion, the algae were allowed to stay in the stationary stage for a long time. This situation increased the level of polyunsaturated fatty acids, while it diminished the level of monounsaturated fatty acids. The biotin addition to the culture media decreased the biomass productiveness compared to the biotin-free control medium; however, it did not influence the total fatty acid context of the biomass. Considering the research in general, the maximum biomass concentration was observed at a low C/N rate, while the maximum total fatty acid context of the biomass was observed at a high C/N rate. The heterotrophic cultivation of the *Chlorella* sp. TISTR 8990 was successfully in glucose (Singhasuwan et al. 2015).

Harvesting of maritime *Chlorella* sp. was investigated by flocculation and autoflocculation by adding coagulator and adjusting pH. The autoflocculation was found to provide low efficiency. The researchers used the response surface methodology to optimize the pH and coagulator dosage for the flocculation. The investigated coagulants were ferric chloride and aluminum sulfate. Empirical models acquired from the RSM (response surface methodology) were found to be compatible with the empirical conclusions. The optimal flocculation was observed at a pH of 8.1, a settling time of 40 min, and the ferric chloride dosing of 143 mg/L. It was reported that biomass concentration had an important influence on harvesting yield. The oil extracted from the maritime alga *Chlorella* sp. cultivated in a urea manure media with a hexane resolvent was reported to be favorable for biodiesel manufacture due to its high context of saturated fatty acids. Before the production of biodiesel, the raw oil should be purified to remove some pollution. Also, a two-stage biodiesel production process was proposed since the free fatty acid context was higher than 1% (Sanyano et al. 2013).

Alhattab and Brooks (2020) studied the surfactant-aided dispersed air flotation technique, which was a low-cost microalgae harvesting technical for biodiesel production, using the surfactant cetyl trimethylammonium bromide (CTAB) and the microalgae *Chlorella saccharophila*. Response surface methodology (Box–Behnken design) was expended to optimize the concentration factor and recovery. The researchers investigated the effects of cetyl trimethylammonium bromide concentration, column height, air flow ratio, and pH. For each response variable, a separate analysis was conducted at the beginning to obtain their models. Only cetyl trimethylammonium bromide and flow ratio were found to be important for the recuperation. Moreover, all well-tried variables were reported to be important for the concentration factor. Subsequently, optimization was conducted to simultaneously maximize the recovery and concentration factor. These optimized processing parameters were reported to be the cetyl trimethylammonium bromide concentration of 100 mg L^{-1}, pH of 10, column height of 795 mm, and flow ratio of 57.9 mL min^{-1}. The expectable values for the concentration factor and recovery were 17.9 $\pm$ 6.19 and 91.1% $\pm$ 1.62%, respectively. According to the results of the experimental tests, the concentration factor was found to be 13.23 $\pm$ 0.98, while the recovery was 94.5% $\pm$ 2.7%. These experimental values were found to be consistent with the expected values of 74% and 96% for the concentration factor and recovery, respectively. The research concluded that the surfactant-aided dispersed air flotation was a hopeful harvesting technique for microalgae biodiesel production processes due to its capacity to allow higher recovery, decrease the working volume of microalgae culture, and require lower energy. However, a secondary dewatering step before the product conversion was recommended for higher concentration.

Large-scale commercialization was desired for biodiesel production from microalgae. However, it was reported that several barriers should be studious to improve the process and render it economic, such as lowly lipid content in the production process and harvesting. The researchers studied the alga *Chlorella pyrenoidosa* and alga *Chlorella minutissima* by altering the nitrogen and carbon content of the media to improve the lipid productivity of the algae. Both algae were grown in the BG11 media for the initial 6 days. Then they were grown on the changed BG11 media without azote content for 2–10 days. On the sixth day, nitrogen deprivation was observed to increase lipid efficiency by 17.6% in alga *Chlorella pyrenoidosa* and by 20% in alga *Chlorella minutissima*. Then, this was combined with carbon supplement in the shape of sodium acetate (5 g/L), natrium carbonate (5 g/L), citric acid (5 g/L), and natrium-potassium tartrate (5 g/L). Thus, the total lipid productiveness of the alga *Chlorella pyrenoidosa* increased by 23% while that of the alga *Chlorella minutissima* increased by up to 24%. The highest lipid efficiency was detected with nitrogen privation combined with sodium acetate. The asset of fatty acid methyl esters, mostly composed of hexadecanoic and octadecanoic acid methyl esters, was revealed by the acidic transesterification. Sodium acetate and nitrogen deprivation employed as a carbon resource resulted in the maximal fatty acid methyl esters of 4% in alga *Chlorella pyrenoidosa* and 3% in the alga *Chlorella minutissima*. Thus, increased sodium acetate as a carbon resource combined with the nitrogen privation in the BG11 medium facilitated the increase in the lipid productiveness of the alga *Chlorella pyrenoidosa* and alga *Chlorella minutissima* (Bharte and Desai 2019).

5.2.6 Chlorococcum oleofaciens

There are very restricted works on biodiesel manufacture from this algae. In their study, Rayati et al. (2020) investigated the impact of light density on lipid accumulation and growth characteristics of the freshwater microalga *Chlorococcum oleofaciens* KF584224.1. This freshwater alga was cultivated for 20 days at 5 diversified light densities of 800, 400, 200, 100, and 50 μmol photons m^{-2} s^{-1}. While the culture curves showed the speedy growth ratio in the algae enlightened with 200 μmol photons m^{-2} s^{-1}, they show the lengthiest exponential growth curve in cultures enlightened with 400 μmol photons m^{-2} s^{-1}. Once the algae achieved the stable stage, the culture illuminated with 200 μmol photons m^{-2} s^{-1} demonstrated the highest biomass productiveness as 367.82 ± 21.63 mg L^{-1} day^{-1}, while the culture enlightened with 400 μmol photons m^{-2} s^{-1} showed the highest oil productivity as 126.72 ± 3.27 mg L^{-1} day^{-1} and highest oil context as 59.18 ± 1.62%. The rate of saturated fatty acids increased significantly with the increase in the density of lighting, while the rate of polyunsaturated fatty acids and monounsaturated fatty acids tended to decrease ($p < 0.05$). It was reported that the peculiarities of biodiesel produced from microalga *Chlorococcum oleofaciens* cultured at 50 μmol photons m^{-2} s^{-1} meet the European standard of EN 14214 and the US standard of ASTM D6751. It was also notified that the increment in the illumination density increased the biodiesel quality. This study revealed that the lipid productivity and biodiesel properties of the potential feedstock of microalgal *Chlorococcum oleofaciens* can be improved for biofuel production, particularly below the light density provisions of 400 μmol photons m^{-2} s^{-1}.

5.2.7 Desmodesmus *sp.*

Desmodesmus sp., which is one of the algae that has attracted caution in last years, has been investigated within the extent of algal biodiesel manufacture. Komolafe et al. (2014) studied the biodiesel production potential of the microalga *Desmodesmus* sp. The study revealed the process of biodiesel manufacture from microalgae grown in wastewater and reported that the microalgae significantly reduced the total coliform and nutritions in the wastewater. In addition, harvesting of the obtained algal biomass by ozone-flotation was studied, and it was found to be feasible and result in FAMEs with greater oxidation stability. Two mixed cultures and the microalga *Desmodesmus* sp. were successfully cultivated in wastewater. Microalga *Desmodesmus* sp. was reported to grow rapidly and have a maximal biomass concentration of 0.58 g/L. The local complex culture that was predominated by *Arthrospira* and *Oscillatoria* attained the concentration of 0.45 g/L and showed the highest lipid and fatty acid methyl ester efficiency. Due to its high degree of saturation, the FAME obtained by the ozone-flotation process showed maximum oxidative stability. Therefore, ozone could be essentially exercised as a united method of ingathering and decreasing fatty acid methyl ester unsaturation. In course of the microalgae treatment, the aggregate nitrogen content of wastewater decreased by 55.4–83.9%. In addition, a high total coliform removal (99.8%) was determined.

In the study conducted by Ji et al. (2015), the use of microalga *Desmodesmus* sp. for nutrients removal was investigated using ADW (anaerobically digested wastewater) for biodiesel manufacture; also, the results of batch cultivation and fed-batch cultivation were compared. The microalga *Desmodesmus* sp. achieved the removal of 236.143 mg/L in TN, 6.427 mg/L in PO_4-P, and 268.238 mg/L in NH_4-N later 40 days of fed-batch cultivation. Conversely, the removal quantities achieved in batch cultivation were found to be 33.331 mg/L in TN, 37.227 mg/L in NH_4-N, and 1.323 mg/L in PO_4-P. Biomass manufacture of microalga *Desmodesmus* sp. was increased in fed-batch cultivation using anaerobically digested wastewater loading each 2 days. Accordingly, the biomass concentration crested at 1.039 g/L. This value was found to be 3-times superior than the value achieved in the batch cultivation (0.385 g/L). Maximum oil manufacture was reported as 261.8 mg/L. It was also reported that fed-batch cultivation of microalga *Desmodesmus* sp. could ensure effective control of ammonia inhibition and nutriments limitation.

5.2.8 Dictyosphaerium *sp.*

The need for environmentally friendly and sustainable fuels has increased the number of studies on biofuels as an alternate to fossil fuels. The research conducted by Okonkwo Ogbonna and Chukwuma Ogbonna (2018) purposed to designate the influences of using dissimilar concentrations of glycerol (0.25 ~ 1.0 mL/L) and glucose (10 ~ 40 g/L) with alga *Dictyosphaerium* sp. on lipid production, heterotrophic growth, and mixotrophic growth. The microalga *Dictyosphaerium* sp. was cultivated in amber bottles of 2000 mL, each of which contains 1000 mL of sterile modified BG-11 medium at a pH of 7.3. Every bottle was inoculated with a 7-day-old pristine isolate culture with an inoculum rate of 15%, and they were incubated in the darkness at 30 °C ± 2 °C for 10 days. The microalga *Dictyosphaerium* sp. showed heterotrophic and mixotrophic cultivation skills on glycerol and glucose as sole carbon substrates. Specific growth rates and biomass productivity did not change with the change in the glucose of the initial medium. Lipid accumulation was reported to be not connected to the glycerole contents of the initial medium. In this study, the average lipid productiveness and context of the organism were found to be high enough to be used in industrial processes. The oil accumulation and growth were found to be better in mixotrophic culture conditions compared to both autotrophic and heterotrophic conditions. In addition to this, both were found to be better than the autotrophic one. The percentage compositions of the major fatty acids obtained from microalga *Dictyosphaerium* sp. grown under different culture conditions were reported to show at least five components each. The eluted carbon skeletons were observed to range between C14 and C22. Oleic acid was reported to be the main component of whole fatty acids, and the convenience of the usage of fat in the manufacture of biodiesel was confirmed.

5.2.9 Dunaliella *sp.*

The alga *Dunaliella* sp. was also examined in the studies on obtaining algae-derived biodiesel. The most costly stage in biodiesel production processes is harvesting the microalgal biomass and extracting the lipids. Therefore, a study was conducted to diminish the cost of lipid extraction exercising the ozone-richer microbubbles technic. In the study, the lipid extraction of the *Dunaliella salina* slurrying (1:2 v/v) was carried out by direct ozonation technique using methanol in a 0.2 L bioreactor at chamber warmth. When small bubbles were observed during extraction at 60 °C, the concentration of the products for 6,10,14-trimethylpentadecan-2-one, stearic acid, and palmitic acid was observed to increase by 156%, 150%, and 88.9%, respectively. The energy used to extract the oil of *Dunaliella salina* biomass with ozone is estimated to be about 36% (2.16 MJ kg^{-1} dry algae). The study concluded that the energy consumption in the ozone extraction method was less than those of solvent and centrifugation extraction methods (Kamaroddin et al. 2020).

Another study examined the microalga *Dunaliella parva* in terms of high biomass productiveness, oil content, and productiveness by optimizing the growth media, mainly for the purpose of obtaining biodiesel. The productivity and content of another biochemical compounds like carbohydrates, proteides, and free amino acids were also examined using the response surface methodology. Maximum biomass productiveness, oil content, and productiveness were determined to be 48.59 mg/L/day, 39.08%, and 19.91 mg/L/day, respectively, in the medium optimized for the response surface methodology and including 0.63 g/L nitrogen, 0.02 g/L phosphore, and 1.61 M sodium chloride. These results were found to be 1.2, 1.5, and 1.4 times higher than the values detected in the normal grow-out media, respectively; also, they were detected to be consistent with the estimated values of 49.85 mg/L/day, 37.51%, and 19.49 mg/L/day. Moreover, polyunsaturated fatty acids were observed to decrease from 54.9% to 40.3%, while saturated fatty acids augmented from 42.8% to 59.7%. Therefore, the study concluded that the microalga *Dunaliella parva* was an alternative source that would contribute to various industrial applications with the production of biodiesel with excellent quality (Fawzy and Alharthi 2021).

Besides their conversion to biodiesel, microalgae should also be promising for the bioremediation of wastewater. In this context, the impact of phenol exposure on algal biomass manufacture and the quality of biodiesel produced from the alga *Dunaliella salina* were investigated in a study. Phenol had an EC_{50} of 155.03 mg L^{-1} on algal growth and led to a decrease in biomass efficiencies and chlorophyll a/b ratios. Conversely, the indicator of phenol-induced oxidative stress increased the superoxide dismutase enzyme activities and malondialdehyde content. *Dunaliella salina* cells that were transferred to culture without phenol content afterward prior to exposure to phenol at 150 mg L^{-1} increased 41% faster. Moreover, lipid content was found to be 26% higher compared to that acquired from the control cohort with no prior phenol exposure. In the research, prior exposure to phenol changed fatty acid methyl ester compounds resulted in the following changes depending on each concentration: increase in methyl linolenate (C18:3(n-3)) and γ-linolenic acid methyl ester (C18:3(n-6)) levels; decrease in cis-10-heptadecanoic acid methyl ester and methyl stearate (C18:0) levels; decrease in cetane count. In addition, the study concluded that the usage of toxic chemicals for prior acclimatization had the potential to support production in terms of efficient microalgal biomass production (Cho et al. 2016).

It has been notified that the use of renewable energy sources can reduce the problems in the energy supply. In this context, the territorially insulated strain of *Dunaliella* sp. was studied by Talebi et al. (2015). The average oil context in cultures enriched with 200 mg L^{-1} myoinositol was found to be 1.5 fold higher compared to the control experiment. Similarly, high oil productiveness values were acquired in cultures treated with 200 and 100 mg L^{-1} myoinositol. According to the results of flow cytometry and microplate fluorescence (fluorometry analysis), the oil accumulation was observed to increase in the Nile red-stained algal specimens. In addition, biodiesel obtained from cells treated with myoinositol was estimated to improve cetane number, oxidative stability, and cloud point values. The research was conducted to develop a strategy for increasing the amount of algal oil and the quality of biodiesel.

5.2.10 Enteromorpha compressa

There are very few studies on this algal biomass, and in one of these studies, in situ transesterification of *Enteromorpha compressa* was carried out for biodiesel production. In this study, the ultrasonic irradiation application provided the highest ME (methyl esters) efficiency of 98.89%. Acid catalyst (H_2SO_4) and tetra hydro furan (THF) co-solvent were found to be suitable for the *Enteromorpha compressa* biomass including high FFA (free fatty acids) to increase the yield of the reagent in situ process. The highest productivity was recorded for the following conditions: ultrasonic irradiation time of 90 min at 65 °C, stirring intensity of 600 rpm, methanol-algae biomass ratio of 5.5:1, 10 wt.% of (H_2SO_4), 30 vol.% of tetra hydro furan as a co-solvent. Characterization of the obtained biodiesel was performed using ^{1}H NMR (^{1}H nuclear magnetic resonance) spectroscopy analyzer. That the reaction traced to the first-degree reaction contraption was revealed by kinetic studies. The research concluded that fast in situ transesterification was an appropriate technic for biodiesel production from maritime macroalgae feedstock (Suganya et al. 2014).

5.2.11 Euglena sanguinea

It was found that *Euglena sanguinea* was one of the algae investigated for algal biodiesel production in recent years. Algal biofuels have catchy worldwide caution owing to their potential to address environmental concerns. Microalgal biomass was reported as a sustained fuel resource with carbon neutrality, where the choice of the strain to be used was very important for productive biofuel production. The microalga *Euglena sanguinea* was found to be one of the robust freshwater species and had high lipid productivity. This alga was studied at the laboratory scale in addition to the mass scale of the raceway pond. The fatty acid value of the extracted fat was reported to be 3.8 mg potassium hydroxide g^{-1} and had a 93% triglyceride content. The acid value was degraded to 0.3 mg potassium hydroxide g^{-1} by the esterification process. Also, optimized parameters were found to have the following values: H_2SO_4, 10 vol.%; methanol/fat rate, 0.36; duration, 42 min. Because of its recyclable, natural, and environmentally friendly properties, the shell of the white mussel was exercised in the subsequent transesterification process. The maximal efficiency of about 98% was achieved for the reaction period of 80 minutes at a 0.35 methanol/oil ratio for 6 wt.% of calcined CaO. The results of the GC-MS analyzer of the biodiesel acquired from algal biomass revealed the existence of saturated fatty acids C16:0, C18:0, C22:0, C24:0 and monounsaturated fatty acids C18:1 at a suitable level to support preferable combustion properties and oxidation resoluteness (Kings et al. 2017).

5.2.12 Halamphora coffeaeformis

The lipid composition, content, and productivity of this diatom were investigated in terms of its potential feedstock availability to obtain biodiesel. It was cultured in the following two stages: (a) increasing biomass like inoculum for larger volumes in photobioreactors (PBRs), (b) naturally increasing the TAG (triacylglycerol) context during the stationary growth stage in the raceway ponds. Biomass concentrations were reached at 0.64 g L^{-1} in the photobioreactor and 0.23 g L^{-1} in the raceway pond. The total oil content was found to be 34% AFDW (ash free dry weight) in the neutral lipid context. On the other part, it was found to be 54.4 (±11.6)% AFDW on the 19th day of reaping in the raceway pond. The triacylglycerol (TAG) productivity was found to be 1.2 mg L^{-1} day^{-1} in the channel pond. Calculations of the fatty acid profile compound and biodiesel indicators revealed that the quality of the oil obtained from the maritime benthic diatom *Halamphora coffeaeformis* is good according to international standards (Martín et al. 2016).

In another study on the same diatom *Halamphora coffeaeformis*, the lipid accumulation, growth, and bioproducts were studied in a two-phase hybrid culture exploiting seawater enriched with nutritions and absent vitamins. In addition, the effect of dissolved and internal nutritions on lipid accumulation and growth was analyzed. The total oil content was found to increase in the descending stage up to 33.4% AFDW (ash free dry weight) owing to an increment in neutral oils with total lipids reaching 87%. The detected tardiness in TAG accumulation was expressed by the accumulation of great internal nitrogen ponds in alga *Halamphora coffeaeformis*. Triacylglycerol, frustules, and solvable exopolysaccharides were analyzed and recommended as commercially important bioproducts. A sustainable biorefinery approximation in terms of being environmentally friendly and economical has been recommended for biodiesel manufacture by a two-phase hybrid culture of alga *Halamphora coffeaeformis* (Martín et al. 2018).

5.2.13 Isochrysis galbana

Lately, works on biodiesel manufacture using microalgae have been the center of attention. In one of the works handled in this context, the potential of the microalga *Isochrysis galbana* was examined. Therefore, the microalga *Isochrysis galbana* was cultivated for biodiesel manufacture. The equipment of the culture system was notified to consist of two systems of methacrylate ponds with a base of 1.40 × 0.40 m, an elevation of 0.4 m, and a thickness of 11 mm. The effects of the stirring, first concentration of inoculum, and CO_2 submission on the growth of algae were analyzed. It was stated that the stirring factor improved the growth rate of algae. However, the inoculum and carbon dioxide supply were reported not to have an important impact on cell density. The acquired biomass was used for fat production by the extraction process, and it was used in the synthesis of biodiesel by the basic-catalyzed reaction. For the transesterification process, microalgae fat was muddled with 1% g NaOH/g fat-sodium hydroxide and methanol (12:1 molar rate of methanol/fat) in a reactor at 62 °C for 3 hours. With a fatty acid methyl esters context of 12.5%, the best harvest was obtained at a biomass concentration of 0.305 g/L (Sánchez et al. 2013).

5.2.14 Micractinium reisseri

The potential of alga *Micractinium reisseri* has been investigated for biodiesel production in recent years. Microalgae cultivation in wastewater to obtain biodiesel is a low-cost approach, and it was noted that wastewater does not contain optimum concentrations of carbon sources and essential nutrients due to the general reduction in lipid and biomass productivity. The goal of this work was to bypass this limitation by processing wastewater separately or in combination using various concentrations of nutrients of Cl^-, NO_3^-, PO_4^{3-}, and SO_4^{2-} and three carbon sources either individually or in combination to cultivate alga *Micractinium reisseri* to obtain biodiesel. First, various dilutions of wastewater were analyzed. The wastewater with a concentration of up to 75% showed the lipid productiveness of 0.014 g L^{-1} day^{-1} and the maximum biomass productiveness of 0.076 g L^{-1} day^{-1}. Optimal manipulating circumstances for highest oil production and maximum productiveness required the addition of 1.0 g L^{-1} glucose in the concentration of the control medium and a 50% reduction in phosphorus. Under these conditions, the lipid productivity and the biomass productivity increased by 4 and 1.7 times, respectively, compared to those observed in the control study. In addition, the phosphorus starvation state in the presence of glucose significantly increased the fatty acid profile in parameters related to biodiesel quality (Elshobary et al. 2019).

In another study, the alga *Micractinium reisseri* was explored by El-Sheekh et al. (2020) for sustainable biodiesel manufacture from this alga with a strategy of combining nutrients stresses with mixotrophic nutrition. The cultivation of alga *Micractinium reisseri* in 25 mg/L improved the lipid efficiency up to 206%. During the mixotrophic nourishment, 0.1 M glycerole increased the dry weightiness by 2.8 times, while 0.05 M glycerole increased the lipid efficiency by 26%. In addition to this, algal culture supplementation of 1 g/L sugarcane molasses increased the total oil yield by 31% and the oil content by 61%. Under the optimization conditions, MUSFA (monounsaturated fatty acids) increased from 14% to 30%, whereas

PUSFA (polyunsaturated fatty acids) decreased from 48% to 31%. Moreover, stearic and oleic acids increased by 284% and 383%, respectively. The conclusions of the analyses of the obtained biodiesel which met the requirements of the ASTM D-6751 and EN 14214 standards revealed that they were high-quality products. According to the result of the research, combining mixotrophic nutrition with nitrogen stresses and salts encouraged the alteration of metabolic pathways toward lipid throughput used as a resource in biodiesel production.

5.2.15 Nannochloropsis *sp.*

One of the works examining biodiesel manufacture using *Nannochloropsis* sp. was carried out by Mitra et al. (2015). The research investigated the impacts of the light density (150 and 60 μmol photons m^{-2} s^{-1}), photoperiod (00 h:24 h, 06 h:18 h, 12 h:12 h, 18 h:06 h, and 24 h:0 h light: darkness) and saltiness (40, 35, 30, and 20 g/L) on the picky nutraceutical peculiarities of the alga *Nannochloropsis gaditana* by focusing on eicosapentaenoic acid manufacture. The maximum biomass and the lipid productivities acquired at a saltiness gradient of 20 g/L were 45.01 ± 1.01 and 14.63 ± 0.79 mg L^{-1} day^{-1}, respectively. Also, the maximum EPA (eicosapentaenoic acid) productiveness was detected at a photoperiod regimen of light:dark (18 h:06 h) and 60 μmol photons m^{-2} s^{-1}, where the EPA content was found to be 32.16 ± 0.40% and 37.83 ± 0.37%, respectively. Low salinity increased chlorophyll-a to 11.71 ± 0.40 μg/mL, while it increased the carotenoid content to 4.32 ± 0.15 μg/mL with respect to high saltiness conditions. The EPA (C20:5) context of strain was determined in the range between 19.13 ± 0.08 and 37.83 ± 0.37%. According to the results of the fundamental constituent analysis, there was a significant correlation thereamong monounsaturated and saturated fatty acid synthesis. The residual fat could be used for biodiesel manufacture. After a substantial extraction of long-chain polyunsaturated fatty acid (PUFA). In this study, the nutrient profile of the microalga *Nannochloropsis gaditana* which was enriched with eicosapentaenoic acid was investigated. The study concluded that microalga *Nannochloropsis gaditana* could be used as a basic material for biodiesel manufacture owing to the high saturated fatty acid and monounsaturated fatty acid content of the oil, which could be used for nutraceutical production, excluding eicosapentaenoic acid and arachidonic acid.

Another study reported that one of the basic steps of obtaining biodiesel from microalgae is flocculation. The flocculation efficiency of the marine microalga *Nannochloropsis oculata* was investigated using eight different flocculants. The maximum flocculation was observed with concentrations of 0.4 g/L $FeCl_3$ and 0.6 g/L $Fe_2(SO_4)_3$ in 180 minutes, where it was 93.80% and 87.33%, respectively. In zinc salts, the second maximum flocculation efficiency reached 89.12% and 84.17% in the concentration of 0.6 g/L $ZnCl_2$ and the concentration of 0.8 g/L $ZnSO_4$ at 210 and 240 min, respectively. On the other part, the flocculation yield of the salts of aluminum at 210 and 240 min with concentrations of 0.6 g/L $AlCl_3$ and

0.4 g/L $Al_2(SO_4)_3$ was observed to be 85.46% and 82.27%, respectively. Although its incubation period was longer, magnesium salts were observed to have less effect on flocculation. Iron salts were excluded from the further flocculation studies because they showed complete cell lysis at low concentrations. The effects of darkness, light, and temperature factors were investigated for Zn and Al salts. It was reported that the optimum temperature of 35 °C and light was required for better flocculation. Under illumination, $ZnSO_4$ and $ZnCl_2$ showed efficient flocculation of 90.49% and 92.34%, respectively, while it was observed to be 80.14% and 83.14% under dark conditions. Also, the cells were observed to remain intact at higher temperature and concentration values. The efficient flocculations of $Al_2(SO_4)_3$ and $AlCl_3$ were 85.91% and 88.49%, respectively, under illumination, while they reduced to 68.79% and 74.77%, respectively, under darkness. Since structural instability is evident in aluminum salts, zinc chloride was suggested as an effective flocculant to flocculate alga *Nannochloropsis oculata* (Surendhiran and Vijay 2013).

In situ transesterification of wet microalgae, which substitutes multiprocesses of cell drying, extraction, and transesterification reagent, has been simplified and has been reported as a hopeful alternate for obtaining biodiesel. In this context, Kim et al. (2017) investigated new methods to enhance biodiesel manufacture from alga *Nannochloropsis gaditana* at high temperatures. The former studies on in situ transesterification process researched the temperature range between 95 °C and 125 °C. This study, however, researched temperatures above 150 °C. This relatively harsh condition did not allow the use of co-solvent during the single extraction-conversion process, or it allowed the use of only a less amount of acid catalyst. It was reported that 0.58% (v/v) of sulfuric acid in the reaction media without any co-solvent could convert 90 wt% of the total oil transformation to biodiesel at 170 °C when the humidity context of wet algal paste was 80 wt%. The impacts of the co-solvent, acid catalyst, and temperature on fatty acid ethyl ester efficiency and specification were investigated. In addition, the reaction kinetics were studied to better comprehend the high-temperature solvothermal in situ transesterification reaction. Having a biphasic system (chloroform/water) in the reaction process and detection of only Ca^{2+}, Mg^{2+}, K^+, and Na^+ cations, and phosphore under 5 ppm facilitated meeting the biodiesel quality requirements of the EN 14214 standard. This research concluded that the application of wet in situ transesterification at high temperatures would reduce the chemical input and subscribe to the retainable manufacture of biodiesel from microalgae.

5.2.16 Navicula cryptocephala

Diatoms have been examined for their high lipid context in recent years. Geologists have suggested that most of the crude oil comes from diatoms. In order to obtain biodiesel, the diatom *Navicula cryptocephala* was insulated from a freshwater resource and grown on a convenient medium for fat characterization and extraction. The following three methods were used for culturing the diatom: photobioreactor, shake flask, and polythene bag. The maximal efficiency of biomass was found on the 5.95 g/100 mL of medium in photobioreactor. Electron microscopy and compound microscopy studies were conducted for the identification of diatoms. The extraction of oil from the diatom *Navicula cryptocephala* biomass was carried out using the sonication and soxhlet methods. The sonication method was found to produce a higher yield of oil of 0.364 g/g of dry biomass. Farther characterization of oil using GC revealed the asset of the major fatty acids of oleic acid, palmitoleic acid, linoleic acid, and palmitic acid. HPLC analysis of the raw oil revealed the asset of OOL (linoleoyldioleoylglycerol) as principal TAGs (triacylglycerols) and OLL (dilinoleoyloleoylglycerol) (Sanjay et al. 2013).

5.2.17 Neochloris oleoabundans

Microalgae, which show higher photosynthetic efficiency compared to plants, are important in biodiesel production. The alga *Neochloris oleoabundans* UTEX 1185, which can be grown in freshwater, has been acknowledged as a potential source for biodiesel manufacture by Banerjee et al. (2019). In this work, various processes of direct oil extraction from wet microalgal biomass were investigated by means of microwave, autoclaving, and ultrasonication pretreatment applications. Autoclaving pretreatment provided further yield for oil extraction with respect to microwave and ultrasonication pretreatment processes. In addition, the transesterification process was fulfilled in the asset of catalyst Fe_2O_3, and it was contrasted with traditional HCl acid and NaOH base catalysts. The used Fe_2O_3 nanoparticles were synthesized from the extract of Hibiscus rosa-sinensis plant by the green procedure. The synthesized catalyst Fe_2O_3 provided a biodiesel yield of up to 81%. It was determined that this value was higher than the values of 64% and 48%, which were obtained with HCl and NaOH, respectively. Then, a biodiesel efficiency of 86% was obtained by optimizing the parameters of the transesterification process like catalyst context, reaction warmth, and reaction time. Fuel properties and FAME (fatty acid methyl esters) profile analysis detected that algal oil was suitable for biodiesel manufacture. 20.2% w/w carbohydrates in oil extracted from microalgal biomass were transformed to biological hydrogen by using acidogenic complex consortium and Saccharomyces cerevisiae (INVSC-1), respectively, by bioethanol and dark fermentation process under anaerobic conditions. The integration of the biorefinery approach with bioethanol and biohydrogen production from degreased microalgal biomass has accomplished biodiesel manufacture more retainable. Life-cycle assessment has appointed that oil extraction from wet microalgal biomass has low peripheral footprints with respect to conventional biodiesel manufacturing processes.

Microalgae with high oil content were recommended as sustainable alternative biomass that could replace conventional vegetable oils produced from oilseed plants used for biodiesel production. Several studies reported that the alga *Neochloris oleoabundans* cultured in anaerobically digested dairy fertilizer or seawater had the potential to produce high amount of monounsaturated fatty acids and triglycerides. In the conversion of biomass into valuable products, supercritical technology has been acknowledged as a green retainable alternate. Therefore, the *Neochloris oleoabundans* biomass was partially dried and subjected to direct supercritical alcohol treatment. Then, the fractionation of the reaction products was investigated by using supercritical carbon dioxide or fluid *n*-hexane. To judge the production of fatty acid ester, direct alcoholysis of the microalgal biomass was fulfilled by increasing the reaction times at 250 °C and 280 °C. Twofold extraction with *n*-hexane was used to obtain up to 35 wt% fatty acid esters from bio-oils acquired from microalgae. Conversely, supercritical carbon dioxide fractionation manufactured improved bio-oils with a fatty acid ester context of up to 74 wt% (Hegel et al. 2017).

The exhaustion of fossil fuels each passing day has increased the attraction of renewable raw materials including microalgae. It was reported that *Neochloris oleoabundans* (*Chlorophyta*), which could grow mixotrophically, had lipid accumulation features, particularly triacylglycerols (TAG), for biodiesel production. In a study conducted in this context, the relation among photosynthesis and oil manufacture was examined, and mixotrophic cultivation and glucose (late exponential: 6 days and late stationery: 14 days, stages of growth) were investigated in two basic steps. According to the results of the study, the usage of glucose led to high biomass productiveness related to fast cell response up to day six, followed by cell expansion by day fourteen. Mixotrophic cells included a large number of stromatic starch grains at day 6, while there was a tendency for starch reduction and a high accumulation of lipids at day 14. Under mixotrophy conditions, protein content and photosynthetic pigment were observed to decrease. It was reported that the degree of photoinhibition was not substantially impressed by mixotrophic cultivation under high light during both experimental periods. The formation of a reduction environment caused by photosynthetic activity tended to increase lipid synthesis with changes in the N:C ratio. The research had a general conclusion that biodiesel production met the requirements (Baldisserotto et al. 2016).

5.2.18 Pennate

In their study, Touliabah et al. (2020) reported that in *Pennate* diatom *Nitzschia palea* could be cultured in alfresco vertical-bed photobioreactors for biodiesel production. To examine lipid and biomass production, impure cultures of *Nitzschia p.* were grown alfresco, and measurements of these cultures were carried out every 2 weeks during the growth process. During the annual period of the algal culture, the dry weight biomass was found to range between 0.11 g L^{-1} and 0.25 g L^{-1}, the culture temperature was found to range between 17.3 °C and 33.5 °C, intracellular lipid content was found to range between 7.1% and 11.4% of biomass weightiness later drying at 60 °C, and the light energy was found to range between 1.94 and 3.9 Wm^{-2}. According to the results of GC/MS analysis of n-hexane extracts, the intracellular oils contained 8.26% C15:0 pentadecylic acid, 9.01% C14:0 myristic acid, and 2 types of C16:0 including 29.25% palmitoleic acid and 41.13% palmitic acid. Gel permeation analysis revealed that 28.9% of lipids, 24.3% of triglycerides, 27.3% of diglycerides, and 16.3% of monoglycerides consisted of carboxylic acids. Alcoholysis of lipids resulted in the transformation of fatty acids to biodiesel or FAME (fatty acid methyl esters), equivalent to about 93.9%. It was also reported that on the base of wt%, it comprised principally of 28.7% C17:1 methyl palmitoleate, 7.2% C16:0 methyl pentadecanoate, 8.3% C15:0 methyl myristate, and 39.8% methyl palmitate.

5.2.19 Scenedesmus *sp.*

In the vast majority of algae-derived biodiesel production studies, alga *Scenedesmus* sp. was used. One of these studies researched the lipid extraction of the microalga *Scenedesmus* sp. using hot compressed hexane (HCH) process. The hexane extraction at room warmth and pressure was compared with the Dyer and Bligh extraction method. It was determined that the extraction performance was around the critique dot of hexane. Experimental results showed that hot compressed hexane (HCH) significantly improved the lipid extraction rate and yield with respect to the usage of hexane under ambiance circumstances. High efficiencies of biodiesel-convertible oil fractions were quickly obtained at the critic dot of hexane, which was comparable to that of the Dyer and Bligh extraction method (Shin et al. 2014).

In another study examining this alga, Hosseini et al. (2016) investigated to improve the operation of top-lift bioreactors with a depth of 1 m using a bubble column or a gas-lift system utilizing weather and CO_2-enriched weather. The researchers aimed to cultivate highly productive algae suitable for biodiesel conversion at high lipid levels. A hydrodynamic model and theoretical energy requirement analysis, which fit well with the experimental measurements, were enhanced to estimate the fluid circulation rates in the gas-lift bioreactor. The effects of the reactor's operational parameters, such as gas flow ratios, bioreactor design, and CO_2 concentration, on the growth and oil volumetrical manufacture of alga *Scenedesmus dimorphus* were assessed by factorial design. With biomass productiveness of 68.2 $g_{dw}\ m^{-2}\ day^{-1}$, the bubble column bioreactor was found to have 12% higher biomass productiveness. On the other part, the maximum oil volumetric production of 0.19 $g_{Lipid}\ L^{-1}$ of the gas-lift bioreactor distributed 6% CO_2 because of light and hydrodynamic stresses.

In another study, carbon sequestration potential and biodiesel productivity were investigated by isolating microalgae in a marble mining area. The microalgal isolates were cultivated in the BG-11 media which was adjuncted with sodium bicarbonate as a carbon resource. As screening parameters, the growth behavior of the microalgal isolates and ratio of $NaH^{14}CO_3$ reception were examined to find the most capable microalgal strain. *Scenedesmus* sp. ISTGA1 was found to be the most productive alga by using the 18SrDNA sequencing procedure. The growth of the isolate was evaluated below dissimilar concentrations of gaseous carbon dioxide (5–15% v/v) and sodium bicarbonate (10–200 mM). The isolate obtained maximal growth under the conditions with 15% CO_2 and 100 mM $NaHCO_3$. In the status of 100 mM sodium bicarbonate, biomass production, chlorophyll context, and oil content were found to be 1508 mg/L, 9 μg/L, and 301 mg/L, respectively. At 15% of the carbon dioxide these properties were found to be 1490 mg/L, 12.1 μg/L, and 268 mg/L, respectively. FAMEs (fatty acid methyl esters) were analyzed by GC-MS and lipids were transesterified. It was reported that C16 or C18 fatty acids, which were convenient for biodiesel manufacture by FAMEs, dominated over 80%, and the addition of inorganic carbon created unsaturated (54–55%) and saturated (33–35.8%) fatty acids in both cases (Tripathi et al. 2015).

The researchers investigated the cultivation of *Scenedesmus* sp. in wastewater system and its usage as a basic material in biodiesel manufacture. In one of these studies, the green microalga *Scenedesmus acutus* was cultivated in pretreated and post-treated urban sewer water discharges. In addition, it was contrasted to a culture media containing 20% of essential nutrients K, P, N to examine the lipid accumulation ability and the potential for simultaneous nutrient removal. The maximum nutrient removal level was found to be 66% phosphorus and 94% organic nitrogen in the pretreated wastewater discharge. Also, the cultures using pretreated wastewater provided better biomass productivity of 79.9 mg/L and lipid accumulation of 280 mg/L, compared to the enriched medium. The pretreated wastewater provided the best results. Therefore, biodiesel preparation was carried out using the mentioned medium. After cultivation, 249.4 mg/L of biodiesel was produced. In reference to the results of the research, the microalga *Scenedesmus acutus* was found to be convenient for wastewater treatment generating biomass with its lipid context appropriate for obtaining biodiesel (Sacristán de Alva et al. 2013). In another study, microalga *Scenedesmus bijuga* was investigated for concurrent wastewater treatment and biodiesel manufacture. In the study, microalga *Scenedesmus bijuga* was grown in the anaerobically digested FWE (food wastewater effluent). To determine the appropriate dilution ratio in biodiesel production, three dissimilar mixing rates with urban wastewater were contrasted. The highest biomass production, which was 1.49 g/L, was achieved with 1/20 diluted food waste effluent (FWE). The highest lipid content of 35.06% was determined in 1/10 diluted food waste effluent. Conversely, the maximum lipid productiveness of 15.59 mg/L/day was determined in 1/20 diluted FWE. It was stated that the fatty acid methyl ester (FAME) compositions in *Scenedesmus bijuga* mainly consisted of C16-C18 over 98.94%. Also, the quality of fatty acid methyl esters was appraised by Bis-Allylic Position Equivalent (BAPE) and Cetane Number (CN) (Shin et al. 2015). In a study in which microalgae were cultivated, microalga *Scenedesmus acuminatus* was cultivated in polyhouse raceway pools and outdoors using a cost-effective ranch manure media. The experimented microalga was grown in both ambiance and polyhouse situations in the winter, rainy, and summer seasons. During the season, the diurnal variations were reported to be in the following ranges: temperature, 26–39 °C; solar density (polyhouse: 28–41 k lux, ambiance: 44–65); humidity, 57–90%; dissolved oxygen, 5.5–13.1 mg L^{-1}; pH of culture suspension, 6.5–10.6. In the study, the microalga *Scenedesmus acuminatus*, which was tested at various culture depths and impeller velocity, reached a tall biomass value of 1.2 g L^{-1} at an impeller velocity of 65 rpm at depth of 30 cm under ambient conditions, while it reached the biomass of 1.11 g L^{-1} below and polyhouse conditions. The experimented microalga was grown in a polyhouse and open pool for two consecutive years maintaining optimum impeller speed and culture depth. The areal biomass productivity under the polyhouse raceway pool conditions in winter, rainy, and summer seasons was surmised as 10.38, 7.83, and 10.15 gm^{-2} day^{-1}, respectively. Accordingly, the areal lipid efficiency was estimated as 1.18, 0.75 and 1.05 gm^{-2} day^{-1}, respectively. Under the ambiance conditions, on the other part, the areal lipid productiveness during the winter, rainy, and summer seasons was found to be 1.30, 0.42, and 1.19 gm^{-2} day^{-1}, respectively. Considering the cultivation conditions, the annual lipid productiveness of the microalga *Scenedesmus acuminatus* cultivated below ambience conditions was surmised as 3.094 ton hectare^{-1} year^{-1}, while it was estimated as 3.168 ton hectare^{-1} year^{-1} for the microalga cultivated below polyhouse raceway pool circumstances. According to the results of the research, annual biodiesel efficiency in the polyhouse and ambience circumstances was estimated as 2.186 and 2.135 tons hectare^{-1} year^{-1}, respectively. These values revealed that the microalga *Scenedesmus acuminatus* was a potential resource for sustainable biodiesel manufacture (Koley et al. 2019).

In a study conducted to enhance the lipid context of the alga, the microalga *Scenedesmus obliquus* was cultured in 2 different cultivation phases: (a) batch using actual wastewater and (b) safekeeping the stationary stage with unlike circumstances of light, CO_2, and salinity pursuant to a factorial design. At the last of the second phase, the asset of these three factors increased the lipid context from 35.8% to 49%. The highest direct effect that increased the lipid context was found to be the entity of CO_2, which is followed by the asset of light and salt. An increase was observed in the context of ω-3 fatty acids with the presence of light and the movement of CO_2 with isolation. Moreover, the effect of the interaction was found to be negative when both factors acted together. The ω-3 eicosapentaenoic acid context of the oil obtained from the microalga *Scenedesmus obliquus* was observed to lightly exceed the maximal 1% rate according to the EU normative to be used as a biodiesel source. As a result of this search fulfilled by Álvarez-Díaz et al. (2015), the picky extraction of ω-3 fatty acids from *Scenedesmus obliquus* oil or mixing this oil with other oils was proposed.

In another study, the impact of monochromatic LEDs (light-emitting diodes) on the growth of the alga *Scenedesmus obliquus* and its biodiesel yield was investigated. During the night period, dissimilar light-emitting diodes were implemented separately or in combination. Among the different individual treatments, red illumination resulted in the highest biomass and lipid productivity due to pigmentation stimulation while the blue illumination resulted in the highest biomass and lipid productivity due to the stimulation of photosystem II. Moreover, the combined blue-red illumination significantly increased biodiesel recovery, microalgal growth, and fat production. Besides, the proportions of the monounsaturated and saturated fatty acids increased in favor of polyunsaturated ones. In addition, blue-red light-emitting diodes increased the clear biodiesel energy output over the control. The overall increment in clear energy output was determined as 5.1 MJ when using red light, 3.8 MJ when using blue light, and 10.8 MJ when using blue-red light. The research notified that the implementation of blue-red light-emitting diodes during the night term is an economic way for microalgae cultivation. It was concluded that this practice may have a potential influence on the futurity of mercantile biodiesel manufacture from microalgae (Abomohra et al. 2019).

Taher et al. (2014) fulfilled a work on biodiesel manufacture from lipid obtained by extraction of microalgae as a hopeful approach for sustainable fuel manufacture. But it was reported that this approach could not be commercialized due to the intense drying energy consumption and high cost of upstream processes associated with lipid extraction processes. In the research, the probability of directly extracting the lipid from the wet concentrate cell was tested using enzymatic degradation to increase extraction, avoiding drying. Cellulase and lysozyme activity were both found to disturb the cell wall structure and increase oil extraction from wet algae specimens. Also, the highest oil extraction efficiency was achieved at 16.6% by exploiting lysozyme. Within the scope of the study, the feasibility of exploiting supercritical carbon dioxide in extracting oil from wet biomass was surveyed, and the maximum efficiency was found to be 12.5% by use of lysozyme. Besides, a 2-pace culturing process was performed exploiting alga *Scenedesmus* sp. to reunite both lipid context and high biomass growth. The strain increased the biomass productiveness up to 174 mg L^{-1} day^{-1} achieving nearly steady oil context in the first phase. Although the biomass productivity was lowered in the second phase, lipid context resulted in a sixfold increase later 3 weeks of nitrogen hunger.

5.2.20 Schizochytrium mangrovei

The alga *Schizochytrium mangrovei* was investigated as a basic material for biodiesel manufacture. In this context, the potential of obtaining biodiesel and precious by-products from algal biomass was investigated by isolating *S. mangrovei* PQ6 from Phu Quoc Island in Vietnam. Because of the high lipid context of algal biomass, till 70% of dry cell weightiness, and the loud level of total fatty acids, this biomass became an ideal source for biodiesel production. Extraction of FAME (fatty acid methyl esters) from the marine microalga *Schizochytrium mangrovei* PQ6 resulted in 88% and 44% yields based on algae oil and algae biomass, respectively. Then, the abruption of the obtained fatty acid methyl esters into a first fraction enriched with SFAME (saturated fatty acid methyl esters) and a second fraction enriched with UFAME (unsaturated fatty acid methyl esters) was effected to utilize valuable by-products. According to the results of the research, the mass fraction was 70% for SFAME and 30% for UFAME. The UFAME fraction was detected to contain high docosahexaenoic acid (DHA) content. According to the saturated fatty acid methyl esters fraction test results, specific gravity, water, and sediment, flash point at 15 °C, kinematic viscosity, sulfur, sulfated ash, cetane number at 40 °C, copper strip corrosion, iodine number, carbon residue at 50 °C parameters were found to meet Vietnam Biodiesel B100 Standard (Hong et al. 2013).

5.2.21 Selenastrum *sp.*

The freshwater microalga *Selenastrum* sp. GA66 was reported to show an important increment in oil content below normal circumstances when exposed to stress (NaCl addition, nitrate, and phosphate starvation) factor. The research in which biphasic optimized conditioning was performed for the first time was conducted by Chakravarty and Mallick (2019). In order to maximize biodiesel production, three optimization experiments were effected under the circumstances with 0.06 g L^{-1} nitrate, 3.5 g L^{-1} NaCl, and an incubation period of 4 days. Besides a significant reduction in the incubation period, the most important result was the threefold increment in oil content. Due to the stress situation, the biphasic optimization strategy was applied to reduce the loss of biomass and oil efficiency. Thus, 2.2 times higher productivity and 2.6 times higher lipid yield were achieved. The analysis results of the fatty acid profile of biodiesel specimens under two-phase optimized conditions revealed higher saturation as opposed to a low quantity of unsaturation. In the study, the fuel features of biodiesel were surveyed by considering international biodiesel standards. In order to generalize this research strategy, six dissimilar freshwater microalgae were cultivated under the same conditions; two-fold higher oil efficiency and productiveness were observed for every alga. The results of the research supported the acceptability of the mass strategy for lipid induction. The optimized environment decreased the nitrate requirement by 16 fold, resulting in cost decrease about five fold under optimized conditions. In addition, 16 fold decreasement in nitrate requirement has made microalga cost-effective and environmentally friendly. Considering the overall results of the research, it was stated that biphasic optimized conditioning was a well lipid amelioration strategy in freshwater green microalgae.

Energy security and the mitigation of the effects of climate change were reported to be the reasons for the increase in demand for biofuels. In this context, while new biomass sources have been discovered, microalgae were reported to be the most promising source. Pugliese et al. (2020) cultivated microalgae in batch conditions in an equipped photobioreactor in the biomass research center laboratory (University of Perugia). The tests were conducted under conditions where the photosynthetic photon flux intensity was 140 $\mu E.m^{-2}\ s^{-1}$ and the temperature was 22 °C. The cultures were characterized for lipid fraction distribution and biomass production. In this research, *Selenastrum capricornutum* was used as a new strain for biodiesel manufacture. In the study, after collecting microalgae and lipid extraction from them, they were transesterified by using methanol/sodium hydroxide solution. After biodiesel production, analysis was carried out on a high-resolution GC to designate the concentration of dissimilar methyl esters. Furthermore, it was observed that the produced fatty acid composition had significant values compared to the most commonly used microalgae. In particular, the concentration of oleic acid was reported to be more compared to the value determined for most of the commonly used microalgae.

5.2.22 Stichococcus

The empirical campaign of autotrophic cultures of *Stichococcus* strains was reported to identify the hopeful strain for biodiesel manufacture. The selected strain, which was *S. bacillaris* 158/11, was cultivated in laboratory-scale bubble colon photobioreactors below semi-continuous and fed-batch circumstances. As nitrogen source, a bold basal media added with sodium nitrate was used. The experiments were conducted under the conditions of an air flow rate in the range of 0.4–4 vvm, the temperature of 23 °C, and 140 μE m^{-2} s^{-1}. Cultures were characterized in periods of total nitrogen concentration, total inorganic carbon, total organic carbon, lipid fraction, biomass, methyl-ester dispersion of transesterified oils, and pH. *S. bacillaris* 158/11 was proven to be the top strain for biodiesel production. The methyl-ester dispersion was characterized by a grand proportion of methyl linolenate, methyl linoleate, methyl palmitate, and methyl oleate as well as phytol. The photosynthetic process yield, which could be defined as the fraction of present light storage as chemical energy, was found to be approximately 1.5%. The tests conducted under semi-continuous conditions revealed that the specific biomass productiveness was about 60 mg_{DM} L^{-1} day^{-1}. The total lipid productiveness was found to be 14 mg L^{-1} day^{-1} at the rarefaction ratio of 0.050 L day^{-1}. According to the results of the research, it was reported that uttermost biomass and total lipid productiveness could be achieved under sunshine (Olivieri et al. 2011).

5.2.23 Tetradesmus obliquus

The alga *Tetradesmus obliquus* has been one of the algae researched for biodiesel manufacture in recent years. In a study carried out in this context, the effect of ferric iron on the lipid production and cultivation of the green microalga *Tetradesmus obliquus* was investigated to obtain biodiesel. The green microalga *Tetradesmus obliquus* was insulated from the freshwater and cultured for 20 days in five kinds of altered BG11 media at the following five ferric iron concentrations: 0, 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4} mmol L^{-1}. The maximum specific growth ratio was observed to be 0.36 ± 0.04 day^{-1} in algae grown in the media which was added with 10^{-2} mmol L^{-1} ferric iron. This stayed in the exponential growth stage for the next 6 days with respect to algae grown in media absent iron addition. In the media added with 10^{-1} and 10^{-2} mmol L^{-1} ferric iron, biomass productivity peaked at 459.20 ± 2.37 mg L^{-1} day^{-1} and oil context peaked at 34.19 ± 1.93% dry weight, respectively. The addition of 10^{-1} mmol L^{-1} ferric iron to the growth media resulted in the highest saturated fatty acids and the minimum unsaturated fatty acids. According to the research findings, the lipid production in the green microalga *Tetradesmus obliquus* may require a two-step process. It was reported that in the first step, it should be discreted from the culture added with ferric iron with a concentration of 10^{-2} mmol L^{-1} to obtain the highest algal biomass. Then, it should be inoculated into the culture added with 10^{-1} mmol L^{-1} ferric iron concentration in the second step. The maximum lipid productiveness was obtained from the green microalga *Tetradesmus obliquus* in the second step. While the quality of biodiesel manufactured from *Tetradesmus obliquus* in all media met the European biodiesel standard of EN14214, it was reported that ferric iron could be supplemented to optimize *Tetradesmus obliquus*'s oil characteristics (Rajabi Islami and Assareh 2019).

5.2.24 Tetraselmis *sp.*

The green marine microalga *Tetraselmis* sp., which has been known to produce lipids, was reported to be a source of biofuel that could be converted to biodiesel. A work was conducted by Teo et al. (2014) to designate the impact of nitrate concentrations of 0.00, 0.10, 0.14, and 0.18 g/L on the growth ratio of *Tetraselmis* sp. In this study, which was carried out using the Nile Red method, the marine microalga *Tetraselmis* sp. was harvested at the exponential stage, and the oil was extracted using the chloroform–methanol solvent. The oil was converted to biodiesel in two ways. The first method was affected by the alkali-based transesterification reaction using NaOH, while the second method was conducted as an enzyme-catalyzed transesterification process, which was the use of immobilized lipase. FAME constituents were detected using GC. Then the results were contrasted with the FAME standard in the way of compliance. Considering the overall results, it was reported that 0.18 g/L nitrate concentration was optimum for growing microalgae biomass. Moreover, the maximum oil context was detected in the test sample without nitrate concentration (0.00 g/L). The yield of biodiesel produced as an outcome of the lipase-catalyzed transesterification process was notified to be seven times more than that of the alkaline-based transesterification process.

In the next process of biodiesel manufacture from microalgae, lipid extraction is a critical step. One of the well-recognized processes for this step is resolvent extraction using admixtures of nonpolar and polar resolvents. Hexane solvent, which is widely preferred in great-scale oil extractions owing to its technic properties, offers economical benefits due to its low cost and loud pickiness, particularly against oils. In a work conducted in this context, extractions using admixtures of hexane and polar solvents were examined in terms of their performance in large-scale lipid extraction processes using microalgae as raw materials to develop a method to improve productivity. The combination of methanol and hexane contributed to the maximum yield of FAME for oils from the alga *Tetraselmis* sp. Impacts of extraction circumstances on extraction efficiency, including the rate of total resolvent volume to dry biomass, extraction time, and methanol-hexane ratios, were evaluated to identify optimized conditions that contribute to higher fatty acid methyl ester and lipid yields. The optimized circumstances were notified as follows: the rate of total resolvent volume to dry biomass, 10 mL/g; extraction duration, 120 minutes; the proportion of hexane to methanol, 1:1. At the end of the research, optimum conditions and the chosen resolvent mixture were applied to great-scale extraction tests with scale-up factors of 10, 50, and 100. The increase in scale-up factors was reported to be almost identical with the fatty acid methyl ester yields of the great-scale extractions. This research concluded that hexane–methanol admixture was a hopeful solvent for great-scale oil extraction from the microalga *Tetraselmis* sp. (Shin et al. 2018).

5.2.25 Tribonema minus

In the literature, there are various studies on biodiesel production from single-celled microalga species with high oil content. However, there are limited studies on biodiesel production from microalga Filamentous oleaginous. The researchers investigated an integrated process of biodiesel manufacture from the filamentous fatty microalgal strain *Tribonema minus*. This microalga was cultivated for 21 days in a 40 L glass panel and harvested by dissolved air flotation (DAF) without any flocculant after the microalgal cells had a lipid content of 50.23%. Then, total oil was extracted from the wet algal paste by subcritical ethanol, and 44.55% of the raw oil was found to be triacylglycerols. For the transformation of crude algal oil into biodiesel, a two-pace catalytic transformation of the pre-esterification and transesterification process was carried out. The conversion rate of triacylglycerols was found to reach 96.52% during the catalysis with 2% potassium hydroxide below the circumstances of methanol/oil molar rate of 12:1, the warmth of 65 °C, and the period of 30 min. The biodiesel produced from *Tribonema minus* was reported to comply with the Chinese National Standards (Wang et al. 2013).

5.2.26 Ulva *sp.*

The marine macroalga *Ulva lactuca* was also studied for biodiesel manufacture. It was notified that the ultrasound-reinforced extraction of lipid obtained from autoclaved algae biomass was influential and provided the highest yield. Optimum conditions for the maximum oil yield by extracting algal biomass were investigated. These conditions were reported to be the moisture content of algae biomass of 5%, solvent:solid rate of 6:1, and biomass dimension of 0.15 mm in 140 min at 55 °C. The n-hexane using co-resolvent methyl tertbutyl ether provided a tall lipid yield respect to another co-resolvents. Then, the extracted lipid was transesterified into biodiesel using silica doped with ZnO as a novel heterogeneous nanocatalyst. The following optimized conditions provided the highest biodiesel yield of 97.43%: reaction temperature of 55 °C, reaction duration of 50 minutes, methanol to oil rate of 9:1, calcination warmth of 800 °C, and catalyst concentration of 8%. The research concluded that the marine macroalga *Ulva lactuca* was a potential resource for biodiesel manufacture (Kalavathy and Baskar 2019).

5.2.27 Studies Using Multiple Algal Biomass Species Together

Yang et al. (2016) conducted a study on combining microalgae growing with advanced wastewater purification and determining the potential for microalgae biomass-based biodiesel manufacture for cost-effective biomass production in photobioreactor (PBR). In the study, the microalga *Scenedesmus obliquus* and microalga *Chlorella vulgaris* were grown in a minor-scale vertical flat-plate PBR added with urban wastewater to simultaneously perform wastewater purification and biomass manufacture for biofuel production. During the process of microalgae cultivation, microalgal nutrient removal and growth were monitored, including total phosphorus (TP), total nitrogen (TN), track elements (Zn^{2+}, Ca^{2+}, Mg^{2+}, and Na^{+}), and total inorganic carbon (TIC). Optimal specific growth rates (μ_{opt}) were found to be 1.39 day^{-1} in the microalga *Chlorella vulgaris* and 1.41 day^{-1} in the microalga *Scenedesmus obliquus*. In addition, total phosphorus and total nitrogen were entirely (>99%) removed from the wastewater in 8 days. Microalgal biomass in the photobioreactor was picked using native flocculant manufactured from the Moringa oleifera seeds. The harvesting yield of Moringa oleifera was found to be 81% and 92% in the alga *Chlorella vulgaris* and alga *Scenedesmus obliquus*, respectively. The following values were calculated in the harvested biomass: (a) the amount of saturated fatty acids: 18.66% in *Chlorella vulgaris* and 28.67% in *Scenedesmus obliquus*; (b) the amount of monounsaturated fatty acids: 71.61% in *Chlorella vulgaris* and 57.14% in *Scenedesmus obliquus*; (c) the amount of polyunsaturated fatty acids: 9.75% in *Chlorella vulgaris* and 11.15% in *Scenedesmus obliquus*. The results of the research revealed that the accumulated fatty acids were appropriate for the manufacture of tall-quality biodiesel with equivalent properties to the biodiesel manufactured from the lipid obtained from crop seeds.

In one study, the symbiotic relation of freshwater microalga species of *Chlorella* sp. HS-2 and *Ettlia* sp. was investigated to increase their biomass productiveness. The species were co-cultivated autotrophically below the Chlorella/Ettlia inoculation rates of 1:16, 1:08, 1:04, and 1:01. Co-cultivation productivity was contrasted with monoculture. Co-cultivation was detected to provide more biomass productiveness ($P < 0.05$) than monoculture at entire inoculation rates. The inoculation rate of 1:08 provided the maximum biomass productiveness, which was 0.70 ± 0.02 g L^{-1} day^{-1}. The biomass productivity was observed to increase to 0.74 ± 0.06 g L^{-1} day^{-1} when the growing mode was switched to the mixotrophic conditions. The biomass productiveness of mixotrophic co-cultivation, which was 0.74 ± 0.06 g L^{-1} day^{-1} ($P < 0.05$), was reported to be higher than that of the mixotrophic uniculture of *Ettlia* sp., which was 0.41 ± 0.06 g L^{-1} day^{-1}, however, it was coequal to that of mixotrophic uniculture of *Chlorella* sp. FACS (fluorescence-activated cell sorter) analysis showed that the biomass acquired after co-cultivation comprised 81% *Chlorella* cells. The biochemical composite of the dry cell weight, co-cultivation (autotrophic) biomass was reported to contain 33% carbohydrates, 11% lipids, 41% protein, and 2% pigments of dry cell weight. It was determined that the main fatty acids obtained by co-cultivation were C16-C18 fatty acids, which were favorable for biodiesel manufacture. The survey was finalized that co-cultivation was again suitable than uniculture application to get a stable biomass composition and high biomass productivity (Rashid et al. 2019). In another work, the biodiesel production potential of the freshwater algae *Micractinium* sp. M-13 and *Coelastrella* sp. M-60 was investigated. In order to increase lipid production and biomass, microalgae were subjected to the following conditions: (a) salinity stress; (b) nutrient starvation such as phosphorus, nitrogen, and iron; (c) nutrient supplements like glucose, vitamin B12, sugarcane industry effluent, and citric acid. The maximum lipid productivity of the isolates was found to be 13.9 $\pm$ 0.4 mg/L/day in the alga *Coelastrella* sp. M-60 and 11.1 $\pm$ 0.2 mg/L/day in the alga *Micractinium* sp. M-13 algae at saltiness stress conditions. The medium added with entire four nutritions was found to show higher lipid productiveness with respect to the control study. The effect of (a) citric acid and (b) sugarcane industry effluent on lipid yield and growth was surveyed using RSM (response surface methodology). The fatty acid profile of the alga *Micractinium* sp. M-13 and alga *Coelastrella* sp. M-60 was reported to be C-18:2, C-18:1, C-18:0, C-16:0, and C-14. Also, it was concluded that their fuel properties met international standards (Karpagam et al. 2015b).

Another study investigated the algae-cork co-culture as an alternate basic material for biodiesel manufacture. The fatty filamentous cork *Aspergillus awamori* was co-cultured with *Chlorella minutissima* UTEX 2219 and *Chlorella minutissima* MCC 27, respectively, in the N11 media containing dissimilar sources of nitrogen and carbon. The lipid and biomass manufacture potential of the *Chlorella minutissima–Aspergillus awamori* co-culture was contrasted with unicultures. Both co-cultures showed significant increases in lipid accumulation and biomass. Potassium nitrate and glycerol were found to be the most effective ones among the supplements of different nitrogen and carbon sources. In the presence of glycerol, co-cultures showed a 3.4–5.1 fold increase in total lipid yield, while they showed a 2.6–3.9 times increment in biomass compared to axenic monocultures. In addition, the major compositions of the co-culture oils were reported to be 31.26–35.02% C16:0 and 21.14–24.21% C18:1 the fatty acids. The research concluded that biodiesel production using this co-culture was a promising strategy (Dash and Banerjee 2017).

A new combinatorial approach integrating carcinogenic and toxic heavy metal arsenic (III, V) reduction due to biodiesel manufacture was adopted by Arora et al. (2017) by using fatty microalgae grown in SSW (synthetic softwater). Four possible microalgae species were tested. Of these species, *Scenedesmus* sp. IITRIND2 and *Chlorella minutissima* were able to tolerate 500 mg L^{-1} of both As (V) and As (III) forms with a loud metal BCF (bio-concentration factor). The results revealed that these strains could be classified as hyper bio-accumulators of arsenic. These arsenic-supplemented microalgae tended to have an important increase in oil manufacture, accumulating great oil dribbles with minimum morphological changes. According to the conclusions of the biochemical composition analysis, there was an important decrease in carbohydrate, protein, and photosynthetic pigments in these microalgal cells. The arsenic taken up by these cells was observed to remodel its cellular constitution to cope up with heavy metal-stimulated loading. With its oxidative stability, loud cetane number, and low chilly flow plugging peculiarities, the biodiesel obtained by using microalgae was reported to be compatible and comparable with herbal oil methyl esters. This remarkable approach offered twofold advantages. The first advantage was the high lipid productivity, and the second was the safe removal of metal arsenic, which has a carcinogenic effect on potable water sources, from water. The study reported that this integrative and innovative technology approach had a strong prospective way in terms of renewable fuel production and being environmentally friendly.

Trace metals, which are vital to microalgae, have been of significant ecological concern when found in high concentrations in habitats. Biodiesel production potential of the microalga *Heterochlorella* sp. MAS3 and microalga *Desmodesmus* sp. MAS1 isolated in the acid-tolerant neutral medium was investigated in synchronous removal of heavy metals like manganese (Mn), zinc (Zn), copper (Cu), and iron (Fe) and growing these microalgae at 3.5 pH. Except for copper, the chosen metals at concentrations of 10–20 mg L^{-1} bolstered the cultivation of both of these strains well. According to the results of the cellular analysis of metal removal, both of the strains seemed to be dominated by the intracellular mechanism. It was determined that Mn and Fe were removed by 40–60% and 40–80%, respectively. In situ transesterification of biomass revealed that the biodiesel efficiency increased when there was an increase in metal concentrations. These acid-tolerant microalgae may be alternative aspirants for sustainable biodiesel and biomass production and simultaneous remediation in settings such as metal-richer acid mine drainages (Abinandan et al. 2019).

The most challenging ways of microalgal biodiesel production have been reported to be the selection of potential alga species and the identification of native alga with high lipid content. For sustainable biodiesel manufacture, hyper lipid-generating algae attracted attention with their maximum triacylglycerols (TAGs) context and preferable fatty acid compound. Therefore, under lipid, cell intensity, triacylglycerols, and fatty acid conditions, a comparative evaluation of *Scenedesmus* sp., *Chlorella vulgaris*, and *Synechococcus* sp. was conducted. A higher biomass efficiency of 0.54 gL^{-1} was acquired in *Chlorella vulgaris* on day thirteen. The highest lipid context was detected to be 36% in *Scenedesmus* sp. which was followed by *Chlorella vulgaris* with 33%. According to the results of the lipidomic analysis, *Synechococcus* sp. had 69% polar lipids content, however, *Chlorella vulgaris* and *Scenedesmus* sp. had higher nonpolar lipids contents, which were 57% and 54%, respectively. In fatty acid profile, C24:0 was dominant with 22.11% in *Chlorella vulgaris*. On the other part, in *Synechococcus* sp. and *Scenedesmus* sp., the predominant ones in the fatty acid profile were C18:2 (22.26%) and C20:0 (31.72%), respectively (Shanmugam et al. 2020). It has been notified that one of the essential factors affecting oil accumulation and growth in microalgae is the cultivation temperature. In this context, the impacts of temperature factor on oil context, fatty acid composition, growth, and biodiesel peculiarities of maritime macroalgae *Tetraselmis suecica* FIKU032, *Nannochloropsis* sp. FIKU036, and *Chaetoceros* sp. FIKU035 were investigated by Chaisutyakorn et al. (2018). These species were cultured at 25, 30, 35, and 40 °C. According to the results of the study, the increment in the temperature resulted in a reduction in biomass, specific growth ratio, and oil context of entire microalgae. In terms of fatty acids in *Tetraselmis suecica* FIKU032 and *Nannochloropsis* sp. FIKU036, the asset of SFAs (saturated fatty acids) decreased with the increase in temperature, unlike PUFAs (polyunsaturated fatty acids). The only species that could grow at 40 °C was found to be *Chaetoceros* sp. FIKU035. In *Chaetoceros* sp. FIKU035, the maximum lipid productivity according to cultivation temperature was found to be 66.73 ± 1.34 mg L^{-1} day^{-1} at 25 °C and 61.35 ± 2.89 mg L^{-1} day^{-1} at 30 °C. The results of the research revealed that the kinematic viscosity, cold filter plugging point, cetane count, and intensity properties of oils acquired from this species met biodiesel standards. Also, *Chaetoceros* sp. FIKU035 was accepted as an appropriate species for obtaining biodiesel in outdoor growing.

Biofuel production industries have been developing using various raw material sources across the world. In this context, in a work conducted by Abomohra et al. (2018), ten species of macroalgae were collected and screened. Because of their high macroalgal biomass manufacture and comparatively high oil context, *Padina boryana*, *Ulva intestinalis*, and *Ulva lactuca* indicated the most important lipids and fatty acid methyl esters areal productivity among the examined species. SAFs (saturated fatty acids) indicated slight differences in the chosen species with PUFAs (polyunsaturated fatty acids) context of 4.2 and 3 times significantly higher in macroalga *Ulva lactuca* compared to macroalga *Ulva intestinalis* and macroalga *Padina boryana*, respectively. The increment in PUFAs was found to be due to the higher content of C18:4n-3, C18:3n-3, and C16:4n-3. *Padina boryana* was found to provide a neutral lipid concentration of 37.7 mg g^{-1} cellular dry weight (CDW), which was 46.7% of the total fatty acids, by lipid fractionation. This value was significantly higher than the neutral lipid fractions of macrolgea *Ulva lactuca* and *Ulva intestinalis*, which were 16% and 17%, respectively. It was reported that the biodiesel properties of the macroalgae examined within the scope of the study met the requirements of international standards. Moreover, when the carbohydrate content is high in the lipid-free remnant biomass, it can be readily transformed to fermentable sugars and biogas. The research proved that macroalgae have been an alternate raw material for the manufacture of biodiesel and other biofuels.

In a work conducted to get maximum oil yield from algae, oil extraction was fulfilled using marine algae *Enteromorpha compressa*, *Ulva lactuca*, and *Gracilaria edulis*. Characterization of algal biomass was performed by Fourier transform-infra red spectroscopy and scanning electron microscopy. In order to obtain the highest oil yield by extraction, six different pretreatment methods were applied to determine the best method. High oil yield was achieved by optimizing the extraction parameters to the following values: solvent/solid rate of 6:1, the particle dimension of 0.10 mm, duration of 150 min, temperature of 55 °C, and agitation rate of 500 rpm. After optimization, oil extraction yield in algae biomass was found to be 9.5%, 12.18%, and 10.50 (g/g), respectively, for the studied algal biomasses. The constant rate for the extraction was acquired as first-order kinetical by using the differential method. Resolute subcellular Cal A and Cal B lipase production of recombinant *Pichia* *pastoris* was established, and it was used as a biocatalyst in the manufacture of biodiesel. Comparison analysis of the lipase activity and biodiesel efficiency was conducted using immobilized Candida antarctica lipase (Bharathiraja et al. 2016).

A study investigating the phycoremediation of wastewater and biodiesel production was carried out by Amit and Ghosh (2018). In this study, microalga strains *Spirulina* sp., *Scenedesmus abundans*, *Nostoc muscorum*, and *Tetraselmis indica* were investigated for sustainable biodiesel manufacture and phycoremediation of wastewater. The algae used in the research were cultivated in photobioreactors with a bulk of 1000 mL and a studying bulk of 750 mL, on four different wastewaters for 14 days under the light density of 94.5 μmol m^{-2} s^{-1}. These microalgal strains showed the highest biomass yield in the STS (secondary treated sewage). In the STS, the highest biomass efficiency of 0.6533 g L^{-1} and the highest oil yield of 25.44 mg L^{-1} day^{-1} were reached for *Tetraselmis indica*. *Tetraselmis indica* removed 60.90–65.97% of phosphate, 63.6–78.24% of nitrate, 71.16–85.70% of TOC, and 61.01–80.01% of ammonia nitrogen in entire four wastewaters. The FAME profile of the microalgal strain *Tetraselmis indica* revealed the entity of myristic acid (1.2%), oleic acid (34.59%), eicosanoic acid (14.88%), linoleic acid (12.38%), palmitic acid (10.32%), and pentadecylic acid (0.28%), in the STS. This research revealed that *Tetraselmis indica* was the best appropriate microalgal species among the microalgal strains of *Scenedesmus abundans*, *Tetraselmis indica*, *Spirulina* sp., and *Nostoc muscorum* for phycoremediation of wastewaters and higher biomass yield for sustainable biodiesel manufacture.

5.3 Conclusions and Future Prospects

Energy is important for ensuring sustainability in economic growth. The increment in the energy request in the world has increased the use of non-renewable fossil fuels, which brought about environmental pollution issues. Therefore, the attraction of environmentally friendly sustainable biofuels as alternative sources has increased. Biodiesel, one of the biofuels, solves the combustion emission problem. In this chapter, a literature review was presented including the recent studies on algal biomass yield, lipid productivity, wastewater treatment, and biodiesel production. Although various raw materials are used in biodiesel production, algal biodiesel production seems promising. The high oxygen content of biodiesel compared to petrodiesel supports early combustion. Biofuels contribute to the reduction of toxic emissions such as unburned hydrocarbons, CO, CO_2, and soot during the combustion process. Moreover, the treatment of domestic wastewater containing various inorganic and organic pollutants by algae species and using the obtained algal biomass for biodiesel production will be both an environmentally friendly and economical approach. According to the studies in the literature, algal biofuel manufacture is in its infancy due to the declining economic profile worldwide. In this context, further research on determining the biofuel potential of unexamined algae is recommended to determine the sustainability line of the process more clearly and for the highest biodiesel productivity.

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Chapter 6
Various Applications to Macroalgal and Microalgal Biomasses for Biohydrogen and Biomethane Production

Nesrin Dursun

Abstract Studies of alternative fuels with renewable properties are increasingly being conducted worldwide owing to the depletion of fossil fuels. In researching these fuel options, care is taken to ensure that they are environmentally friendly fuels. The fact that after combustion, only water vapour is produced from biological hydrogen which is indirect proof that biohydrogen is an environmentally friendly biofuel that does not pollute the air. It is also known that biological methane has a reducing effect on greenhouse gas emissions. For this reason, researchers have pointed out the importance of biogas. Researchers have targeted cheap, easily accessible waste or residual biomass for sustainable and environmentally friendly fuel in this context. While competition between first-generation carbohydrate-rich food and second-generation lignocellulosic biomass continues, researches on third-generation algal biomass with (a) rapid growth, (b) high biomass production, and (c) high photosynthetic efficiency have accelerated. Current applications of algal biomass for biohydrogen and biomethane production using macroalgae and microalgae-based grouping are examined in this section.

Keywords Algal biomass · Biofuel · Biogas · Production · Pretreatment

6.1 Introduction

Biofuels, which cost less than fossil fuels, have the latent to cushion dependence on non-renewable fossil fuels (Zittelli et al. 2013; Chen et al. 2015). However, depending on the raw materials used in their production, they may require wide area of lands and competition for food production. The advantages of biofuels is that they do not affect arable land and compose little or no greenhouse gas emissions (Zittelli et al. 2013; Sikes et al. 2011). While competition continues between

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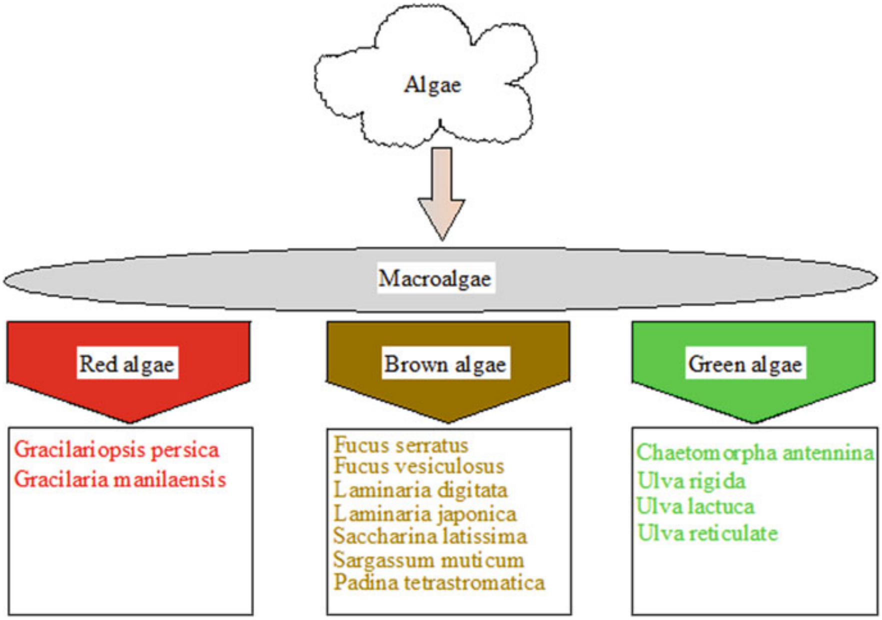

Fig. 6.1 Classification of macroalgae according to their pigmentation and some species

first-generation edible products, carbohydrate-based feedstocks, and second-generation lignocellulosic biomass, researchers have focused on third-generation biomass such as algae (Chen et al. 2015). Because of their rapid growth, efficient use of nutrients from wastewater, including phosphorus and nitrogen, high photosynthesis efficiency, and high biomass production, algae used as raw materials are commonly used in fuel production technologies (Chen et al. 2015; Kim 2015). Algae can be grown on unused or barren land. Biofuel production using algae has focused on one-stage techniques such as biogas production from macroalgae and the use of microalgae (Chen et al. 2015). Algae have carbohydrates, fat, and protein. Algae with a high carbohydrate content can be converted into hydrogen and methane and processed using biochemical conversion. Biochemical processes can be grouped into anaerobic digestion and fermentation (Kim 2015). Furthermore, algae that can be considered biofuel sources can be forked into two groups according to their extent: macroalgae and microalgae. According to their pigmentation, macroalgae are also divided into three groups, red, brown, and green algae, as shown in Fig. 6.1. On the other hand, microalgae are divided into three groups according to their pigmentation, as shown in Fig. 6.2.

With the movement of water in macroalgae, gases are transferred to the surface and the dissolved nutrients are distributed. In addition, increased water movement has been reported to increase the rate of photosynthesis and thus support growth. Hydrodynamic forces can separate or damage the thallus of algae from the substrate when water movement is rapid. When water movement is slow, growth may be

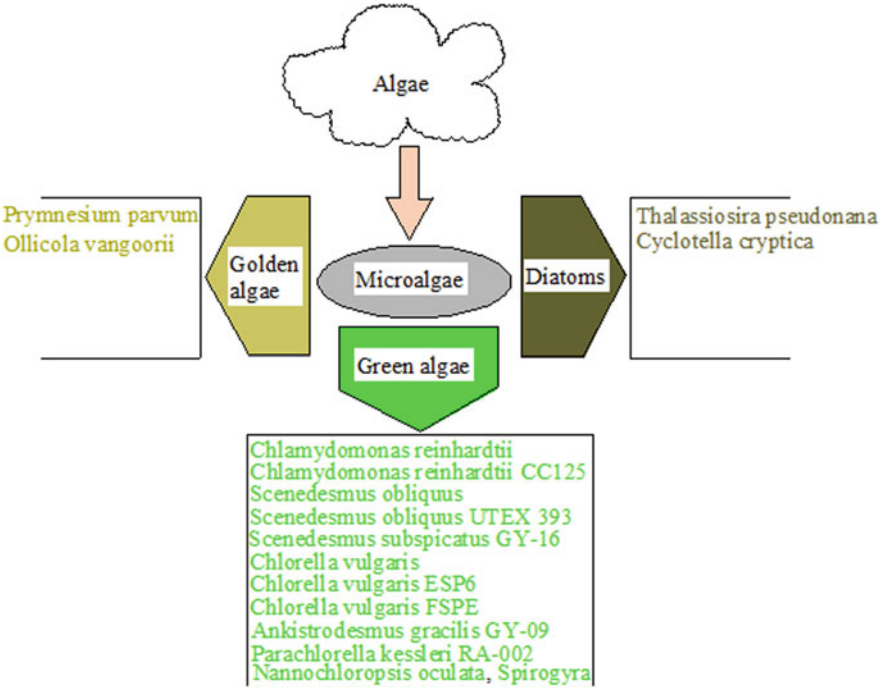

Fig. 6.2 Classification of microalgae according to their pigmentation and some species

nutrient-limited. In addition, photosynthesis may be limited by the mass transfer of carbon and other essential nutrients (Stewart and Carpenter 2003). Among macroalgae, red algae grow mainly in the intertropical regions, brown algae in cold waters, and green algae in all types of aquatic environments. The average photosynthetic efficiency was reported as 6–8%, higher than territorial biomass, which is 1.8–2.2% (Chen et al. 2015). Compared to terrestrial plant biomass, macroalgae reportedly contain no lignin and very little cellulose and therefore are more easily hydrolysed. Because the biochemical content of marine algae can vary with the seasons, so does the gas yield. Gas yield is related to both stored carbohydrate level and ash content. Furthermore, the C:N ratio is critical for optimizing the digester regime. In this context, the co-digestion of nitrogen (N)-rich agricultural sludges and food waste substrates, and marine algae strengthens the argument. Biogas yield also depends on many variables, such as the configuration of the digester process, the inoculum, and the composition of the feedstock (Hughes et al. 2012). According to life cycle assessment (LCA), macroalgae may generate 11,000 MJ/t of net energy from bare algae, compared to 9500 MJ/t from microalgae gasification. Compared to microalgae, macroalgae are wort-like and on wort property in multicellular, making them easy to harvest (Chen et al. 2015). Environmental effects of marine algae in large-scale areas include (a) changes in nutrient availability in the water column and seabed shading in shallow areas, (b) benthic effects due to increased supply of organic matter, (c) sedimentation patterns due to local hydrodynamic changes (Hughes et al. 2012).

Microalgae can accumulate fat and fermentable carbohydrates. It can also synthesize some valuable by-products that can be commercialized to integrate in feed and food, such as proteins, polyunsaturated fatty acids (PUFAs), and vitamins. Finally, (a) all algal biomass and (b) residues from the extraction of carbohydrates, oil, or other specific products for biogas production can be anaerobic digested and gasified to outturn syngas (Zittelli et al. 2013). Microalgae, used as a third-generation biofuel raw material, offer a significant advantage because they can be grown in salt water or brackish water and on land unsuitable for agriculture (Zittelli et al. 2013; Murphy et al. 2015). The productivity of algae cultures based on the supply of CO_2, which is why they need to be supplied with CO_2. The need to dissolve significant amounts of carbon dioxide (CO_2) in the growth medium, perceived as a false advantage, results from algae cultures expensive and energy-intensive requirements (Zittelli et al. 2013). The use of sea algae as a biofuel is essential to eliminate the sea algae thrown onto beaches and the resulting unpleasant smell, improve the aquatic environment, reduce CO_2 associated with energy production, and reduce nitrogen pollution of the water (Murphy et al. 2015). Microalgae (a) require less freshwater compared to conventional farming methods so that they can be grown in arid regions, (b) are less affected by seasonal changes, (c) can support wastewater treatment by using nutrients in wastewater for their growth, (d) can fix CO_2 coming from various sources and reduce available CO_2 (Chen et al. 2015; Zittelli et al. 2013).

Current research on biohydrogen and biomethane generation using macroalgae and microalgae-based groups for biogas production, as well as anaerobic digestion and fermentation applications, is presented in this section. There is a wealth of data in the literature on the potential biohydrogen and biomethane production from algae. However, research on microalgae has focused on the production of biohydrogen and biomethane from green algae. According to the studies conducted in this context, no studies on diatoms and golden algae were found in the literature.

6.2 Biohydrogen Production from Algae

Biological hydrogen manufacture can be accomplished by various methods, including extraction, chemical, and ultrasonic treatment. Biological hydrogen production using degreased algae cake and enriched acidogenic consortium as potential feedstock after lipid extraction was studied in one of the researches conducted with the acidogenic consortium. When compared to other conditions, algae pretreatment extraction (AP-E), cumulative hydrogen production (CHP), specific hydrogen yield (SHY), and maximum hydrogen production rate (HPR) are essential for waste recovery, with a high substrate degradation of 65% in terms of COD elimination productivity. The consortium produced a noticeable quantity of volatile fatty acids (VFAs) in addition to substrate removal and biohydrogen generation. The production of VFA during fermentation resulted in a drop in pH in the reactor. The research determined that using degreased algal biomass as a feedstock for biological hydrogen generation is feasible (Venkata Subhash and Venkata Mohan 2014). In another study, algae and sewage sludge were fermented together to increase hydrogen production. For this purpose, the influence of Fe^{2+} on the co-fermentation processing was investigated. With the addition of 600 mg/L Fe^{2+}, 2.15 times the sole fermentation of sludge, 2.00 times the sole fermentation of algae, and 1.87 times the co-fermentation absent Fe^{2+} adjunct, the highest biohydrogen manufacture was obtained by the co-fermentation group with 14.8 mL H_2/g VS_{added} (28 mL/100 mL). The co-fermentation process stimulated both protein degradation and volatile solids. Upon examining the microbial analysis results, it was found that the co-fermentation clique with the adjunct of Fe^{2+} contained enriched *Terrisporobacter*, *Clostridium sensu stricto 13*, and *Clostridium tertium* bacteria which had an affirmative impression on the cumulative biological hydrogen production. Both Fe^{2+} addition and co-fermentation were found to support hydrogen generation in the study. However, it has been found that the presence of Fe^{2+} and the co-fermentation of algae and sludge can significantly increase biological hydrogen production (Yin et al. 2021).

In a study by Kim et al. (2021) in which hydrogen production by pretreatment of algal biomass was investigated, the impression of magnetic Fe_3O_4 addition on biohydrogen production by anaerobic fermentation using extracted marine biomass and *Clostridium butyricum* DSM 10702 was examined below the possible complex sugar compound and several toxic substance concentrations. Extraction of marine biomass with varying sugar content, from which tall added-value energy can be derived, products by-produces such as levulinic acid, formic acid, and 5-hydroxymethylfurfural. In this context, the utility of conductive bearer was investigated to surpass the inhibition of hydrogen production by using by-products. The presence of each toxic material, including levulinic acid, formic acid, and 5-hydroxymethylfurfural (5-HMF) organic acids, had a different inhibitory effect on hydrogen production. Biological hydrogen production by magnetic addition was higher than nonmagnetic at all toxicant concentrations. Under mixed sugar conditions, where metabolic delay may occur, the addition of magnetic Fe_3O_4 was situated to increase total hydrogen manufacture by 64%. The hydrogen manufacture efficiency was determined with 1.7 mol-H_2/mol of sugar consumed, and the maximum manufacturing ratio was 15.5 mL/h. Magnetic supplementation using marine biomass hydrolysate was found to enhance hydrogen production under inhibitory conditions. In addition, magnetic supplementation resulted in more than 20% increased hydrogen production at lower concentrations than levulinic acid, formic acid, and 5-hydroxymethylfurfural (5-HMF) for 2, 3, and 2 g/L, respectively, irrespective of the sort of toxicant. Another study in which 5-HMF was formed as a by-product was conducted by Park et al. (2011) by studying the red alga *Gelidium amansii*. The feasibility of biological hydrogen production was investigated for the red alga *Gelidium amansii*. The chief sugar monomer in red algae, galactose, is easily transformed to biohydrogen by dark fermentation. A maximum biohydrogen production ratio of 2.46 L H_2/g VSS/day and a galactose outturn of 2.03 mol H_2/mol $galactose_{added}$ were determined. These results were higher than the values of

1.48 mol H_2/mol galactose$_{added}$ and 0.914 L H_2/g VSS/day addition determined for glucose. The distribution of soluble products indicated that biohydrogen manufacture is the most basic stage in galactose uptake. The cause of non-competitive inhibition in the hydrogen fermentation process was reported as 5-HMF, resulting from acid hydrolysis of red algae. At 1.37 g/L, 5-HMF reduced biohydrogen manufacture by 50% collated to the check. When red algae were hydrolysed at 150 °C for 15 min and detoxified with activated carbon, 53.5 mL of hydrogen production was determined from 1 g of bare algae with a biohydrogen production ratio of 0.518 L H_2/g VSS/day. The study found that red algae grown in large marine areas with sunlight and without nitrogen fertilizers are suitable for biological hydrogen production. A new inoculum was tested in another study to minimize the inhibition of 5-hydroxymethylfurfural (5-HMF). In this study, a new inoculation method is proposed to reduce the inhibition of 5-HMF. Acidic algal hydrolysate at a rate of 1.5 g 5-hydroxymethylfurfural/L and 15 g hexose/L hexose was fed into the partly packaged continuous fixed-bed reactor containing hybrid-immobilized beads. Due to reduced inhibition by 5-HMF and increased biofilm formation, the inoculation method promoted high biohydrogen production. The furthest hydrogen manufacture was achieved at 6 h HRT. The biological hydrogen production ratio was determined as 20.0 ± 3.3 L H_2/L-day and the biohydrogen yield as 2.3 ± 0.4 mol H_2/mol hexose$_{added}$. Real-time quantitative polymerase chain reaction analysis showed that *C. butyricum* bacteria accounted for 94.3% of the sum bacteria (Anburajan et al. 2019).

Some hydrogen production standards have been tested in some studies. In one of these studies, algal biomass of *Chlamydomonas reinhardtii* and cellular Nicotinamide Adenine Dinucleotide Phosphate (NADPH) fluorescence and the β-NADPH standard were examined as a possible indicator of biological hydrogen production. NADPH fluorescence was used to compare cultures educated in TAP-S (Tris-acetate-phosphate minus sulfur), TAP +3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), and TAP (Tris-acetate phosphate). Hydrogen production from sulfur depletion correlated directly ($r = 0.941$) with Nicotinamide Adenine Dinucleotide Phosphate during the 10 days. The adjunct of leachate was intended to multiply hydrogen efficiency; this application increased Nicotinamide Adenine Dinucleotide Phosphate concentration by 50–70%. It was found that there is a direct correlation ($r = 0.929$) between Nicotinamide Adenine Dinucleotide Phosphate and hydrogen when the medium mixed with leachate is used. The electron acceptor in the photosynthetic chain, Nicotinamide Adenine Dinucleotide Phosphate, plays a critical role in hydrogen synthesis as a bearer molecule. The study discovered that cellular NADPH fluorescence could be used to indicate hydrogen production in sulfur deficiency (White et al. 2014). In another study, algae isolated in a freshwater pool were examined for hydrogen production. In a freshwater pond in the Pathumthani province of Thailand, unicellular green algae capable of producing hydrogen were isolated and examined under a light microscope, and the algae were found to belong to the species *Tetraspora*. *Tetraspora* sp. CU2551 was found to have the briefest doubling time when grown in TAP (Tris-acetate-phosphate) medium at 36 °C and 48–92 μE/m^2/s light intensity. An increase in hydrogen production was observed when pH increased from 5.75 to 9.30. The adjunct of 0.5 mM b-mercaptoethanol to the medium Tris-acetate-phosphate expostulated hydrogen production by nearly twofold. Using both sulfur and non-nitrogen-containing TAP media resulted in a 50% rise in biohydrogen production in the hydrogen generation phase. Under sulfur and nitrogen-free conditions, expostulation of hydrogen generation with 0.5 mM b-mercaptoethanol occurred at a light intensity of fewer than 5 μE/m^2/s with no adverse impacts on the cells. The maximum hydrogen production was determined to be 17.3–61.7 μmol/mg Chl a/h, and it was found to have a sky-high production ratio compared to another green algae (Maneeruttanarungroj et al. 2010).

Sivagurunathan et al. (2018) handled a new view on the combined use of macro and microalgae for biological hydrogen production. This study aimed to digest microalgal and macroalgal biomass together to enhance hydrogen production. The biomass of the red macroalga *Gelidium amansii* and the green complex microalgal biomass were studied by mixing at a rate of 8:2, with an first substrate concentration of 10 g/L and different addition rates of the inoculum of 3–15% (v/v). Thus, the aim was to effectively digest the algal biomass and determine the appropriate substrate-inoculum ratio. In the study, digestion with the addition of 6% inoculum and 45 mL/g dry biomass resulted in tall hydrogen ingredient of 24% in the gas grade, indicating that this result represents the maximum hydrogen output. Middle hydrogen ingredient in the backlash of 17–22% was observed in other conditions studied. The research conclusions signified that anaerobic co-digestion of microalgae and macroalgae biomass is required for enhanced hydrogen production with initial biomass loading of 6%.

Below is a detailed description of current research on biological hydrogen production by macroalgae and microalgae.

6.2.1 *Biohydrogen Production from Macroalgae*

Biomass species with high carbohydrate content are commonly used in biogas production studies. Accordingly, Table 6.1 shows the chemical compound of some macroalgae used as biomass.

Although marine algae provide an alternative source of bioenergy, their polymeric nature requires them to be pretreated with dilute acid hydrolysis before fermentation. In this study, the check versions of batch dilute acid hydrolyses of algae biomass were optimized by dark fermentation process. The powdered *Gelidium amansii* biomass was hydrolysed at 120–180 °C with a solid/liquid (S/L) ratio of 5–15% (w/v) and sulfuric acid (H_2SO_4) concentrations of 0.5–1.5% (w/w) and then used in a batch biohydrogen fermentation. It was found that the furthest hydrogen production ratio was 0.51 L H_2/L/h in 37.0 mL H_2/g dry biomass at a hydrolyses temperature of 161–164 °C, 0.50% H_2SO_4, and an S/L ratio of 12.7–14.1%. Research results showed that optimized hydrolysis with dilute acid increased the feasibility of hydrogen fermentation of red algal biomass from *Gelidium amansii* (Park et al. 2013). In another study, using only acid pretreatment

Table 6.1 Chemical compound of some macroalgae (% dry matter) (Syrpas and Venskutonis 2020)

Species	Carbohydrates	Lipids	Proteins
Anadyomene brownii	25.8	6.2	9
Boergesenia forbesii	21.38	11.42	7.43
Caulerpa peltata	45	11.42	6.41
Chaetomorpha antennina	27	11.45	10.13
Gracilaria gracilis	46.6	0.60	20.2
Microdictyon agardhianum	27	9.4	20.93
Osmundea pinnatifida	32.4	0.9	23.8
Saccorhiza polyschides	45.6	1.1	14.44
Sargassum muticum	49.3	1.45	16.9
Ulva lactuca	11.6–13.2	9.6–11.4	11.4–12.6
Ulva reticulate	16.88	8.5	12.83
Valoniopsis pachynema	31.5	9.09	8.78

as a pretreatment application, hybrid immobilized cells were used as a microbial catalyst by pretreating the red alga *Gelidium amansii* with 2% H_2SO_4 dilute acid and studying its conversion to biological hydrogen by anaerobic fermentation in a continuous stirred tank reactor (CSTR). The process was examined for 85 days at 15 g/L hexose equivalent feed concentration, 24 and 16 h hydraulic retention time, to evaluate the continuous system stability and hydrogen production performance. In the 24 h hydraulic retention time, the highest hydrogen production ratio was 2.7 L/L/day, and the hydrogen yield was designated to be 1.3 mol/mol substrate $\text{hexose}_{\text{added}}$. The hydrogen production ratio was determined to be 1.8 L/L/day during the 16 h hydraulic retention time, and the hydrogen yield was 0.7 mol/mol substrate $\text{hexose}_{\text{added}}$, resulting in an important drop in hydrogen production. Bacterial community analysis was characterized by 454 pyrosequencing, and *Firmicutes* was represented by over 98% at the 24-h hydraulic retention duration, while *Proteobacteria* was the dominant population at 84% at the 16-h hydraulic retention duration. The research results show that hydraulic retention time significantly affects the composition of the prevailing microflora, so it is vital to control hydraulic retention time as a prerequisite for influential hydrogen production (Kumar et al. 2018).

There are studies in the literature that combine microwave pretreatment with other pretreatment applications. One of these recent studies was conducted by Kumar et al. (2019a). This study investigated the enhancement of biological hydrogen production from the marine macroalga *Ulva reticulate* subjected to induced microwave pretreatment (AHMW) by acidic H_2O_2. Microwave (MW) pretreatment resulted in a highly soluble chemical oxygen demand (SCOD) release of 1450 mg/L and a liquefaction ratio of 30.2% for a pretreatment time of 15 min. To increase the organic release caused by H_2O_2 during microwave pretreatment (HMW), H_2O_2 concentrations between 0.003 and 0.03 g/g TS were investigated at 40% optimal microwave power. The maximum liquefaction ratio of 33.9% was achieved at an hydrogen peroxide concentration of 0.024 g/g TS. Microwave pretreatment was carried out when the pH was in the range of 4–6.5. Microwave pretreatment induced with acidic hydrogen peroxide (H_2O_2) at optimum pH of 5.0 was found to release 1850 mg/L SCOD with 38.5% liquefaction in 10 min. Compared to HMW and MW, the results showed that AHMW pretreatment considerably reduced pretreatment time and enhanced liquefaction. The highest biological hydrogen production with the AHMW pretreatment was 92.5 mL H_2/g COD. The same macroalgae *Ulva reticulate* biomass was pretreated in another study by Kumar et al. (2020). Hydrogen peroxide (H_2O_2) under alkaline circumstances and microwave pretreatment were applied to marine macroalgal biomass *Ulva reticulata* to improve hydrogen production. The COD solubilization rate was determined to be 27.9% by pretreating macroalgal biomass at optimal microwave power for 15 min. When combined with hydrogen peroxide (microwave–H_2O_2 disintegration (MH)), this optimal microwave power increased COD solubility of 24 mg H_2O_2/g macroalgae dose. When microwave and hydrogen peroxide (microwave–H_2O_2 under alkaline (MHA)) were combined under alkaline conditions at pH 7–12, the results were superior to MH. Under optimal alkaline conditions, where the pH is 10, MHA pretreatment was found to have a solubility of 34% COD. Microwave application under alkaline conditions resulted in increased OH radical synthesis and H_2O_2 decomposition. In addition, this synergistically enhances dissolution. The MHA processes significantly reduced the time and specific energy required to degrade macroalgal *Ulva reticulata* biomass. When comparing the samples, the highest H_2 yield for MHA was 87.5 mL H_2/g COD. Yin and Wang (2018) conducted a study in which fermentative biohydrogen production from algal biomass can be enhanced by suitable pretreatment. In this context, the macroalgae *Laminaria japonica* was decomposed using a combined microwave–acid pretreatment, and batch hydrogen production was examined using dark fermentation. Proteins and polysaccharides are consumed in the fermentation process. The results obtained by simultaneously applying microwave pretreatment at 140 °C and 2450 MHz with 1% sulfuric acid (H_2SO_4) acid pretreatment for 15 min showed that *Laminaria japonica* cells were effectively degraded, organic matter was released, soluble chemical oxygen demand (SCOD) concentration was increased 1.92-fold and 5.12 g/L was reached. Proteins and polysaccharides are consumed in the fermentation process. It was found that acetate-like fermentation dominates in hydrogen production. Moreover, the domination of the genus *Clostridium* contributed to the efficiency of biohydrogen production. After pretreatment, the hydrogen yield augmented from 15 mL/g TS_{added} to 28 mL/g TS_{added}. It was found that the energy transformation efficiency augmented from 9.5% to 23.8%. The application of a combined microwave–acid pretreatment of the macroalgae *Laminaria japonica* biomass was found to be important in increasing biohydrogen production. The biomass of the marine macroalgae *Chaetomorpha antennina* was investigated in a study with the support of microwave pretreatment and surfactants to increase and improve biological hydrogen production with the support of surfactants (ammonium dodecyl sulfate) supported by surfactant helped microwave disintegration. Microwave disintegration was performed by varying the energy density in the range of 10–70% for a cycle of

0–30 min. Microwave disintegration showed a solubility rate of 14.6% and the highest dissoluble organic release of 1.260 mg/L within 15 min. At optimum MD power, the soluble organic release was increased by 40% to 1.490 mg/L with the help of surfactants. When 0.0035 g/g TS was dosed, maximum solubilization of 17.3% was obtained. Higher biohydrogen production of 74.5 mL/g COD was obtained when surfactant-aided microwave disintegration (SMD) was applied. Therefore, the use of SMD was found to reduce the pretreatment time and increase the organic release respect to microwave disintegration (Kumar et al. 2019b).

Margareta et al. (2020) conducted one of the current investigations in which acid pretreatment is combined with thermal, alkali, or ultrasonic pretreatment. The goal of the study was the effective release of fermentable sugars by a combined pretreatment of green macroalgal biomass (*Ulva* sp.) with a small amount of acid and heat. Hydrolysis achieved the best efficiency among the H_2SO_4 acid concentrations studied, with 0.21 g RS/g biomass reducing sugar yield at 4% H_2SO_4 concentration at 121 °C for 40 min. The concentration of furfural and 5-hydroxymethylfurfural, which are fermentation inhibitors, was less than 1 g/L. From *Clostridium butyricum* CGS5 bacteria used as the first reducing sugar concentration of 12 g/L and pH of 5.5, the highest hydrogen efficiency was determined to be 208.3 mL/L/h, the maximum cumulative biohydrogen production was 2340 mL/L, and the hydrogen yield was 1.53 mol H_2/mol RS. In the study that used continuous fermentation, the maximum hydrogen efficiency increased to 782.45 mL/L/h in 6 h of hydraulic retention duration. In addition, the yield of 1.52 mol H_2/mol hexose hydrogen was detected. Another research examined algal biomass for biohydrogen production using acid, heat, alkali, and ultrasonic pretreatments. In this research, fermentative biohydrogen production by anaerobic complex bacteria using the macroalga *Laminaria japonica* was investigated. The saccharification efficacy and hydrogen production of *Laminaria japonica* alga were examined using acid, heat, alkali, and ultrasonic pretreatment in the study. The saccharification efficiency of *Laminaria japonica* was highest in acid pretreatment, and the saccharification efficiency for reducing total sugars was 350.54 ± 19.89 mg/g. Cumulative hydrogen production was determined as 66.68 ± 5.68 mL/g in heat pretreatment *of Laminaria japonica*, 43.65 ± 6.87 mL/g in acid pretreatment of *Laminaria japonica*, 15.00 ± 3.89 mL/g in alkaline pretreatment of *Laminaria japonica*, 23.56 ± 4.56 mL/g in ultrasonic pretreatment of *Laminaria japonica*, and 10.00 ± 1.21 mL/g in the control sample. In addition, the impacts of initial pH and substrate concentration on hydrogen production in thermally pretreated *Laminaria japonica* were investigated. The research results evinced that at the first pH of 6.0 and a substrate concentration of 2%, a hydrogen concentration of 28.4% and a maximum biohydrogen production of 83.45 ± 6.96 mL/g were obtained from the heat-pretreated macroalgae. Moreover, it was found that *Laminaria japonica* used as substrate only by heat pretreatment was the best efficient method to increase fermentative biohydrogen production (Liu and Wang 2014).

Chemical pretreatment of macroalgal biomass may (a) destroy microbial inhibitors, (b) increase the accessibility of process microorganisms to the substrate, (c) increase product yields. The study aimed to remove phenolic content and increase biological hydrogen production by applying various chemical pretreatment procedures to *Padina tetrastromatica* algae. In the study, various mineral acids (H_2SO_4, HNO_3, and HCl) and bases (KOH and NaOH) were used for efficient pretreatment of algae. Application of diluted sulfuric acid to macroalgal biomass resulted in a maximum cumulative biological hydrogen production of 78 $\pm$ 2.9 mL/0.05 g VS and a decrease in phenolic ingredient of 1.6 $\pm$ 0.072 mg gallic acid equivalent (GAE)/g. Using response surface methodology, the optimization of three pretreatment variables, namely acid concentration, substrate concentration, and reaction duration, was investigated. After optimizing the pretreatment conditions, the phenol ingredient was reduced to 0.06 mg gallic acid equivalent (GAE)/g, and an improvement in biological hydrogen production was observed. The constructional change owing to pretreatment was determined by XRD and FTIR analyses. It was found that the dilution of sulfuric acid and pretreatment can effectively remove the phenolic content and increase biological hydrogen production (Radha and Murugesan 2017). Some searches have examined the impact of the addition of elements on hydrogen production. In this context, the research conducted by Yin and Wang (2019a) surveyed the enhancement of biohydrogen production from macroalgae with Fe^{2+} adjunct. At 400 mg/L Fe^{2+} supplementation, the maximum hydrogen efficiency of 19.47 mL/g VS_{added} was determined to be 6.25 times that of the control experiment. Microbial distribution, substrate degradation, and metabolite formation analyses were performed. In the analysis results, 67.2% *Clostridium butyricum* and 24.2% *Ruminococcus gnavus* were the predominant species in the group to which Fe^{2+} was added, affecting hydrogen production and volatile organic acid accumulation. In the group without Fe^{2+} addition, 29.0% *Exiguobacterium* sp., 24.5% *Acinetobacter lwoffii*, and 23.4% *Clostridium stricto* 13, species were predominant. Thus, higher efficiency was obtained in both mineralization and hydrolysis of biomass. Fe^{2+} has been found to perform a significant role in the fermentation process of macroalgae by affecting the structure of the microbial community and subsequently altering metabolic pathways.

In another study, the effect of nanoparticles on fermentation was examined. The impacts of Fe^0 NPs (Fe^0 nanoparticles) on macroalgae fermentation were investigated in this context. With the adjunct of 200 mg/L Fe^0 NPs, 20.25 mL H_2/g VS_{added} was obtained, and when compared with the control test, it was determined that hydrogen production increased 6.5 times. In evaluating substrate conversion, both acid accumulation and hydrogen formation were supported by the adjunct of Fe^0 NPs. The results of the microbial analysis showed that with the addition of Fe^0 NPs, both the hydrogen-producing strains of the genus *Terrisporobacter* sp. and *Clostridium*, which are suitable for acid emergence, were enriched, and the species *Acinetobacter lwoffii*, which was beneficial for organic mineralization, was eliminated. After analysing the research findings, it was determined that Fe^0 NPs influence microbial dispersion and play a key role in macroalgae fermentation (Yin and Wang 2019b).

Although algal biomass is treated differently in almost all studies, few studies examine biological hydrogen production without pretreatment. In this context, the fermentative hydrogen production potential of the brown algae *Laminaria japonica* was examined below mesophilic conditions of 35 ± 1 °C absent any pretreatment. Initially, the applicability study was carried out in a row of batch systems. At a carbohydrate-based substrate concentration of 20 g COD/L, the hydrogen efficiency was 71.4 mL H_2/g TS, with 0.92 mol H_2/mol hexose$_{added}$ at the first pH of 7.5 and a cultivation pH of 5.5. Following that, the hydraulic retention time (HRT) was 6 days, and the anaerobic sequential batch reactor (ASBR) was continuously operated for 80 days. After nearly 30 days of operation, a steady hydrogen efficiency of 0.79 ± 0.03 mol H_2/mol hexose$_{added}$ was determined (Shi et al. 2011).

6.2.2 Biohydrogen Production from Microalgae

Figure 6.3 shows the generalized structure of a potential biomass microalgae cell in biogas production studies.

Biological hydrogen production from microalgae was carried out based on various strains, different pretreatment applications, and some parameters. The bacteria *Clostridium butyricum* DSM 10702 and *Enterobacter aerogenes* ATCC 13048 were examined for biological hydrogen production exploiting *Scenedesmus obliquus* microalgal biomass as a substrate for fermentation in one of the strain-based studies. The quantity of biological hydrogen produced by every strain was examined in both wet (69% moisture) and dried (5% moisture) biomass of *Scenedesmus obliquus*. The utmost biological hydrogen production efficiency was 57.6 mL H_2/g VS$_{alga}$ at 2.5

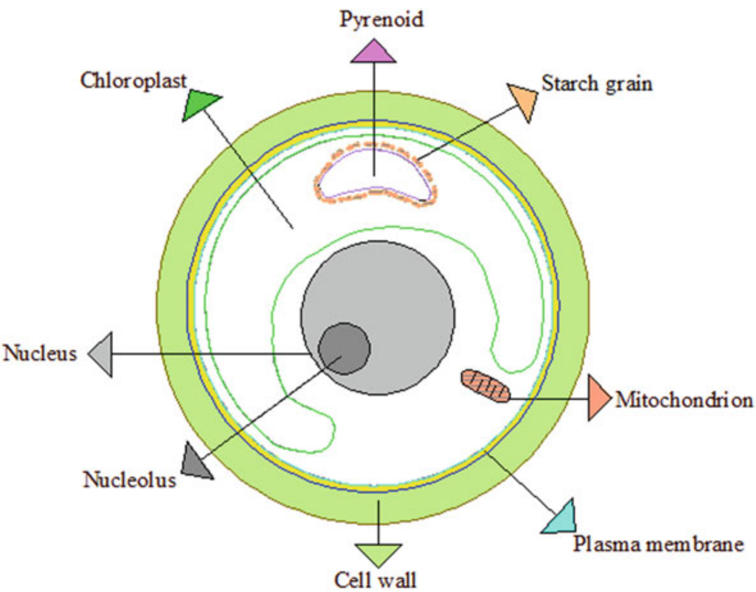

Fig. 6.3 Generalized schematic representation of a microalgae cell

g_{alga}/L by *Enterobacter aerogenes* ATCC 13048 and 113.1 mL H_2/g VS_{alga} at 50.0 g_{alga}/L by *Clostridium butyricum* DSM 10702. It was found that the purity and biohydrogen production rates of biogas obtained with soggy biomass as fermentation substrate are higher or equal to those obtained with dried microalgae. Considering that the use of *Scenedesmus obliquus* biomass in fermentation for biohydrogen production is one of the stages that require much energy and that no drying stage is needed, it is concluded that significant energy savings can be achieved with *Scenedesmus obliquus* biomass (Batista et al. 2014). Mutant strains were also used in a study handled by Oncel et al. (2014). Because of their sustainable and environmentally friendly features, microalgae are one of the utmost substantial energy sources in biological hydrogen production. Despite various types of microalgae in biohydrogen production, the eukaryotic *Chlamydomonas reinhardtii* has been recognized as one of the utmost promising hydrogen producers. Biological hydrogen production could not be conducted directly in these processes, even though metabolic and environmental variables were clearly defined. Genetic engineering tools have been successful in increasing biological hydrogen production in mutant strains in this context. This study examined CC124 and D1 protein mutant strains (D240-41, D239-40, D240) for diversified light densities, lighting patterns, and *Chlamydomonas* strains for biological hydrogen production. It was found that increasing light density shortens the lag stage in hydrogen production, and smaller differences in the illumination pattern affect biological hydrogen production. It was found that the highest biohydrogen production of 490 ± 10 mL L^{-1} was achieved with the dual deletion mutant strain D239-40. Another double deletion mutant, strain D240-41, followed this strain, with a biohydrogen production of 388 ± 10 mL L^{-1}.

Dark fermentation may be hindered by substrates' tough degradable cell wall structures and insufficiently bioavailable nitrogen and carbon sources. Microalgae can optimize volatile fatty acids and biological hydrogen production with different mixing ratios of carbohydrate-rich and protein-rich rice residues. At 140 °C, under optimal hydrothermal conditions for 10 min, it achieved 187.3 mg/g volatile solids (VSs) with a hydrolyses efficacy of 54% in 1% H_2SO_4 pretreatment of microalgae and a decreasing sugar yield of 924.9 mg/g VS with a hydrolyses efficacy of 100% in 0.5% H_2SO_4 of rice residues. Characterization of the solid hydrolytic remnant affirmed significant injury to both substrates. As a result of the co-fermentation of pretreated substrates (brass residues, microalgae) at a mixing rate of 5:1, the highest hydrogen efficiency of 201.8 mL/g VS was obtained, with an increase of 10.7-fold collated to mono-fermentation of pretreated microalgae. It was found that the 25:1 mixture had a maximum conversion of 96.8% carbon to volatile fatty acids. It was found that this result corresponds to the highest energy transformation productivity of 90.8% (Sun et al. 2018). In this study handled by Sun et al. (2018), algal biomass and carbohydrate-rich substrate were mixed in specific proportions for biohydrogen production. In another similar study, the photosynthetic green microalga *Chlamydomonas reinhardtii* was investigated in co-culture with ragi tapai immobilized in chitosan for biological hydrogen generation in a two-stage process. As a result of the assessments, it was found that immobilization and the presence of ragi tapai did not prevent biological hydrogen production by microalgae. Indeed, green microalgae increased biological hydrogen yield and facilitated the transition process between microalgal cell growth and hydrogen production modes. Co-culture with ragi-tapai showed 650,000 ppm maximum biological hydrogen at the best ratio of *2 g microalgae:0.5 g ragi-tapai* (Nomanbhay et al. 2017).

Some studies have investigated biological hydrogen production by acidic, thermal, and alkaline pretreatments of microalgal biomass. The biomass of the microalgae *Chlorella vulgaris* was chosen for use as a substrate in this study, together with sewage digested sludge (DS) as an inoculum. Thermal and acidic pretreatments have been applied to boost algal biomass hydrolysis and increase biological hydrogen production. Thermal pretreatment of microalgal biomass gave better consequences than acid pretreatment. Thermal pretreatment at 100 °C for 60 min was determined to be the most suitable circumstance for *Chlorella vulgaris* microalgae. When the optimal substrate to inoculum ratio was investigated, it was repetitious that the highest hydrogen efficiency was 190.90 mL H_2/g-VS at a rate of 8. The results showed that sludge and microalgae biomass could effectively enhance biological hydrogen production by optimizing the substrate/inoculum ratio and pretreatment applications (Stanislaus et al. 2018). In Liu et al. (2012) study, the carbohydrate-rich microalgae *Chlorella vulgaris* ESP6 was photoautotrophically grown to sequester CO_2. The microalgal biomass obtained was hydrolysed by acidic or enzymatic/alkaline pretreatment. Later, microalgae and *Clostridium butyricum* CGS5 were used for biological hydrogen production. While acidic pretreatment effectively hydrolysed *Chlorella vulgaris* ESP6 biomass, combining alkali pretreatment and enzymatic hydrolysis achieved similar hydrolysis efficiency. *Chlorella vulgaris* ESP6, containing 57% carbohydrates on a nonirrigated weight basis, was efficaciously hydrolysed by acid treatment with 1.5% HCl, yielding nearly 100% reducing sugars (RS). The reducing sugar in *Chlorella vulgaris* ESP6 can be used for biohydrogen production without the need for an adjunct organic carbon source. Optimum conditions for biohydrogen production were achieved at a controlled pH of 5.5, 37 °C and loading of 9 g RS/L microalgal hydrolysate. Under optimal conditions, a cumulative hydrogen production of 1476 mL/L, a hydrogen production ratio of 246 mL/L/h, and a hydrogen efficiency of 1.15 mol/mol RS were obtained. In another study by Liu et al. (2013), *Chlorella vulgaris* ESP6 microalgae were also used. This study examined the consumption and conversion of COD and CO_2 by-products from darkness fermentation into microalgae biomass using a mixotrophic culture of insulated *Chlorella vulgaris* ESP6 microalgae. To improve the assimilation efficiency of soluble metabolites, the beam intensity was 150 μmol m^{-2} s^{-1}, and the nutrient/microorganism ratio (F/M) was 4.5. Over 9 days, the mixotrophic culture reduced the CO_2 level of the darkness fermentation effluents from 34% to 5%, with roughly 100% depletion of the solvable metabolites (acetate and butyrate). The acquired microalgal biomass was pretreated with 1.5% hydrochloric acid hydrolysis. The hydrolysed *Chlorella vulgaris* ESP6 biomass was used as a substrate for biological hydrogen production later being processed. The cumulative hydrogen rate, hydrogen production ratio, and hydrogen efficiency were 1276 mL/L, 240 mL/L/h, and 0.94 mol/mol of sugar, respectively. In another research, acid-pretreated biomass of *Spirogyra* sp. was investigated in sequential batch reactors for biohydrogen production. *Spirogyra* sp. biomass was pretreated with 1N and 2N H_2SO_4 acid hydrolysis followed by fermentation with *Clostridium butyricum* DSM 10702. It contains 1N H_2SO_4 hydrolysate 37.2 g/L total sugar, 2N acid hydrolysate 40.8 g/L sum sugar, a small amount of furfural and hydroxymethylfurfural (HMF). These composition did not prevent biohydrogen production in untreated hydrolysates of *Spirogyra* sp. Cumulative biohydrogen production for 1N and 2N hydrolysates was determined similarly, and hydrogen production ratio were also specified to be 438 and 288 mL/L h, respectively. These results indicated that the 1N hydrolysate was favourable for successive batch fermentation. Sequential batch reactors were operated unremittedly for 13.5 h with 1N acid hydrolysate in a sequential three-batch system. The total ratio of hydrogen production was 324 mL/L h, with an efficiency of 2.59 mol/mol. The research results showed that *Clostridium butyricum* DSM 10702 is effective for biohydrogen production from the microalgal biomass of *Spirogyra* sp. (Ortigueira et al. 2015).

In some studies, the effect of waste leachate on microalgae cultivation and biohydrogen production was studied. In this regard, the influence of landfill seepage water on *Chlamydomonas reinhardtii* CC125 biomass for biological hydrogen production was investigated. The growth of microalgae was studied in the control culture (TAP) and then in media enriched with 10%, 12%, 14%, 16%, 18%, and 20% leachate. Cell liveliness and maximum biomass were found in a 16% seepage water medium with a growth ratio of 927 μg/L chl a day^{-1} with respect to 658 μg/L chl a day^{-1} tris-acetate phosphate (TAP) control culture. *Chlamydomonas reinhardtii* CC125 cultured in a medium to which waste leachate was added was then stimulated to produce more than 37% biological hydrogen compared to the control culture. It has been reported that the reason for the increase in growth could be the excess of basic elements in the dilute seepage water. According to energy distributive X-ray analysis, 16% of waste leachate media cells accumulated the most Mn, Cr, Ni, Mo, Cd, Co, and Fe. The advantages of the seepage water environment are also evident in the hydrogen production stage with Pulse Amplitude Modulated Fluorometry (PAMF). The research results showed that waste leachate increased the biomass and biological hydrogen yield of *Chlamydomonas reinhardtii* CC125 (White et al. 2013).

Recent studies in the literature investigating biohydrogen production from microalgae using anaerobic solid-status fermentation, photofermentation and darkness fermentation are present. In this context, the hydrogen conversion of *Chlorella* sp. TISTR 8411 microalgae were examined sequential using anaerobic solid-status fermentation (ASSF) processes followed by darkness fermentation. Initially, *Chlorella* sp. TISTR 8411 was photoautotrophically cultured in rectangular glass tanks of 80 L volume. It was then grown in open ponds with a volume of 240 L for biomass production. The supreme biomass concentration achieved was 4.45 g L^{-1}. With a pH of 11.5 and a biomass concentration of 2.6 g/L, the biomass was harvested with more than 90% flocculation efficiency. Sequential processes yielded a total hydrogen efficiency of 16.2 mL/g volatile solid (VS), of which 11.6 mL/g VS was obtained by anaerobic solid-state fermentation. The conclusions displayed that the high hydrogen yield obtained by anaerobic solid-state fermentation is efficient and can be integrated into conventional hydrogen production processes to develop biomass energy production (Lunprom et al. 2019). In another study, the green microalgae *Parachlorella kessleri* RA-002, newly insulated in Armenia, was investigated for its hydrogen production. This research aims to determine the conditions for optimizing the illumination regime and organic carbon sources to enable light-dependent hydrogen production. It has been enounced that the lighting regime and organic carbon sources have an impact on hydrogen generation. Hydrogen production was realized with the carbon source used in the study. In the presence of acetate, the maximum hydrogen yield was detected, which was twice as high as the hydrogen yield in the presence of glycolyses. The rise in hydrogen production could be related to stimulating the synthesis of the enzyme-[Fe]-hydrogenase, which produces hydrogen. The results showed that acetate is suitable as an efficient carbon resource for hydrogen production. When the microalgae *Parachlorella kessleri* were illuminated for 24 h and then moved to the dark area, hydrogen production by the presence of glucose and acetate increased 1.5–2.5 times compared to the algae cells under continuous illumination (Gabrielyan et al. 2017). In the research by Lo et al. (2010), photofermentation, autotrophic microalgal growth, and dark fermentation were integrated to create a CO_2-free, highly efficient biological hydrogen production system using various feedstocks. Comparing the four-carbon sources studied, sucrose proved to be the best and efficient for successive darkness fermentation with *Clostridium butyricum* CGS5 and photofermentation with *Rhodopseudomonas palustris* WP3-5. Sequential dark photofermentation was carried out stably for 80 days. In the study, the maximum hydrogen yield was determined to be 11.61 mol H_2/mol saccharose, and the biohydrogen production ratio was determined to be 673.93 mL/h/L. The CO_2 content of biogas fed directly with a *Chlorella vulgaris* C-C microalgae culture grown at 30 °C below the illumination of 60 μmol/m^2/s and produced by sequential dark fermentation was determined to be 40.0%. During the autotrophic growth of *Chlorella vulgaris* C-C microalgae, the CO_2 produced during the fermentation process was wholly consumed. The results yielded a microalgal biomass concentration of 1999 mg/L consisting of 23.0% carbohydrates, 12.3% lipids, and 48.0% protein.

Some studies have used strategies to optimize single or multiple parameters. Optimization of physicochemical parameters attempted to maximize the biomass and carbohydrate productivity of the microalgae *Scenedesmus obliquus* UTEX 393. The effects of pH, temperature, nitrogen weld concentration, and carbon weld concentration parameters were studied. In multi-parameter optimization, maximum biomass was found to be 491 mg L^{-1} day^{-1} and carbohydrate efficiency 270 mg L^{-1} day^{-1} at an initial pH of 6.69, 27.65 °C, glucose concentration of 3.33 mg L^{-1}, and urea concentration of 126.77 mg L^{-1}. The impact of the photobioreactor system on carbohydrate and biomass efficiency was enquired. Using the airlift photobioreactor, carbohydrate and biomass productiveness were found to increase to 309 and 560 mg L^{-1}day^{-1}, respectively. Microalgae were extracted and transesterified at 22.6% w/w lipid ratio before being subjected to fermentative biological hydrogen production for biodiesel manufacture. Skimmed carbohydrate-rich microalgal biomass was studied for dark fermentation using acidogenic mixed consortia. It was found that the degreased algae biomass is suitable as a basic materials for biohydrogen production, and the cumulative hydrogen manufacture is 68.9 mL g^{-1} cell dry weightiness (Singh et al. 2019).

Currently, there are studies investigating biological hydrogen production from microalgae by adding chemicals. The microalgal strains *Scenedesmus subspicatus* GY-16, *Ankistrodesmus gracilis* GY-09, and *Chlorella vulgaris* FSPE were examined for biological hydrogen production concerning carbohydrate accumulation in this context. When the three strains were evaluated for photoautotrophic growth, *Chlorella vulgaris* FSPE was found to have the highest biomass productivity at 825.6 mg/L/day and the highest carbohydrate productiveness at 365.8 mg/L/day. *Chlorella vulgaris* FSPE carbohydrate and biomass yields increased to 498.5 and 1022.3 mg/L/day, respectively, when grown mixotrophically with 2.0 g/L sodium acetate supplementation. In addition, the half-batch operation of the photobioreactor increased equilibrium for extended incubation of carbohydrate-richer *Chlorella vulgaris* FSPE. Accordingly, the carbohydrate efficiency was determined to be 384.8 mg/L/day, and the biomass efficiency was determined to be 1063.3 mg/L/day. Following that, *Chlorella vulgaris* FSPE biomass was used as raw material for separate fermentation and hydrolysis processes to generate biological hydrogen. One percent acidic H_2SO_4 hydrolysate fermented with *Clostridium butyricum* CGS5. Consequently, the hydrogen production ratio was calculated to be 176.9 mL/h/L, with a maximum hydrogen efficiency of 2.87 mmol H_2/g biomass. Research results have shown that carbohydrate-rich microalgae feedstock has the potential for biological hydrogen production (Chen et al. 2016). In another study, the green microalgae *Parachlorella kessleri* RA-002 insulated in Armenia was investigated for biological hydrogen production during the oxygen photosynthesis stage. The hydrogen yield of *Parachlorella kessleri* was increased by the addition of the protonophores 2,4-dinitrophenol (DNF) and carbonyl cyanide *m*-chlorophenylhydrazone (CCCP). In the entity of 15 μM CCCP, the maximum hydrogen yield was determined as ~2.20 mmol L^{-1}. In the entity of 50 μM DNF, the furthest hydrogen efficiency of 2.08 mmol L^{-1} was achieved. Even in the entity of protonophores, hydrogen production by *Parachlorella kessleri* microalgae was not observed under dark conditions. This situation showed that light conditions contribute to the manufacture of hydrogen from algae. In the alga *Parachlorella kessleri*, the upsizing impact of protonophores can be combined with the distribution of proton-conducting strength opposite the thylakoid membrane. This situation facilitated the availability of [Fe-Fe]-hydrogenase, which promotes hydrogen production from protons and electrons. However, in the entity of diuron (3-(3,4-dichlorophenyl)-1,1-dimethylurea), a specifical inhibitor of the photosystem (PS) II, hydrogen production did not occur. Moreover, diuron showed an inhibitory impact on hydrogen efficiency in *Parachlorella kessleri* when protonophores were present. The inhibitory effect of diuron was attributed to the suppression of electron transfer from PS II. As a result of the research, it was found that the way of hydrogen production from *Parachlorella kessleri* microalgae depends on PS II (Manoyan et al. 2019).

6.3 Biomethane Production from Algae

Biomethane was produced through various applications such as ozone, extraction, chemical treatment, ultrasonic treatment, and co-substrate applications. Banu et al. (2020) used low-cost surfactant coupled ultrasonic homogenization (SCUH) to increase the biomethanation potential of mixed microalgae. Mixed microalgal biomass was harvested using alum as a coagulant in a water channel pond. After harvesting the algal biomass, an ultrasonic homogenization (UH) method was applied, the power of which was changed in the range of 100–180 W. At 25,200 kJ/kg TS ultrasonic input energy (UIE), the highest soluble organic oscillation of 2131 mg/L was reached. To reduce energy waste and increase the release of soluble organic matter, the optimized circumstance of ultrasonic pretreatment was combined with a different dosage of sodium dodecyl sulfate (SDS). At ultrasonic input energy (UIE) of 4200 kJ/kg SS and a surfactant dosage of 0.02 g sodium dodecyl sulfate/g SS, a resolution of more than 30.5% was achieved in surfactant-coupled ultrasonic homogenization. Surfactant-coupled ultrasonic homogenization resulted in superior methane production at 358 mL/g COD with respect to 185.9 mL/g COD ultrasonic homogenization. According to the findings, surfactant-coupled ultrasonic homogenization is more economically feasible than ultrasonic homogenization. In the study by Miura et al. (2015), a methanogenic microbial community from maritime deposits was developed to enhance the methane productiveness of brown algae below high salinity conditions. Discontinuous feed cultivation was performed with a dry algal admixture of 1 wt% TS (total solids), essential on the fluid weight of the NaCl-including sediment, per growth frame. The salinity level rose 1.6 times, and the methane production rate increased eight times by the tenth cultivation period. Moreover, the methane manufacture rate stayed high as well at the tenth cultivation time due to salts from marine algae deposition at 10 wt% TS. It was found that the salt content in the tenth time culture was equivalent to 5% sodium chloride (NaCl). Increased methane production was associated with enhanced acetoclastic methanogenesis because acetate is rapidly converted to methane during cultivation. In addition, bacteria from the *Fusobacteriaceae* family and archaea from the genus *Methanosaeta* predominated after cultivation.

Another study on biomethane production used fresh materials (anaerobic water and substrate) corresponding to cultured and untreated brown algae from marine sediments adapted to different climates. In this context, half-continuous methane production was studied below sequential circumstances at various organic loading rates (OLRs) and hydraulic retention time (HRT) without nutrient source and salinity. Methane manufacture remained stable at 2.0 g VS/kg/day in 39 days of hydraulic retention time (HRT). Still, it became irresolute at 2.9 g VS/kg/day in 28 days due to propionate and acetate accumulation. Subsequently, the OLR was reduced to 1.7 g VS/kg/day with a HRT of 46 days, stabilizing methane production beyond a steady state. At all organic loading rates, methane yields of more than 300 mL/g VS were obtained. According to the research findings, a varied climate-adapted marine sediment culture could generate half-continuous methane from untreated brown algae below steady-state circumstances without nutrient supply and dilution. Microbial community analysis revealed that hydrogenotrophic methanogens predominate among archaea in irresolute methane production (Miura et al. 2016). In the study handled by Kumar et al. (2017), electrolysis and ultrasonication pretreatment applications were performed. The combined effect of electrolysis and ultrasonication pretreatment applications for maximum carbohydrate and sCOD recovery and lowest energy input using mixed microalgae biomass was investigated in this study. It was found that soluble chemical oxygen demand (COD), protein and carbohydrate composition, and hydrolysis method positively affected the amount and supported the increase in methane yield. The result of the study was that the maximum soluble carbohydrate yield was 309 mg/L, and the maximum protein yield was 279 mg/L with the combination of the two pretreatments applied. These values were found to allow recovery of about 90% and 85% of the total content, respectively. Biomethane potency tests displayed a peak methane production efficiency of 257 mL/g VS_{added} for the hydrolysate of the combined pretreatment, with respect to the check sample with 138 mL/g VS_{added}.

Current research on biomethane production with macroalgae and microalgae is discussed in more detail below.

6.3.1 *Biomethane Production from Macroalgae*

Generally, the blade parts of macroalgae which can be massive have been examined in biogas production studies. Figure 6.4 depicts a generalized schematic representation of macroalgae in the aquatic environment.

After a freshwater washing pretreatment, marine algae were used for biofuel research. In this context, it was noted that there are few studies on washing algae before the anaerobic digestion process. The study investigated the effects of *Sargassum muticum* macroalgae washed with freshwater on methane production, salinity, and leachate loss due to deposition from the anaerobic digestion process. Washing with freshwater increased the moisture content by 89.1% in the washed algae and 85.6% in the unwashed algae. This treatment reduced the salt content (washed 42.5%, unbathed ash containing 51.5%) and ash content (washed 30.6% dw, unbathed 32.7% dry weight dw). The high heating value of the dry biomass increased significantly with bath owing to the low ash coverage of 11.5–12.6 kJ g^{-1} dw. Washing increased nitrogen content by 3.85–4.77% dw, while lipid and protein content did not change significantly. Washing increased leachate losses, and total leachate losses after washing increased by 12.7–25.2%. During anaerobic digestion (28 days), there were no significant differences in methane efficiency, 0.177 L CH_4 g^{-1} VS for washed samples and 0.225 L CH_4 g^{-1} VS for unwashed samples. However, washing was found to retard biological methane production (Milledge et al. 2018).

There are various studies in which biomethane is obtained by applying different pretreatment processes to algal biomass. Ozone application, a new pretreatment

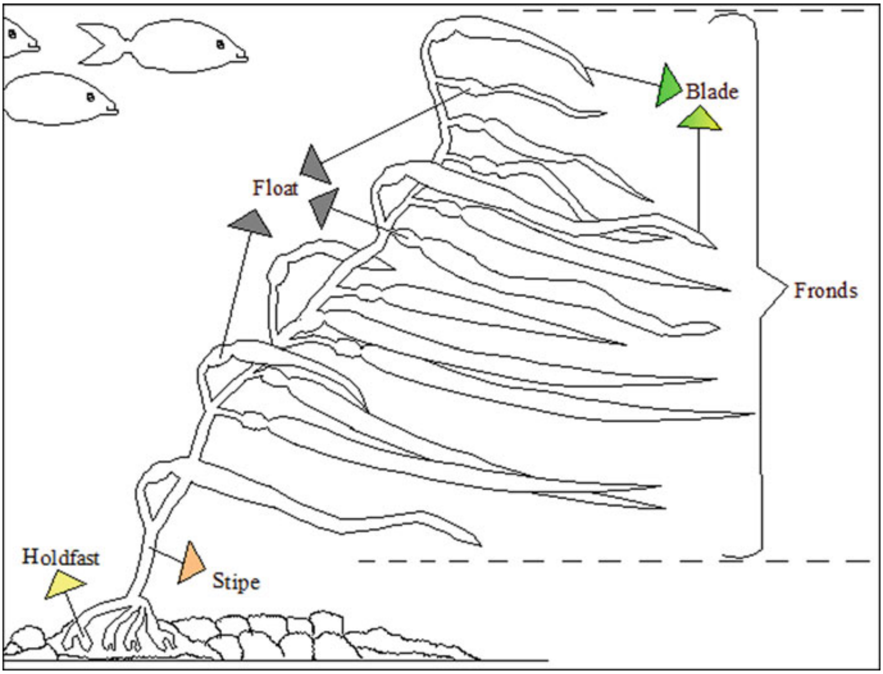

Fig. 6.4 Generalized schematic representation of macroalgae

application in the literature, was performed by Hassaan et al. (2021), and methane production from macroalgal biomass was studied. *Ulva lactuca*, the green macroalgae, has been described as someone of the predominant kind of algae on the Mediterranean shore of Egypt. The ratio-limiting phase in the anaerobic digestion process is the hydrolysis of the algal cell wall. For this reason, pretreatment applications have been reported to have a considerable impact on biogas output. In this study, the biomass of the green macroalgae *Ulva lactuca* was used to increase biogas production. In this context, biomass was investigated using the ozone application, a novel pretreatment method. The effects of ozonation at various doses and the use of two inoculums (activated sludge and cow dung) on the performance of the mesophilic anaerobic digestion process were utilized. With the use of cow manure inoculum, a 249 mg O_3 g^{-1} VS ozone dose was consumed on the macroalgae *Ulva lactuca*, and the maximum biogas output was 498.75 mL/g VS. TGA, FTIR, XRD, and SEM results revealed the effect of ozone (O_3) on the algal cell wall structure and integrity breaking.

Some studies have investigated biological methane production by applying various pretreatment methods such as milling, ultrasound, microwave, enzymes, ball milling, and beating to algal biomass. In this context, an action was conducted to search the impact of ball milling (BM), beating (BT), and microwave (MW) pretreatment applications for biogas recovery from *Laminaria* spp. macroalgae by anaerobic digestion (AD). Anaerobic digestion was effected intermittently at 38 ± 1 °C during an incubation period of 25 days. Following a 3-day digestion process, the samples were pretreated with beating, and a 37% rise in methane was noticed compared to the untreated algae. Both microwave and ball mill pretreatment were observed to minimize methane yield in 25 days compared to untreated marine algae. Beating (BT) was included in the energy balance analysis because it leads to higher methane yields than untreated samples. After a 3-day anaerobic digestion process, a 28% energy gain was obtained by beating (BT) (Montingelli et al. 2016). Energy demand and global warming have increased efforts to find clean and renewable energy sources. It has been reported that algae used as third-generation biofuel can prevent rivalry for agricultural land and can be used in biogas production through anaerobic digestion (AD). Accordingly, a study described the predominant marine brown algae *Fucus serratus* and *Fucus vesiculosus* overtaken in the Baltic Sea in southwestern Sweden and evaluated them for biomethane production. The research used these brown algae and studied the effects of pretreatment on biogas production. Four preprocessing methods were applied to the algae in this study: (a) mechanical (milling), (b) 600 W, 2 min under microwave conditions, (c) 15 min 110 V ultrasonic conditions, and (d) 600 W, 2 min 15 min under microwave conditions combined with conditions 110 V ultrasonic. Subsequently, the biogas plant was co-digested with leachate. In the anaerobic digestion process, cell wall hydrolysis of algae was the rate-limiting stage, and pretreatment affected biogas production. In this study, methane yield from the anaerobic digestion process after pretreatment applications was investigated. Compared to mechanical pretreatment alone, microwave pretreatment increased cumulative methane yields by 156%, ultrasonic pretreatment by 167%, and microwave combined ultrasonic pretreatment by 185%. In the combined pretreatment, the highest methane efficiency was 260 mL/g VSs after 20 days of digestion (Wu et al. 2019). Enzyme pretreatment, one of the pretreatments for macroalgal biomass, was effected by Lamb et al. (2019). The study found that the increase in environmental risks was due to fossil fuels and reported that the orientation towards alternative energy sources increased. Some polysaccharides such as cellulose, mannitol, and laminarin were found in the macroalgal structure. These polysaccharides, which are not easily accessible for biological degradation, have been found to have alternate possibilities for biofuel production. Enzymatic pretreatment of macroalgal biomass may facilitate access to these polysaccharides. It has been reported that this situation may allow efficient use of the anaerobic digestion process. *Saccharina latissima*, a brown macroalga with high carbohydrate content, has been found to be abundant on the Norwegian coast. The target of this study was to view the biogas production capability of the brown macroalga *Saccharina latissima* after an anaerobic digestion process. The analyses showed a reducing sugar content of 30.11 ± 2.30 g per 100 g of the dry sample when harvested from the brown macroalga *Saccharina latissima* on enzymatic hydrolysis. Anaerobic digestion produced a biogas yield of 459 ± 30 mL/g VS with a methane content of 56%. The study found that the brown macroalga *Saccharina latissima*, which grows efficiently on the seabed, has 1760 m^3/ha biological methane potential. An action by Barbot et al. (2015) enounced that marine biomass causes problems on beaches by hitting the shore.

Furthermore, it was noted that the current governance of waste biomass does not contain a retainable disposal policy. Interest in macroalgae has increased in the area of biofuel manufacture. This marine waste biomass can be considered a potential feedstock in biogas facilities as "spare" biomass. For this study, the waste biomass of the macroalga *Fucus vesiculosus* was picked from the coast. The biomass was subjected to thermo-acidic pretreatment, and its potential for methane production was investigated to increase the hydrolysis of the polymer molecules. Acidic industrial waste crop flue gas condensate (FGC) has been reported to accumulate in the plants. It has been reported that this waste product can be used for acid hydrolyses and technical hydrochloric acid (HCl) and water. Biological methane potential experiments were performed on the pretreated specimens. Under 24 h reaction duration conditions, 80 °C temperature, and 0.2 M HCl pretreatment, methane output ascended by +140% in biogas production. Flue gas condensate and warm water pretreatment at 80 °C for 24 h recovered more methane from the untreated macroalgal biomass *Fucus vesiculosus*, +38% and +51%, respectively. No additional methane recovery occurred when the pretreatment reaction time was spread over 24 h the reaction time spread over 2 h. The studies revealed that a curative combination of temperature and acidity is required to achieve the highest efficiency in the pretreatment process.

Some studies have examined biomethane productivity in the main body of macroalgae and growing seasons. It was reported that the overgrown *Ascophyllum nodosum* brown marine algae demonstrated a considerable seasonal change in biogas generation and chemical composition in a research studying seasonal breeding differences in Ireland. For biogas production, polyphenol contents were found to be again critical factor than ashes contents. In the summer months, the high polyphenol contents had a negative impact on biogas production. In addition, two possible harvest dates are recommended: March and October. In this context, the brown alga *Ascophyllum nodosum* was harvested in October, partially yielding small amounts of polyphenols (2% of TS) and 23% ash of volatile solids (VS). Moreover, a specific methane efficiency of 215 L CH_4 kg VS^{-1} was obtained, 44% of the theoretical efficiency. In October, the maximum output per wet weightiness was determined as 47 m^3 CH_4 t^{-1}. This was 2.9 times superior than the least yield, which was 16 m^3 CH_4 t^{-1} in December. The results noted that the gross energy efficiency of *Ascophyllum nodosum* marine brown algae could be 116 GJ ha^{-1} $year^{-1}$ in October under optimal biogas manufacture (Tabassum et al. 2016). According to a study conducted by the same authors, brown algae could be one of the raw material alternatives for biogas production. The study examined the effects of various parts of the main body of algae on biological methane production. It was found that there are significant differences in terms of final, biochemical composition, and affinity in various parts of the thalli of *Laminaria hyperborea*, *Saccorhiza polyschides*, *Laminaria digitata*, *Ascophyllum nodosum*, and *Saccharina latissima* algae. While the maximum biological methane potential of 286 L CH_4 kg VS^{-1} was determined from the stem of the algae *Laminaria digitata*, the least biomethane worth of 118 L CH_4 kg VS^{-1} was determined in the holdfast zone of the algae *Laminaria hyperborea*. Biological methane performance was lowered in the holdfast zone due to salt deposition compared to stem and leaf. The specific yield of algae per fresh weightiness was gauged in the range of $10 - 32\,m^3\,CH_4\,t_{wwt}^{-1}$. The leaf was indicated as the most crucial part for biogas manufacture from algae, considering the predominance of the fresh thallus (Tabassum et al. 2018).

Karray et al. (2017) surveyed the influence of some inoculum types on biomethane generation from macroalgae. In this study, a green macroalga, *Ulva rigida*, abundant in the Mediterranean Sea, was investigated for its methane production by anaerobic digestion tests in batch mode. The purpose of this study was to see how different inoculations and a interference of fresh algae, fungi, bacteria, and sediment gathered from the shores of Sfax affected biogas manufacture from *Ulva rigida* macroalgae. According to the test results, the optimal inoculum for feeding an anaerobic reactor and producing biogas was obtained in 408 mL of biogas by shuffling dissociated macroalgae with water and anaerobic sludge. The processing was then studied in a sequential batch reactor. Biogas manufacture of 375 mL with 40% methane was achieved. Co-digestion works were carried out using sugar waste water as co-substrate in an anaerobic upstream bioreactor. The maximum efficiency of biogas production was determined to be 75% methane in 114 mL g^{-1} VS_{added}. The use of green macroalgae grown in Tunisia has offered a new alternative for methane recovery by co-digestion, which has been proposed.

Biological methane production from a mixture of macroalgae and other substrate sources has been studied. One such study was handled by Ohlsson et al. (2020). The study reported that the macroalgae *Phragmites australis* cane and *Laminaria digitata* have the potential to soak CO_2 and nutrients during growth and be a biogas energy source. The study aimed to look into the nutrient recycling and biogas production of cane and algae using a two-phase pilot-scale process. The total volume of the facility, which occurs of an up-flow anaerobic sludge blanket (UASB) reactor and a hydrolysis bed, is reported as 430 L. Two tests were effected, one for *Laminaria digitata* macroalgae and the other for a mixture (*Phragmites australis* cane + *Laminaria digitata* macroalgae). At 305 K, frozen substrates were installed in the hydrolyses bed and digested for 70 days for the macroalgae *Laminaria digitata* and 100 days for the *Phragmites australis* cane + *Laminaria digitata* macroalgae mixture. In both the experiments, the methane yield obtained was determined to be about 170 L kg^{-1} volatile substances (101.3 kPa, 273.15 K). The research results reported that the use of *Laminaria digitata* macroalgae as the single substrate in the anaerobic digestion process allows high methane yield and has the potential to contribute to sustainable energy studies in the future. Another study on the mixture of macroalgae and other substrates investigated the factors affecting the anaerobic digestion of maritime macroalgae and terrestrial plant biomass as a co-substrate for biogas manufacture. The research was conducted using *Laminaria digitata*, brown maritime algae, and green peas. In the study, 2% of the feedstock from the reactor where green peas were operation was removed, and marine algae with an organic loading ratio (OLR) of 2.67 kg VS m^3 day^{-1} was added instead. This change inhibited methane production, which was less detrimental to the acidogenesis stage and the excessive accumulation of volatile acids. After this application, it became hard to reach reactor stability. The test was first rehearsed with a lower organic load of 0.70 kg VS m^3 day^{-1} of a green peas before adding algae. Although similar symptoms were observed in the first test, process stability was improved by controlling alkalinity and organic loading ratio. These controls resulted in an increment in the total organic loading ratio of 1.25 kg VS m^3 day^{-1} containing 35% marine algae. The study showed that specific marine algae components, even at trace concentrations, inhibit the methanogen group more than other microbial groups of the anaerobic digestion process. It was concluded that an suitable adaptation strategy, including the first fallen proportion of marine algae relative to the total organic load and a whole fallen ratio of organic load, is essential to provide efficient adaptation of microorganisms to the inhibitory components of marine algae (Akunna and Hierholtzer 2016).

The influence of warmth on the biomass of macroalgae *Laminaria digitata* on biogas production in the anaerobic digestion process was searched in a study examining the temperature factor in biological methane production from macroalgae. The potential of biogas production was searched in batch reactors with temperatures of 25, 35, 45, and 55 °C and a hydraulic retention time (HRT) of 40 days. First of all, modified Gompertz and Logistic patterns were used to determine the kinetical parameters of the biogas manufacture process. As a result of the evaluations, it was found that the chemical composite of the macroalgae substrate was $C_{316}H_{613}O_{289}N_{13}S_1$, and the theoretical methane efficiency was 336 ± 0.86 L CH_4 kg VS^{-1}. Methane yields of 318 ± 1.58, 293 ± 1.11, 271 ± 0.98, and 352 ± 0.63 mL CH_4/g VS were obtained in the reactors operated at 25, 35, 45, and 55 °C, respectively. In the results, the fit of $R^2 > 0.90$ was found for both models to estimate methane production kinetics (Membere and Sallis 2018).

In a study applying thermo-chemo disperser (TCD) pretreatment for biomethane production from macroalgae, a new experiment on efficient biological methane production from the marine macroalgae *Chaetomorpha antennina* with the combination of thermochemical liquefaction and thermo-chemo disperser (TCD) liquefaction was conducted. For efficient methane production and effective solubility, optimal parameters were accepted as at pH 11, 80 °C, and a dispersing g-force of 1613 g. This combinative liquefaction application reduced specific energy from 6294 kJ/kg TS to 800 kJ/kg TS and treatment time from 60 to 15 min. In the anaerobic fermentation process, high volatile fatty acid (VFA) saving of 890 mg/L in thermo-chemo disperser (TCD) was observed when comparing thermo-disperser (TD) (750 mg/L) and disperser (D) (322 mg/L). Thermo-chemo disperser (TCD) was found to increase the methane manufacture potential of macroalgal biomass. In addition, higher methane manufacture (215 mL/g VS) was observed in TCD with respect to samples D (100 mL/g VS) and TD (149 mL/g VS). The cost analysis affirmed the feasibility of applying TCD liquidization in practice with a net gain of 90 USD/t for marine macroalgae. For these reasons, it was concluded that the marine macroalga *Chaetomorpha antennina* is a suitable basic materials for methane manufacture (Tamilarasan et al. 2018).

Fan et al. (2015) conducted a study to investigate by addition chemical the biological methane production from macroalgae. Marine sediments from sublittoral and littoral sites have been used as inoculum to gain methane from the giant *Macrocystis pyrifera* algae growing in the seawater system by anaerobic fermentation. High biological methane yield was obtained as 217.1 ± 2.4 mL/g-VS. Both the concentration and methane yield increased with the addition of 0.8 mM Na_2MoO_4 (sodium molybdate), while the lag-time was significantly reduced. This suggested that sulfate might be one of the main inhibitors. Microbial community analysis recovered that the deterioration of the algal species *Macrocystis pyrifera* requires the cooperation of complex microbial populations. Absolute dominance in distribution was observed for hydrogenotrophic methanogens compared to acetotrophic ones. This showed that syntrophic acetate oxidation in conjunction with hydrogenotrophic methanogenesis might have an essential role in the marine anaerobic fermentation process. The research conclusions demonstrate that untreated marine algae found in seawater systems have the potential to produce biological methane.

In a study where the residues and total biomass obtained after macroalgae extraction were analysed for biomethane production, the widespread use of macroalgae in the industry to obtain valuable products such as carrageenan and agar was reported. In this context, it was noted that extraction processes produce large quantities of residues in the form of biomass waste. In the study, red marine algae, *Gracilariopsis persica* and *Gracilaria manilaensis* were investigated for their biological methane potential as industrial residues and whole biomass. In addition, batch analyses were compared at 100 °C, 1 h, and pH 2 conditions after minor acid pretreatment. The pretreated residual biomass of macroalgae *Gracilariopsis persica* and *Gracilaria manilaensis* gave 62% and 70% theoretical yields. The study concluded that wastes from microalgal biomass are suitable as raw material for anaerobic digestion processes than whole plant biomass (Hessami et al. 2019).

6.3.2 *Biomethane Production from Microalgae*

Table 6.2 displays the chemical composition of some microalgal biomass used in biogas production studies.

In producing biomethane from microalgae, efficient production was carried out based on operating parameters and pretreatments (electroporation, thermal, alkaline, and chemical treatment).

In wastewater treatment plants present microalgae, nutrients can be recovered from contaminated wastewater via microalgal biomass, and algae can be used as a cost-effective alternative for conversion to bioenergy. Anaerobic digestion processes are known as viable and simple technologies for bioenergy recovery. In addition, anaerobic biodegradability can be increased by pretreatment applications to microalgae. So far, full-scale systems have been implemented only on a limited scale, accepting disadvantages such as lowly biomass concentration, later traditional harvesting and inadequate processing of the precious crops. This research was conducted in a demonstration-scale microalgal biorefinery to study pretreated

Table 6.2 Chemical compound of some microalgae (% dry matter) (Singh et al. 2012; Becker 2007; Syrpas and Venskutonis 2020)

Species	Carbohydrates	Lipids	Proteins
Aphanizomenon flos-aquae	23	3	62
Botryococcus braunii	8–20	25–75	8–17
Chlamydomonas reinhardtii	17	21	48
Chlorella pyrenoidosa	26	2	57
Chlorella vulgaris	12–17	14–22	51–58
Dunaliella salina	32	6	57
Euglena gracilis	14–18	14–20	39–61
Neochloris oleoabundans	20–60	35–54	20–60
Scenedesmus obliquus	10–17	12–14	50–56
Spirogyra sp.	33–64	11–21	6–20
Spirulina maxima	13–16	6–7	60–71
Spirulina platensis	8–14	4–9	46–63
Tetraselmis maculata	15	3	52

microalgal biomass in anaerobic digestion processes, compare the obtained results with previous laboratory-scale studies, and evaluate in detail the scalability of these bioprocesses. In laboratory-scale tests, urban wastewater was purified in high-ratio algal ponds with a volume of 2×0.47 m^3, and the harvested microalgal biomass was thickened and digested to manufacture biogas. Thermal pretreatment was carried out at 75 °C for 10 h, and it was found that methane yield increased by 67%. Herewith, the identical preprocessing was exercised for the demonstration-scale research. In addition, semi-closed tubular photobioreactors with a volume of 3×11.7 m^3 were processed. The harvested microalgal biomass was thickened and thermally pretreated above entering the anaerobic digestion process to manufacture biogas. In the study, 70% VS was removed in the bioreactor, and methane yields of up to 0.24 L CH_4/g VS were obtained, consistent with laboratory-scale results. Furthermore, the improvement of photosynthetic biogas promoted biological methane production. The plan was to make biofertilizer from the digestion product obtained, so a wetland was created. Digestion of microalgal biomass in anaerobic processes at demonstration-scale and using systems with microalgae proved the feasibility of recovering biofertilizer and biomethane from agricultural flow (Díez-Montero et al. 2020).

In another study, using thermal pretreatment of microalgal biomass, the effect of pretreatment by thermal pretreatment of degreased *Scenedesmus dimorphus* algal biomass was investigated, and it was aimed to increase the biological methane potential. In this regard, 1, 3, and 5 g L^{-1} concentrations of degreased microalgal biomass were pretreated at 100 °C (20 min), 120 °C (40 min), and 150 °C (60 min). The dissolved TOC was developed up to 71 mg L^{-1} after pretreatment at 150 °C and a reaction time of 60 min. The biodegradability was improved, and the methane output was raised by up to 60% by using thermal pretreatment. As a result of the research, it was found that the optimization of the integrated process of thermal pretreatment and bio-methanation contributed to an increase in methane yield by 1.6 times (Chandra et al. 2014). In a research, Mendez et al. (2015) optimized the demanding cell wall degradation pretreatments for anaerobic digestion efficiency using microalgae as substrate. This research surveyed the possible increase of methane efficiency by thermal pretreatment of *Chlorella vulgaris* microalgal biomass in semi-continuous feeding operations. After thermal pretreatment, it was discovered that organic matter hydrolysis resulted in a tenfold increment in soluble chemical oxygen demand (COD). In reactors nurtured with untreated and thermally pretreated microalgal biomass, the total COD removal varied in the range of 36.5–49.7%. Despite nitrogen mineralization of 52% in untreated *Chlorella vulgaris* microalgae and 78% in thermally pretreated *Chlorella vulgaris* microalgae, no ammonium/ammonia inhibition was observed. It was determined that the reactor nurtured with thermally pretreated microalgal biomass showed a 50% increase in methane efficiency compared to the reactor nurtured with untreated microalgal biomass. Although no co-inhibition was determined, it was found that the methane efficiency obtained was relatively lower than that acquired during batch mode digestion. As a result of the study, it was highlighted that semi-continuous feeding is promising for anaerobic digestion.

There are various studies in which pretreatments such as chemical, thermal, and lime applications are applied to microalgal biomass. In a study conducted in this context, anaerobic digestion was tested directly as the primary process and the hydrothermal application applied to the untreated algae feedstock was then applied to the anaerobic digestion. For the hydrothermal processes, hydrothermal carbonization (HTC) and wet oxidation (WetOx), the comparisons were performed without waiting time after the process attained the desired warmth of 200 °C at first pressures of 0.1 and 0.82 MPa, respectively. At a pressure of 0.82 MPa, the conversion of solids to soluble products by hydrothermal carbonization was 47–62% and by wet oxidation 64–83%. In addition, carbon losses of 20–39% in the solid–liquid phases were determined based on a specific total chemical oxygen demand. This resulted in high-soluble product concentrations in the 6.2–10.9 soluble COD/L range. It was found that these hydrothermal processes contribute to a fourfold improvement in anaerobic biodegradability of digestion using potential biomethane tests. Hydrothermal treatments augmented the methane efficiency of the untreated digestion product by 200 L_{STP} CH_4/kg VSs compared to nontreated 66 L_{STP} CH_4/kg VSs (Nuchdang et al. 2018). In a study in which thermo-alkaline pretreatment with CaO (lime) was applied to microalgae, the effectiveness of thermo-alkaline pretreatment with CaO on the anaerobic digestion of microalgal biomass was investigated. Pretreatment was effected at 25 °C room temperature, 55 and 72 °C with the addition of 4% and 10% lime doses, respectively. The exposure time for pretreatment at different temperatures was tested at 4 days at 25 °C and 24 h at 55 and 72 °C. Following that, untreated and pretreated microalgae were tested for biochemical methane potential. According to the results, the pretreatment application was found to increase the carbohydrate solubility by 31.4% and protein solubility by 32.4% at the highest CaO dose of 10% (lime) at 72 °C. In addition, the kinetics of anaerobic digestion was improved from 0.08 to 0.14 day^{-1} for nontreated and pretreated microalgae, respectively. The maximal increase in biochemical methane potential (25%) was obtained at 10% lime (CaO) and 72 °C, where biomass solubility was highest. The research results showed that the use of lime pretreatment is a possible strategy to cure the anaerobic digestion of microalgal biomass (Solé-Bundó et al. 2017). Microalgal biomass has been accepted as a renewable, promising raw material in anaerobic digestion processes due to its lignin-free structure and sufficient carbohydrate content, as it binds CO_2 within itself. In a study in which microalgal biomass was pretreated with dilute acid, *Chlorella* sp. biomass was used and optimized for biological methane manufacture. In this context, the variables used were temperature, solid–liquid ratio, pretreatment time and centrical composite design, and reaction surface methodology. Optimal pretreatment conditions were determined to be a temperature of 64.1 °C, a solid–liquid rate of 0.29, and a pretreatment time of 1.2 h. In this study, 110.04 mL CH_4/g VSS-d methane manufacture ratio and 302.22 mL CH_4/g COD methane yield were obtained. It has been reported that a weight factor of 1.5–1.6 is sufficient for high methane yield and biomass *Chlorella* sp. bioavailability. It was found that the test results were consistent with the predictions of the model. It was concluded that this research supports the effective use of algal biomass in biological methane production and enables scale up with the closed-loop concept (Park et al. 2020).

In another study, a new application, solar hydrothermal pretreatment, was investigated. In this regard, hydrothermal pretreatment applications have been reported to be effective in increasing methane production by using microalgal biomass with anaerobic digestion. However, high energy consumption has been reported to hinder the development of hydrothermal pretreatment applications. Therefore, solar hydrothermal pretreatment has been recommended for energy conservation. In this context, the microalgae sludge was hydrolysed by absorbing solar energy, flowing directly from the parabolic trough collector. The study examined the effectiveness of operational parameters such as flow rate, direct normal illumination, mass fraction, and residence time on organic matter yield. With the pretreatment of microalgae biomass, the highest carbohydrate yield of 267.3 mg/g TS and the highest protein yield of 265.2 mg/g total solids were obtained. This result was 7.4 and 3.7 times higher than untreated microalgae biomass, respectively. Moreover, methane production from solar-hydrothermally pretreated microalgal biomass incremented by 57% in the anaerobic digestion phase respect to that acquired with untreated microalgal biomass. As a result of the research, microalgae sludge was suitable for use in solar hydrothermal pretreatment applications as an alternate energy-saving approximation for hydrothermal pretreatment applications (Xiao et al. 2019).

Calicioglu and Demirer (2016) applied autoclave, heat, and thermochemical pretreatments to microalgal biomass to boost biomethane production and anaerobic digestion. In the study, a semi-continuous photobioreactor was used to specify nutrition removal efficiency in a culture with a single alga, *Chlorella vulgaris*. The highest azote and phosphorus removal efficiencies of 99.6% and 91.2% were reached in the photobioreactor. Microalgal sludge formed in photobioreactor effluent was subjected to autoclave, heat, and thermochemical pretreatment to enhance biogas production and anaerobic digestion, followed by biochemical methane potential analysis. Heat pretreatment was shown to be the most efficient pretreatment application in terms of upsizing anaerobic digestibility at a load of 19 ± 0.5 g L^{-1} COD after assessing the three pretreatment applications. This method was found to increase methane efficiency by 83% from 223 to 408 mL CH_4 g VS_{added}^{-1} with respect to the nontreated *Chlorella vulgaris* microalgae sludge reactor with the same COD loadings. The maximum methane output was obtained with 356 mL CH_4 g VS_{added}^{-1} in the autoclave-pretreated *Chlorella vulgaris* microalgal sludge among the reactors with the first concentration of 35 ± 1.5 g L^{-1} COD. This value was 43% higher than the worth found in the reactor nourished with nontreated microalgal sludge. Thermochemical pretreatment led to the formation of inhibitory compounds. In addition, lower biological methane manufacture and COD levels were found with respect to nontreated microalgae. The results of this research revealed that anaerobic and combined microalgal biotechnologies could be retainable alternatives for nutrition removal and biofuel manufacture applications.

A study was conducted by Garoma and Shackelford (2014) in the context of biomethane production from algal biomass pretreated by electroporation. This study investigated the applicability of electroporation (EP) for the pretreatment of algal biomass in the anaerobic digestion process. The study found that biomass pretreatment by electroporation improved soluble COD (SCOD) and increased it to over 830% at a treatment intensity (TI) of 28 kWh/m^3. Apart from treatment intensity, culture circumstances also affected the performance of the electroporation process (EP). In one sample, optimum electroporation conditions were identified using SCOD (soluble COD) at a pH of 7.0 and a cell concentration of 13.2 g/L. Even though there was a direct link between ionic strength (IS) and treatment intensity (TI), soluble COD decreased as ionic strength increased. At 35 kWh/m^3 treatment intensity (TI), biomethane manufacture augmented by up to 110%. Compared to higher treatment levels, lower treatment intensity (TI) levels were found to support higher gain rates per energy input.

Proteases and carbohydrates were investigated as biocatalysts to increase methane yield from microalgal biomass and organic matter solubility in a study conducted to evaluate the influence of biocatalysts on biomethane efficiency. The carbohydrate accumulation in *Chlorella vulgaris* algae growing in urban wastewater was determined to be 40% on a VSS basis. Despite the fraction dominated by carbohydrates, a higher hydrolysis efficiency of organic matter of 54% was achieved with protease pretreatment. Microscopic examination revealed that the proteases biocatalyst was not selective to cell wall components, while the carbohydrate biocatalyst had a minor effect on the cell wall. Untreated and pretreated biomass was digested at a hydraulic retention time (HRT) of 20 days and an organic loading ratio (OLR1) of 1.5 kg t COD m^{-3} day^{-1}. The maximum methane yield in the reactor pretreated with proteases and fed with *Chlorella vulgaris* was determined to be 137 mL CH_4 g COD in^{-1}. In addition, anaerobic digestion was performed at a HRT of 15 days and an organic loading ratio (OLR2) of 3 kg t COD m^{-3} day^{-1}. Compared to the nontreated biomass, methane efficiency was augmented 5 and 6.3

times in organic loading ratio 1 and organic loading ratio 2, respectively. In the anaerobic digestion process, no inhibitor was detected (Mahdy et al. 2016).

There are some studies investigating microalgae + different substrate sources for biomethane production from microalgae. In this context, a study that included pretreatment with ultrasound (US) investigated anaerobic digestion (AD) processes and agricultural waste under suitable conditions for biomethane and biogas production. Pretreatment with ultrasound (US) was applied to corn stalks with a lignocellulosic structure. It has been reported that the reason for this is that lignocelluloses are insoluble in water, have a stable structure, and are resistant to the harmful effects of mechanical action and enzymes. After adsorption with activated carbon, the digestion product was used to purify for algae growth following the anaerobic digestion process. The cost-effective cultivation of photosynthetic microalgae is considered to have considerable potential for most of their applications. In the study, ultrasonic pretreatment was applied to corn stalks, which were the only substrate, and their digestive (co-digest) effects were detected along with algal biomass. The total daily biogas efficiencies of ultrasound pretreated, untreated, and microwave pretreatment corn stalks were 1350.5, 1116, and 1293.25 cm^3/L, respectively, from three independent biodegradation processes. This study also demonstrated the possibility of algal biomass accumulation in the anaerobic digestion process environment. Pretreatment with ultrasound (400 W) showed higher efficiency compared to extracts acquired per unit energy inlet. The adjunct of 4 g/L microalgal biomass as co-substrate supported increased biogas yield compared to natural stems. The research framework consisted of anaerobic digestion of lignocellulose structured substrates, pursued by growing microalgae in the digestion product, and finally returning the microalgae biomass to the bioreactor as co-substrate. With the research conducted, the framework is a simplistic and cost-effective technology that demonstrates the ability to grow algae in degradation products, the renewability of the substrate used, and the low-cost availability (Hubenov et al. 2020). A search by Ahmad et al. (2015) investigated the co-cultivation of oil palm empty fruit bunch (OPEFB) and *Nannochloropsis oculata* microalgae for the treatment of palm oil mill effluent (POME) and anaerobic biological methane production. Under optimal conditions, co-cultivation of oil palm empty fruit bunch (0.12 g/mL palm oil mill effluent) and *Nannochloropsis oculata* (2 mL/mL palm oil mill effluent) algae resulted in methane efficiency 4606–5018 mL CH_4 L^{-1} POME day^{-1} and high specific biogas production ratio 1.13–1.14 m^3 kg^{-1} COD day^{-1}, as predicted by response surface methodology. Though the particular biogas production ratio stayed stable as 1.13–1.16 m^3 kg^{-1} COD day^{-1} when OPEFB and *Nannochloropsis oculata* microalgae were not grown together, the biological methane efficiency was 1.3 times lower. Anaerobic and aerobic treatment of POME with *Nannochloropsis oculata* microalgae after 7 days compared to BOD (77–86%), COD (58–68%), and TOC (58–68%) without microalgae, resulting in high removal yield for BOD (84–98%), COD (90–97%), and TOC (65–80%).

Microalgae have been recommended as retainable raw materials for biomethane manufacture by anaerobic digestion. In this regard, the operating parameters are essential. The effects of algal culture medium on pretreatment efficiency and biomethane potential at 60–80 °C were investigated. On synthetic media, wastewater, and decay product from anaerobic digestion of paper stock and paper bio-sludge (non-sterile and sterile), local algae species dominated by *Scenedesmus* sp. and a mixed culture of *Chlorella vulgaris* were grown. The biological methane potential of microalgal biomass varied in the range of 154–252 L CH_4 kg^{-1} VS depends on the conditions of the culture medium. The yield of pretreatment at 80 °C for 3 h to dissolve 9–12% of the biomass of native algae and green microalgae *Chlorella vulgaris* was similar for algae grown in sterile and non-sterile wastewater media. Pretreatment augmented the biological methane potential of the local algal biomass by 11–24% (Kinnunen and Rintala 2016). Another study investigated the mesophilic anaerobic digestion of a microalgal mixture in a continuous stirred tank reactor (CSTR), where the temperature parameter played a role. In this context, green algae *Scenedesmus* spp. were first cultivated continuously over 6 months in a 100 m^2 water channel reactor equipped to recording dissolved oxygen, pH, and warmth. In the water channel, additional carbon, 10% CO_2 in the form of chimney gas, was extracted from the diesel cauldron and introduced into the 1 m deep chamber to maintain the pH in the range of 7.8–8.0. The dilution was optimized considering biomass efficiency. Results of 10–15 g total suspended solids (TSS) m^{-2} day^{-1} in wintertime (December–February) and 20–25 g total suspended solids (TSS) m^{-2} day^{-1} in spring (April–May) were obtained. The culture was harvested in February to test for anaerobic digestion. Centrifugation was then performed to obtain an algal sludge with a volatile solids (VS) content of 4.3%. Semi-continuous digestion achieved volumetric biogas production of ~0.99, ~0.83, and ~0.66 L L^{-1} day^{-1} at OLRs of 3.50, 2.75, and 2.00 g VS L^{-1} day^{-1} , respectively. The specific methane efficiency ranged from 0.13 to 0.14 L CH_4 g^{-1} VS_{added} with ~62% methane contents in the biogas. In the general evaluation of the study, it was found that the anaerobic digestion process was steady. Still, there was ~30% VS ravage with low biodegradability owing to the brief residence times and the poorly degradable nature of the microalgal biomass (Tran et al. 2014).

In one of the studies, two digesters were examined depending on the kind of feed. Anaerobic digestion tests with the industrial alga *Nannochloropsis salina* were carried out in two identical 5 L putrefaction processes over nearly 300 days. One digester was nutrition with lipid-extracted biomass (LEB) of algal residues, while the other digester was nutrition with whole-cell algal biomass (WCB). The whole-cell algal biomass digester process obtained a higher specific methane efficiency, ranging from 0.59 to 0.65 m^3 CH_4/kg VS. Methane efficiency in the range of 0.29 to 0.42 m^3 CH_4/kg VS was achieved in the lipid-extracted algal biomass digester process. Based on the results, it was found that an organic loading rate (OLR) of up to 5.0 g VS/L/day can be achieved in the lipid-extracted algal biomass residue digester. In comparison, the whole-cell algal biomass digestion process failed at 3.0 g VS/L/day. Since algal biomass's long-chain fatty acids (LCFA) have been described as the significant inhibitors during the anaerobic digestion process, these two highest organic loading rates have volumetrical methane manufacture rates of 1.40 m^3 CH_4/m^3/day can be achieved in the LEB and WCB digester processes. Illumina MiSeq sequencing targeting 16S rRNA genes, *Tissierella,* *Methanomethylovorans, Proteiniclasticum*, and *Methanosaeta*, species were preponderant at low organic loading rates, and *Levilinea Methanosaeta, Trichococcus*, and *Methanobacterium* species were dominant at high organic loading rates (Ma et al. 2021).

It has been enounced that biological methane production from microalgae by anaerobic fermentation is getting more attention day by day as a CO_2-neutral energy source. In addition, studies have shown that microalgae are challenging substrates for anaerobic digestion due to their unfavourable protein content and high cell wall recalcitrance. Some studies reported that using microalgal biomass with a low N ratio in continuous anaerobic digestion processes significantly supports biological methane efficiency. This research investigated the productive transferability of a low-N fermentation/growth strategy for a wastewater-derived microalgal isolate that can overcome microbial contamination and tolerate light conditions and high temperatures. Long-time continuous anaerobic digestion was characterized by steady and specific biogas efficiency 765 ± 20 mL_N g^{-1} volatile solids (VS) day^{-1} and biomethane efficiency 478 ± 15 mL_N g^{-1} volatile solids (VS) day^{-1}, corresponding to volumetrical methane efficiency 1912 mL_N L^{-1} day^{-1}. This study established the applicability of biomass as a uni-substrate for high-efficiency methane production at low N ratios in wastewater-derived microalgae (Klassen et al. 2020).

6.4 Production of Biomethane and Biohydrogen from Algae in a Two-Phase Process

Biomass can be harvested from algal blooms and used to produce biofuel from gas. However, untreated algae may prevent productive microbial transformation to make biological hydrogen and biological methane due to their rigid cell structure. Biomass from the algal bloom formed on Lake Dianchi was pretreated with hydrothermal/vapour acid before consecutive darkness hydrogen fermentation and anaerobic digestion to enhance the energy transformation yield of algae. When the X-ray diffraction and Fourier transform infrared spectroscopy results were evaluated, it was determined that the amorphous construction, including hemi cellulose and amorphous cellulose, was more damaged by the acid pretreatment, as evinced by the higher crystallinity index. After pretreatment, the screening electron microscopy analysis results indicated larger cell spaces (~1 mm) and smaller fragments (~5 mm) formed on the algal cell surfaces. Compared to vapour acid pretreatment, hydrothermal acid pretreatment was found to have a maximum energy transformation yield of 44.1%, producing 299.88 mL CH_4/g total volatile solids (TVS) and 24.96 mL H_2/g TVS (Cheng et al. 2019). Wu et al. (2020) investigated the biomass of *Chlorella* sp. for energy recovery by hydrothermal pretreatment. This research investigated the impact of hydrothermal treatment on the energetic utilization of the biomass of the green alga *Chlorella* sp. in a two-stage anaerobic fermentation (TSAF) process. The control group (CG) achieved the highest biological hydrogen yield as

8.29 ± 0.33 mL H_2/g VS. The group obtained the highest biological methane yield with the feeblest hydrothermal treatment severity (HTS) (2.49) with 434.38 ± 5.72 mL CH_4/g VS. Energy recovery increased by 2.49 hydrothermal treatment severity group 12.78% from the groups respect to the control group (CG). The severity of hydrothermal treatment decreased by 6.05% and 32.09% in groups 4.06 and 5.21, respectively. The energy recycle in the two-phase anaerobic fermentation process is 22.23–146.78% more than in TSAF with single anaerobic digestion. After the first stage of biological hydrogen fermentation, furfural and 5-HMF were reduced by 46.58–82.20% and 17.65–71.08%, respectively. The study also analysed the microbial structure and found that *Desulfovibrio* and *Peptococcaceae* bacteria were associated with inhibitor degradation and enriched with increasing severity of hydrothermal treatment during the first fermentation stage. During the second phase of biological methane fermentation, the genus of *Enterobacteriaceae* was reduced as a symbiosis with hydrogenotrophic methanogens, reducing the genus of *Methanobacteriaceae*. As a result, it was determined that due to the pretreatment effect of the first fermentation stage, there were more detoxifying microorganisms in the initial fermentation phase and fewer acidogenic and hydrolytic bacteria in the second fermentation stage. Excess acetoclastic methanogens were able to metabolize VFA-rich effluents more efficiently. Moreover, biorefinery conversion of microalgae to biological hydrogen and biological methane can be effectively carried out by hydrothermal treatment (HTT) combined with a two-stage anaerobic fermentation (TSAF) process.

Another study looked into the impact of parameters including HRT, OLR, and C/N on the co-production of two-stage continuous fermentative methane and hydrogen. Ding et al. (2018) examined the co-production of two-phase constant fermentative methane and hydrogen using *Arthrospira platensis* and *Laminaria digitata* algae at a C/N rate of 20. In the initial phase, the hydrogen reactor was operated with a hydraulic retention time (HRT) of 4 days. The maximum specific hydrogen efficiency was achieved at 55.3 mL/g of volatile solids (VS) with an organic loading rate (OLR) of 6.0 gVS/L/day. In the second phase, the methane reactor was operated with a hydraulic retention time of 12 days, and a specific methane efficiency of 245.0 mL/gVS was obtained with an organic loading rate of 2.0 g VS/L/day. These organic loading rates contributed to the two-stage continuous systems process stability and energy efficiency of 9.4 kJ/g VS, 77.7 % of the idealized batch system. However, increasing the OLR resulted in lower methane and hydrogen output in both the reactors. A single-stage anaerobic co-digestion of algal mixtures with a hydraulic retention time of 16 days was compared to the process. Very high saltiness of 13.3 g/L was reported. In addition, an accumulation of VFAs (volatile fatty acids) was found in the single-stage methane reactor. During the investigations, two-stage systems were found to perform better in both process stability and energy recovery.

One of the studies examined the hydrogen and methane yields of a thermal acid-treated microalgae consortium. Two-stage sequential processes were used to apply thermal acid hydrolysis conditions to the native microalgal consortium to produce hydrogen and methane. By performing tests at varying acid concentrations, 45 mL H_2 g VS^{-1} yield and 432 mL CH_4 g VS^{-1} yield were obtained. The hydrogen manufacture stage dissolved COD up to 30%, forming volatile fatty acids (VFAs) up to 10 g COD L^{-1}. The study identified that high hydrogen and methane production potential is present at low acid concentrations. The results demonstrated that thermal-acid hydrolyses using the local microalgae consortium is a simplistic yet efficient technique for sequential methane and hydrogen production processes (Carrillo-Reyes and Buitrón 2016). A study was exercised to examine the impact of hydrothermal dilute acid pretreatment (HTDAP), hydrothermal pretreatment (HTP), and enzymolysis pretreatment. In this context, the brown macroalga, *Laminaria digitata*, is widely distributed in the seas. This study explored *Laminaria digitata* as a basic material for biogas manufacture by successive dark fermentation and anaerobic digestion. Methods such as hydrothermal dilute acid pretreatment (HTDAP), hydrothermal pretreatment (HTP), enzymolysis and their combinations for depolymerization of *Laminaria digitata* brown macroalgae were used to evaluate the effects on biological hydrogen and biological methane efficiencies. Screening electron microscopic images showed that the smooth and flawless construction of the algae was seriously damaged. Moreover, after hydrothermal pretreatment (HTP) at 140 °C for 20 min, some micropores and debris formed while intact constituents remained filamentous constructions. Mixed carbohydrate polymers in the brown macroalga *Laminaria digitata* restricted the catalytic action of glucoamylase, resulting in a restricted increment in the efficiency of carbohydrate monomers. With the help of H_2SO_4 (1 v/v%) in the hydrothermal pretreatment (HTP), the depolymerization of biomass and its more transformation into carbohydrate monomers were greatly enhanced. The efficiency of total carbohydrate monomers afterwards hydrothermal dilute acid pretreatment (HTDAP) was reported to be 3.5 times that of the untreated biomass. In the initial phase of darkness fermentation, this resulted in a 60.8% increase with a biological hydrogen production of 57.4 mL/g VS. However, the production of by-products such as hydroxymethylfurfural beneath such hard circumstances compromised the second phase of anaerobic digestion of the hydrogenogenic effluent, eventuating in a 25.9% reduction in biological methane efficiency. In the study, hydrothermal pretreatment (HTP) was found to increase the biogas energy conversion efficiency of marine algae by 26.7% compared to untreated *Laminaria digitata*. Therefore, hydrothermal pretreatment has been admitted as the optimum pretreatment (Ding et al. 2020).

Wieczorek et al. (2014) compared non-enzymatic pretreated biomass to pretreated biomass in their study. This research investigated hydrogen fermentation by combining darkness fermentation and hydrogen manufacture from microalgae, followed by methane fermentation from remnants in a two-phase combined fermentation process. In the study, *Chlorella vulgaris* microalgae culture was evaluated as a substrate. Microalgae *Chlorella vulgaris* was used without enzymatic pretreatment, and hydrogen was found to be produced at substrate dosages ranging from 1.75 ± 1.50 to 19 ± 2.94 mL H_2 g-VS^{-1}. Pretreatment of microalgae with Onozuka R-10 and Macerozyme R-10 enzymes eventuated in a sevenfold increment in hydrogen production: 19 ± 2.94 to 135 ± 3.11 mL H_2 g-VS^{-1}. When the results of methane fermentation were evaluated, it was found that the enzyme pretreatment was able to increment the methane efficiency to 245 ± 2.46 to 414 ± 2.45 mL CH_4 g-VS^{-1}.

6.5 Conclusions and Future Prospects

By reducing greenhouse gas emissions, biofuels minimize other negative environmental impacts. Since the fossil fuels that continue to be used are not sustainable, dependence must be reduced. For this reason, it is necessary to develop and use biohydrogen and biomethane as alternative gas biofuels to reduce their production costs and increase their feasibility. The basis of these studies is determined by the first-, second-, and third-generation biomass type factor to be used. This section examines recent developments in biohydrogen and biomethane resulting from methods applied to third-generation algal biomass. In the vast majority of the studies reviewed, various types of biomass pretreatment were used. These applications were found to increase biogas yield. Hydrolysis of algal biomass requires hybrid pretreatment processes characterized by CO_2, wastewater treatment, no arable land, growth under very harsh environmental circumstances, and high biomass potential. The range of data on biogas production from algal biomass is wide. However, research in microalgal biomass has focused on biogas production from green algae. According to the studies, no study on biogas production from diatoms and golden algae was found in the literature. It is expected that more research on this algal biomass will be conducted and developed in the future.

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Chapter 7
Algal Biofuels: Clean Energy to Combat the Climate Change

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Abstract Algal biofuels are an indispensable tool for combating climate change as they are the clean sources of energy prevalent in nature and recognized as third-generation biofuels. The different types of algae are renewable sources of energy that can be cultured at low cost as compared to first- and second-generation crops and thus reducing the pressure on agricultural and water land use. The biomass production of algae has been increased up to 32.67 million tonnes worldwide. The algae have maximum light use efficiency and produce 2–15 folds higher lipids in comparison to other oilseed crops, viz. soybean and rapeseed. Oil-producing algae are 100–200 times greater than soybean. The production of oil yield from soybean was 446 L ha^{-1} when grown on 594 mha area while microalgae produce 136,900 L ha^{-1} oil on 2 mha land area. Algae biomass production is helpful to minimize the pollutants and heavy metals such as ammonium nitrates and phosphates from wastewater and soil. According to IPCC climate change is real and happening around the world. The concentration of carbon dioxide was 415 ppm in 2020 has been increased by 44% from 278 ppm in 1960. The cellulosic and algal biofuel production would be important biological approaches to store and convert atmospheric carbon dioxide into bioenergy resources in the coming future. On a large scale, basis microalgae have the potential to entirely replace petrodiesel with biodiesel. The production of bioenergy resources from microalgae have the ability to lower the amount of GHG emissions by 4–5%. The production technology of algal biofuels is still somewhat new and extravagant. The chapter attempted to comprehend the role of algal biofuels in the bioenergy sector, and how they can play a crucial role in combatting climate change.

Keywords Climate change · Pollutants · Algal biofuels · Biodiesel · Clean energy · Harvesting methods

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7.1 Introduction

Algal biofuels do not depend upon agricultural land and drinking water resources as these are third-generation biofuels. The algae have the potential to be harvested in outdoor cultivations, viz. wastewater (urban, industrial, and agriculture), saline, and freshwater. Algae are simplest to culture and harvest in fresh, saline, or wastewater cultivations (Verma and Mishra 2020). These are made up of organic compounds. The different types of algae are renewable sources of energy that can be cultured at low cost as compared to the first- and second-generation crops and thus reducing the pressure on agricultural and water land use. Algae's maximum biomass yield is due to its rapid growth and development and also has the potential to produce higher carbohydrate and lipid content. The cultivation of different kinds of algae, i.e. macroalgae and microalgae provides biodiesel, biomethane, bioethanol, and biogas as a by-products. Bioethanol can be produced by using algal carbohydrates, while biodiesel production can be done by algal oil (Saad et al. 2012). There are different algal harvesting methods used to obtain useful biofuels which can be used in daily life routine, viz. biochemical, thermochemical, and transesterification.

According to IPCC climate change is real and happening around the world (IPCC 2014). The air temperature is increased by 1.0 °C in 2020 and is likely to reach 1.5–2.0 °C from 2030 to 2050 due to adverse weather conditions that cause global warming (IPCC 2018). Global warming is caused by various environmental, economic, and social impacts. Influences of both natural and human origin climate change refer to variability of weather parameters, viz., temperature, wind speed, and rainfall over time. Man-made greenhouse gases from the burning of fossil fuels for power, vehicles, trains, aircraft, and buildings, as well as oil and gas production from oil fields, have been proved to be the primary causes of changing climate. Humans have emitted 2400 billion tonnes of CO_2 since the late 1800s (IPCC 2021). Furthermore, deforestation and land-use changes add more pressure to greenhouse gases (Onoja et al. 2011). The rise in global average temperature is merely one sign of major changes, which include high temperatures, rising sea levels, storms, drought, flooding, food production impacts, and infectious epidemics (Anonymous 2015).

The trend of urbanization is linked with increase in income. The higher urban incomes are related with the maximum use of fossil fuel-based energy and release of gases in the atmosphere causes the rise in temperature in metropolitan cities. Fossil fuels, carbon monoxide, particulate matter, smog, and noxious CFC's amount has been rise continuously in recent years, due to man-made activities that have led to increase the concentration of GHG's and range of air temperature thus causes global warming.

Several variables have an impact on the earth's climate. The energy emitted from the surface of the earth, volcanic eruption, release of gases in the atmosphere, and particulate matters are the variables impacts of the climate of earth. Carbon dioxide (CO_2) has been the most significant contributor to global warming as compared to methane since the Industrial Revolution (i.e., 1750). The levels of CO_2 have risen 44% from 278 parts per million in 1960 to 401 parts per million in 2015

(Anonymous 2015). During the Conference of the Parties (COP-21) at the Paris Climate Conference (2015), a legally enforceable and multilateral agreement on climate change to keep global warming below 2 °C was accomplished. By the middle of the century, large-scale changes in energy systems and land use can achieve considerable reductions in anthropogenic GHG emissions. If manufactured sustainably, biofuels may eliminate the equivalent of 2.1 Gt of CO_2 emissions each year, according to the International Energy Agency (IEA). This is nearly the same amount of carbon dioxide absorbed by the oceans.

By harnessing algae to absorb carbon dioxide and enabling algae to sink into deep waters, algal production aims to minimize the greenhouse effect in the atmosphere. Carbon, together with algal remains, would be kept for several centuries in deep waters, on the seafloor. The carbon recycling method can only absorb a little amount of CO_2, however, algae have a tremendous potential to lower CO_2 from throughout the atmosphere. Land-based plants absorb 52% of total carbon dioxide absorbed by the biosphere, while ocean-based algae absorb 45–50%, indicating that algae may absorb carbon dioxide efficiently despite their small size owing to their relatively short life cycles (Haoyang 2018). If we provide algae with the appropriate environment, including temperature, nutrients (iron is one of the most important and effective ones), sunlight, and the abundance of animals that consume them, algae can even decrease the CO_2 concentration even more. As a consequence, algae cultivation is a promising strategy for reducing greenhouse gas emissions.

Biological strategies to sequestering and converting CO_2 include second-generation cellulosic biofuels and third-generation algal biodiesels. When compared to petroleum fuels, algae-derived biofuels can lower CO_2 emissions by 50–70% over their lifetime (Liu et al. 2013). The race is on to develop the technology for producing biofuels from lignocellulose sources, and biotechnology innovation is anticipated to make this happen eventually for the benefit of humanity.

7.2 Algal Biofuels and Climate Change

The different species of algae are the photosynthetic creatures with the highest growth rates, doubling their biomass in far less than 24 h. It has the potential to provide more energy m^2 of land than the other crops produces biofuel. Algae are differentiated into three groups, viz., microalgae, macroalgae and micro- and macroalgae (Table 7.1). The growth and development of species are fast and may be harvest on a regularly; algae provide an endless supply of energy, according to Hundt and Reddy (2011). To flourish, algae need sunlight, moisture, CO_2, and mineral elements. Inorganic nutrients include nitrates, phosphates, iron, and trace elements. The optimum temperature for growth is between 20 and 30 °C (Demirbas 2010). Plants on land absorb 52% of the total carbon dioxide absorbed by the biosphere, while algae absorb 45–50%. Because of their brief life cycle, algae can efficiently absorb carbon dioxide. As a result, algae farming is a possible strategy for

Table 7.1 Different groups of algae

S. no	Microalgae	Macroalgae	Micro- and macroalgae
1.	Pyrrophceae	Phaeophceae	Rhodophyceae
2.	Chrysophyceae	Chlorophyceae	
3.	Euglenophyceae		
4.	Cyanophyceae		
5.	Xanthophyceae		

reducing greenhouse gas emissions and global warming. Algae, unlike higher plants, lack an embryo, vascular structures, or a protective shell.

Wastewater treatment, energy generation, bioremediation, natural fertilizer, animal fodders, pharmaceutical compounds, and nutraceuticals are all examples of algae's economic value. Algae can also be utilized to produce a range of products, such as proteins, vitamins, dyes, health supplements, and oils (omega-3) [European Biofuels Technology Platform (2016)]. Light use efficiency of algae is higher and thus increases the growth and development of algae. The production of lipids from different types of algae is 2–15 folds more than first- and second-generation crops, viz., Soybean and Jatropha. In mass culture, >30,000 L of algal oil is produced annually per hectare of land. Algae that produce oil are 100–200 times more powerful than soybeans (Lam and Lee 2011). Diatoms, which are single-celled algae with silica cells, are crucial in the industrial production of oils. Algal lipids can be isolated and refined, then trans-esterified to produce biodiesel. Algae biomass production increased globally in 2016, reaching 32.67 Mt (fresh weight).

Aquaculture provides the majority of algal biomass (96.5% in 2016), while wild stock collection provided 98% of total algae biomass in Europe over the same year (Araujo et al. 2019). Seaweed cultivation has recently reappeared in Bali, after being slowed for nearly a decade. The tourist sector has plummeted as a result of the Corona crisis, and seaweed farming has resurfaced, providing workers with up to $400 per month in income. Although this is only little more than half of what individuals had prior to the pandemic, it highlights how diversity can help in the context of such a pandemic (Channel News Asia 2020).

7.3 Different Steps That Regulate and Encourage Production of Algal Biofuel

1. Roles and responsibilities within government institutions, including different government sectors and non-governmental organizations (NGOs).
2. Collaboration between government and industry, including low-impact development, operations, and maintenance techniques for biofuel production.
3. To produce algae-based biofuels key ecological challenges are identified at all levels.

4. Compile a list of each production process's known and unknown environmental implications.
5. Establishing a framework for environmental assessment of algal biofuels.

7.4 Different Groups of Algae for Production of Biofuels

(a) **Macroalgae:** One of the two important species of algae are macroalgae and microalgae. Macroalgae are multicellular and enormous. The term "seaweeds" is widely used to describe them. They are marine animals that resemble large sea plants. Without the use of a microscope, macroalgae can be observed. It can fix GHG's (CO_2) through photosynthesis, it is a promising renewable energy source. The average photosynthetic efficiency is 6–8%, which is much higher than the terrestrial biomass (1.8–2.2%) (Aresta et al. 2005). Algal biomass can be converted into biofuels such as biogas, bioethanol, biodiesel, and biooils through anaerobic digestion, fermentation, transesterification, liquefaction, and pyrolysis (Fig. 7.1). Macroalgae, unlike microalgae, is a multicellular plant with plant-like features, making extraction more convenient. It is mostly composed of carbohydrates, making it an excellent option for biofuel generation such as biogas, bioethanol, and biooils (Maceiras et al. 2011). Based on their photosynthetic pigmentation features, macroalgae are classified as red, brown, or green. Brown algae flourish in cold or very cold seas, while red algae survive in the intertropical zones. Chlorophyta can grow in a wide range of water conditions (Bucholc et al. 2014). In Asia, macroalgae is currently cultivated for food, fertilizers, and hydrocolloid extraction, with China, Korea, the Philippines, and Japan accounting for roughly 72% of global yearly production (Roesijadi 2010). Macroalgae productivity varies between 150 and 600 t ha^{-1} $year^{-1}$ fresh weight, with global production exceeding 12 million tonnes dry matter $year^{-1}$. Macroalgae have slow growth rates and less energy yield than microalgae, but they are more premium. With a biomass yield of about 73.5 t dry mass ha^{-1} $year^{-1}$ and a methane yield of 285 m^3 t^{-1}, it is one of the most productive

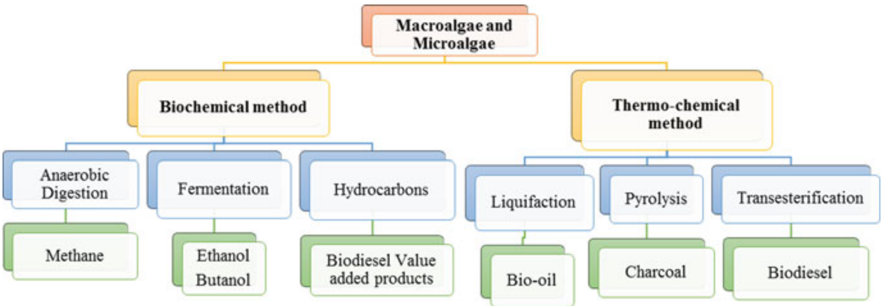

Fig. 7.1 Macroalgae biofuel conversion through biochemical and thermochemical methods

Table 7.2 Characteristics of different groups of microalgae

Microalgae	Habitat	Storage	Pigment	Cell wall
Pyrrophceae	Marine/ freshwater	Starch	Chlorophyll (A&C)	Cellulose
Chrysophyceae	Marine/ freshwater	Leucosin	Chlorophyll (A&C)	Cellulose (silicate frustules)
Euglenophyceae	Freshwater	Paramylon	Chlorophyll (A&B)	N/A
Cyanophyceae	Marine/ freshwater	Starch and protein	Chlorophyll (A&D)	Mucopeptide (carbohydrates and amino acids)
Xanthophyceae	Marine/ freshwater	Leucosin	Chlorophyll A	Cellulose and hemicellulose

ecosystems on the planet, electricity production from macroalgae would be economically viable (Gao et al. 2020).

(b) **Microalgae:** Microalgae are tiny plant-like organisms found in rivers, seas, ponds, and lakes. As a result, need a microscope to study microalgae. They are mostly unicellular organisms, and some create colonies by clumping together and form multiple cells. The various groups of microalgae are characterized by its habitat, photosynthetic pigments, and cell wall as depicted in Table 7.2. They are photosynthetic organisms with photosynthetic pigments and accessory pigments, similar to plants. They come in blue-green, yellow, brown, and orange colours. Phytoplankton is the prominent name for microalgae. Microalgae are microscopic aquatic plants that can perform photosynthesis, whereas prehistoric photosynthetic plants that are mostly found in water. Mehta et al. (2018) concluded that by 2024, microalgae-derived products are anticipated to reach USD 1143 million in the worldwide market. Microalgae are a feasible renewable biomass feedstock for producing a variety of bioproducts and biofuels. The algae business for biofuels and other technologies seems to be on the positive track. Several microalgae species, including *Botryococcus braunii* and *Chlorella vulgaris*, have been found to contain >50% lipids by total dry biomass, indicating a significant prospect for biodiesel generation (Najafi et al. 2011).

Keffar and Kleinheinz (2002) inferred that about 3000 characteristics were discovered to be beneficial for CO_2 sequestration and biodiesel extraction out of a total of 200,000 traits examined. CO_2 mitigation through storing CO_2 fixation, biofuel generation, and wastewater treatment are all viable options for microalgae. Storage and fixing of CO_2 by photosynthetic algae production has the ability to reduce CO_2 emissions into the atmosphere, thereby assisting in the mitigation of the global warming trend. Biofuel is made from microorganisms that can exist on terrain that is not suitable for growing crops and produce substances that are ready for use in engines. These are known to be as third-generation biofuels (New Scientist 2011). The carbon dioxide concentration (12–14%) released from various industries and chimneys can be sequester by growing various species of microalgae, viz., *Chlorella* spp., *Botryococcus* spp. as shown in Fig. 7.2. *Chlorella vulgaris* was the first

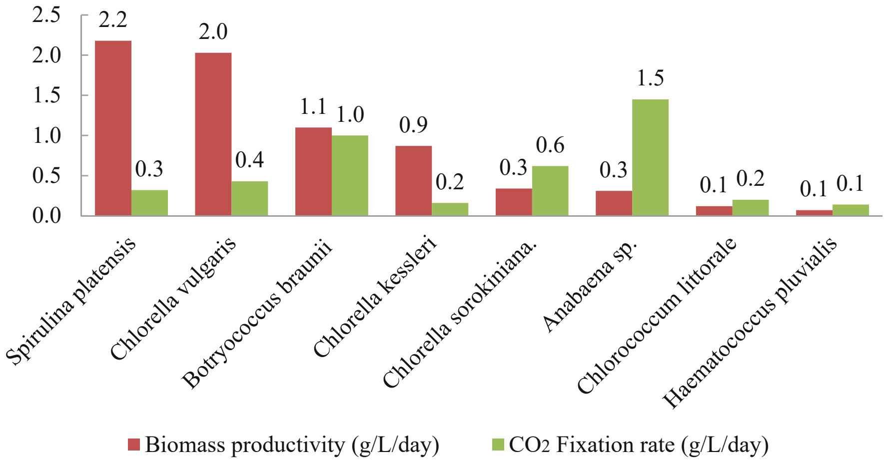

Fig. 7.2 Different species of microalgae carbon dioxide fixation rate and biomass production

microalgae to be commercially cultivated, originating in 1960 in Japan (Mobin and Alam 2017).

Global microalgae productivity in 2004 was estimated to be around 5000 tonnes (dry weight) and US$ 1.25 billion year^{-1} (Pulz and Gross 2004). The most prevalent kind of algae, chlorophytes, have use in bioremediation, sewage water treatment, food production, medicine, and energy generation. Saad et al. (2019) inferred that to make algae biofuel production more appealing, new and efficient processes are required. Increased biofuel production will aid in the conservation of natural resources, hence protecting the environment. The biomass of microalgae has a potential to yield 100 times more oil to convert to biodiesel. The different species of microalgae and their oil content are depicted in Table 7.3 (Chutia et al. 2017; Culaba et al. 2020). Oil-producing microalgae are 100–200 times greater than soybean and other first- and second-generation crops. The oil content yield from biomass of microalgae was 58,700 L/ha/year as compared to corn (172 L/ha/year), soybean (446 L/ha/year), sunflower (952 L/ha/year), jatropha (1892 L/ha/year), rapeseed (1190 L/ha/year), etc. (Fig. 7.3). The production of biodiesel from algal biomass has been reported (Fig. 7.4) to be 51,927 kg/ha/year which is more than corn (152 kg/ha/year), soybean (562 kg/ha/year), sunflower (946 kg/ha/year), jatropha (656 kg/ha/year), rapeseed (862 kg/ha/year), and oil palm (4747 kg/ha/year) (Bosnjakovic and Sinaga 2020).

Table 7.3 The production of oil contents through different species of microalgae

Microalgae	Oil content (wt% of dry basis)
Botryococcus braunii	25–75
Chlorella species	28–32
Crypthecodinium cohnii	20
Cylindrotheca species	16–37
Dunaliella primolecta	23
Isochrysis species	25–33
Monallanthus species	>20
Nannochloris species	20–35
Nannochloropsis species	31–68
Neochloris oleabundans	35–54
Nitzschia species	45–47
Phaeodactylum tricornuturn	20–30
Schizochytrium species	50–77
Tetraselmis sueica	15–23

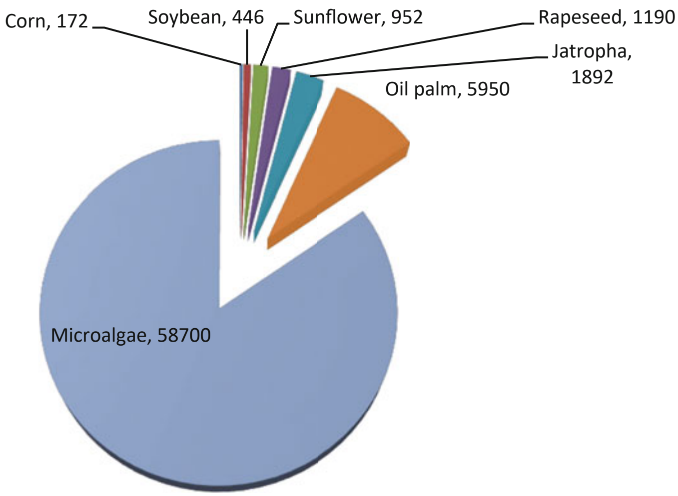

Fig. 7.3 Evaluation of various crops with microalgae in terms of oil content production (L/ha/year)

7.5 Algae Production and Their Socio-Economic Impact in the Future

The producers of algae (micro and macro algae) are presently the foremost drivers of growth and development. The algae industry helps the world's population to give employment and to enhance the economy. The harvesting of Eucheuma seaweed accounts for 7.6% of Zanzibar's gross domestic product (GDP), while Tanzania's seaweed sector employs 30,000 people (The Fish Site 2020). The algae industry is an

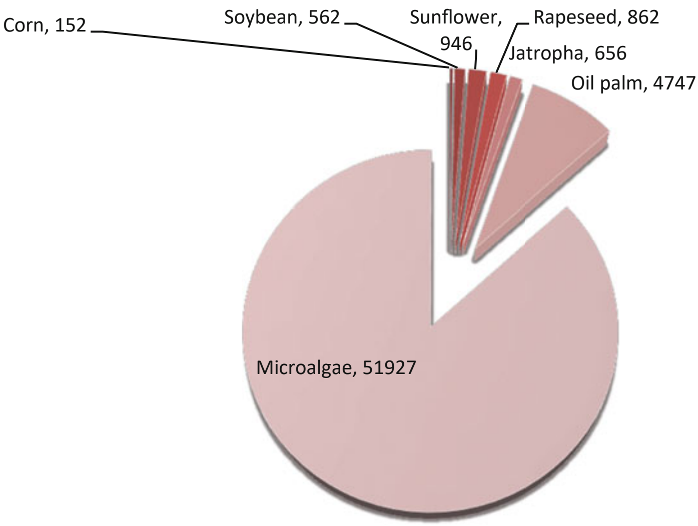

Fig. 7.4 Evaluation of various crops with microalgae in terms of biodiesel production (kg/ha/year)

important step towards the development of economy and environmental conservation. There should be important strategic points for development of economy and natural conservation are as following:

- New educational opportunities in schools and universities. Provides information on a new topic (Algal Biotechnology).
- Establishment of algae industries and assistance to start-up businesses.
- Establishment of a digitalized network for marketing techniques.
- Tool development and research achievements.
- Financial assistance for new initiatives (Algal funds).

The European Union also invests to algae culture development projects. The European Union is assisting Kanembu communities in Chad by contributing €8 million to a project aimed at mitigating the effects of climate change, preserving natural resources, and developing renewable energy sources by providing technology for more efficient algal cultivation and Spirulina drying (European Union 2020). For the optimal production system to create biomass, algae require not only carbon dioxide and water, but also nitrogen and phosphorus and a variety of macronutrients. A considerable number of macronutrients are used in agricultural production for plant growth and development, and runoff of these nutrients into the rivers and lakes frequently results in poisonous algal blooms (Michalak et al. 2013).

Algal farming in the sea, rivers, and lakes serves to diminish the effect of nutrient runoff caused by agricultural operations, as well as being economically beneficial and ecologically sustainable. Algae vertical farming technique utilized on rooftops of greenhouses, utilizing closed or semiclosed reactors, could be a promising alternative for nutrient recycling. The “Algenhaus” in Hamburg, Germany, is one example of how algae farms may be integrated into modern design (Abmus et al. 2018).

7.6 Land-Based Algae Cultivation

The farming of algae helps to provide food security for future generations, as existing cropland is anticipated to be insufficient owing to population expansion. Algae cultivation reduces greenhouse gas emissions and nutrient losses, resulting in green power generation. In dry places, such as Israel's Arava desert, Chile's Atacama Desert, or Morocco's coastal desert, open pond systems or photobioreactors are used. The production of marine microalgae depends solely on coastal deserts. Freshwater algae cultivation in urban areas should take place in moist climatic regions with plenty of water. Outdoor ponds, greenhouses, light bioreactors, fermenters, and hybrid systems combining bioreactors are all used to cultivate algae. Dunaliella is a species of algae that in ponds with minimal mixing, such as deep saline ponds. The species *Spirulina* is cultivated in shallow racetrack ponds using a paddlewheel to keep the culture moving around the track. Algal cells are transported to the surface by turbulence, where they absorb sunlight. Micro-screening, filtering, centrifuging, and flocculation are all methods for harvesting algae biomass (Algae Industry Magazine 2013). There are several processes, viz., cultivation, harvesting, screening, thickening, dewatering, filtering, drying, and oil extraction involved in converting microalgae to biodiesel (Fig. 7.5). The selection of harvesting technique plays an important role for high harvesting rate with low cost (Fig. 7.6 and Table 7.4). Dewatering and thickening harvesting processes plays a principal function in microalgal harvesting (Ananthi et al. 2021).

7.7 Sea-Based Algae Farms

Commercially produced microalgae and macroalgae (seaweed) can provide biomass for biofuel production, specialized chemicals for food processing, beauty products, and medicines, soil supplements and fertilizers, cattle feed, and other end products. Algae grown at sea provides competitive edge over terrestrial-based crops grown for biofuels. Since it does not require terrestrial platform, irrigation infrastructure, and additional fertilizers. Microalgae may be cultivated in semiporous containers near the coast to conserve space on land, minimize the need for supplementary synthetic fertilizers, and benefiting from natural sunlight (Hoffman et al. 2017).

Seaweed farming is done along the coasts either as a single seaweed farm or as an integrated multitrophic farm that combines fish production with algae production at the same location and time. This could also aid in catching fertilizer stream from densely populated agricultural regions, preventing harm to aquatic habitats near the coast.

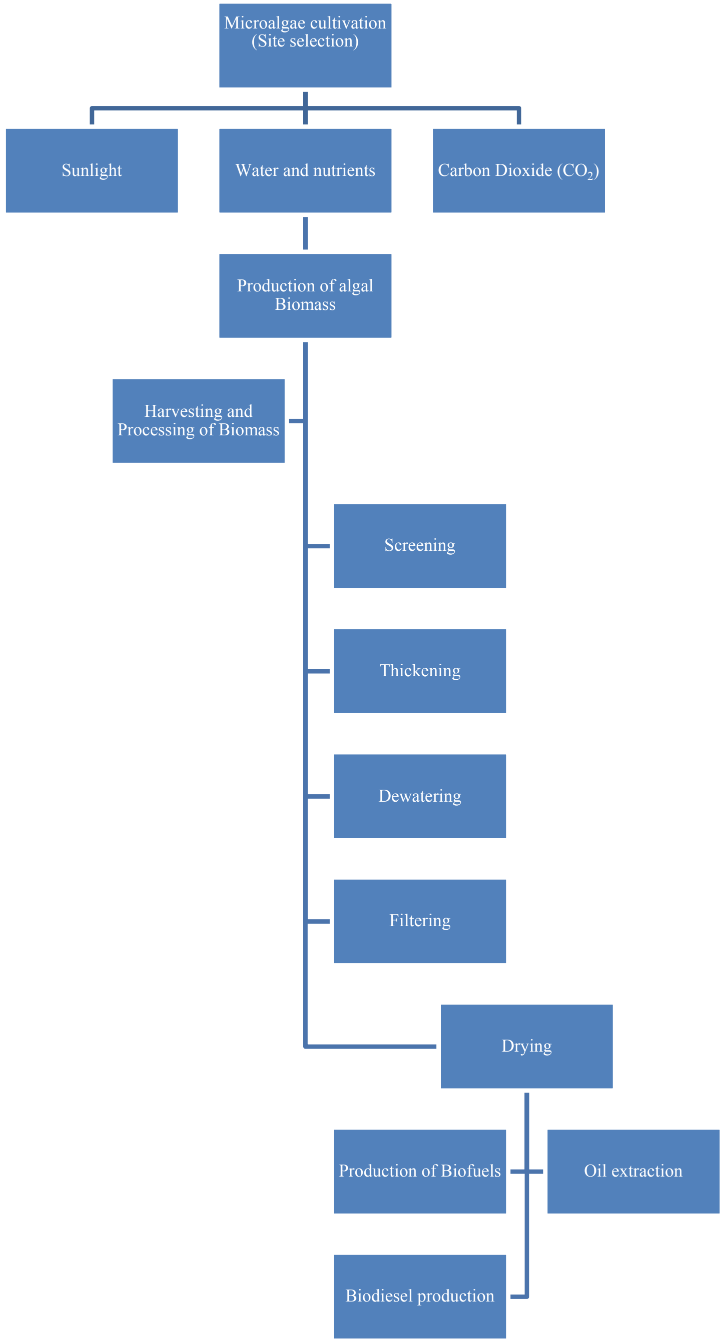

Fig. 7.5 Harvesting and drying methods of microalgal biomass for production of biodiesel

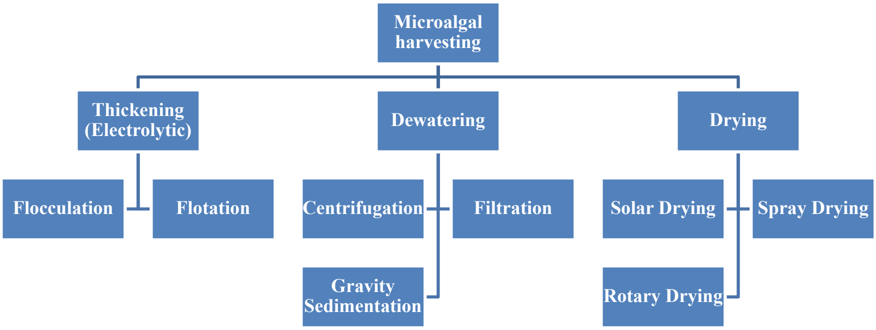

Fig. 7.6 Different harvesting techniques for biodiesel production from microalgal biomass

Table 7.4 Advantages and disadvantages of algal harvesting methods

Harvesting method	Advantages	Disadvantages
Chemical flocculation	Simple and quick method	Chemical flocculants can be costly and harmful to the biomass of microalgae
Bioflocculation	It is a low-cost approach. Allows for the recycling of culture media. Biomass of microalgae is unaffected	Changes in the structure of cells
		Microbiological contamination is a possibility
Gravity sedimentation	Simple and low-cost method	Time-consuming. There are chances of biomass deterioration. The algal cake has a low concentration
Flotation	Large-scale applications are possible. Method with a low cost	Chemical flocculants are usually recommended. Harvesting marine microalgae is not possible
Electrical based processes	It can be used on a diverse range of microalgae species. Do not imply the use of chemical flocculants	Poorly dispersed. Costs of energy and equipment are high
Filtration	High efficiency of restoration	The risk of fouling or blockage raises operating costs. Membranes should be cleaned on a regular basis
	Allows sensitive species to be separated	

7.8 Reduction of Greenhouse Gases Through Algal Biofuels

Biofuels are energy sources derived from plant biomass harvested subsequently. Although biofuels have long been accessible, petroleum and coal have typically been used as sources of energy owing to inherent abundance, high calorific value, and reduced prices. Biomass is mostly used to manufacture fossil fuels such as coal and petroleum, even though process requires millions of years. As a result of rising oil prices, depleting fossil fuel resources, the need for a renewable, sustainable source of energy, and as a strategy to address climate change, biofuels are regaining momentum. Biofuels are a renewable resource since they are replenished in the environment on a continuous basis. Fossil fuels, on the other hand, are not renewable because their formation takes millions of years. They are distinguished by their biomass sources, limits as a clean energy source, and technological advancement.

Fermentation, distillation, and transesterification are characteristics of well-known technologies and processes used to make first-generation biofuels. For hundreds of years, these approaches have been used in a variety of applications, including the manufacturing of alcohol. The primary disadvantage of first-generation biofuels is that they are made from food source biomass. When there is not enough food to feed everyone, this becomes an issue. Since extensive bioenergy necessitates more fertile agricultural lands, resulting in less lands available for human and animal food production, first-generation biofuels pose massive financial, ecological, and social challenges (Alam et al. 2015; Saad et al. 2012; Litvak and Litvak 2020). First-generation biofuels, often known as conventional biofuels, are derived from sugar, starch, and vegetable oil. Carbohydrates are fermented to generate bioethanol, biobutanol, and biopropanol in smaller quantities. Bioethanol has the property of burning clean than gasoline and so emitting less greenhouse emissions. Transesterification is the prominent method to produce biodiesel by extraction of oils from plants. In this process, lipids are exposed to an alcohol such as methanol in the presence of a catalyst. Distillation process has been used to isolate the primary product from any reaction by-products. In many diesel engines, biodiesel or a combination of the two can be used instead of petroleum diesel.

Since second-generation biofuels are derived from non-food biomass, they still compete with food cultivation for land. These biofuels overcome many of the difficulties that first-generation biofuels have, and they do not compete with food crops or fuels since they originate from different biomass. They also produce more energy per acre than first-generation fuels. They enable the use of poor quality land where food crops may fail to thrive. Because the technology is still in its early stages, it has the potential to reduce costs and enhance manufacturing efficiency when scientific breakthroughs are accomplished. However, because certain biomass for second-generation biofuels grows in the same environment as food crops, some biomass for second-generation biofuels competes with land use. As a result, farmers and policymakers are faced with the difficult task of deciding which crop to produce. Corn stover, for instance, is a cellulosic source that grows alongside food crops and may be utilized for biomass (leaves, stalk, and stem of corn). However, this would deplete the soil nutrients, which would need to be replenished with fertilizers. Furthermore, producing second-generation biofuels is more difficult than producing first-generation biofuels since it involves pretreating the biomass to release the trapped sugars. This demands additional resources and energy. Even though third-generation biofuels do not contend with food or land use, they are the most environmentally friendly alternative fuel. However, there are still several challenges to overcome before they can be widely popular. As an energy source, these biofuels exploit exclusively adapted crops like algae. These algae are produced and harvested for the purpose of extracting oil from them. The oil can then be processed into alternative fuels to replace petroleum-based fuels, or turned into biodiesel in the same way as first-generation biofuels.

These biofuels have a higher energy density per harvest area than the first- and second-generation biofuels (Alam et al. 2015; Litvak and Litvak 2020). They have been cultivated as low-cost, high-energy, and 100% renewable energy sources. Algae have the benefit of being able to thrive in places that are unsuitable for first- and second-generation crops, reducing the amount of water and arable land required. Wastewater, sewage, and saltwater from seas or salt lakes can all be used to cultivate it. There would be no need to utilize water that would otherwise be used for human consumption as a result of this. Algae have numerous benefits over land-based plants as a possible source of renewable fuel (Anonymous 2012).

The globe is currently dealing with a serious challenge of global warming. By utilizing algae to absorb carbon dioxide and allowing algae to sink into deep seas, algae may be cultivated to decrease the greenhouse effect in the atmosphere. Carbon, together with algal remains, would be preserved for several generations in deep seas, on the bottom (Haoyang 2018). Because of their short life cycles, algae can effectively absorb carbon dioxide. As a result, they may become a necessary tool for reducing greenhouse gas emissions.

Microalgae have a photosynthetic efficiency of 11–20%, which is higher than terrestrial plants (1–2%), and they produce a considerable amount of air oxygen and absorb carbon dioxide, accounting for 50% of all photosynthesis on the planet (Banerjee et al. 2020). The utilization of microalgae for carbon sequestration is a recent and innovative technique to transform CO_2 into biomass through photosynthesis. Algae, like other renewables, absorbs CO_2 during growth and dissipates it when the fuel is combusted. They may not reduce carbon dioxide levels in the atmosphere by themselves, but they can contribute in the reduction of emissions when fossil fuels are replaced.

Emissions come from a variety of sources, including:

- Building materials, construction, and the effect of land-use change;
- Providing fertilizer, stirring or pumping the algae to ensure access to nutrients and light, or providing artificial lighting;
- Harvesting, drying, and processing;
- Transport, consumers supply, and fuel usage.

When compared to C_4 plants, microalgae have a greater ability to fix CO_2 (Banerjee et al. 2020). Microalgal biomass is also utilized to make sustainable biofuels, food, animal, and aquaculture feed, as well as other high-value goods including cosmetics, medicines, nutraceuticals, bioactive compounds, and biofertilizers. The mitigation of greenhouse gases (GHG) emissions by cultivating microalgae plays an prominent role in storage of carbon from industries, vehicular pollutants, and burning of agricultural residues (Delrue et al. 2016).

Litvak and Litvak (2020) investigated at the trends of carbon dioxide emissions in planetary energy production and revealed that the adoption of renewable energy sources by industrialized countries has resulted in stable carbon dioxide emission over the last 3 years. Total greenhouse gas emissions are obtained by calculating emissions over the course of a biofuel's life cycle. The raw materials used to make biofuel determine its impact on climate change, which has a substantial impact on the chemical composition and performance of the fuel. Biofuel made from lignocellulose raw material may be more efficient in terms of cutting greenhouse gas emissions. Microalgae's ability to bind carbon dioxide from the atmosphere could contribute in tackling the greenhouse effect problem.

The biomass of microalgae or heterotrophic microorganisms is used to make third-generation biofuel. Microalgae are grown in closed and open ponds, as well as photobioreactors. The expenses of setting up and maintaining open ponds are negligible, but the biomass production is poor. In this scenario, only regions with warm temperatures and high solar radiation can effectively cultivate algae in open sea. Growing microalgae in photobioreactors, on the other hand, may provide substantially greater biomass yields but has high infrastructure and maintenance costs. In bioreactors, an optimum temperature should be maintained in addition to the requirement for circulation and gas exchange in the growth medium. There are already many operations that produce biofuels from microalgae grown in photobioreactors (USA, Brazil). During cultivation another advantage is that algae absorb instead of emit CO_2, which is a key nutrient (building material) for photosynthesis. Microalgae's incredible potential to bind carbon dioxide from the atmosphere might help to solve the greenhouse effect problem.

7.9 Algal Biomass Production for Generation of Biofuels

The different forms of fuel that may be made from algae are determined by the algae species and the portion of the algae that is utilized (Fig. 7.7).

Biodiesel Different chemical processes are utilized to produce biodiesel (fatty acid methyl ester; FAME) and glycerol through the extraction of oils (lipid) from the microalgae cultivation.

Bioethanol Bioethanol is a substitute for gasoline that is made by microorganisms or yeasts fermenting the carbohydrate content of algae. Most automobiles can run on gasoline with 10% ethanol, while flexible-fuel vehicles can run on 85% ethanol blends.

Biobutanol The carbohydrates contained in micro- and macroalgae are fermented to produce biobutanol. It has more energy per molecule than ethanol and is less damaging to internal combustion engines.

Hydrocarbons Unprocessed algae treated with high pressures and temperatures to create hydrocarbons or microalgal oil can be chemically converted to produce hydrocarbons. Hydrocarbons are produced directly by some algae species. Aviation fuel is made up of hydrocarbons.

Biogas Biogas is generated during anaerobic digestion when microorganisms break down into organic materials in the absence of air (or oxygen). It can be burned directly to generate heat and electricity, or processed to make biomethane, a natural

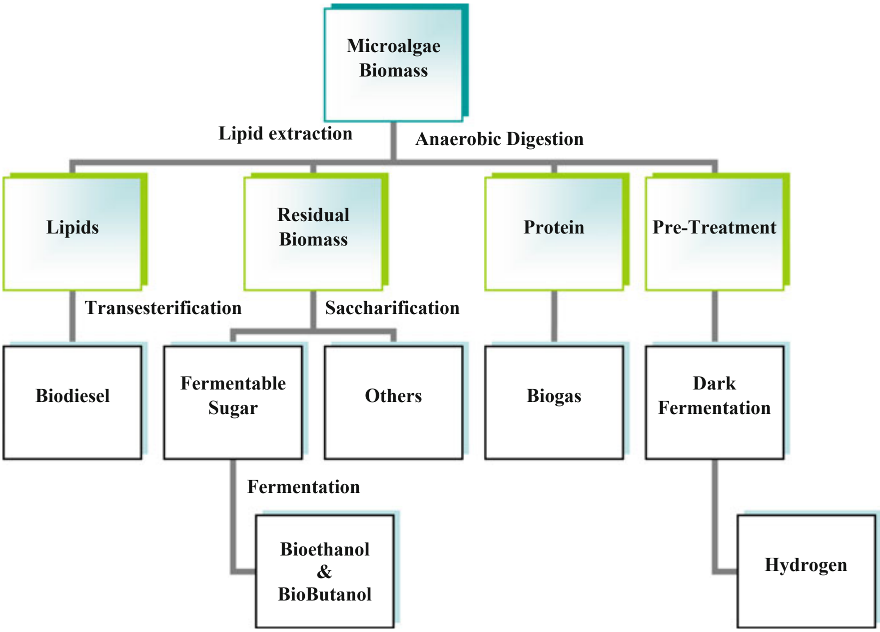

Fig. 7.7 Algal biomass production for generation of biofuels

gas alternative for energy generation, heating, and transportation. Untreated algae or waste left over following the extraction of other products can be used to make biogas.

Hydrogen Several species of algae and bacteria produce hydrogen from water in the absence of oxygen.

7.10 Algal Biofuels for Removal of Pollutants from Water

Algal growth requires organic and inorganic additives, which can be found in waste water. The combination of microalgae and wastewater is a cost-effective waste treatment and microalgal biofuel production method. Transportation is one of the fastest-growing industries, absorbing 27% of primary energy in the current environment. India's annual oil demand is approximately 5.5%, with expectations of rising over the next 10 years. Bhatt et al. (2014) accounted that diesel now satisfies around 73% of India's transportation fuel demand, with gasoline accounting for the remaining 20%. By the end of this decade, average transportation fuel consumption is anticipated to rise from 117 billion litres (2013) to 167 billion litres by 2023. It is well known that global warming rises in accordance to greenhouse gas (GHG) emissions and rise in air temperature highlighting the critical need for a more eco-friendly alternative to fossil fuels. There is a need for an alternative, scalable, and sustainable energy source due to the continuous depletion of natural resources and growing fuel prices. Furthermore, the globe confronts two major challenges: fresh and drinking water shortage and an energy crisis.

Renewable energies: hydropower, solar energy, and wind energy as a viable substitute to conventional energy sources. The renewable energy sources have the potential to meet the needs of transportation sector. They are environmentally friendly and have the potential to decrease the pollution in the atmosphere. Microalgae as an energy source also have the potential to become a new source of petrodiesel. They have high photosynthetic efficiency, environmentally friendly approach, and can be cultivated on non-arable land or industrial wastewater. Combining wastewater with microalgae growing could be a feasible biofuel production strategy. Algal cultivation systems (wastewater) can recycled huge amounts of water and nutrients for algae growth. The wastewater cultivation approach will be commercially viable and eco-friendly method for the generation of algal biofuel. Microalgae have two applications: biomass production for long-term biofuels and phycoremediation. The cultivation of algae will be very useful for production of biodiesel, biohydrogen and bioethanol due to higher lipid content (Fig. 7.8).

Wastewater is a term that refers to the final output of residential, municipal, agricultural, and industrial sources. Organic matter as well as inorganic matter are found in wastewater. Eutrophication or algal blooms are caused due to the presence of organic and inorganic matter (heavy metals) in nearby water bodies, which is commonly caused by anthropogenic waste production. According to recent data from the Central Pollution Control Board (CPCB) in New Delhi, India, the metropolitan cities are the major source of wastewater production in the country. The total of 40 billion L day^{-1} wastewater are produced from metropolitan areas. The waste water treatment in sewage treatment plants is about 20–30%. The household, urban, agricultural, and industrialized activities are the primary sources of polluted water production in the majority of developing countries, and wastewater is released into the environment without adequate treatment.

7.11 Urban Waste Water Treatment

The three main contaminants in urban waste water are carbon, nitrogen, and phosphorus. Microalgae can remediate pollution by ammonium, nitrate and phosphate. Prof. Oswald and his co-workers since the 1950s at the California University have undertaken numerous investigations. In two 1000 m^2 pilot-scale high-rate of algal ponds reported very good removal rates for ammonia (85–90%) and phosphorus from wastewater (99%) (HRAPs). In urban wastewater treatment, a pilot-scale HRAP operating hydraulic retention time (HRT) for 4 days was able to provide a mean biomass productivity of 16.7 g/m^2/day (maximum of 24.7 g/m^2/day) (Park and Craggs, 2010).

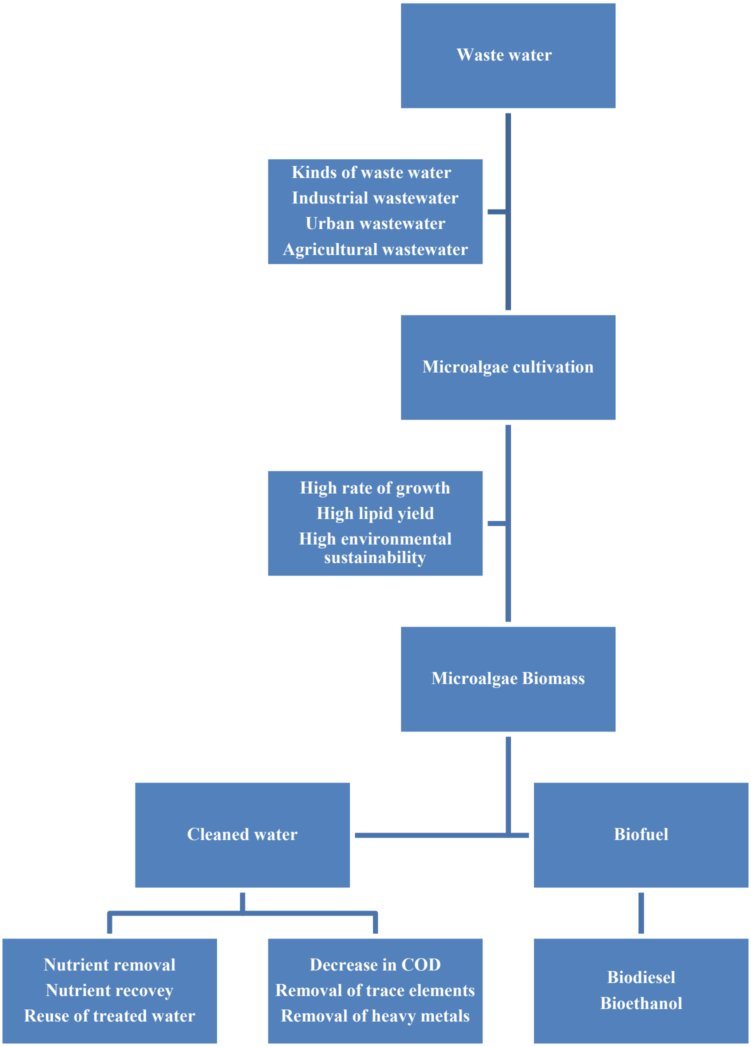

Fig. 7.8 Cultivation of algae through different kinds of wastewater for environmental sustainability

The organic loading rate can also be decreased by the cultivation of microalgae. On a centrate from an urban wastewater treatment facility, 70% COD decrease (3000 to 400 mgO_2/L) was achieved in total 13 days using a PBR infected with *Chlorella* spp. (Min et al. 2011). In a 16 m^2 open field pond, *Chlorella* spp. and *Scenedesmus* spp. production can remove 90% of COD from a wastewater stream (Hammouda et al. 1995). Microalgae have been proven to thrive on a variety of carbon substrates, ranging from glucose and lactose to alpha-cyclodextrin (Tian-Yuan et al. 2014). On treated wastewater exposed to pretreatment, primary settling, activated sludge, and secondary settling, three strains (*Scenedesmus obliquus*, *Chlorella vulgaris*, and *Chlorella kessleri*) and a natural bloom were effectively produced (Arbib et al. 2014). The most common method for treating activated sludge generated during urban wastewater treatment is anaerobic digestion.

7.12 Industrial and Agricultural Waste Water Treatment

Several microalgae strains have been tested for their ability to remove contaminants from industrial and agricultural waste waters, which are challenging to treat using traditional activated sludge techniques. Industrial wastewaters from molasses-based distilleries have high BOD and COD (ranges from 40 to 50 $gO_2\ L^{-1}$ and 80 to 100 $gO_2\ L^{-1}$, respectively) and are produced in large volumes. One liter of alcohol production produces 15 L of effluents (Sankaran et al. 2014). In a 50 L PBR, *Chlorella sorokiniana* cultivated for 3 days and reduced the pH = 6.0–7.0 of COD of alcohol distillery sewage. The use of *Chlorella sorokiniana* culturing reduced the amount of NO_3^- to 95% and 35% to 77% PO_4^{3-} and Sulphate (Solovchenko et al. 2014). Microalgae can be used to remediate wastewater from the pulp sector and paper sector. A consortium from a pond was able to remove up to 58% COD, 80–84% colour, organic halogens from diluted pulp sector and paper sector effluent (Tarlan et al. 2002). Microalgae treatment of dairy and livestock wastewaters has also been investigated. *Chlamydomonas polypyrenoideum* cultivated in 250 mL flasks for 10 days in dairy and livestock wastewater, could reduce 90% NO_3^- and NH_3 and 60–70% PO_4^{3-} and (Kothari et al. 2013). Mining industry such as acid mine drainage (AMD) is another type of wastewater that causes significant environmental harm to the society. To treat the waste from mining industry, biological treatment test cells were used. A cyanobacterial-microbial consortium was used to generate a microbial mat (Sheoran and Bhandari 2005). A consortium of bacteria used to reduce the amount of metals. Ninety-five percent for Fe and Pb, 79–97% for Cu and Zn, 59–83% for Co, 22–62% for Ni and Mn. Agricultural wastewaters can also be treated with microalgae.

7.13 Contaminant Removal from Soil Through Algae

Extreme sensitivity because of the existence of hazardous substances, algae are usually applied as indicators to evaluate the ecotoxicity, neurotoxic effects, and environmental impact of pollutants in soil and sediments (Fu and Secundo 2016). Scientists have been paying a lot of attention to the exploitation of algae in recent years because it is effective in reducing pollution. The issue of environmental contamination is much more critical than before, and among them soil pollution has a serious consequences. Many contaminants must be to responsible, but pollution comprised of harmful organic compounds and toxic elements are at the top of priority list.

7.13.1 Effective Removal of Organic Contaminants by Algae

The kitchen, gardens, and other biodegradable organic wastes are some examples of biodegradable waste. These wastes originated from living beings and can be oxidized by microorganism found naturally. Non-biodegradable organic wastes, often referred as persistent organic compounds (POPs), are carbon-enrich compounds and withstand chemical, biological, and photolytic breakdown in the environment.

POPs' physical and chemical characteristics enable them to travel over great distances, biomagnify in living tissues and food chains. Persistent bioaccumulative and toxic chemicals, persistent toxic substances, and persistent environmental pollutants are also used to describe them (Priya et al. 2014).

7.14 Biological Degradation of Organic Pollutants by Algae

Microalgae degrade biological compounds as a result of facultative chemoautotrophy. Microorganisms can use biological compounds as their primary source of growth, which are essential for them to survive and advance (Priya et al. 2014).

7.14.1 Toxic Metals Elimination Through Algae

The eradication of toxic elements is based on a simple mechanism. Peptides that can bind toxic substances are synthesized by microalgae, other eukaryotic photosynthetic organisms, and some fungi.

$$\text{Peptides} + \text{toxic metals} = \text{Organometallic Complexes}$$

These compounds are further differentiated within vacuoles to enable for more specific control of heavy metal ion concentrations in the cytoplasm, minimizing or neutralizing their potentially detrimental effects.

The important approaches of heavy metal removal by microalgae are biosorption and bioconcentration. Bioconcentration leads to the adsorption of pollutants (e.g., toxic ions) on mostly non-living algal biomass, whereas biosorption confined to the adsorption of contaminants (e.g., metal ions) on mostly live algal biofuel. Microalgae can metabolize heavy metals like cadmium and mercury into sulphides having poor solubilities and therefore negligible toxicities since they are physiologically inactive (Raven 2017). The potential of microalgae to bind and assimilate heavy metals is attributed to their large interfacial ratios, high affinity metal binding groups on cell surfaces, and efficient metal absorption and storage mechanisms.

7.15 Conclusion and Future Perspectives

In recent years, biofuels obtained from algae have provided an alternate to fossil fuels; however, the technologies related to these biofuels have to overcome several hurdles before their broad deployment. The different kinds of algae are utilized as renewable resources for the production of biofuels. But there are many problems and barriers for biofuel commercialization. The more emphasis should be taken for mass cultivation of algae by agricultural, industrial, and urban-sewage waste water. The different techniques and feasible methods were developed for lowering the cost of biofuel production. The genetic modification techniques in the mass cultivation of algae in waste water should be improved. However, still there is much work and potential required in bioenergy resources sector for the production of biofuels.

Downstream process should be used for production and commercialization of oil produced by algae due to low energy consumption and increase in the yield of oil. The downstream method consumed less energy and produces high yield of algal oil. The biomass production of algae has been increased up to 32.67 million tonnes worldwide. The algae have maximum light use efficiency and produce 2–15 folds higher lipids in comparison to other oilseed crops, viz. soybean and rapeseed. Oil-producing algae are 100–200 times greater than soybean. The production of oil yield from soybean was 446 L ha^{-1} when grown on 594 mha area while microalgae produce 136,900 L ha^{-1} oil on 2 mha land area. Hence, still, innovative research is needed for algal biomass production and the successful co-extraction of multiple products.

Bioenergy resources play are key element in the sustainability of the environment. The cultivation of algae helps to store the carbon and reduces the impact of global warming on the planet.

The development of technology for the production of biofuel of the second, third, and future generations, which are independent to the risks of agricultural production, plays a vital role in maintaining energy security. Producing such environmentally favourable biofuels would not only allow for the avoidance of competition between renewables and the food sector of the economy, but will also partially solve the problem of waste disposal from various industries and reduce greenhouse gas emissions substantially. Effective breakthrough technologies in the field of algae biofuels, as well as complete low-carbon development policies, will enable the Paris Agreement's targets for mitigating climate change impacts to be accomplished.

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Chapter 8
Thermo-kinetic Study of *Arthrospira Platensis* Microalgae Pyrolysis: Evaluation of Kinetic and Thermodynamics Parameters

Satya Prakash Pandey, Achyut K. Panda, and Sachin Kumar

Abstract Pyrolysis kinetics of microalgae *Arthrospira platensis* was investigated via thermogravimetric analysis in the nitrogen atmosphere at diverse heating rates of 20, 40, 60, 80 and 100 °C/min. Kissinger-Akahira-Sunose (KAS), Ozawa-Flynn-Wall (OFW), Friedman (FRM), Coats-Redfern (C-R) and Vyazovkin (VYZ) methods were applied on thermogravimetric data of microalgae to evaluate the different kinetic parameters such as activation energy, pre-exponential factor and thermodynamic parameters. The results obtained in thermal degradation process represents three main zones such as evaporation, active and passive pyrolysis zone. Activation energy obtained from KAS, OFW, Friedman, C-R and VYZ methods were 132.63, 175.65, 312.67, 216.87 and 149.26 kJ/mol, respectively. The average pre-exponential factor obtained through Kissinger approach were 4.045×10^{10}, 1.16×10^{10}, 7.89×10^{9}, 1.05×10^{10} and 1.157×10^{10} s^{-1} at 20, 40, 60, 80 and 100 °C/min, respectively. Experimental results reveal that the values of kinetic parameters from five different models free isoconversional methods are in good agreement and *Arthrospira platensis* can prove to be a promising alternating source for bio-fuel production.

Keywords *Arthrospira platensis* microalgae · Non-isothermal pyrolysis kinetics · Activation energy · Pre-exponential factor · Thermodynamic parameters

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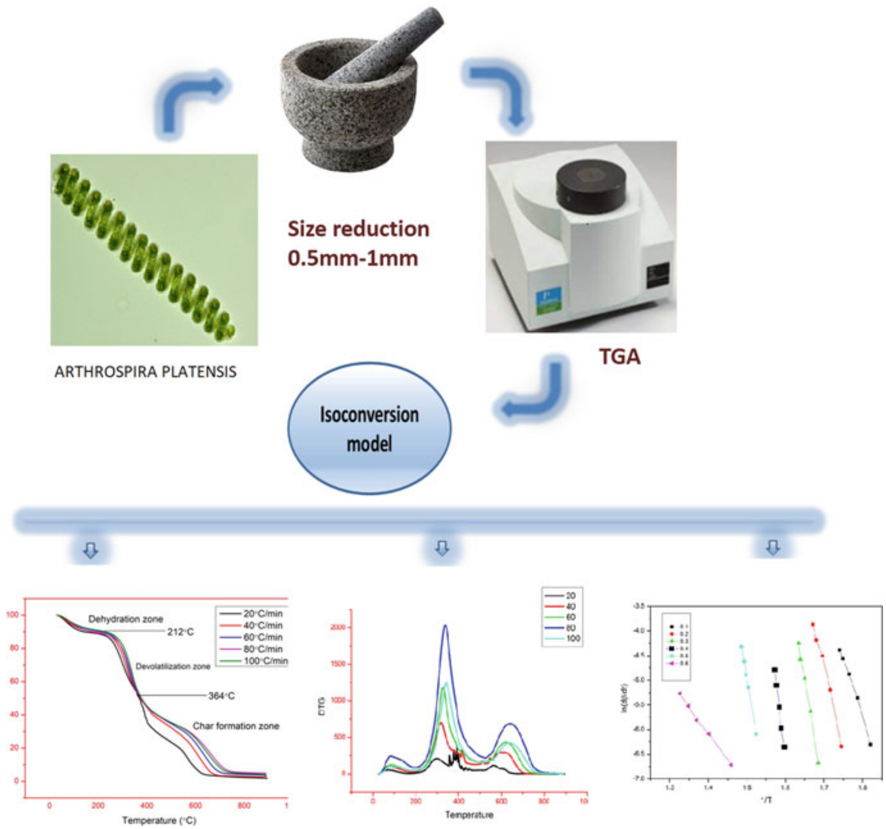
Abbreviations

TGA	Thermo-gravimetric analysis
DTG	Derivative thermogravimetry
Ea	Activation energy
KAS	Kissinger-Akahira-Sunose
OFW	Flynn-Wall-Ozawa
FRM	Friedman
VYZ	Vyazovkin
ΔG	Change in Gibbs free energy
ΔH	Change in enthalpy
ΔS	Change in entropy



8.1 Introduction

Consumption of fossil fuels in recent years has increased the amount of greenhouse gases in our atmosphere imposing serious concern to living species. There is a need of mankind to glance beyond fossil fuels in order to sustain our upcoming generation. Renewable sources of energy being clean source of energy present an attractive alternative to fossil fuels due to several advantages such as minimum environmental damage minimum secondary wastes in comparison to fossil derived energy. Among renewable sources, biomass is gaining attention as it contains large amount of volatile matter and extractive components (Rodionova et al. 2017). Biomass is considered as a renewable source of energy which is readily available all around the globe (Ramage and Scurlock 1996). Biomass is considered carbon neutral source as carbon dioxide emission resulting from bio-fuel combustion can be recycled back to plant for photosynthetic activity (Tsai et al. 2007). Biomass is directly or indirectly obtained from green plants utilizing sunlight for photosynthetic activity (McKendry 2002). Biomass is the only renewable energy source which can be transformed into solid, liquid and gaseous fuels as per convenience (Sinha et al. 2012). Microalgae can be described as photosynthetic microorganisms which require sunlight and fertilizers for efficient growth. Bio-fuel obtained from microalgae offers several advantages such as non-toxicity, no requirement of fresh water and arable land (Chernova et al. 2020). Microalgae can be converted to different value added products through thermochemical conversion process. Pyrolysis is the process through microalgae can be converted to energy fuels with lower feed to fuel ratio making it suitable for employing as a substitute for conventional sources of energy (Demirbas and Arin 2002). In pyrolytic experiment, thermal degradation of components present in biomass initiates at 350–350 °C and completes at 700–800 °C (Fisher et al. 2002). Studies on macro and microalgae pyrolysis and kinetic study that have been reported in the literature are *S. platensis* and *C. potothecoides* (Peng et al. 2001), *Dunaliella tertiolecta* (Shuping et al. 2010), *Enteromorpha prolifera* (Li et al. 2010a), *Laminaria japonica* and *Sargassum pallidum* (Li et al. 2010b), *Chlorella vulgaris* (Chen et al. 2011), *Pophyra yezoensis*, *Plocamium telfairiae* Harv and *Corallina pilulifera* (Li et al. 2011), *Saccharina japonica* (Kim et al. 2013), *Botryococcus braunii* and *Hapalosiphon* sp. (Liu et al. 2012), *M. pyrifera* (Zhao et al. 2013), *Chlorella pyrenoidosa* and *Spirulina platensis* (Gai et al. 2013), *Sargassum* sp. (Kim et al. 2012), *Chlorella vulgaris* (Agrawal and Chakraborty 2013) and *Polysiphonia* elongate (Ceylan et al. 2014) are summarized.

Biomass can be transformed to renewable forms and other chemical products through various biochemical and thermochemical conversion processes. The thermochemical methods include gasification, liquefaction and pyrolysis. Among these, pyrolysis is a simplest and widely used thermochemical conversion process. Estimation of kinetic parameters using thermogravimetric analyser has very significant for the assessment of energy potential in any organic material. Microalgae can prove to be a promising source for bio-fuel production via thermochemical processes due to its favourable characteristics such as high yield with respect to area, larger and rapid growth rate and more lipid productivity (Zabed et al. 2019; Mutsengerere et al. 2019; Chen and Lin 2019; Mathimani et al. 2019). 39.6 wt% bio-oil was reported from pyrolysis of *Scenedesmus* microalgae (Bordoloi et al. 2016). *Spirulina platensis* (SP) and *Chlorella protothecoides* (CP) microalgae were compared for different properties such as activation energy, bio-char and bio-oil production (Peng et al. 2001). 55.3 wt% bio-oil was obtained through pyrolysis of *Chlorella protothecoides* at 502 °C and yield decreased with rise in temperature (Demirbaş 2006). 40 wt% liquid yield from *Chlorella* sp., 60.7 wt% from *Chlorella vulgaris*, 64.9 wt% from *Dunaliella salina* and 46 wt% from *Spirulina* sp. at 425 °C, 500 °C, 500 °C and 550 °C, respectively (Koniuszewska et al. 2020; Oasmaa et al. 2012; Li et al. 2012; Bhola et al. 2011). A fundamental study of pyrolysis kinetics of *Arthrospira platensis* microalgae can help us to evaluate the pyrolysis behaviour and to design efficient reactor for thermochemical process. The results obtained by thermogravimetric analyser are used to define the kinetic triplets through various model free and model fitting approaches (White et al. 2011). Model free approaches are utilized to evaluate the activation energy value at different conversion rates (Slopiecka et al. 2012) while the model fitting methods are used to determine activation energy value throughout the process and it is difficult to determine the reaction mechanism with increasing conversion changes, therefore, unrealistic values of kinetic parameters are obtained (Hu et al. 2015).

A fundamental study of pyrolysis kinetics of protein rich microalgae *Arthrospira platensis* can help us to evaluate the pyrolysis behaviour and design efficient reactor for thermochemical process. The pyrolysis process of *Arthrospira platensis* could be subdivided into three phases, namely water evaporation stage, devolatilization stage and decomposition stage. Kinetic analysis of raw material is necessary as it can provide information regarding thermal behaviour based on which parameters can be optimized as well as pyrolysis reactor can be efficiently designed. The parameters involved in kinetic analysis include activation energy, frequency factor or pre-exponential factor and thermodynamic parameters. The activation energy was determined using Kissinger-Akahira-Sunose (KAS), Ozawa-Flynn-Wall (OFW), Friedman, Coats-Redfern and Vyazovkin (VYZ) methods. Thermogravimetric analysis was carried out at various heating rates of 20, 40, 60, 80 and 100 °C/min.

8.2 Materials and Methods

8.2.1 Materials

The microalgae were procured from the collection facility of Algae culture in Aban Infrastructure Pvt. Ltd. (biotechnology division) Chennai, Tamil Nadu, India. The cultures were maintained in Central Food Technology Research Institute (CFTRI) medium ($NaHCO_3$—4.5 g/L: $NaNO_3$—1.5 g/L; K_2HPO_4—0.5 g/L; K_2SO_4—1.0 g/L; $MgSO_4 \cdot 7H_2O$—1.2 g/L; $CaCl_2 \cdot 2H_2O$—0.04 g/L; $FeSO_4 \cdot 7H_2O$—0.01; NaCl—1.0 g/L; Seawater—1000 L) with 140 μmol photon m^{-2} s^{-1} light intensity and 25 ±

1 °C temperature in the growth room. This algae was nourished through seawater (Salinity: 30 ppt) in a 140 m^2 raceway pond. After culture, 40% of the pond volume was harvested through filtration using 200 mesh filter cloth.

The biomass harvested was washed using freshwater and the pH level was reduced to 7 in the biomass. Washing also helped in removing the salts. Thereafter, the wet algal biomass was dried under sunlight. The dried biomass was then pulverized using a mini flour mill and the biomass was stored in a cool dry place for further usage.

Biochemical analysis of algae biomass for proteins, carbohydrates and lipids was estimated using the standard test methods. Proximate analysis of the dry algae powder sample has been conducted through recommended methods of American standards such as ASTM D 4442, ASTM D 3172, ASTM D 3177 and ASTM D 3175.

Elemental analysis of algal biomass feedstock was carried out using the CHNS elemental analyser (Elementar Vario ELIII). Sulphanilamide was employed for calibration of instrument. Amount of oxygen was obtained by eliminating the combined proportions of carbon, nitrogen, hydrogen and ash from total weight percentage. H/C, O/C and N/C of raw feed stock were derived from the elemental composition. The higher heating value of microalgae was calculated through the Dulong's formula as mentioned below.

$$\text{Higher heating value (MJ/kg)} = 0.338\ \mathrm{C} + 1.428(\mathrm{H} - \mathrm{O}/8) + 0.095\ \mathrm{S}$$

where C, H, S and O represent the wt.% of carbon, hydrogen, sulphur and oxygen, respectively.

8.2.2 TGA

Thermogravimetric analysis (TGA) of the powdered algae sample was carried out using a Shimadzu DTG-60/60H instrument. Fixed amount of sample was taken and heated up to 900 °C in nitrogen atmosphere with flow rate of 35ml/min at different heating rate of 20 °C/min, 40 °C/min, 60 °C/min, 80 °C/min and 100 °C/min.

8.2.3 Analysis of Results Obtained by TGA

The weight loss with respect to temperature, statistical parameters and kinetic modelling were examined utilizing MS Office Excel 2016 (version 16.0.12325.20344), origin 2020 and RStudio (version February 1, 5033) software.

The derivative of TGA data (DTG) and TGA data were standardized through Eqs. (8.1) and (8.2) for normalization of different heating rates, standardized data of TGA and DTG are symbolized by M and dM/dt, respectively,

$$M = m_t / m_o \tag{8.1}$$

and

$$dM/dt = (dm_t/dt)\ (1/m_o) = \mathrm{DTG}\ (1/m_o) \tag{8.2}$$

where m_o represents the initial mass at 20 ± 3 °C and m_t is the instantaneous mass at any particular time t.

The coupling of these parameters facilitate the seed conversion and estimate the energy required for increasing conversion. These data prove to be beneficial in process design of pyrolysis bio-fuel feedstock. The α and $d\alpha/dt$ denote the conversion and rate of conversion, respectively, whose values can be obtained from Eqs. (8.3) and (8.4). M_i, M_t and M_∞ are the initial mass, mass at specific time and final mass, respectively. The activation energy and pre-exponential factor have been evaluated as kinetic parameters.

$$\alpha = (M_i – M_t)/(M_i – M_\infty) \tag{8.3}$$

$$d\alpha/dt = –(dM_t/dt)\ (1/M_i – M_\infty) \tag{8.4}$$

8.2.4 Kinetic Theory

Pyrolysis is the process of thermal degradation of biomass in the absence of oxygen and pyrolysis kinetics is beneficial for investigation of reaction mechanism. Better understanding of biomass reaction mechanism will ease the optimization of process parameters and efficient design of reactor for commercial scale bio refineries. Multiscale complexity of biomass structures and reaction occurring in pyrolysis process makes it difficult to investigate the mechanism. A general pyrolysis reaction for any biomass can be described as:

Biomass volatile matter (condensable + non condensable) + char (solid residue)

The rate of kinetic process is generally represented in the form of equation as

$$\frac{d\alpha}{dt} = kf(\alpha) \text{ or } \frac{d\alpha}{dt} = \frac{A}{\beta} \exp\left(-\frac{E}{RT}\right) f(\alpha) \tag{8.5}$$

where $\frac{d\alpha}{dt}$, $kf(\alpha)$, A, E, β and R are the conversion rate of reaction, rate constant, reaction model, pre-exponential factor (s^{-1}), activation energy (kJ/mol), heating rate (°C/min) and the universal gas constant which is constant as 8.314 J mol^{-1} K^{-1}, respectively.

Kinetic parameters can be obtained through multi heating rate model free methods. KAS, OFW, Friedman, Coats-Redfern and VYZ methods are employed to obtain the activation energy in the present work.

The conversion in pyrolysis process can be obtained from

$$\alpha = \frac{W_i - \mathrm{W}}{W_i - W_f} \tag{8.6}$$

where W_i, is the initial mass, W is the instantaneous mass and W_f is final masse is the above equation.

The rate equation can be represented as

$$\frac{d\alpha}{dt} = k(T)f(a) \tag{8.7}$$

where $k(T)$ and $f(a)$ are rate constant and reaction model, respectively.

Arrhenius law can be written as

$$k(T) = \mathrm{A}\ \exp\left(-\frac{E}{RT}\right) \tag{8.8}$$

where T is the absolute temperature. Putting Eq. (8.8) in Eq. (8.7), we will get

$$\frac{d\alpha}{dt} = Ae^{\left(-\frac{E}{RT}\right)} f(\alpha) \tag{8.9}$$

β can be written as a function of temperature in the following form:

$$\beta = \frac{dT}{dt} = \frac{dT}{d\alpha}\frac{d\alpha}{dt} \tag{8.10}$$

Combining Eq. (8.9) and Eq. (8.10) gives a new equation

$$g(\alpha) = \int_0^\alpha \frac{d\alpha}{f(\alpha)} = \int_0^T \frac{A}{\beta}\ e^{\left(-\frac{E}{RT}\right)} dT = \frac{AE}{\beta R}\int_x^\infty u^{-2}\ e^{-u}\ du = \frac{AE}{\beta R}\ p\ (x) \tag{8.11}$$

where the value of x can be calculated from $\frac{E}{RT}$

$g\ (\alpha)$ represents the integral form of the reaction models. The right-hand side of Eq. (8.11) denotes the integral of temperature without an exact integral solution. This equation can be solved through numerical approximations.

8.2.5 Determination of Kinetic Parameters Through Model Free Methods

Five different isoconversional methods such as Kissinger-Akahira-Sunose (KAS), Flynn-Wall-Ozawa (OFW), Friedman (FRM), Coats-Redfern (C-R) and Vyazovkin (VYZ) have been utilized to calculate the activation energy.

In KAS method, the following equations were applied to determine the values of kinetic parameters:

$$\frac{dT}{\beta} = dt \tag{8.12}$$

$$\frac{d\alpha}{f(\alpha)} = \frac{A}{\beta} \exp\left(-\frac{E}{RT}\right) dT \tag{8.13}$$

$$\int_0^{\alpha} \frac{d\alpha}{f(\alpha)} = \int_0^{T} \frac{A}{\beta}\, e^{\left(-\frac{E}{RT}\right)} dT \tag{8.14}$$

$$g(a) = \int_0^{\alpha} \frac{d\alpha}{f(\alpha)} = \int_0^{T} \frac{A}{\beta}\, e^{\left(-\frac{E}{RT}\right)} dT \tag{8.15}$$

$$g(a) = \frac{AE}{\beta R}\, p\,(x) \tag{8.16}$$

The Kissinger-Akahira-Sunose (KAS) method employs approximation of $p(x) = x^2 e^{-x}$

$$\ln \frac{\beta}{T^2} = \ln\left[\frac{AE}{Rg(a)}\right] - \frac{E}{RT} \tag{8.17}$$

$\ln \frac{\beta}{T^2}$ and $\frac{1}{T}$ were considered as the dependent and independent variables in the equation. The value of activation energy, E was calculated from the slope of the linear equation.

The Ozawa-Flynn-Wall (OFW) method uses Doyle's approximation (Doyle 1961) of

$$\log\left(\mathrm{P(x)}\right) = -2.315 + 0.457x \tag{8.18}$$

and

$$\log\left(\beta\right) = \log\left[\frac{AE}{Rg(a)}\right] - 2.315 - 0.457\frac{E}{RT} \tag{8.19}$$

Slope is given by $-E/R$ in the plot of log (β) vs. $1/T$ where R is the universal gas constant. Activation energy for the progressive values can be obtained by calculating in conversion range from 0 to 1.

8.2.6 Determination of Kinetic Parameters by Model Fitting Methods

Coats and Redfern method is a model fitting approach applied to evaluate the pre-exponential factor and activation energy for calculating the order of reaction.

$$X = e^{-\int_0^t k(T)dt} \tag{8.20}$$

General solution for kinetic integral assuming Arrhenius dependence for k (T) is described by

$$\int_0^t k\,(T)\,dt = \left(\frac{ART^2}{\beta E_a}\right)\left(\left(1 - \frac{2RT}{E_a}\right) + \ldots.\right)e^{\left(-\frac{E_a}{RT}\right)} \tag{8.21}$$

The RT/E_a can be neglected and substituting Eq. (8.21) in Eq. (8.20), we get

$$X = e^{-\left(1 - \frac{2RT}{E_a}\right)\left(\frac{ART^2}{\beta E_a}\right)e^{\left(-\frac{E_a}{RT}\right)}} \tag{8.22}$$

Taking log on both sides and rearranging Eq. (8.22), we get

$$\ln\left(\ln\left(\frac{X}{T^2}\right)\right) = \frac{-E_a}{RT} + \ln\left(\frac{-AR\left(1 - \frac{2RT}{E_a}\right)}{\beta E_a}\right) \tag{8.23}$$

Again rearranging Eq. (8.23), we get

$$\ln\left(\frac{\beta}{T^2}\right) = \ln\left(\frac{-AR\left(1 - \frac{2RT}{E_a}\right)}{E_a \ln(X)}\right) - \frac{E_a}{RT} \tag{8.24}$$

Friedman method (FRM) is the most straightforward technique to determine the activation energy as a function of the extent of reaction. The magnitude of rate constant is usually represented by the following equation:

$$kf(\alpha) = \beta\left(\frac{d\alpha}{dT}\right) = Af(a)\exp\left(-\frac{E}{RT}\right) \tag{8.25}$$

where $f(\alpha)$ is the reaction model, α denotes the conversion function, β represents the heating rate, A specifies the pre-exponential factor and R denotes the universal gas constant.

Applying logarithmic function on both sides of Eq. (8.25), we get

$$\ln\left(\beta\frac{d(\alpha)}{dT}\right) = \ln\left(\frac{d\alpha}{dT}\right) = \ln\left[Af(\alpha)\right] - \frac{E}{RT} \tag{8.26}$$

The slope lines between $\ln\left(\frac{d\alpha}{dT}\right)$ verses $\left(\frac{1}{T}\right)$ at the same conversion for different heating rates are utilized for finding the activation energy.

Vyazovkin (VYZ) method is a non-linear numerical integration approach employed to determine activation energy with a better accuracy as compared to FRM, OFW and KAS methods. Minimizing Eq. (8.19), we get

$$\Omega\ (E) = \sum\nolimits_{i=1}^{n}\sum\nolimits_{j\neq \mathrm{i}}^{n}\frac{I(E,\ \mathrm{Ti})\beta\mathrm{j}}{I(E,\ \mathrm{Tj})\beta\mathrm{i}} = n(n-1) \tag{8.27}$$

where n represents the number of experiments performed at different rates of heating and

$$I(E,\ T) = \int_0^T e^{\left(-\frac{E}{RT}\right)}dT = (E/R)\ p(x) \tag{8.28}$$

where $I\ (E,\ T_i)$ and $I\ (E,\ T_j)$ indicate the integral temperature $p(x)$ with respect to the heating rate β_i and β_j, respectively. $I\ (E,\ T)$ can be solved through numerical integration method as it has no exact solution. In case of *Arthrospira platensis* microalgae, following 8th-order rational approximation was applied

$$p(x) = \frac{e^{-x}}{x^2}\left(\frac{x^7 + 70x^6 + 1866x^5 + 24920x^4 + 170136x^3 + 577584x^2 + 844560x + 357120}{x^8 + 72x^7 + 2024x^6 + 28560x^5 + 216720x^4 + 880320x^3 + 1794240x^2 + 1572480x + 403200}\right) \tag{8.29}$$

where x = E/RT.

8.2.7 Analysis of Pre-exponential Factor by Kissinger Approach

The Kissinger method has been employed for determining the pre-exponential factor by using the kinetic analysis data. Following equation has been developed for Kissinger approach:

$$\ln \frac{\beta}{T^2} = \frac{-E}{RT} + \frac{AR}{E} \tag{8.30}$$

After solving Eq. (8.25), for the estimation of pre-exponential factor, we get

$$\mathrm{A} = \frac{\beta E e^{\left(-\frac{E}{R\mathrm{Tp}}\right)}}{R\mathrm{Tp}^2} \tag{8.31}$$

where T_p denotes the peak temperature on the DTG graph and E represents the activation energy evaluated from VYZ approach.

8.2.8 Thermodynamic Parameters

By utilizing the equations mentioned below, thermodynamic parameters such as Gibbs free energy change (ΔG), enthalpy (ΔH) and change in entropy (ΔS) can be calculated

$$\Delta G = E + \mathrm{R}T_m \ln\left[\frac{k_b T_m}{hA}\right] \tag{8.32}$$

$$\Delta \mathrm{H} = \mathrm{E} – \mathrm{RT} \tag{8.33}$$

$$\Delta \mathrm{S} = \frac{\Delta \mathrm{H} - \Delta \mathrm{G}}{T_m} \tag{8.34}$$

where k_b and h are the Boltzmann constant and Planck constant having constant values of 1.381×10^{-23} J/K and 6.626×10^{-34} J/s, respectively, A denotes the pre-exponential factor (s^{-1}) calculated from the Kissinger (KAS) method, E specifies the activation energy (kJ/mol) and T_m corresponds to peak temperature at that corresponding heating rate.

8.3 Results and Discussions

8.3.1 Characterization of Arthrospira platensis *Microalgae*

The proximate (moisture, ash, volatile and fixed carbon), biochemical and ultimate analysis (carbon, hydrogen, nitrogen, sulphur and oxygen) of *Arthrospira platensis* microalgae is presented in Table 8.1. Moisture content of biomass species affects the pyrolytic behaviour and influences the quality of pyrolytic products. Dry feed biomass may result in the production of highly viscous bio-oil, especially at higher temperatures (Singh et al. 2014). In case of higher moisture content, thermal pre-treatment is required to remove the moisture and to improve the product quality and thermal efficiency of process (Kan et al. 2016). *Arthrospira platensis* microalgae has a moisture content of 7.25 wt % which could result in increase in total pyrolytic oil, Volatile matter is an important parameter influencing H/C ratio and bio-oil yield (Wani et al. 2020; Mythili et al. 2013), the volatile content of the microalgae was found to be 74.65 wt%. According to the literature, cellulose and hemicelluloses are the major components that results in the generation of volatile matter (Soltes and Milne 1988). *Arthrospira platensis* microalgae has a fixed carbon content of 10.8 wt %. Fixed carbon content of the biomass enhances with the increase in pyrolytic temperature leading to better heating value of the biomass (Demirbas 2006). Hydrogen content was calculated to be 6.76 wt % indicating more production of H_2 and CO as a result of pyrolysis process (Zhao et al. 2010). Higher proportion of nitrogen

Table 8.1 Comparison of biochemical, proximate and ultimate analysis of *Arthrospira platensis* microalgae with Scenedesmus, microcystis and dunaliella salina microalgae

		Scenedesmus (Kim et al. 2014)	Microcystis (Miao et al. 2004)	Dunaliella salina (Gong et al. 2014)
Biochemical composition (wt%)				
% Protein	62.2	36.4	30.8–59.93	58.8
% Lipid	11.21	19.5	5.22–12.5	10.5
% Fibre	14.57	29.3	11.6–20.19	11.9
Proximate analysis (wt%)				
% Moisture	7.25	4.59	9.59	4
% Volatile matter	74. 65	75.33	70.13	76.3
% Ash content	7.3	7.3	6.14–13.26	7.2
% Fixed carbon	10.8	12.78	14.14	12.5
Ultimate analysis (wt %)				
% C	43.98	50	42.26	48.1
% H	6.76	7.11	6.27	7.1
% N	10.67	7.25	7.88	9.4
% S	0.71	0.54	0.52	0.9
% O	31.58	30.7	43.07	23.3
Gross calorific value (MJ/kg)	18.95	21.1	16.2	21.2

(10.67 wt %) and sulphur (0.909 wt %) was obtained for the chosen biomass which is an indication higher NO_x emissions as a result of application of produced bio-fuel. *Arthrospira platensis* was found to be having 31.58 wt % oxygen content, thus less amount of CO will be produced due to complete combustion of bio-oil. Higher amount of volatile matter (74.65 wt %) suggests the suitability of this biomass feedstock for higher production of bio-oil and bio-char (Pandey and Kumar 2020). The algae sample has high of percentage of protein content (62.2%) and less content of fibre (14.57%). The lipid content was found to be 11.21% which is less than Scenedesmus microalgae (19.5) and more than dunaliella salina microalgae. Larger lipid concentration in nitrogen depletion may result due to longer cultivation period of the microalgae (Granata 2017). Compounds containing nitrogen appear to be in more amount in the low lipid/high protein sample in comparison to the high and medium lipid microalgae samples (Adamakis et al. 2018). Lipid content in biomass tend to enhance the energy content leading to variation in the physiochemical properties of products obtained through the microalgae pyrolysis (Hong et al. 2020). Fibre composition (14.57 wt %) was comparable to the fibre content of microcystis and less than scenedesmus microalgae.

8.3.2 TG-DTG Analysis

The thermal degradation profile of the *Arthrospira platensis* microalgae has been obtained through thermogravimetric analyser. Figure 8.1 shows the weight loss (%) with increasing temperature during the thermal conversion of microalgae at different heating rates under N_2 atmosphere. Lignocellulosic biomass comprises of three components, hemicelluloses, cellulose and lignin. Hemicellulose decomposition generally takes place in the temperature range of 40–202 °C while cellulose decomposition occurs of 212–530 °C while lignin decomposition occurs around 900 °C. The TGA patterns of the lignocellulosic biomass can be attributed to the convolution of the TGA profiles and extent of evolution depends on the percentage composition of the individual components (Dhyani and Bhaskar 2018). Thermal degradation of microalgae occurs in three stages, namely stage I, II, and III. Drying stage or dehydration stage is the initial weight loss process in biomass pyrolysis which occurs from room temperature to around 160 °C (Cai and Liu 2007). In stage I, the amount of moisture present in the microalgae dropped rapidly with appearance of subsequent weight loss in the temperature range of 50–140 °C due to evaporation of moisture with rise in temperature. The TG curve presented a flat line indicating that 175 °C is the drying end temperature in the temperature range of 150–175 °C. In case of *Arthrospira platensis* microalgae, stage I resembling dehydration stage appears in the range of temperature between 40 and 202 °C accompanied by the evolution of moisture and some extractive compounds. The second stage corresponds to devolatilization process which occurred in between 212 and 530 °C temperature range accompanied by removal of volatile substances. The volatile matter yield depends on temperature and gas atmosphere (Roque-Diaz et al. 1985).

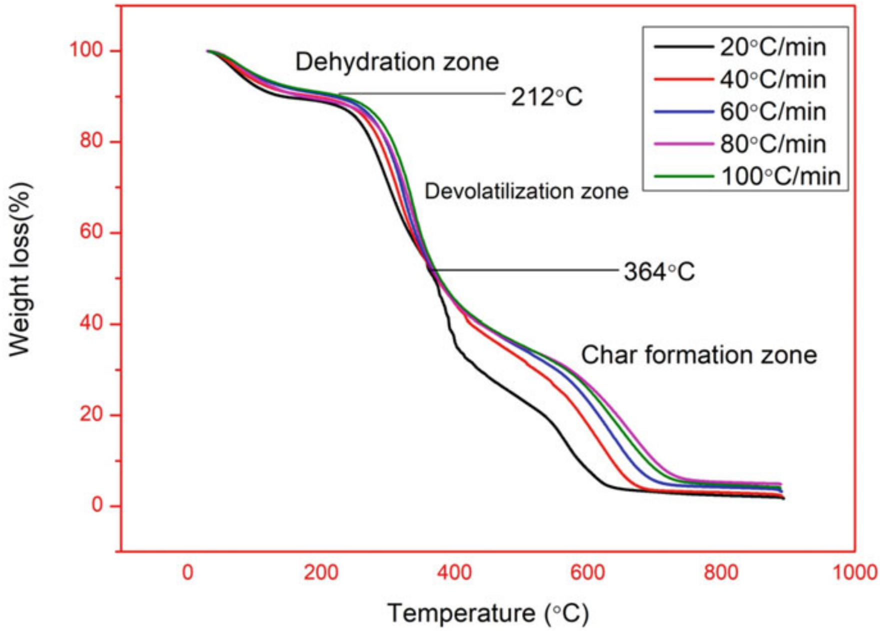


Fig. 8.1 TGA curve of microalgae at various heating rates of 20, 30, 40, 60 and 100 °C/min

Devolatilization stage is the main breakdown phase in which volatile substances were released due to decomposition of hemicelluloses, lignin and cellulose decomposition. Majority of the devolatilization occurred in the second stage of the TG curve due to thermal breakdown of weak bonds and formation of stronger bonds (Zhang et al. 2006). Compounds of higher molecular weight break into lower molecular weight compound as a consequence of continuous heat supply. The mass loss rate as resembled by the derivative thermogravimetry is an indication of reactivity of the biomass feedstock. The temperature at resembling maximum weight loss is represented by the peak position of the curve. The third stage (≤512 °C) known as lower decomposition zone is characterized by the end of cellulose decomposition, volatile decomposition, C–C bond cracking and ultimately, char formation. In this stage, the pyrolysis residue gradually decomposes as the velocity of weight loss becomes lower and lower and the residue ratio seems to be constant towards the end (Antal and Varhegyi 1995). As indicated in Fig. 8.1, the trend of the degradation profile of microalgae is not largely governed by heating rate programmes of 20, 40, 60, 80 and 100 °C/min, however, there is a gradual fetch in the upper zone in the temperature range from 212 °C to 530 °C, commonly described as the active pyrolysis region. The major peak at higher temperatures may be ascribed to cellulose pyrolysis and shoulder peaks at lower temperatures in the devolatilization stage was due to hemicelluloses pyrolysis.

First peak in the DTG curve was observed due to light volatile matter and moisture removal up to 202 °C, while second peak was obtained due to the degradation of hemicellulose and cellulose in the devolatilization stage. The peaks in the DTG curve shifted towards higher temperature zone without influencing the biomass conversion at higher heating rates. Figure 8.2 of DTG thermograph for *Arthrospira platensis* microalgae at five different heating rates (20, 40, 60, 80 and 100 °C/min) in N_2 atmosphere indicated the endothermic process and unstable curve below 212 °C. The second and third peaks emerged due to degradation of hemicelluloses and cellulose in the second stage whereas the lignin decomposed at a lower rate and temperature greater than 530 °C. As observed from the thermograph, the peaks obtained in DTG curve are much closer to each other which may be due to the catalytic characteristics of mineral components found in the biomass (Varhegyi et al. 1997). DTG peaks of the cellulose and hemicelluloses overlap each other due to shifting of cellulose decomposition to lower temperatures by inorganic salts (Shafizadeh 1982). As observed from Fig. 8.2, degradations are symbolized by downward slope indicating the extractives. In case of *Arthrospira platensis* microalgae, the first tiny peak of DTG curve indicates the drying of the sample which is not of much importance. The only subsequent peaks in the temperature range of 230–900 °C indicate biomass pyrolysis temperature range. The initial decomposition temperature is around 250 °C. A lower temperature shoulder signalizes at various heating rates of microalgae at about 270 °C indicating degradation of hemicelluloses, while higher temperature shoulder characterizes at around 490 °C at all the heating rates for the AM sample because of cellulose devolatilization and depolymerisation reaction (Sahoo et al. 2021). Hemicellulose decomposes in identical manner to cellulose, i.e. by dehydration at lower temperatures and depolymerisation at elevated temperatures (Alen et al. 1996). Dehydration results in the formation of water soluble acids, gases, anhydride fragments, char and water whereas volatile organics, levoglucosan, levoglucosenone, furans and other anhydrohexoses are obtained from depolymerisation (Sánchez-Jiménez et al. 2013). Above 900 °C, the DTG profile was almost stationary indicating the completion of the pyrolytic reaction of residual carbonaceous solid components.

8.3.3 Analysis of Kinetic Parameters

8.3.3.1 Estimation of Activation Energy

Isoconversional methods such as; KAS, OFW, C-R, FRM, VYZ were employed to determine the activation energy (E_a) with respect to conversion. The steady value of the linear regressions obtained through these models were investigated by coefficient of regression (R^2). Conversion ranges from 0.1 to 0.85 are selected to calculate activation energy. From Table 8.2, it can be seen that higher the conversion (>0.85), lower the coefficient of determination providing obstacle in model fitting. The evaluation of activation energy (E_a) with increasing conversion rate calculated

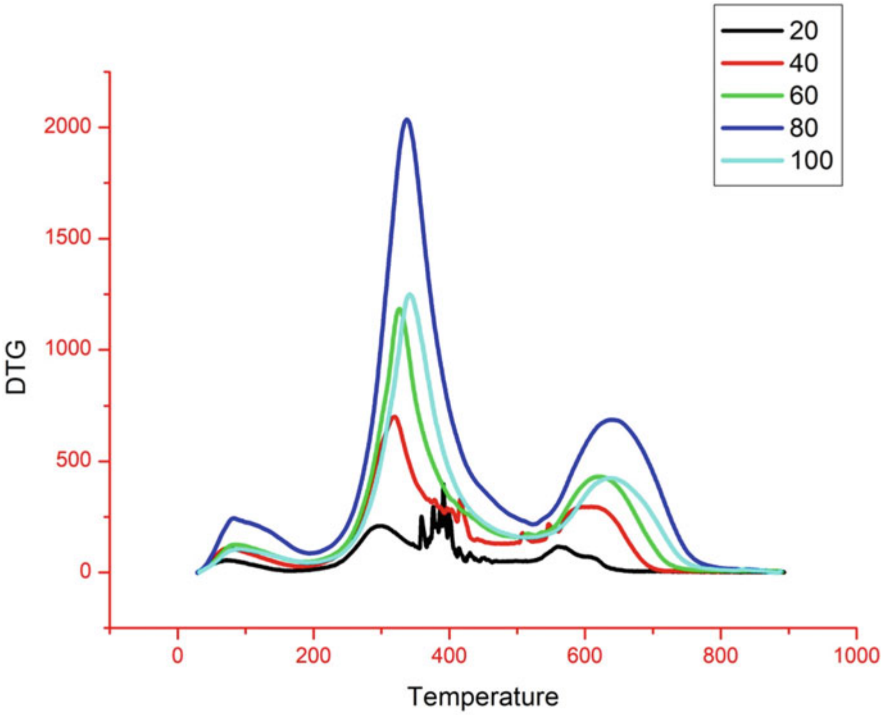


Fig. 8.2 DTG curve of microalgae at different heating rates

from the models have been shown in Figs. 8.3, 8.4, 8.5 and 8.6 for KAS, FRM, OFW and C-R methods respectively.

In the pyrolysis of *Arthrospira platensis* microalgae, the activation energies evaluated with the help of KAS, OFW, C-R, FRM and VYZ methods were constant at 0.3–0.45 conversion ranges. The differences in between maximum and minimum activation energy as a conversion for *Arthrospira platensis* microalgae pyrolysis were: 51.94% (181.52–87.24 kJ/mol), 74.05 % (264.4079–68.60994 kJ/mol), 81.28% (384.153–71.919 kJ/mol), 83.29% (533.894–89.217 kJ/mol), 49.71% (189.74–95.42KJ/mol) for KAS, OFW, C-R, FRM and VYZ methods, respectively. The mean of activation energy is 174.36 kJ/mol while the standard deviation of activation energy (E_a) is 55.02 kJ/mol from the VYZ model. In all the models, value of activation energy resembled an increasing trend with corresponding increase in conversion rate. From Table 8.2, it can be seen that the average value of errors in activation energy increased according to the order of FRM $<$ C-R $<$ KAS $<$ OFW. The evaluated activation energies are compatible with the activation energies of similar biomasses.

Table 8.2 Estimation of kinetic parameters based on KAS, OFW, C-R, FRM and VYZ models

Conversion (α)	KAS			OFW			C-R			FRM			VYZ
	Adj R^2	E_a (kJ/mol)	E_a error (%)	Adj R^2	E_a (kJ/mol)	E_a error (%)	Adj R^2	E_a (kJ/mol)	E_a error (%)	Adj R^2	E_a (kJ/mol)	E_a error (%)	E_a (kJ/mol)
0.1	0.9975	87.24	2.95	0.9972	156.444	1.99	0.99848	155.241	2.12	0.99173	198.238	1.23	95.42
0.2	0.9965	99.82	5.29	0.9965	168.871	5.23	0.99343	167.914	7.05	0.98072	273.183	3.71	125.1
0.3	0.9983	128.52	2.62	0.9987	191.198	2.51	0.98	302.082	4.57	0.99291	388.432	2.04	147.57
0.4	0.9995	139.48	2.76	0.9997	264.407	5.02	0.99446	384.653	1.72	0.98217	533.894	2.11	162.53
0.5	0.9998	159.54	2.15	0.9996	204.361	1.76	0.99579	219.937	3.34	0.99865	393.052	3.01	175.21
0.6	0.9854	181.52	14.81	0.9931	68.6099	15.77	0.98786	–71.919	9.57	0.99698	89.217	14.54	189.74
	Avg.	132.63	5.09	Avg.	175.64	5.38	Avg.	216.87	4.72	Avg.	312.67	4.44	149.26

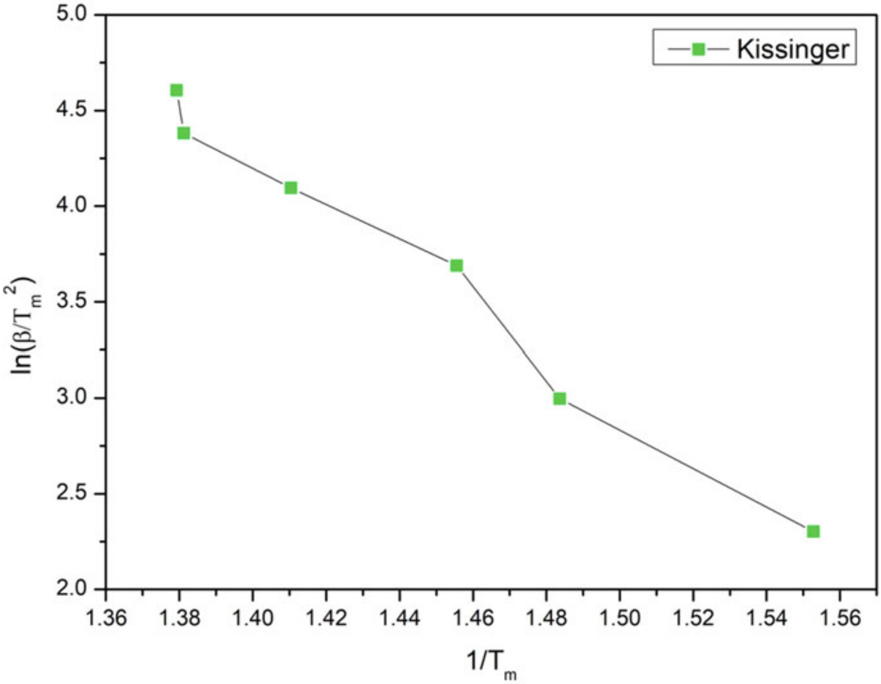


Fig. 8.3 KAS model of microalgae at various conversion rates of 20, 30, 40, 60 and 100 °C/min

8.3.3.2 Determination of Pre-exponential Factor

Estimation of pre-exponential factor at 20 °C/min heating rate was calculated by Kissinger method using the above mentioned Eq. (8.31) and the values are summarized in Table 8.2. The lower heating rate was selected due to the impact of interactions between the constituents during the pyrolysis process was high at higher heating rates (Sait et al. 2012). The range of pre-exponential factor for the microalgae biomass pyrolysis was in between 4.65 s^{-1} and 1.33 × 10^{24} s^{-1} at a single heating rate of 20 °C/min. Unanticipated rise of pre-exponential factor after 0.7 conversion rate can be attributed as the impact of noisy character at the end of conversion or due to energy barrier formed as a result of interference of minerals or heavy metals present in the ash (Raveendran et al. 1996). Higher value of pre-exponential factor in case of *Arthrospira platensis* microalgae pyrolysis symbolizes more collision that occurring between the molecules and initiation of a new reaction (Daugaard and Brown 2003).

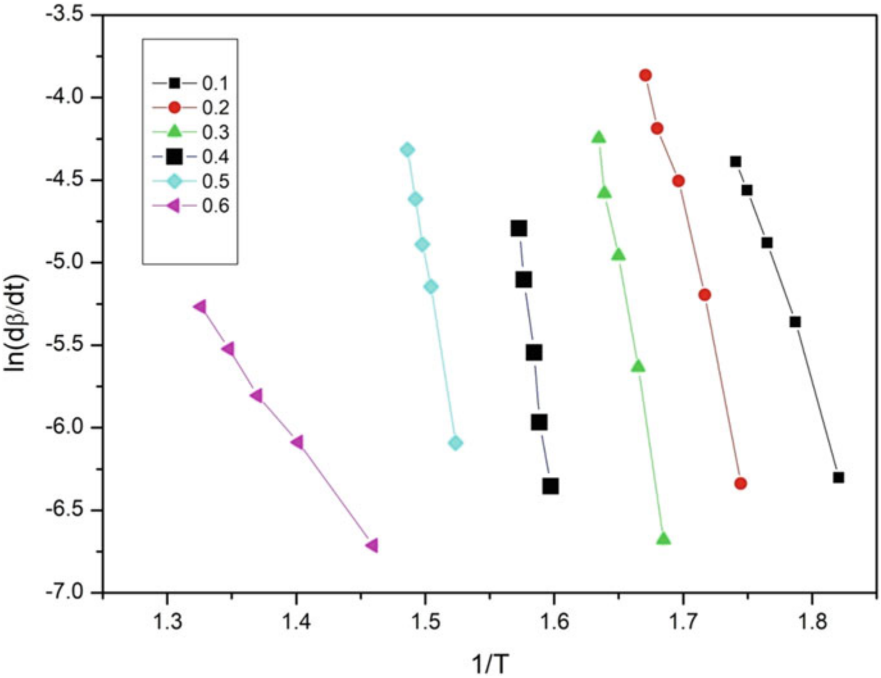


Fig. 8.4 FRM model of microalgae at different conversion rates

8.3.3.3 Determination of Thermodynamic Parameters

The change in Gibbs free energy change (ΔG), entropy (ΔS) and enthalpy (ΔH) was calculated from the Eqs. (8.32), (8.33), and (8.34) and presented in Table 8.3. The activation energy with respect to conversional rate evaluated using the Vyazovkin method (VYZ) was employed to obtain three thermodynamic parameters; ΔG, ΔS and ΔH. This analysis is very crucial for determining the feasibility of the pyrolytic process as well as performing the energy analysis. Gibbs free energy change (ΔG) is the maximum amount of mechanical work obtainable from the specified quantity of chosen biomass. At 20 °C/min heating rate, the variation in ΔG from 217.32 kJ/mol to 211.15 kJ/mol was observed while at heating rate of 40 °C/min, the Gibbs free energy change varied from 220.78 kJ/mol to 215.76 kJ/mol whereas for 60 °C/min, similar trend of variation in ΔG from 225.41 kJ/mol to 218.95 kJ/mol was found. The energy required to raise temperature from room temperature to the reaction temperature is known as enthalpy and transform the biomass into value added products (Kaur et al. 2018). Positive value of enthalpy changes signifies endothermic reactions prevalent in the specified temperature ranges (Tahir et al. 2020). Change in enthalpy increased with increase in conversion rate and it varied from 87.92kJ/mol to 194.42 kJ/mol, 87.12 kJ/mol to 193.72 kJ/mol, 87.72 kJ/mol to 191.57 kJ/mol, 76.69

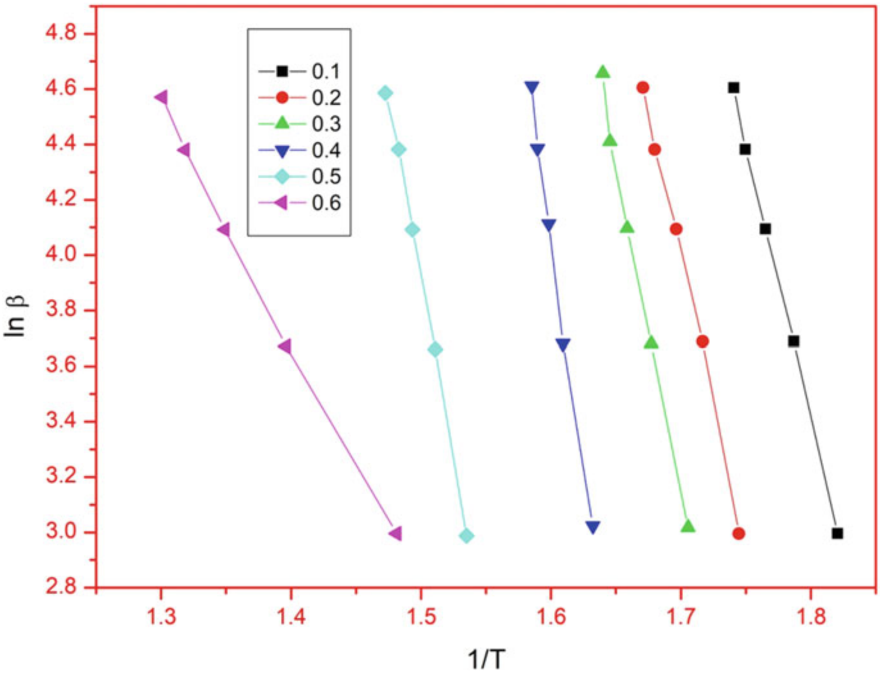


Fig. 8.5 OFW model of microalgae at different conversion rates

kJ/mol to 191.24 kJ/mol, 83.24 kJ/mol to 189.25 kJ/mol at 20, 40, 60, 80 and 100 °C/min heating rates, respectively. Change in entropy varied from –218.41 J/mol to 39.25 J/mol, –221.67 J/mol to 68.45 J/mol, –212.523 J/mol to 47.52 J/mol, –242.26 J/mol to 52.54 J/mol, –247.65 J/mol to 53.36 J/mol at 20, 40, 60, 80 and 100 °C/min heating rates, respectively. Negative value of change in entropy signifies characterization of active complex by a much higher degree of arrangement whereas higher value of change in entropy represents higher reactivity and rapid activated complex formation (Tahir et al. 2020) (Table 8.3).

8.4 Conclusion

In order to convert a biomass reaction process from laboratory scale to industrial scale, it is beneficial to understand kinetic behaviour of selected microalgae. Kinetics of algae is considered a very complex task due to several reactions occurring simultaneously. Isoconversional methods are capable to ascertain the multiple reactions occurring inside the material. In the present work, an experimental investigation of kinetic behaviour of *Arthrospira platensis* microalgae was carried out where

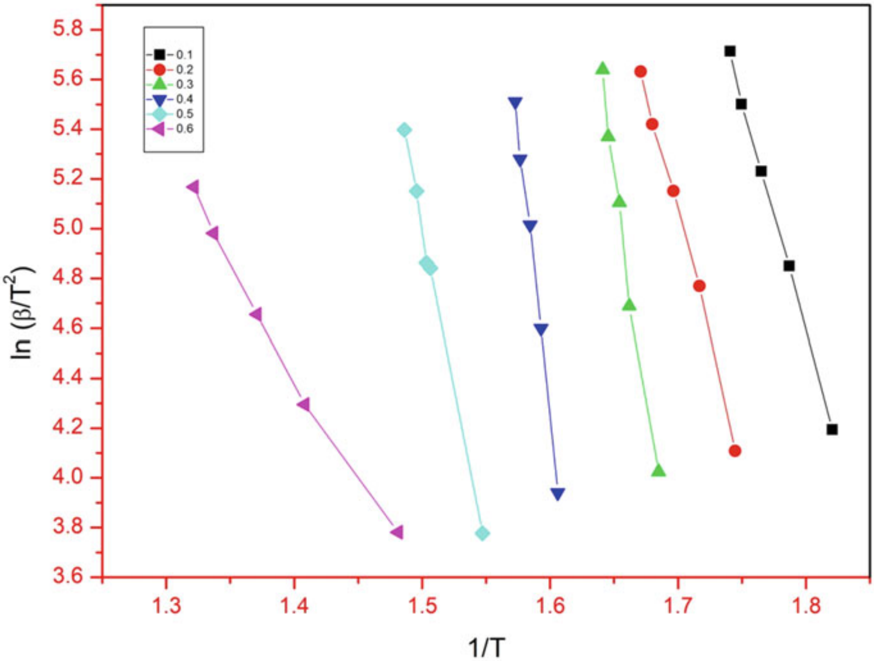


Fig. 8.6 C-R model of microalgae at different conversion rates

thermogravimetric analysis was accomplished under nitrogen atmosphere at five different heating rates. Thermal degradation of *Arthrospira platensis* occurred in three steps, namely; water removal, passive and active pyrolysis stages. It can be observed from the TGA data that major pyrolysis occurred in between 212 and 530 °C temperature. Impact of heating rate on TG and DTG profiles was analysed. Activation energy was calculated through model free methods, whereas pre-exponential factor was obtained through Kissinger approach. Gibbs free energy change, enthalpy and entropy change were calculated through appropriate equations. The average values of activation energy obtained from different kinetic models such as KAS, OFW, C-R, FRM and VYZ methods were 132.63, 175.65, 216.87, 312.67 and 149.26 kJ/mol, respectively. The obtained results from kinetic and thermodynamic study suggest the suitability of *Arthrospira platensis* microalgae for pyrolysis process.

Table 8.3 Thermodynamic parameters obtained at 20, 30, 40, 60 and 100 °C/min heating rates

Conversion (α)	A (s^{-1})	ΔH (kJ/mol)	ΔG (kJ/mol)	ΔS (J/mol)	ln A
At 20 °C heating rate					
0.1	3.49×10^{1}	87.12	220.78	–221.67	5.767823
0.2	2.46×10^{4}	112.64	219.72	–176.34	7.258845
0.3	2.78×10^{6}	134.85	217.12	–151.57	12.61484
0.4	7.34×10^{6}	149.54	217.57	–135.45	14.48701
0.5	5.16×10^{8}	162.34	216.24	–87.12	21.57124
0.6	6.95×10^{10}	193.72	215.76	–68.45	23.84934
Average	1.16×10^{10}	140.04	217.86	–140.1	14.25157
At 40 °C heating rate					
0.1	3.49×10^{1}	87.12	220.78	–221.67	5.767823
0.2	2.46×10^{4}	112.64	219.72	–176.34	7.258845
0.3	2.78×10^{6}	134.85	217.12	–151.57	12.61484
0.4	7.34×10^{6}	149.54	217.57	–135.45	14.48701
0.5	5.16×10^{8}	162.34	216.24	–87.12	21.57124
0.6	6.95×10^{10}	193.72	215.76	–68.45	23.84934
Average	1.16×10^{10}	140.04	217.86	–140.1	14.25157
At 60 °C heating rate					
0.1	2.43×10^{1}	87.72939985	225.4392257	–212.523	3.874013
0.2	2.45×10^{4}	112.7693923	223.6272323	–176.474	6.428974
0.3	6.78×10^{5}	123.5193941	222.7790725	–153.512	12.60671
0.4	5.32×10^{6}	144.8193932	221.3491011	–121.457	16.42274
0.5	1.04×10^{8}	169.7193954	220.8375914	–84.7623	20.5172
0.6	4.67×10^{10}	191.5693972	218.9515717	–47.5178	23.78424
Average	7.81×10^{9}	138.348333	222.161667	–132.708	13.934
At 80 °C heating rate					
0.1	2.09×10^{1}	76.69939962	227.2392268	–242.259	5.724017
0.2	1.95×10^{4}	115.4693963	226.5272374	–189.339	7.618929
0.3	6.94×10^{5}	134.57939921	225.5790777	–143.142	12.8 0784
0.4	3.67×10^{6}	152.7193431	224.2491026	–138.597	14.52241
0.5	7.04×10^{8}	167.9693985	223.5375918	–94.2929	18.8924
0.6	6.23×10^{10}	191.239343	222.7515724	–52.5347	25.65473
Average	1.05×10^{10}	139.779	224.9753333	–143.3595	14.195
At 100 °C heating rate					
0.1	3.18×10^{1}	83.23939976	229.5392219	–247.652	3.874016
0.2	1.56×10^{4}	113.5393993	228.8272342	–189.234	7.818922
0.3	7.74×10^{5}	124.7793994	227.1790743	–143.749	12.10781
0.4	5.72×10^{6}	139.1693992	226.7491056	–139.294	14.72244
0.5	3.45×10^{8}	162.6693992	225.2375954	–91.823	13.7923
0.6	6.91×10^{10}	189.2393995	224.6515711	–53.3641	21.4479
Average	1.157×10^{10}	135.433	227.023333	–144.185333	12.28833

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Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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Chapter 9
Growth of *Chlorella Minutissima* Microalgae from Fruit Waste Extract for Biodiesel Production

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Abstract Fruit peel extracts are ample source of several nutrients such as minerals and carbon, necessary for microalgal cultivation. In the present study, fruit (orange and banana) peel extracts are utilized as a source of essential inorganic and organic supplements for the cultivation of microalgae. Chemical composition of fruit (orange and banana) peel extract was determined by AAS analysis and elemental analysis. The HPLC analysis discloses that the obtained extracts contains major quantity of glucose and fructose. The presence of number of functional groups was observed by the FTIR analysis. *Chlorella minutissima* was used as the microalgae strain for the culture of algal biomass. For each prepared media, the fixed algae concentrations were incubated in seven different concentrations ratios 1:2.5, 1:5, 1:7, 1:10, 1:15, 1: 20 and 1:25. The growth of algae in media of both the fruit peel extracts was detected by spectroscopy. Furthermore, Nile red staining method through fluorescence microscopy was carried out to estimate the lipid accumulation and found that the lipid accumulation is higher in banana peel media. GC-MS analysis of transformed FAME biodiesel demonstrates the high content of essential fatty acids.

Keywords *Chlorella minutissima* microalgae · Orange and banana peel extracts · GC-MS · HPLC · FAME biodiesel

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9.1 Introduction

A demand of food for human consumption is increasing globally day by day. The world population is estimated to 9 billion in the year of 2050 which requires a 70% increase in the production of food to meet its future requirement. It is also estimated that a quarter to one-third of food produced for human consumption is wasted (Guo et al. 2020). Food waste refers to the disposal or another use of food which is safe and nutritious for human consumption. Energy can be produced from food waste through various such as incineration and anaerobic digestion or the waste can be used in cattle-feed to maintain the nutrient equilibrium. There is also one alternate method of utilizing the food wastes in which these wastes can be used as a feedstock for the cultivation of micro-organisms. This method is further beneficial in the production of chemicals, materials and energy from biomass. A renewable and environmental friendly fuel may be obtained from algae termed as algal fuel but the production of algae in large scale is still has substantial economic and technical limitations (Havlik et al. 2013). Citrus sinensis or sweet orange is the most demanding citrus fruits in the world with the production more than 50 million tons. Half of its total production is utilized in the preparation of juice and rest 50% such as peel, pulp and seeds are termed as waste materials. The orange peels have the different chemical compositions due to the climatic conditions and other factors like stages of growth and time for harvesting (Xinni et al. 2019). Banana is also an important food crop in tropical and subtropical region. The global production of banana was estimated around 118 million ton per year in 2013. *Musa acuminata* comes under the category of sweet bananas or dessert bananas which are accounted as 68% of the total banana production while 32% are available as hybrids of *M. acuminata* and *M. balbisiana*, these are categorized as plantains or cooking bananas (Waghmare and Arya 2016). One recent study compared the alternative culture media for microbial growth with conventional culture media and suggested that the alternative culture media presented satisfactory results in terms of microbial growth efficiency and production cost (dos Santos et al. 2021). The potential of using papaya, pineapple and mango fruit wastes as nutrient mediums was investigated for the cultivation of *C. vulgaris* and *H. pluvialis*. The higher yield of biomass concentration (4.133–4.533 g/L) was obtained with the use of 20% tropical fruit waste medium for *C. vulgaris* whereas the similar biomass concentration was yielded using 10% mango waste medium for *H. pluvialis*. The study demonstrated the feasibility of tropical fruit wastes to cultivate microalgae (Tan et al. 2021). The waste obtained from vegetable market was transformed into a best suited fermentation medium for cultivation of oleaginous yeast *Rhodosporidium toruloides*. The prepared culture medium can increase in the lipid and carotenoid contents by 24.17% and 8.77%, respectively, as compared to synthetic medium. The experiments were upscaled to fermenter for the production of lipids and biodiesel and suggested that these waste products would lead to waste management as well as the production of value-added commodities (Singh et al. 2018). The extracts of ten different fruits were successfully utilized as the substrates for single cell protein production to be used as food or animal feed (Dunuweera et al. 2021). The single cell protein was also produced efficiently using apple and cucumber fruit peels as substrate for *Saccharomyces cerevisiae* and *Spirulina*. Apple peel was the promising substrate for *Saccharomyces cerevisiae* as compared to cucumber peel (Shukla et al. 2017). A fungal medium can be produced from watermelon peel waste due to its high nutritional contents and low-cost production. The mechanical pre-treatment of watermelon peel waste was found to be the best suitable for fungal growth (Hasanin and Hashem 2020). Another application of fruit peel waste extract is the production of bacterial nanocellulose. A low-cost medium of rotten apple was used as a nutrient and source of carbon for the production of bacterial nanocellulose. A strain isolated from rotten apple and classified as *Komagataeibacter xylinus* IITR DKH20 using 16 s rRNA sequencing analysis was utilized for this purpose (Khan et al. 2021).

The extract obtained from fruit waste contains mostly glucose and fructose, make it very striking source of carbon for microalgal biomass production. Several applications of orange peel and banana peel extracts have been investigated by different researchers. Orange peel extract (OPE) was used to inhibit corrosion for the replacement of current toxic inhibitors. OPE restricted the corrosion attack by forming a protective film. Four variables, such as solvent ratio, acid concentration, temperature and contact time, were utilized to study the inhibitive performance of OPE. The maximum inhibition effects were observed at acid concentration less than 1M, temperature at 30 °C, contact time of 2 h and orange peel to solvent ratio of 10: 100. Temkin, Freundlich and Langmuir isotherm models were employed to validate the experimental data. Langmuir adsorption isotherm model with the highest regression coefficient value was followed by orange peel media. The obtained results suggested that the orange peel media may be used as a potential alternative in corrosion inhibition (Hong and Kiew 2019). Sweet orange (Citrus sinensis) peel was used for the preparation of gold nanoparticles. Spherical gold nanoparticles with narrow size distribution were acquired by controlling pH and adjusting sequence. The stability of ultra-small gold nanoparticles and its mechanism of synthesis from fruit peels extract was easily examined by the obtained results (Yang et al. 2019). Orange peel was used as a substrate for yield of bio-surfactant with 1.796 g/L and emulsification activity of 75.17% against diesel. The fatty acid residue contained lipopeptide which was observed in FTIR analysis of bio-surfactant. The bio-surfactant produced by *B. licheniformis* can be used as a potential source of eco-friendly biodegradable product (Kumar et al. 2016). Banana peel extract (BPE) was utilized in synthesis of bio-inspired silver nanoparticles. The content of banana peel extract, concentration of silver nitrate, incubation temperature and pH was selected as the parameters of reaction conditions for the formation of silver nanoparticles. The synthesized silver nanoparticles exhibited antimicrobial activity against bacterial cultures and fungal (Bankar et al. 2010). The potential of Banana peel waste of *Musa balbisiana*, *Musa acuminate* and *Musa Paradisiaca* as substrate has been investigated for the production of citric acid by Aspergillus niger. Different values of citric acid production, total biomass and pH were obtained at differences of banana peel waste media. The obtained percentages of citric acid using media of *Musa balbisiana*, *Musa acuminate* and *Musa paradisiaca* were 46.80, 58.80 and

69.84%, respectively (Khairan et al. 2019). Banana peel has been utilized for the removal of cadmium cation from environmental and industrial wastewater. Rapid attainment of phase equilibration and high sorption capacity values are major advantages of banana peel fruit waste. The important parameters such as temperature, initial metal ion concentration, contact time and pH were taken for the experiments. pH played an important role in binding of metal ions. The partitioning behaviour of the system was estimated by Langmuir adsorption isotherm at room temperature. The pseudo-first-order rate equation was followed for kinetics of sorption. The spontaneous reaction and endothermic nature of the sorption process was observed by thermodynamic parameters; Gibbs free energy and enthalpy. It has been concluded that banana peel can effectively be used for the removal of cadmium cations from environmental and industrial wastewater samples (Jamil et al. 2008). One more study has shown the application of orange peel and banana peel as adsorbents for the removal of heavy metal ions. FTIR analysis was employed for the characterization of various native and surface modified adsorbents. Heavy metals such as Cu^{2+} and Ni^{2+} were effectively adsorbed by the adsorbents prepared from orange and banana peels. It was confirmed that orange and banana fruit waste peels can act as effective adsorbents as compared to commercially available activated carbon (Thirumavalavan et al. 2011). Microwave energy was applied in the alkali pre-treatment on banana peel waste to achieve optimal reducing sugar. The percentages of lignin and hemi-cellulose in banana peel waste were reduced drastically by microwave assisted alkali pre-treatment process whereas the high amount of cellulose was found. This process can be used as an energy-saving technology for bioethanol production from sugar rich banana peel waste in a cost-effective way (Tiwari et al. 2019). The *Chlorella Vulgaris* microalgae was cultivated in a low-cost plantain (*Musa paradisiaca*) peel extract medium. Furthermore, the cultivated biomass was utilized as a carbon source for bioethanol production through separate hydrolysis fermentation (SHF) and separate hydrolysis co-culture fermentation (SHCF) techniques (Agwa et al. 2017). The banana peel hydrolysate as a fermentation medium was chosen as a low-cost feedstock for the production of Torula yeast (*Cyberlindnera* sp.) biomass which can provide a vital source of high protein content as compared to natural protein (Jiru and Melku 2018).

The algal biomass composition can be optimized by varying the limiting factors for growth to obtain the maximum yield of preferred compounds such as; carbohydrate, proteins, lipid and polyunsaturated fatty acids. Only few researchers utilized different fruit waste extracts for the production of these desired compounds. The extracts of *Musa acuminata Colla* (LFB), *Musa paradisiaca* (BB), *Josapine* (JP) and *Ananas comosus* MD2 (MD2) fruits were utilized for the production of high valued omega-3 essential fatty acid (DHA). The extract of MD2 fruit was found to be a potential source of carbon and a better material as compared to the commercially available glucose and fructose for biomass, lipid and DHA production (Nazir et al. 2020). The extracts of kinnow peel separately and in the combination with dairy wastewater, sewage and distilled water were used to prepare three economically feasible cultivation medium for algal biomass production. The maximum yield of lipid (17.52 mg/L-d) was obtained using KEMS as cultivation medium. The result suggested that the kinnow peel extract with different wastewaters formed high amount of biomass and a cost-effective medium for biodiesel feedstock production (Uttam and Ghosh 2019). Peel extract of orange fruit was utilized as an inorganic and organic source of nutrient for the cultivation of *Chlorella vulgaris*. *C. vulgaris* cells grown in orange peel extract medium had shown 3.4 and 4.5 times maximum yield of biomass and of FAME biodiesel. Orange peel extract was the best suitable for economically cultivation of microalgae and for the production of microalgal biodiesel (Park et al. 2014). The potential of orange peel extracts as a nutrient substrate for cost-efficient biomass and DHA production from the oleaginous microalgal strain *Aurantiochytrium* sp. *KRS101* was assessed. It was found that the DHA productivity using *Aurantiochytrium* cells grown under a heterotrophic culture condition using OPE combined with an optimized inorganic nitrogen source was increased by 2.5 times as compared to the previous studies using conventional media (Park et al. 2018). Orange fruit peel waste was used to produce α-amylase enzyme under submerged conditions by *Streptomyces* sp. KP314280 (20r). Highest yield of α-amylase enzyme (12.19 U/mL) was obtained at the optimum operating conditions (Ousaadi et al. 2021). The orange peel extract based liquid medium was utilized for its ability to check the growth and the production of lipid in submerged cultures of 18 yeast strains. The biodiesel yields from the lipids of *R. toruloides* and *C. laurentii* strains were 36.9 and 31.9%, respectively. The FAME compositions of these two biodiesels are similar to conventional biodiesels such as; jatropha and palm oils (Carota et al. 2020).

Microalgae seems to be a promising feedstock for production of biofuel. Microalgae follows the same mechanism of photosynthesis as followed by the other plants, but this can be converted into biochemical energy with the help of solar energy more efficiently and in the liquid product due of its simple cellular structure. It has more ready access to water and nutrients as the cells grow in aqueous media. Several varieties of algal strains are available which are capable of transforming more than 50% of their biomass as lipids (Metting 1996). Therefore, more liquid/oil can be produced from microalgae per unit area of land as compared to terrestrial energy crops such as jatropha, karanja and rapeseed or canola. Algal cells have the lipid content from which the microalgal biofuels are obtained, these biofuels can act as the potential feedstocks for alternative fuels for transportation, comprising biodiesel and green diesel, green jet fuel and green gasoline; the rest of the algal biomass can also be used for the conversion into biofuels via biochemical or thermochemical conversion process (Pienkos and Darzins 2009). High quality biofuel may also be produced by increasing the quality of extracted lipids with the higher amount of polyunsaturated fatty acids in algal biomass (Laraib et al. 2021).

Chlorella minutissima (eukaryotic alga) is very easy to culture and has a fast rate of growth. It also has high content of amino acids and polyunsaturated fatty acid to be utilized potentially in diet complements and in pharmaceuticals (Seto et al. 1984). Additionally, *Chlorella minutissima* has a high acceptance of carbon dioxide and can be supportive in utilizing waste carbon dioxide for cultivation of algae (Santos-Ballardo et al. 2015). *Chlorella minutissima* can also be used as a possible feedstock for biodiesel production (Papazi et al. 2008). Hence, *Chlorella minutissima* was selected in this work to estimate the optimum growth conditions on fruit waste extracts. For the growth of microalgae, the orange and banana peel extracts have been utilized effectively in the present study. The curves of growth are determined with the help of optical density as a parameter of biomass growth. The fruit peels media is analysed using various chemical characterization methods and compared with traditionally used BB media. The fatty acid content in microalgal lipid was also estimated to assess its feasibility for biodiesel production. The aim of the present study is to determine the suitability of fruit peel extract as a media for cell growth and lipid accumulation of *Chlorella minutissima.*

9.2 Materials and Methods

9.2.1 Preparation of Aqueous Media from Banana and Orange Peel

The banana and orange fruit peels were taken as base for the production of fruit extract. These peels were collected from a juice centre nearby NIT Rourkela, Odisha, India and grounded into a wet slurry using distilled water. The aqueous media from fruit peel was synthesized as per the process available in literature (Razaghi et al. 2016). Approximately 25 g of the slurry was mixed in 50 ml distilled water and sterilized. The media was then extracted in an autoclave at 121 °C above 1 atm pressure for 15 min. After that, it was taken out and cooled to room temperature. The synthesized aqueous media was then filtered and centrifuged to remove the solid particles. Finally, this aqueous extract was stored at 4 °C to be used in future.

9.2.2 Characterization of Fruit Peel and Its Aqueous Extract

The percentages of protein, lipid and fibre were determined from the dried raw peel of banana and orange fruits as available in our earlier work (Kumari and Singh 2019). Elemental analysis of banana and orange peel was determined using an Elemental Analyser System, make Germany Vario EL. The oxygen percentage was calculated by the difference between hundred and percentage of carbon, nitrogen, sulphur, hydrogen. A pH metre was also used to determine the acidity and alkalinity range of orange and banana peel aqueous extracts.

A microwave digester was used for acid digestion of dried and powdered fruit peel samples. Concisely, 0.5 g of fruit peel sample was digested in a solution of nitric acid (65%) and hydrogen peroxide (30%) for 15 min at a temperature of 200 °C. The obtained product after digestion was cooled, filtered and stored for the spectro-analysis. Atomic absorption spectroscopy (AAS) make Perkin-Elmer Intensitron was used to estimate the percentage of Iron, Zinc, Manganese, Copper and

Magnesium. Residual concentration of some specific metal ions such as sodium, potassium and calcium were determined using flame atomic absorption spectrometer make Systronics, India. The phosphorus concentration was estimated by Murphy Riley reagent method as available in the literature (Emaga et al. 2007).

Perkin-Elmer RX Fourier transform infrared spectroscopy (FTIR) was used to identify the functional groups present in fruit peels and aqueous extracts in the range of 400–4000 cm^{-1}. The aqueous extracts obtained from banana and orange fruit peels were also characterized to determine the sugar and acid content by high-performance liquid chromatography (HPLC) of Shimadzu LC solution, DGU-20A. 10 μL of aqueous extract sample was inserted in the column having flow rate of 0.4 mL/min and mobile phase of 100% DI water with temperature 85 °C, and the peaks were identified by RI detector.

9.2.3 Algal Strain and Conditions for Culture Media

Chlorella minutissima was used as the microalgae strain in this study, procured from Indian Agricultural Research Institute, New Delhi. The received strain was first cultured in modified BB media. For further experiments, the obtained exponentially growing culture was kept in an incubator shaker with the shaking speed of 120 rpm, at 25 ± 2 °C under the light intensity of 50 ± 5 μmol m^{-2} s^{-1} and the light dark ratio of 18:6 h photoperiod. The microalgae was then separately incubated in synthesized culture media of fruit peels and BB media as control. After that, the same concentration of algae inoculum were incubated in seven different concentrations and the ratios of algae inoculum: media are 1:2.5, 1:5, 1:7, 1:10, 1:15, 1:20 and 1:25. The ratio was altered by altering the concentration of stock solution with fixed amount of distilled water. The average pH was maintained in between 6.5 and 7 for all the culture media. The growth monitor and trend was analysed by method described by Santos-Ballardo et al. (Santos-Ballardo et al. 2015). Briefly, chlorophyll concentration was estimated by UV-VIS spectrophotometer between 664 and 690 nm.

9.2.4 Determination of Lipid Content and Its Characterization

Neutral lipid detection of algae cells in vivo was observed by Nile red (Himedia Pvt. Ltd.) staining method. A minimum 200 μg/ml concentration of Nile red solution synthesized in acetone was used for incubation of the algal suspension. Stained algal cells were witnessed under fluorescence microscopy (IX-71, Olympus). For lipid determination, the dried algal biomass was mixed with the extraction solvents such as methanol, chloroform and 1M NaCl as described in our earlier work (Kumari and Singh 2019). The solvent was then collected after centrifugation, and the residual biomass was also separated. This method of lipid extraction was repeated three times and the average value was taken as final. The extracted lipid was then dried and weighed. Transformation of lipid into fatty acids methyl ester (FAME) was carried out using a molar ratio of 1:82:4 for oil, methanol and HCl at a reaction time of 6.5 h and 65 °C reaction temperature. The organic phase at the top contained fatty acids methyl ester was pipetted out for further analytical characterization.

Gas chromatography–mass spectrometry (GC-MS) analysis was used to determine the composition of obtained algae biodiesel using Agilent 7890B GC–MS with flame ionization detector and a capillary column of DB-5MS. One microlitre sample was inserted into the column with carrier gas, helium. The details of experimental conditions were found in our earlier work (Kumari and Singh 2019).

9.3 Results and Discussions

9.3.1 Characterization of Fruit Peels and Their Aqueous Extracts

Protein, lipid and fibre content were determined according to the methods mentioned in the previous section. Protein content as per AOAC procedures of orange and banana peel were found to be 6.8% and 8.3%, respectively. Lipid extraction through conventional petroleum ether were determined to be 3.1%, 5.7% for orange and banana peel. Similarly, the fibre content estimation for the present study of orange and banana peel were 38% and 45.4% while the others contribute as 52.1% and 40.6%, respectively. The results indicate that the peels are having high nutritional value. Therefore, these human waste could be further utilize for many purposes. Similar findings were reported and suggested that these fruit waste could be utilized for such purpose (Seto et al. 1984).

The result of chemical composition of orange and banana peel has been represented in Table 9.1.

As per obtained result, almost all elements have comparatively higher percentage in banana peel than orange peel. The potassium being highest, i.e. 44,252 ± 139 mg/kg; 16,459 ± 718 mg/kg in banana and orange peel, respectively. The peels were also rich source of calcium and phosphorous nutrient. Similarly, magnesium and sodium were also obtained in moderate quantities. However, Iron, Zinc, Manganese content were low and Copper content was observed in very trace ratio. The similar trend of macro- and micromineral nutrients are commonly found in banana, orange fruits and peels (Emaga et al. 2007; Özcan et al. 2016). However, seasonal and soil nutrient variation effects the quality of fruits. Apart from carbon source, algae cultivation could be enhanced by mineral supplement. Similar as plant, NPK elements act as source of fertilizer for efficient algae cultivation. Trace minerals also requires for vital metabolic activities within cells (Costa et al. 2020). CHNS analysis also attributes the higher carbon percentage in the feedstock. High content

Table 9.1 The chemical composition of orange and banana peels

Metal	Banana	Orange
Iron (mg/kg)	16.7 ± 2.5	10 ± 1
Zinc (mg/kg)	15.3 ± 0.4	2.44 ± 0.22
Manganese (mg/kg)	7.6 ± 0.4	1.87 ± 0.01
Copper (mg/kg)	0.9	0.44
Phosphorus (mg/kg)	1761 ± 13	1087 ± 63
Magnesium (mg/kg)	695 ± 8	562 ± 27
Flame photometry		
Sodium (mg/kg)	359 ± 86	252 ± 18
Potassium (mg/kg)	44,252 ± 139	16,459 ± 718
Calcium (mg/kg)	8152 ± 210	5644 ± 240
CHNS analysis		
Carbon (%)	45.87	43.98
Hydrogen (%)	6.123	5.850
Nitrogen (%)	0.94	1.74
Sulphur (%)	0.273	0.609
Oxygen (%)	46.794	47.821

of carbon and low content of nitrogen is desirable for a promising culture medium (Güzel and Akpınar 2020). The carbon nitrogen ratio in banana peel is 45:1 while it is 25:1 in orange peel media. Similar range of carbon nitrogen ratio 30:1 was obtained in orange fruit peel, sufficient for the growth of fungus *Blakeslea trispora* (Kaur et al. 2019). The pH values of orange peel media, banana peel media and BB media are 6.70, 6.60 and 7 ± 0.2, respectively, which show that these media are almost neutral in nature. One more advantage of using BB media that it does not contain the glucose percentage.

Table 9.2 represents the functional groups present in the fruit peels and their media. As it can be observed from FITR table, major functional groups alcohol, alkanes, ketones, aldehydes and carboxylic acids, alkenes, phenyl rings, alkanes, alcohols, phenols, esters and ethers, aromatic compounds are present in the fruit peels and peels media. It shows the alkanes group C–H at frequency 3288.05 cm^{-1}, 2940.03 cm^{-1}, 1604.46 cm^{-1} and 1368.71 cm^{-1} in orange peel. Whereas, for orange peel media, 2930.56 cm^{-1}, 1600.23 cm^{-1}, 1369.50 cm^{-1} are the frequency for alkanes group in orange peel media. Ketones, aldehydes or carboxylic acids functional groups are found at frequency 1734.42 cm^{-1} and 1738.29 cm^{-1} in orange peel and media, respectively. The major peaks observed at frequency of 1231.73 cm^{-1} and 1009.42 cm^{-1} due to the presence of alcohols, phenols, esters or ethers in orange peel. Similarly, for orange peel media, the frequency is 3519.25 cm^{-1}, 1270.67 cm^{-1} and 1078.67 cm^{-1}. The additional phenyl rings at 1600.23 cm^{-1}, 764.55 cm^{-1} and aromatic ring at 649.45 cm^{-1} are also evident in orange peel media. Deviations at this frequency usually result from the combination of individual atom groups of carboxyl oxygen.

Table 9.2 Functional groups present in orange and banana fruit peel and their media

Name of the functional group	Type of vibration	Frequency Orange peel	Frequency Banana peel	Frequency Orange peel media	Frequency Banana peel media
Alcohol, phenol	O–H	X	X	3519.25	3438.98
Alkanes	C–H	3288.05	3294.28	X	3077.11
Alkanes	C–H	2940.03	2921.48	2930.56	X
Ketones, aldehydes and carboxylic acids	C=O	1734.42	1731.43	1738.29	X
Alkenes	C=C stretching	1604.46	X	1600.23	X
Phenyl rings	X	X	X	1515.17	1571.42
Alkanes	C–H bending	1368.71	1375.19	1369.50	1412.54
Alcohols, phenols, esters and ethers	C–O and O–H stretching	1231.73	X	1270.67 and 1078.67	1065.44
Alcohols, phenols, esters	C–O and O–H stretching	1009.42	X	1461	1458
Phenyl rings	X	X	X	764.55	X
Aromatic compounds	O–H bending	X	X	649.45	674.07

It can be seen from Table 9.2, the alkane functional group is observed at frequency band of 3294.28 cm^{-1}, 2921.48 cm^{-1}, 1375.19 cm^{-1} for banana peel while for banana peel media, it is at 3077.11 cm^{-1}, 1214.54 cm^{-1}frequency. At band 1731.43 cm^{-1}, the vibrations of C=O functional groups was found only in banana peel. The presence of alcohols, phenols, esters and ethers functional groups at frequency 1031.79 cm^{-1} and 1065.44 cm^{-1} are due to the C–O and O–H stretching type of vibrations for the banana peel and banana peel media, respectively. The presence of alcohols, weak amines and aromatic compounds were found at absorption bands of 3438.98 cm^{-1} and 674.07 cm^{-1}, respectively. Banana peel extract can be a good inhibitor due to the presence of organic moieties which are rich in oxygen atoms and aromatic rings (Ji et al. 2015).

The result of HPLC analysis for both orange and banana peel media is represented in Table 9.3. Highest concentration 2.125 of xylose was found at 6.613 retention time and concentration 2.05 of glucose was observed at 8.313 retention time for orange peel extracts. Similarly, highest concentration 4.9 of glucose was found at 8.811 retention time for banana peel extract. Some other compounds such as fructose, maltotriose, arabinose, acetic acid, propionic acid, citric acid and butyric acid are also observed in fruit peel extracts. High concentration of glucose in both the fruit peel extracts implies that these can be a vital source of microalgae biomass cultivation. The presence of carotenoids such as b-carotene, lutein and violaxanthin

Table 9.3 Peak table for HPLC analysis of the OPM and BPM samples

Retention time	Possible structure	Concentration in OPE	Concentration in BPE
6.479	Glucose	X	0.6509
6.613	Xylose	2.125	X
8.817	Citric acid	0.00301	0.03789
7.643	Maltotriose	X	0.01884
8.313	Glucose	2.05	2.206
8.811	Glucose	X	4.9
9.768	Glucose	0.625	0.456
10.375	Xylose	X	0.4173
10.441	Fructose	0.2854	X
11.134	Arabinose	0.0126	0.000543
13.79	Acetic acid	X	0.59012
15.532	Acetic acid	0.00025	0.1128886
18.453	Propionic acid	0.0009	0.0077935
22.921	Butyric acid	X	0.01051

in mango peel extract reveals that it can be used in pharmaceutical applications (Majila et al. 2010).

9.3.2 Growth Curve

Growth of microalgae in the media of varying concentrations was observed. The growth of cell was analysed and measured on daily basis. The patterns of light absorbance for different concentrations (1:2.5, 1:05, 1:07, 1:10, 1:15, 1:20 and 1:25 media with the time in days) are shown in Fig. 9.1a, b. It was found that the maximum absorbance was in between 680 and 690 nm for the evaluated microalgae out of the total scanning range of wavelength from 660 to 720 nm. The maximum absorbance was observed at different stages of cell growth and showing the same pattern in all experiments. Due to the presence of different contents of pigments such as chlorophyll and carotenoids, the difference in the maximum absorbance for the microalgae species was observed (Özcan et al. 2016). The growth of *Chlorella minutissima* microalgae in orange peel and banana peel media was supervised by the estimation of optical density and altering the concentration ratios of the stock solutions. At highest concentration ratio (1:2.5) of orange peel extract media, the growth is the fastest, reaches a peak and then falls rapidly as shown in Fig. 9.1a.

The less concentrated ratios (1:05, 1:07, 1:10, 1:15 and 1:20) display a persistent growth over an extended time period. The minimum concentration ratio of 1:25 shows a relaxed growth and withstands it for a longer period of time. Similarly, in banana peel extract media, the growth is sustained for a longer duration of time as compared to the orange peel extract media as shown in Fig. 9.1b. The rate of growth is highest and is constant for a longer duration of time in highly concentration ratio (1:2.5) media.

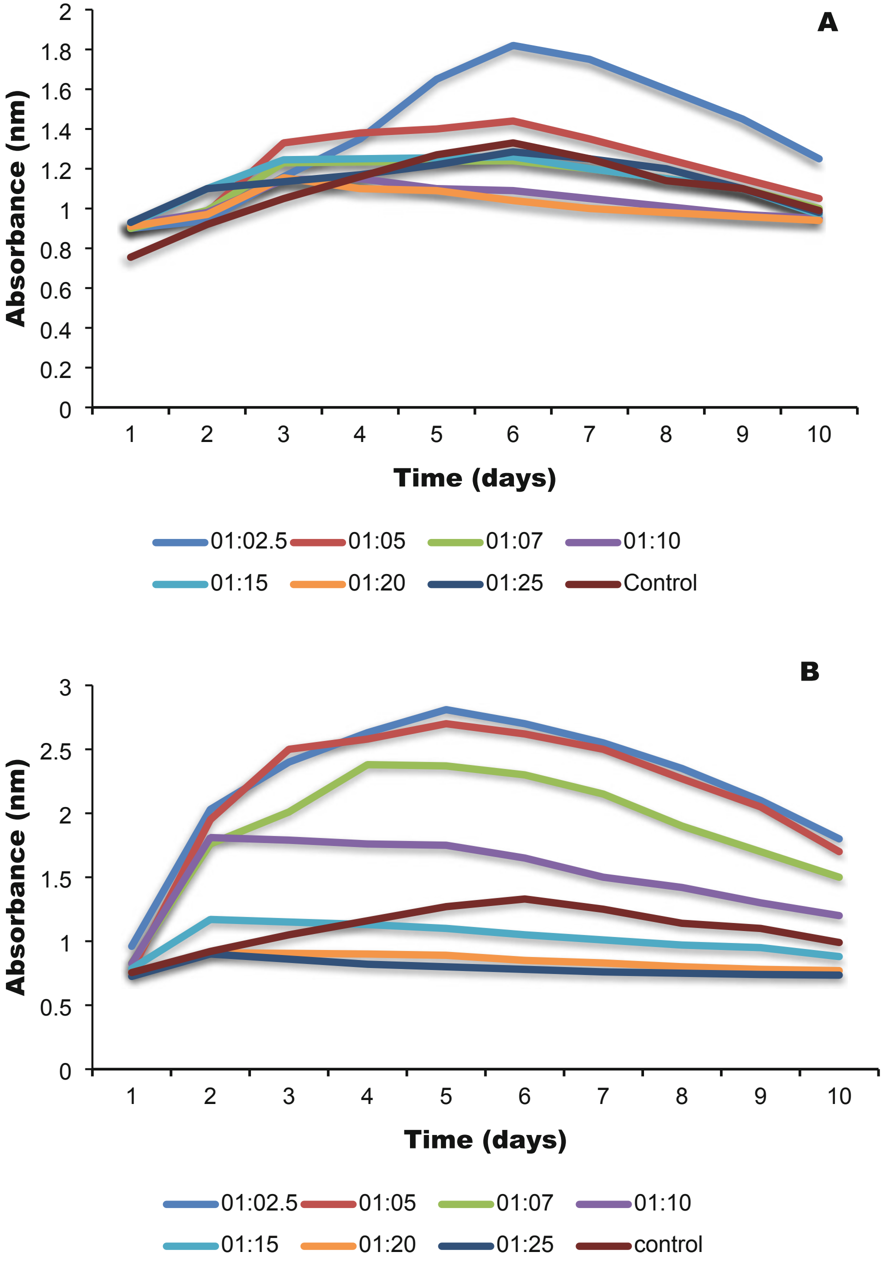

Fig. 9.1 Growth curve of microalgae in (**a**) orange peel media, (**b**) banana peel media

9.3.2.1 Growth of Microalgae

Growth of microalgae generally contains six phases in batch culture like the growth of micro-organisms. These six phases are named as lag phase, exponential phase, linear phase, declining growth phase, stationary phase and death phase. In the first phase or lag phase, the physiological adjustment in new environment is responsible for the initial growth. Second phase is an exponential phase, in which the cells nurture and distribute as a function with a rate of change proportional to the time. The intensity of light and nutrients do not constrain the growth of microalgae during this phase. In the third or linear growth phase, the separation of micro algal cells slows down due to the limiting presence of light. Therefore, accumulation of microalgae biomass takes place at a constant rate until the nutrients or inhibitors become a limiting factor in the culture medium. The declining growth phase or fourth phase is introduced by the reduction in cell division rate due to limiting factors such as nutrients, carbon dioxide and others. During the stationary phase or fifth phase, the growth rate becomes zero due to consumption of all the nutrients in the culture medium. In the last phase or death phase, the cell concentration decreases exponentially due to the depletion of all the limiting factors such as nutrients, temperature rise, pH differences and contamination from other sources. Figure 9.2a shows the peel media of banana fruit and the algae growth in banana peel media after 7 days has been shown in Fig. 9.2b. Similarly, Fig. 9.2c shows the orange peel media and the algae growth in orange peel media after 7 days has been shown in Fig. 9.2d.

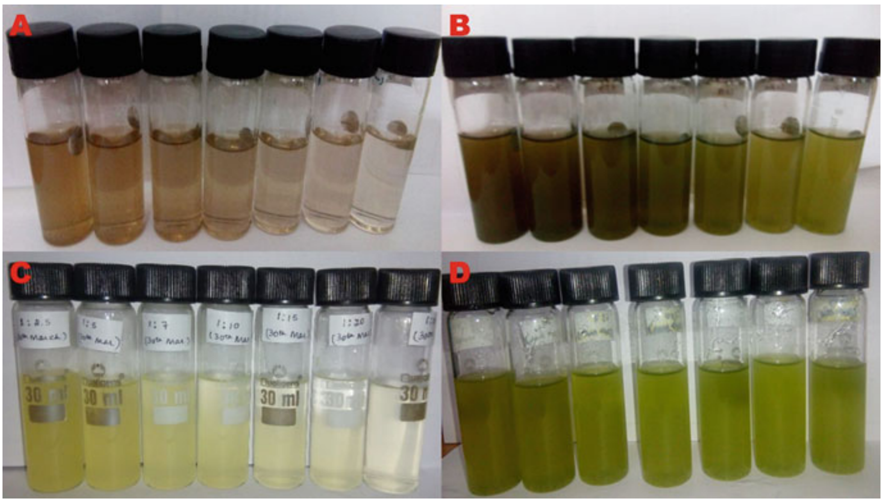

Fig. 9.2 (**a**) Banana peel media. (**b**) Algae growth in BPM after 7 days. (**c**) Orange peel media. (**d**) Algae growth in OPM after 7 days

9.3.3 Estimation of Lipid

The Nile red microscopy method was used to observe the lipid accumulation within algae cells. It is helpful to plan further experiment set and saves labour and time of experiment. Figure 9.3a–c shows the micrograph of fluorescence image in three different media conditions, banana peel media, BB media and orange peel media, respectively.

The red stain shows the algae cells while yellow reflection shows the presence of lipid accumulation. As much yellow reflection was not observed from algae cells grown in BB media so that samples were not analysed further for lipid extraction. In orange and banana fruit peel media, the cells are found in bundled form and well connected with adjacent cell aggregates while the cells of *Chlorella minutissima* microalgae cultured in BB media are not connected to each other. Similar morphological structure of Sphaerocystis sp. microalgae has been observed when lugol was added as reagent (Kumari and Singh 2019).

The yield of lipid was estimated by Folch's method of lipid extraction. Algal cells were used for the extraction of lipid content, however, lipid extraction from the cell much more influenced by the solvent polarity due to the presence of various polar and non-polar lipid within cell. The lipid yield was 16.1% in banana peel media and 12.7% in orange peel media. The lipid content was higher in banana peel media due to high percentage (74.9%) of insoluble dietary fibre (Ji et al. 2015).

9.3.4 FAME Characterization by GC-MS Analysis

Gas chromatography and mass spectrometry (GC-MS) technique was used to characterize the fatty acid methyl ester (FAME) biodiesel obtained by transformation of lipids. Figure 9.4a shows the GC-MS chromatogram of FAME biodiesel obtained from *Chlorella minutissima* microalgae cultured in banana peel media. A total of around five different fatty acid substrate and one alcohol ranging from C_{16} to C_{20}

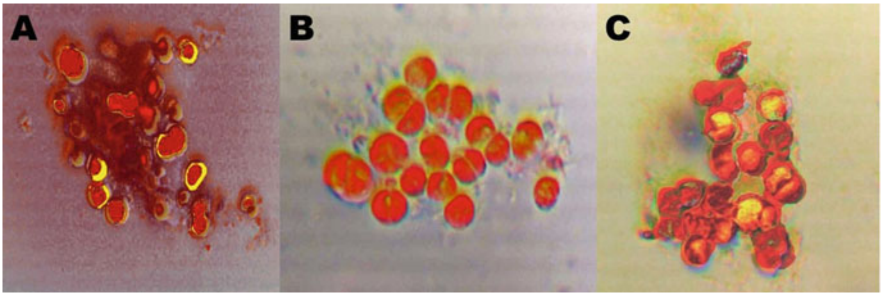

Fig. 9.3 (**a**) Chlorella minutissima cultured in BPM. (**b**) Chlorella minutissima cultured in BB media. (**c**) Chlorella minutissima cultured in OPM

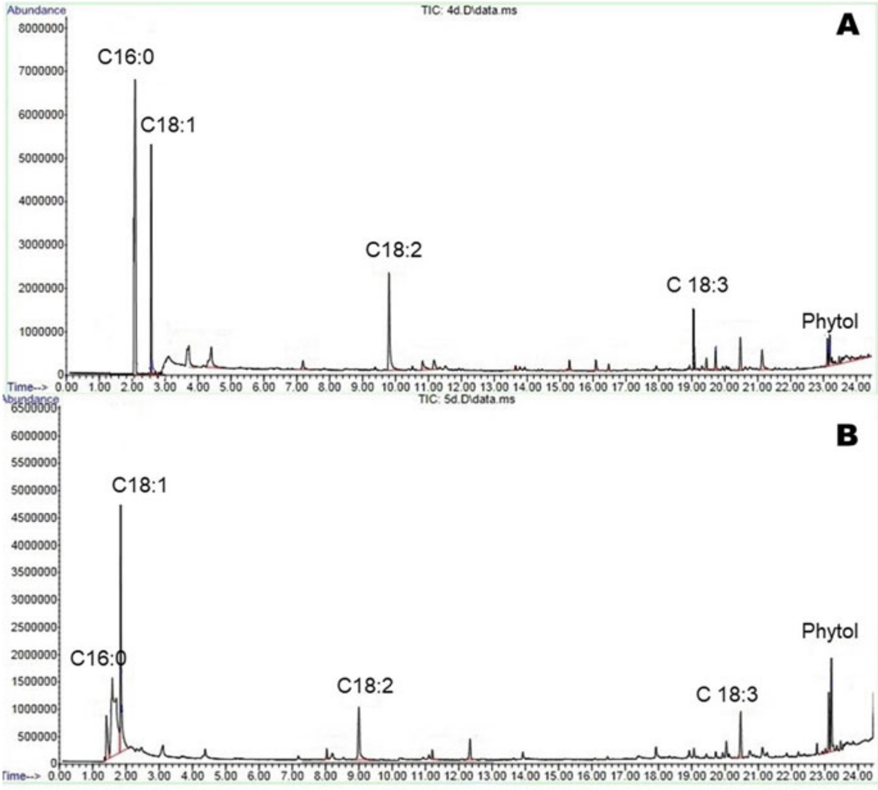

Fig. 9.4 GC-MS chromatogram of obtained FAME. (**a**) *Chlorella minutissima* cultured in BPM. (**b**) *Chlorella minutissima* cultured in OPM

were recognized by mass spectrometry. The major fatty acids are low carbon chain compounds, appropriate for biodiesel production. Oleic acid (C18:1), linoleic acid (C18:2) and linolenic acid (C18:3) comprises more than 70% of total fatty acids as per the obtained retention time. These acids are widely used in making of soaps, emulsifiers, beauty products and anti-inflammatory agent. Only one alcohol Phytol ($C_{20}H_{40}O$) was found which can be used as a precursor for the manufacture of synthetic forms of vitamin E and vitamin K1. Phytol is a major component of plant chlorophyll, which is then converted to phytanic acid and deposited in fats. Similar fatty acids were found in FAME biodiesel obtained from local mixed algal culture (Majila et al. 2010). Similarly, the total fatty acid methyl ester (FAME) concentrations from lipids extracted by two different techniques; PBR+CL and open tray+CL, were estimated as 50.59% and 38.31%, respectively when the *Citrus limetta* (CL) residue was used for cultivation of *Chlorella* sp. to obtain the biodiesel (Tibolla et al. 2014).

Palmitic acid (C16:0) is also found along with the other three fatty acids (C18:1, C18:2, C18:3) and phytol in the GC-MS chromatogram of FAME biodiesel obtained from *Chlorella minutissima* microalgae cultured in orange peel media as shown in Fig. 9.4b. The important application of palmitic acid is in manufacturing of cosmetics and biofuels.

9.3.5 Fuel Properties of the FAME Biodiesel

Biodiesel Analyzer, an online analytical tool, is used for the prediction of FAME biodiesel fuel properties based on fatty acid profile determined by gas chromatography (GC) analysis (Kumari and Singh 2016, 2021). Table 9.4 represents the FAME biodiesel obtained from *Chlorella minutissima* microalgae cultured in banana and orange peel media. Saturated fatty acids are the simplest fatty acids having unbranched structure with no double bonds in a carbon chain, palmitic acid and stearic acid bearing carbon number C_{16} and C_{18} are the most common saturated fatty acids which are generally found in animal lipids.

The percentage of saturated fatty acids is higher 40% in banana peel media as compared to FAME biodiesel obtained from *Chlorella minutissima* microalgae cultured in orange peel media whereas the percentage of monounsaturated fatty acids (MUFA) is higher about 55.290% in orange peel media. Monounsaturated

Table 9.4 Properties of FAME biodiesel

Biodiesel properties	Banana peel media (BPM)	Orange peel media (OPM)
Saturated fatty acids % (SFA)	40.000	17.640
Monounsaturated fatty acids % (MUFA)	31.400	55.290
Polyunsaturated fatty acids % (PUFA)	28.540	27.050
Degree of unsaturation (DU)	88.480	109.390
Saponification value (SV) mg/g	206.763	202.325
Iodine value (IV)	90.482	109.599
Cetane number (CN)	52.339	48.617
Long-chain saturated factor (LCSF)	4.000	1.764
Cold filter plugging point (CFPP) (°C)	–3.910	–10.935
Cloud point (CP) (°C)	16.048	4.287
Pour point (PP) (°C)	10.600	–2.168
Allylic position equivalent (APE)	88.480	109.390
Bis-allylic position equivalent (BAPE)	39.940	38.810
Oxidation stability (OS) (h)	6.723	6.950
Higher heating value (HHV) (MJ/Kg)	39.329	39.445
Kinematic viscosity (μ) (mm^2/s)	3.672	3.735
Density (ρ) (kg/m^3)	0.876	0.878

fatty acids contains a single bond and are generally found in oils nuts, seeds, fruits and meat. These are completely absorbed by the intestine and oxidized for energy production, or combined with tissue lipids (Schwingshackl and Hoffmann 2012). The percentages of polyunsaturated fatty acids (PUFAs) are nearly the same in FAME biodiesel obtained from *Chlorella minutissima* microalgae cultured in both the media. PUFA are the chain of hydrocarbon consisting one or more double bonds, found in particularly marine microalgae (Morales-Sánchez et al. 2020). Additionally, unsaturated fatty acids will also be increased by the mass cultivation of *Chlorella vulgaris* in citrus peel amino acid supplemented medium (Nateghpour et al. 2021; Katiyar et al. 2019). The PUFA, major sources of fungi and microalgae, was increased efficiently by using various residues as fermentation substrates (Kothri et al. 2020). The saponification value was calculated to 206.763 and 202.325 mg/g for banana and orange peel media cultured microalgae biodiesel respectively. The estimated saponification values are very close to the saponification value of 198.85 mg/g for jatropha biofuel (Gopinath et al. 2009). Cetane number for FAME biodiesel obtained from *Chlorella minutissima* microalgae cultured in BPM is 52.339 while in case of OPM, it is 48.617. The minimum cetane number should be 51 as per the European specification for biofuel obtained from any biomass feedstock. The major difference is observed in cloud point and pour point which are very less in case of FAME biodiesel obtained from *Chlorella minutissima* microalgae cultured in OPM. This may be due to the presence of less amount of saturated fatty acids in orange peel media which makes it unsuitable to be used in colder regions (Hazrat et al. 2020). Higher heating values of both the biodiesel are less as compared to conventional diesel fuel due to higher amount of oxygen in biomass feed stock (Singh et al. 2014). Kinematic viscosity and density are slightly higher for both the biodiesel when compared with diesel fuel due to their chemical structure comprising fatty acids (Islam et al. 2013).

9.4 Conclusions

The appropriateness of fruits extracts (orange peel and banana peel) was studied for the cultivation of *Chlorella minutissima* microalgae. The characterization of fruit peels and their aqueous extracts by elemental analysis, AAS analysis and FTIR spectroscopy was carried out to determine the presence of elements, metals and functional groups. The result of FTIR analysis shows the existence of alkanes, phenyl rings, alcohol, phenol and esters in both the peel extracts which is an indication their suitability for microalgae cultivation. The ratio of 1:2.5 for both peel extracts shows highest cell density while the ratio of 1:25 for orange peel extract shows fastest rate of increment in growth density. Therefore, it can be observed that banana peel media will be considered better as comparison to orange peel media due to its cell density and prolonged survival of *C. minutissima*. A total of around five different fatty acids and one alcohol ranging from C_{16} to C_{20} were identified by the GC-MS analysis of FAME biodiesel obtained from *Chlorella minutissima* microalgae cultured in both the media. The findings of the present study suggest that fruit peel extracts are suitable for the cost-effective cultivation of microalgae and transformation of FAME biodiesel from the lipids. However, the future work is required to maximize the lipid content.

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Conflict of Interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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Chapter 10
Microalgae: A Way Toward Sustainable Development of a Society

Komal Agrawal, Tannu Ruhil, and Pradeep Verma

Abstract The growing needs of the human population have come across as a global challenge. More population means more reliance on fossil fuels and more burden on available land for food, thus creating more pollution. In a recent scenario, a microscopic organism that has attracted the attention of scientists across the globe is microalgae. They are found in a varied range of habitats, and their easy availability, renewability, and sustainability have made them an alternative to conventional products and services. Microalgae have found applications in bioremediation, biofuel and biohydrogen production, food, health, and cosmetics industries, etc. However, one main problem faced while using microalgae is its commercialization for mass production as more research need to be done for its effective implementation. Thus, the present chapter gives an insight into the applications of microalgae in a concise manner. An effort to unravel the limitations has also been made along with the prospects.

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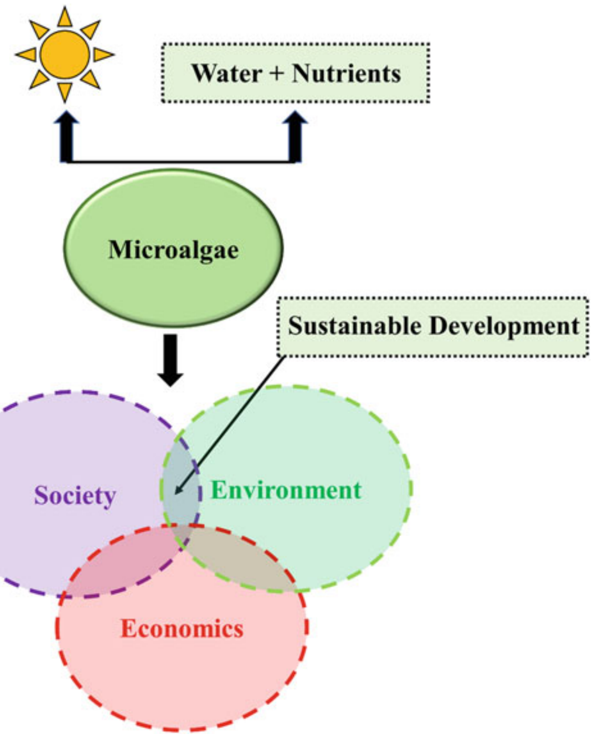
Graphical Abstract

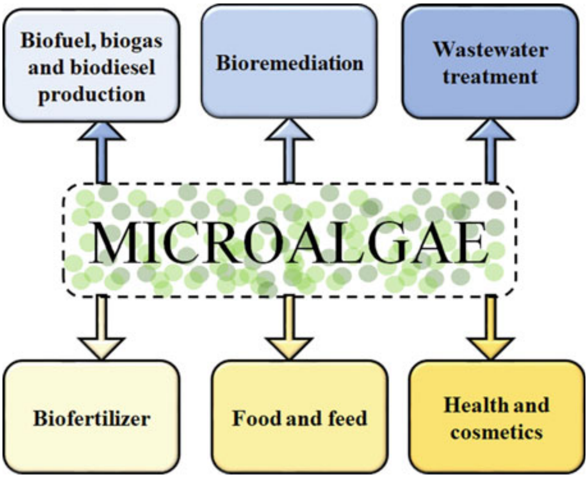


Keywords Microalgae · Biomass · Bioremediation · Application · Sustainability

10.1 Introduction

The population of humans is increasing alarmingly and is expected to cross nine billion by the year 2050 (Godfray et al. 2010), and to meet the increasing needs is a global challenge. Thus, the focus of scientists has shifted toward renewable sources to effectively meet people's current and future needs. This shift will be beneficial for society as well as the environment (Vuppaladadiyam et al. 2018). In the last few years, air pollution and water pollution are the most critical issues faced worldwide as emerging medical/clinical problems, e.g., COVID-19 and restriction in normal day-to-day lives. Conventional water treatment technologies are not economically feasible and fully sustainable. On the other hand, biological treatment processes that involve microorganisms not only treat water but also produce metabolic products that are of greater value (Costa and De Morais 2011). Among various microorganisms, the best choice is microalgae as they can easily grow in non-arable land, use brackish water, and wastewater and do not compete with food crop production (Arun

Fig. 10.1 A summary of microalgae benefits discussed in the review



et al. 2018). Also, they have high growth rates and photosynthetic efficiency (Rosenberg et al. 2011). They are used in various industries such as pharmaceuticals, fertilizer, etc. It can be used in place of conventional wastewater treatment technologies, for efficient nutrient removal and water treatment. It is also effective for heavy metal stress mitigation and photosynthetically transform inorganic carbon and water to organic compounds in a sustainable manner (Arun et al. 2017; Bilad et al. 2014) (Fig. 10.1). Further, the major concern globally is greenhouse gas (CO_2), and its emission can be significantly reduced using microalgae as it utilizes CO_2 during growth during photosynthesis (Rosenberg et al. 2011). Thus, the present chapter gives an insight into the applications of microalgae. Further, its limitations have also been discussed along with the prospects.

10.2 Microalgae: A Microscopic Miracle

Microalgae can be prokaryotic or eukaryotic and are found in varied habitats (Mata et al. 2010). They reproduce through cell division and grow faster than other plants and thus makes them the most productive ones (Kumar et al. 2011). There are over three lakh microalgal species present but thirty thousand have been documented (Mata et al. 2010). For cultivation, it uses light energy which takes place in chloroplast and form microalgal biomass (Ozkurt 2009; Deviram et al. 2020). The media in which the microalgae are cultivated should consist of nitrogen and phosphorus to fulfill algae's nutrient requirements along with macro- and micronutrients like Na, Mg, Ca, K, Mo, Mn, B, Co, Fe, and Zn aid in growth (Chisti 2007). Microalgae due to their easy availability and immense role in biotechnology, microalgae is the current topic of interest throughout the world (Gonçalves et al. 2016).

10.3 Microalgae in Wastewater Treatment

There are many sources of wastewater, e.g., industrial, agricultural, and municipal (Goswami et al. 2020a, b, c; Bhardwaj et al. 2020), and are a source of huge amounts of carbon, nitrogen, and phosphorus. By treating this wastewater, the nutrients can be recovered and water can be further used for different purposes. One approach we can use for this is microalgae which are sustainable and economical. The microalgae-based treatment of wastewater results in nutrients recovery and treatment and has gained considerable attention presently (Li et al. 2019; Agrawal et al. 2020). The addition of microalgae to the wastewater system provides two advantages: first it prevents eutrophication and second the algal biomass can be used in biorefinery industries (Chinnasamy et al. 2010; Wang et al. 2010). However, numerous parameters have to be considered for the treatment of wastewater using microalgae such as fast growth, high biomass, enhanced removal of pollutant/contaminant, and flexible adaptability. The increased growth rate of the microalgae represents increased adaptability, enhanced robustness, and efficiency in treating wastewater. This added advantage also has the potential to overcome the limits such as low lipid (Cai et al. 2013; Pires et al. 2013; Li et al. 2019). In the study by Yadav et al. (2021), some of the parameters which need to be followed for efficient treatment like microalgae growth in wastewater and is the most important parameter for selection, its nutrient use, and elasticity to adapt to diverse settings has been discussed. For wastewater treatment, *Chlorella* sp. and its members like *C. sorokiniana, C. emersonii* are preferred (Arita et al. 2015) (Table 10.1). Further, microalgae have been used for the remediation of macronutrients and heavy metals and are discussed as follows:

10.3.1 Macronutrients

Microalgae is also beneficial to remove macronutrients from different types of wastewaters so it is used in remediation too. One of the major macronutrients is

Table 10.1 Role of microalgae in the treatment of wastewater

S No	Type of wastewater	Microalgae used	Reference
1	Piggery wastewater	*Chlorella sorokiniana*	Leite et al. (2019)
2	Poultry wastewater	*Chlorella* sp. and *S. platensis*	Wang et al. (2018)
3	Municipal wastewater	*Scenedesmus Chlorella* sp. 227	Olsson et al. (2014) Cho et al. (2011)
4	Domestic wastewater	*Spirulina platensis*	Laliberte et al. (1997)
5	Cheese factory anaerobic effluent	*Micractinium pusillum*	Blier et al. (1995)

Table 10.2 Microalgae in nutrient removal and heavy metal mitigation

S No	Microalgae used	Heavy metals	Reference
1	*Chlorella vulgaris*	Cu, Cd, Pb	Sharma et al. (2021)
2	*Euglena gracilis*	Pb, Cd, Hg	Khatiwada et al. (2020)
3	*Chlorella sorokiniana*	Dissolved inorganic carbon, orthophosphate, and ammonia	Leite et al. (2019)
4	*Chlorella* sp. and *S. platensis*	NH_4^+, total phosphorous, and total organic carbon	Wang et al. (2018)
5	*Halochlorella rubescens*	Phosphate and nitrate	Shi et al. (2014)
6	*Arthrospira platensis*	Nitrogen and phosphorous	Hadiyanto et al. (2013)
7	*Chlorella vulgaris*	Cobalt, copper, and zinc	Afkar et al. (2010)
8	*Scenedesmus vacuolatus*	Cd	Le Faucheur et al. (2005)

ammonia and is mainly found in agriculture wastewater as some synthetic fertilizers like nitrogen fertilizer add it to water bodies. Ammonia is toxic for human consumption as well as for aquatic organisms, so it becomes important to treat this water. Microalgae use ammonia first in the wastewater before other nitrogen sources and thus an important factor in wastewater treatment (Hussain et al. 2020) (Table 10.2). Further phosphorus is one of the components used in agricultural practices. It gets discharged to the water bodies. It is a component of various metabolic activities, energy, lipid, protein, and coenzymes of the microalgal body and that's why needed for microalgal growth. It can be biologically recovered, but it will still need further treatment to be able to use again in agriculture. Microalgae can be used for the recovery, and it will act as a P enrichment biofertilizer for the crops (Solovchenko et al. 2016). Microalgae can use $H_2PO^-{}_4$ and $HPO^-{}_4$ as substrates to form ATP, the main energy source through phosphorylation. Microalgae can also accumulate phosphate as polyphosphates from ponds, but this will depend on certain environmental factors like concentration of phosphate, the intensity of light, and temperature (Hussain et al. 2021) (Table 10.2).

10.3.2 Heavy Metals

It accounts for the largest component in wastewater. They have medium density and are toxic at lower concentrations They are toxic for both aquatic plants and animals and are placed under three groups, i.e., precious metals, radionuclides, and toxic metals (Wang and Chen 2009). Heavy metals become a nuisance for human beings by being a part of the food chain as they are resistant to decomposition (Hussain et al.

2021). Heavy metal-tolerant microalgal strains like *Nitzschia palea* and *Nitzschia* per minute can accumulate metals and then can be used to treat wastewater (Chen et al. 2013) (Table 10.2). Two methods have been reported for the removal of heavy metal using microalgae, i.e., using living microalgae and biosorption. In the case of using living microalgae two steps are involved in the process, i.e., extraction of metal in initial cell surface and slow indoor transport. The other method, i.e., biosorption of heavy metals using microalgae, has paved new ways for the future where the live algal and inert biomass has been employed for the remediation of heavy metals (Ibrahim 2011; Romera et al. 2007). In addition, the microalgae biochar too contributed to the remediation of heavy metals (Pavithra et al. 2020.). Thus, the microalgae have tremendous potential, and detailed study in developing a system for treating polluted water can provide major/groundbreaking utility in the field of environmental sustainability.

10.4 Algal Biomass Utilization

The harvested microalgae biomass has biomolecules that can be further used for bioenergy production (Fig. 10.2).

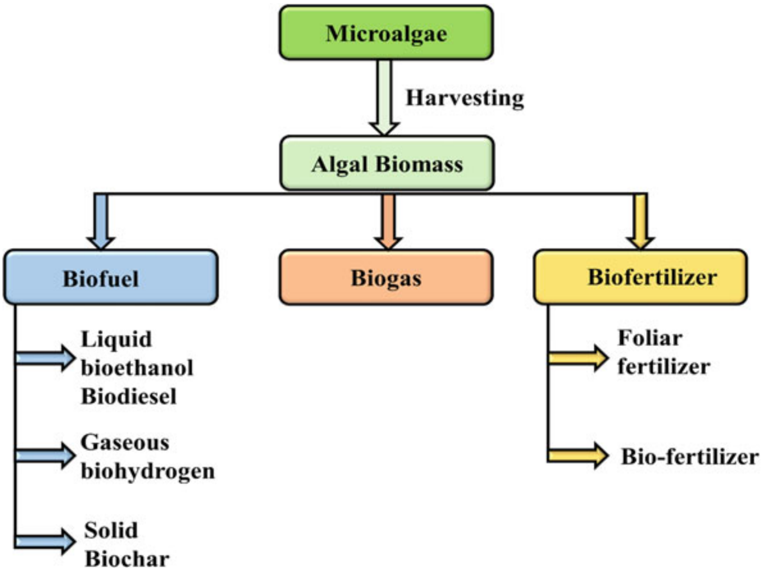


Fig. 10.2 Microalgal culture in sustainable fuel, gas, and fertilizer production

Table 10.3 Different microalgae used for biogas production

S No	Microalgae	Biogas production	Reference
1	*Chaetoceros muelleri*	521 mL CH_4 g VS^{-1}	González-González et al. (2021)
2	*P. cruentum*	130 mL CH_4/g VS	Mudimu et al. (2014)
3	*G. verrucosa*	279 mL biogas/g VS	Jard et al. (2013)
4	*C. reinhardtii*	587 ± 8.8 mL g VS^{-1}	Mussgnug et al. (2010)

10.4.1 Biofuel, Biogas, and Biodiesel

Biofuel can be defined as non-toxic and eco-friendly. It can be produced from various feedstocks and are renewable. The bio-oils from microalgae and vegetable oils have similar physio-chemical properties and are great substrates for biofuel production. Microalga readily absorbs carbon dioxide discharged after the biodiesel combustion process and thus reduces its impact on the environment (Hussain et al. 2021). Also, microalgae contain high oil contents and thus are suitable for high-quality biodiesel production (Chisti 2007), and an effort to increase the biofuel-producing ability has been performed globally using numerous chemical and mechanical methods (Mubarak et al. 2015). For example, sonication increases the microalgal cell's lipid yielding ability (Drira et al. 2016). Algal biomass can be easily converted to biogas due to the presence of low lignin and cellulose contents (Harun et al. 2011). Further, the biogas from algal biomass is renewable and can be further used for various purposes, e.g., electricity, liquid fuel, etc. (Hussain et al. 2021). Much fresh water and saltwater microalgae are utilized for biogas generation as shown in Table 10.3, and various papers reported for biofule has been represented in Table 10.4.

10.5 Bio-Fertilizer

Being environment friendly, microalgae can be easily used as a biofertilizer for crop plants (Antizar-Ladislao and Turrion-Gomez 2010; Brooijmans and Siezen 2010) (Table 10.5). Today the modern agriculture depends extensively on fertilizers to meet the growing call for food. Also, the extensive and uncontrolled use of chemical fertilizers have resulted in soil, air, and water pollution. In fertilizers, phosphorous (P) is the major component (Chen et al. 2018; Solovchenko et al. 2016), though in the mineral form it is found only in phosphate rock reserves, thus restricting its use. Thus, an alternate strategy that has gained importance is the environmentally friendly fertilizers (Chen et al. 2018) and is a balance between the nutrient uptake and provided by an external source, i.e., biofertilizers. The wastewater consists of huge concentrations of P and can be used for resource recovery. Thus, the microalgae biomass obtained after it is cultivated in wastewater can be used for various purposes in the biorefinery industry (González-Delgado and Kafarov 2011). This microalga grown in wastewater allows the recovery of nitrogen (N) and P, consists of various macro and micronutrients, and can be effectively used as a biofertilizer to enhance plant growth (Renuka et al. 2016) (Table 10.5).

Table 10.4 Microalgae in biofuel production

S No	Microalgae used	Method used	Biodiesel yield	Specific conditions	Reference
1	*Amphiprora* sp.	Soxhlet extraction method	81.47 ± 1.59%	24 μmol m^{-2} s^{-1} of light intensity 2% of catalyst amount with 1.5:1 methanol/oil, 3 h at 65 °C	Jayakumar et al. (2021)
2	*Chlorella vulgaris*	Soxhlet extraction	61.4%	Microalgae were treated with methanol and castor oil containing pressurized CO_2 Microalgae 10 g, water 20 wt%, castor oil 4.5 g, methanol: Microalgae 1.5 (g/g) at 220 °C and 9.7 MPa for 30 min	Chang et al. (2020)
3	*Nannochloropsis* sp.	Transesterification with organic solvent extraction	600 m^3	The system consumes 2800 tons of CO_2 for microalgae production, produces 1400 tons of algal biomass	Bhadra et al. (2020)

Table 10.5 Different microalgae used as bio-fertilizers for different plants

S No	Microalgae	Plant used	Reference
1	*Chlorella sorokiniana*	Brassica rapa chinensis	Vinzon et al. (2021)
2	*Chlorella vulgaris*	*Lactuca sativa*	Faheed et al. (2008)
3	*Acutodesmus dimorphus*	Roma tomato plants	Garcia-Gonzalez and Sommerfeld (2016)
4	*Scenedesmus sp.*	*Triticum aestivum L.* var. Gemmiza	Shaaban et al. (2010)
5	*Azolla microphylla*	*Oryza sativa*	Ventura and Watanabe (1993)

10.6 Food and Feed

Microalgae have emerged as a sustainable food source for both humans and animals and can be a potential solution for global food security via mitigating environmental issues and are discussed as follows.

10.6.1 Food

Microalgae are a rich source of protein and carbohydrates and have a high polyunsaturated fatty acids (PUFA) and omega-3-fatty acid content. Eicosapentaenoic and docosahexaenoic acids have proven to be very important fatty acids for the health of humans and have been detected in microalgae. They are rich in colorful pigments like carotenes, chlorophylls, and phycobiliproteins which can act as antioxidants and natural colorants. *Spirulina, Chlorella, Dunaliella,* and *Haematococcus* account for the most consumed microalgae in the world (Fernández et al. 2021).

10.6.2 Feed in Aquaculture

High protein and lipid content in microalgal biomass makes it ideal for aquaculture. It has been reported for its utility as animal feed, enhanced immune response, better growth, and weight and also livestock product enrichment with bioactive compounds (Kusmayadi et al. 2021). It can also be used in fish diets as it aids in improving health and growth. Two mainly used omega-3 long-chain PUFA in aquaculture are docosahexaenoic acid and eicosapentaenoic acid. These omega-3 fatty acids are mainly produced by marine photosynthetic microalgae, but DHA can also be produced heterotrophically by *Schizochytrium, Crypthecodinium*, etc.

10.6.3 Feed for Animals

Microalgae are feed for some domestic animals, e.g., horses, chickens, and cows. They play various other functions in them along with being feed, like they boost their immune response and also help in improving gut function which further will have positive effects on their appetite, and these animals have also found to have more weight and eggs (Kovač et al. 2013). It has been reported that they have the potential to replace conventional proteins up to 5–10% if they are used in poultry feed and up to 33% if used for pigs (Spolaore et al. 2006; Sousa et al. 2008). Ruminants are found best among all animals for algae feed as they can even digest unprocessed algal biomass (McCauley et al. 2020).

10.7 Microalgae in Health and Cosmetics

Microalgae are also sources of many pigments, toxins, lipids, vitamins (Saide et al. 2021; Mehariya et al. 2021), bioactive carbohydrates, antioxidants, and fatty acids which are used in many fields especially biomedical (Table 10.6).

10.7.1 Health

They have anti-cancer activity and can aid in treating/controlling diabetes (Kang and Kim 2013; Lauritano et al. 2016). Some carotenoids even protect against diabetic retinopathy like lycopene, lutein, and zeaxanthin (McClinton et al. 2020). Fucoxanthin and neoxanthin have anti-obesity activity by suppressing adipocyte differentiation (Okada et al. 2008). Fucoxanthin can also be used as a food supplement and for weight loss (Sathasivam and Ki 2018). Carotenoids also help in preventing vision loss by preserving healthy eye cells by reducing oxidative damage as they absorb UV light and other forms of solar radiation (Krinsky 2002; Goswami et al. 2021). PUFAs from microalgae are widely used in pharmaceuticals especially in cardiac and neurological disorders. Widely used PUFAs like docosahexaenoic acid and eicosapentaenoic acid are found in baby foods, dairy products, and non-alcoholic drinks. PUFAs are obtained from a wide range of genera like *Nannochloropsis, Tetraselmis, Crypthecodinium*, and *Isochrysis.*

10.7.2 Cosmetics

Arthrospira and *Chlorella* microalgae genera are widely used in the skincare market (Morone et al. 2019). Metabolites obtained from microalgae like β-carotene, flavonoids, phenols, phycobiliproteins, steroids, saponins, terpenes, tannins, and vitamins are found to be useful (Stengel et al. 2011).

Table 10.6 Microalgae benefits in health and cosmetics

S No	Microalgae	Bioactive compound	Effect	Reference
1	*Nannochloropsis* sp.	Canthaxanthin	Tanning pills	Chandra et al. (2017)
2	*Haematococcus pluvialis*	Astaxanthin	Super antioxidant, lower blood pressure	Shah et al. (2016); Hussein et al. (2005)
3	*Spirulina porphyridium*	Phycocyanobilin phycoerythrobilin	Antioxidant pigment for eyeliner and lipsticks	Hamed (2016)
4	*Phaeodactylum tricornutum*	Fucoxanthin	Anti-obesity effects	Kim et al. (2016)
5	*Scenedesmus, Muriellopsis*	Lutein	Prevent age-related macular degeneration (ARMD)	Fernández-Sevilla et al. (2010)

10.8 Biohydrogen Production

Biohydrogen is a sustainable energy source via the utilization of renewable resources like solar energy and organic substrates. The production of biohydrogen occurs in the presence of light and electron (e^{-1}) transfer to hydrogenase and nitrogenase via hydrogenesis involving the splitting of water (H_2O) molecules (Manis and Banerjee, 2008; Show et al. 2018). The above-mentioned enzyme has been reported for its function in aiding biohydrogen production in both prokaryotes and eukaryotes (Show et al. 2019). One main advantage of using hydrogen as a fuel is its oxygen-free combustion and water as an end product (Limongi et al. 2021). Additional advantage of using microalgae is that they are more resilient, stable, and cheaper than subcellular chloroplast preparations used earlier (Benemann 2000) and do not produce any dangerous by-products while running at low temperature (Dalena et al. 2017). Microalgae uses various processes for biohydrogen production (Wang et al. 2021) as shown in Fig. 10.3. The use of a closed reactor system has been studied for the production of biohydrogen but is still at an early stage, and much work and study have to be carried out for developing a better and enhanced system. The major challenge is the low yield, though research is being carried out globally for increasing the yield by optimizing physiological parameters, and recently the use of molecular tools has significantly contributed toward enhanced biohydrogen production (Mathews and Wang 2009; Chaturvedi et al. 2020). Thus, the use of advance research would pave new ways in the future for the production and utilization of biohydrogen for the welfare of the society and the various mechanism involved in biohydrogen production has been discussed as follows.

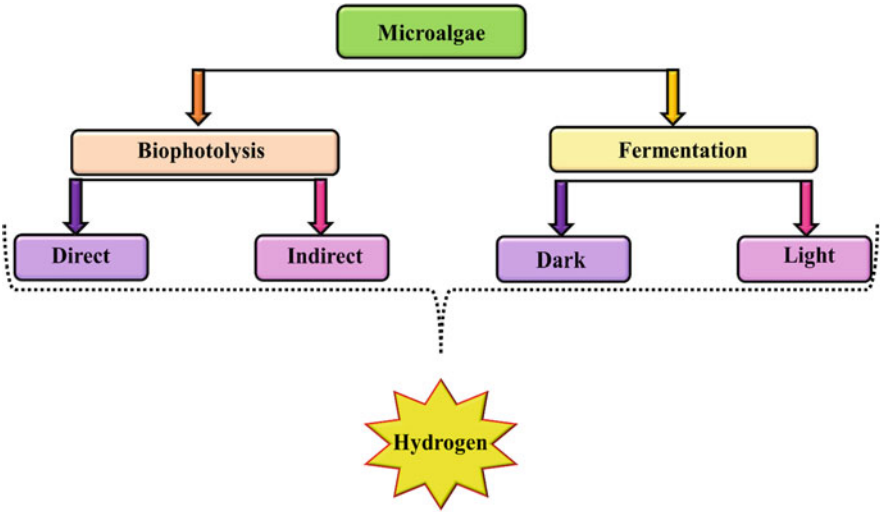


Fig. 10.3 Various mechanisms reported for hydrogen production in microalgae

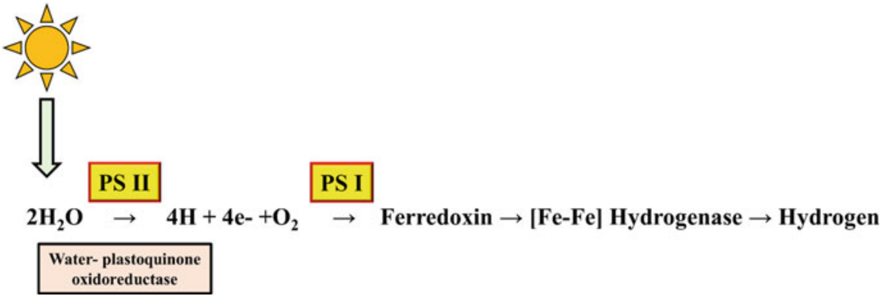


Fig. 10.4 Direct photolysis leading to hydrogen production

10.8.1 Direct Photolysis

It is a light-dependent process for hydrogen production and can occur in green microalgae (Benemann 2000). It is a photochemical oxidation reaction completed in two steps as shown in Fig. 10.4. Hydrogenase is sensitive to oxygen so it is important to maintain its low level (Zhang et al. 2014a, b). The main advantage of direct photolysis is that water is used as primary feed which is inexpensive (Holladay et al. 2009), and one limitation is that microalgae produce oxygen that inhibits [Fe]-hydrogenase and further hydrogen production.

10.8.2 Indirect Photolysis

This is mainly designed to overcome oxygen limitation indirect photolysis. Electrons here are derived from carbohydrate metabolism that further leads to hydrogen production (Prince and Kheshgi 2005). Here photosynthesis is spatially separated from the photosynthesis process through sulfur depletion/repletion (Kruse and Hankamer 2010). Sulfur-deprived medium is generally used to favor this type of photolysis (Kumar et al. 2020) (Fig. 10.5).

10.8.3 Fermentation

Many fermentative bacteria like *Escherichia coli, Thermococcales* spp. transform carbon sources for hydrogen production. For photo fermentation, they use organic substrates in the presence of sunlight to yield hydrogen and other by-products. For dark fermentation, the above process is the same just that the light is not used. To use algal and bacterial culture simultaneously is more beneficial for hydrogen production than to use algal culture alone as observed in the case of *Chlamydomonas reinhardtii* and *Escherichia coli* co-culture where hydrogen production was increased by 60% (Limongi et al. 2021).

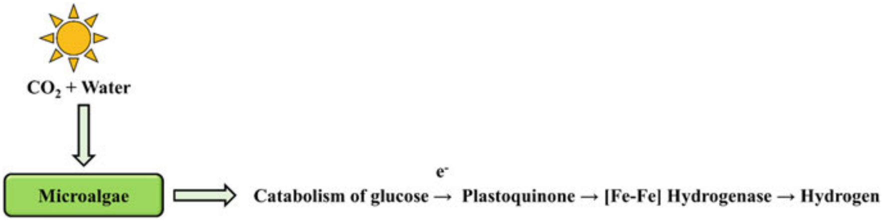


Fig. 10.5 Indirect photolysis leading to hydrogen production

10.9 Limitations and Future Prospects

Sustainability is itself a challenge, to begin with, and meeting the needs of the growing population is another. However, due to technological advancement, people have become much aware of their environment and the need to preserve it. Practically for a microalgal production system, sustainability is evaluated based on techno-economic assessment, life cycle analysis, and socio-economic impact (Vuppaladadiyam et al. 2018). Microalgae produce several bioactive metabolites which are beneficial for humans, but the limitation lies in their complete knowledge, utilization, and scale-up process. At the laboratory, the cultivation of microalgae is easy, but at large scale, it becomes difficult as various other factors like cost, contamination, and maintenance also have to be considered. Microalgae being diverse, its cultivation can be done in both open and closed systems. The open system is mainly preferred due to its easy operation, low investment, and maintenance cost, but they are also very prone to contamination by other microflora which further leads to system failure and limits carbon dioxide from the atmosphere, thereby resulting in low biomass productivity and eventually affecting biomass harvesting and the costs associated with the process (Bilad et al. 2014). In a closed system, though it is free from contamination, it is more complex in operation. Further, the usage of microalgae is limited for animal feed because of production scale and costs which is proved by comparing production volume values of microalgae against soy oil, meal, and fish oil which are the commonly used feed for animals (Fernández et al. 2021). For microalgae-derived biodiesel production, there are many proposed processes, but these are still not economically feasible due to production costs. The limitations of biohydrogen production are production, storage, and cost (Limongi et al. 2021). Along with all these limitations, one main limitation is proper government support. Policies need to be made for sustainable microalgae production. Thus, researchers around the world are interested in developing processes that are cheap, sustainable, and able to meet the current and future needs.

Thus, detailed and continuous research activities are very much essential and need of the hour to identify the limitations and further explore the undiscovered benefits of microalgae and their role in sustainable development.

10.10 Conclusion

It is indeed amazing to see how these microscopic organisms are used in various fields. They are great tools to solve problems sustainably and economically. Though there are still a few limitations to their commercial use, with the advancement of technology, it has numerous possibilities in the future. Although it can be individually used too for wastewater treatment, nutrient removal, and biofuel production, its simultaneous use and technological advancement with an interdisciplinary approach will help in improving the efficiency and utilization of the system. For example, its use as a biofertilizer has been reported, thus more intensive research can be done followed by commercialization. Microalgae can meet the needs of the present without compromising the future generations, and, in no doubt, it is indeed a way toward sustainable development.

Conflict of Interest The authors declare no conflict of interest.

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