Chapter 13
Biodiesel from Microalgae

Rozita Madadi, Meisam Tabatabaei, Mortaza Aghbashlo, Mohammad Ali Zahed and Ahmad Ali Pourbabaee

Abstract Widespread application of non-renewable energy resources such as fossil fuels is limited mainly due to their adverse environmental impacts by increasing the amount of greenhouse gas (GHG) emissions. A solution to limit fossil-fuel pollution is the use of renewable energy resources. In the recent years, microalgae have received considerable attention as a suitable feedstock for biofuel production. Microalgae can grow in various aquatic wastewater media and are able to produce biomass, lipids, and hydrocarbons. Using different types of wastewaters as media for algae cultivation could not only reduce their freshwater footprint but also the costs associated with algae cultivation and biofuel production. This chapter presents an overview on various algal cultivation systems as well as on optimization of algal

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R. Madadi
Department of Agricultural Biotechnology, University College of Agriculture
and Natural Resources, University of Tehran, Karaj, Iran

M. Tabatabaei (✉)
Biofuel Research Team (BRTeam), Karaj, Iran
e-mail: meisam_tab@yahoo.com; meisam_tabatabaei@abrii.ac.ir

M. Tabatabaei
Department of Microbial Biotechnology, Agricultural Biotechnology Research,
Institute of Iran (ABRII), Agricultural Research, Education and Extension Organization
(AREEO), Karaj, Iran

M. Aghbashlo (✉)
Department of Mechanical Engineering of Agricultural Machinery,
Faculty of Agricultural Engineering and Technology, College of Agriculture
and Natural Resources, University of Tehran, Karaj, Iran
e-mail: maghbashlo@ut.ac.ir

M. A. Zahed
Faculty of Biological Sciences, Kharazmi University, Tehran, Iran

A. A. Pourbabaee
Department of Soil Science, Faculty of Agricultural Engineering and Technology,
University College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran

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cultivations, while downstream processes including harvesting and drying of microalgae and lipid extraction systems are also reviewed and discussed. Subsequently, different microalgae biofuel production pathways are presented. Finally, the applications of microalgae in integrated systems, i.e., in wastewater treatment and biodiesel production systems and biofixation of carbon, are scrutinized.

Keywords Biodiesel · Algae · Waste treatment · CO_2 fixation

13.1 Introduction

Energy resources are divided into non-renewables and renewables, with the latter having a minor contribution to the global energy market at the present time. However, due to the limitations on non-renewable energy resources and increasing greenhouse gas (GHG) emissions as a result of using these fuels, the share of renewable energy resources such as biofuels, hydro, wind, solar, and geothermal energies is bound to increase (Bwatanglang et al. 2015). Among the above-mentioned renewables, only biofuels are being used globally in the transportation sector, the main GHG emitter. Based on their feedstocks, biofuels are classified into first-generation biofuels (FGBs) produced from sugar, starch, animal fats, and vegetable oils; second-generation biofuels (SGBs) produced from non-food crops, agro-forest residues, and wastes; and third-generation biofuels (TGBs) produced from microalgae (Demirbas and Demirbas 2011; Laghari et al. 2015).

Microalgae, photosynthetic microorganisms, could be grown on non-arable land and have an acceptable growth rate (20–30 times faster than other conventional energy crops) and high photosynthetic conversion efficiency (Ullah et al. 2014). Cultivation of microalgae consumes less water than land crops, and unlike corn, soybean, and palm as main sources of biofuel production, algae is not used as a primary food source for human being, affirming that they can be used distinctively as fuel while having less impact on food security. Moreover, due to their ability to withstand high CO_2 contents in gas stream, microalgae have high efficiency for CO_2 mitigation as well (Demirbas and Demirbas 2010; Mata et al. 2010; Wang et al. 2008; Zhang 2015).

Microalgae are classified into four main taxonomic groups: diatoms (*Bacillariophyceae*), green algae (*Chlorophyceae*), cyanobacteria or blue-green algae (*Cyanophyceae*), and golden algae (*Chrysophyceae*). These microorganisms contain high lipid, high protein, and low carbohydrate content. Nowadays, the main interest is in cultivating microalgae to produce lipid as feedstock for biodiesel (Markou and Nerantzis 2013), while other types of biofuels are also of some interest. The lipid content, lipid productivity, and different types of biofuel reportedly produced from different microalgae are shown in Table 13.1. Moreover, a comparison among different biodiesel feedstocks in terms of their oil properties is also presented in Table 13.2.

Table 13.1 Lipid content, lipid productivity and different types of biofuel production from different microalgae species[a]

Microalgae	Algae type	Lipid content (% dry wt. biomass)	Lipid productivity (mg L^{-1} d^{-1})	Volumetric productivity of biomass (g L^{-1} d^{-1})	Areal productivity of biomass (g m^{-2} d^{-1})	Major biofuel type
Arthrospira maxima	Blue-green	20.34	–	–	–	Hydrogen, biodiesel
Chlorella emersonii	Green	25.0–63.0	10.3–50.0	0.036–0.041	0.91–0.97	–
Chlorella protothecoides	Green	14.6–57.8	1214	2.00–7.70	–	Biodiesel
Chlorella sorokiniana	Green	19.0–22.0	44.7	0.23–1.47	–	–
Chlorella vulgaris	Green	5.0–58.0	11.2–40.0	0.02–0.20	0.57–0.95	Ethanol
Chlorella	Green	18.0–57.0	18.7	–	3.50–13.90	Biodiesel
Chlorococum sp.	Blue-green	19.3	53.7	0.28	–	Biodiesel, ethanol
Dunaliella salina	Green	6.0–25.0	116.0	0.22–0.34	1.6–3.5/20–38	–
Dunaliella sp.	Green	17.5–67	33.5	–	–	Ethanol
Haematococcus pluvialis	Red	25.0	–	0.5–0.6	10.2–36.4	Biodiesel
Nannochloris sp.	Green	20.0–56.0	60.9–76.5	0.17–0.51	–	–
Nannochloropsis oculata	Green	22.7–29.7	84.0–142.0	0.37–0.48	–	–
Nannochloropsis sp.	Eustigmatophytes	12.0–53.0	37.6–90.0	0.17–1.43	1.9–5.3	–

(continued)

Table 13.1 (continued)

Microalgae	Algae type	Lipid content (% dry wt. biomass)	Lipid productivity (mg L^{-1} d^{-1})	Volumetric productivity of biomass (g L^{-1} d^{-1})	Areal productivity of biomass (g m^{-2} d^{-1})	Major biofuel type
Neochloris oleabundans	Green	29.0–65.0	90.0–134.0	–	–	Biodiesel
Scenedesmus obliquus	Green	11.0–55.0	–	0.004–0.74	–	Methanol, hydrogen
Spirulina platensis	Green	4.0–16.6	–	0.06–4.3	1.5–14.5/24–51	Hydrogen

Mata et al. (2010), Maity et al. (2014)

Table 13.2 Comparison of different biodiesel feedstocks[a]

Conventional feedstocks	Oils or fats	Oil content (% oil by wt. biomass)	Physicochemical properties of biodiesel feedstocks							Water footprint (m^3 GJ^{-1})	Land use (m^2 GJ^{-1})	Biodiesel yield (L ha^{-1} a^{-1})
			Density (kg/m^3)	Kinematic viscosity at 40 °C (mm^2 s^{-1})	Cetane no. (°C)	High heating value (MJ kg^{-1})	Flash point (°C)	Saponification value	Iodine value			
Edible	Canola	41	911.5	34.72	37.6	39.7	246	189.80	–	383	258	1190
	Soybean	18–22	913.8	28.87	37.9	39.6	254	195.30	128–143	383	689	446
	Sunflower	25–35	916.1	35.84	37.1	39.6	274	193.14	125–140	61	323	951
	Palm	30–60	918.0	44.79	42.0		267	208.63	48–58	75	52	5906
	Peanut	42–52	902.6	39.60	41.8	39.8	271	191.50	84–100	58	220	1396
	Corn	48	909.5	30.75	37.6	39.5	277	183.06	103–128	–	–	152 (kg biodiesel ha^{-1} $year^{-1}$)
	Coconut	63–65	918	27.26	–	–	–	267.56	7.5–10.5	49	128	2399
	Cottonseed	18–25	914.8	33.50	–	39.4	234	198.50	103–115	135	945	325
Non-edible	Jatropha	30–40	940	33.90	–	38.65	225	200.80	82–98	383	258	1190
	Castor	48	955	251.20	42.3	37.4	–	191.08	83–86	–	–	1156 (kg/ha year)
	Microalgae *Chaetoceros* sp.	70	1.305 gm/ml	6.2	–	–	–	173.56 mg/mg of oil	–	<379	2–13	24355–136886
	Spirogyra	14.82	884	4.4	–	–	–	–	–	–	–	–
	Cladophora	11.76	892	3.8	–	–	–	–	–	–	–	–
	Tolypothrix	12.78	857	4.1	–	–	–	–	–	–	–	–
	Mahua	35	960	24.50	–	36.0	232	190.5	58–70	–	–	–
	Neem	30	918.5	50.30	–	–	–	209.66	65–80	–	–	–

[a] *Sources* Demirbas (2009), Demirbas and Demirbas (2010), Karmakar et al. (2010), Mata et al. (2010), Kumar et al. (2011), Moser and Vaughn (2012), Ananadhi Padmanabhan and Stanley (2012), Atabani and Silva César da (2014) and Singh et al. (2016)

Microalgae can grow in various aquatic environments, such as freshwater or marine water (Zhou et al. 2011a, b), industrial wastewaters (Wang et al. 2010, Zhou et al. 2013), municipal wastewaters (Kong et al. 2010), animal wastewaters (Wang et al. 2010; Zhou et al. 2012; Hu et al. 2012), and agricultural wastewaters. Accordingly, many studies have strived to promote biofuel production using wastewater resources as a means of improving the economic aspects of algal fuels production (Pittman et al. 2011; Wu et al. 2012). However, in a rather recent critical review, Chisti (2013) pointed out the constraints to microalgal biofuel commercialization. Among those was the calculations concerning the inadequacy of wastewater as a source of nitrogen and phosphorus for microalgal cultivation and that the algal biofuels produced using the wastewater generated in a metropolitan area such as New York city could only be sufficient to replace 1–3% of the petroleum demands of the city. As shown in Table 13.3, presenting the potential of algal fuel production from wastewater in major cities in the world, this is absolutely true and such a scenario would be totally inefficient if one places the main focus on biofuel production using wastewater.

To the contrary and by highlighting algal-based wastewater treatment instead of biofuels production, i.e., by looking at this scenario other way around, different conclusions could be made. Accordingly, biofuel production using wastewater would come second as a strategy to further justify, or economize, the algal-based treatment process of various types of wastewater. Table 13.4 tabulates the pros and cons of microalgal-based wastewater treatment systems and compares them with the conventional wastewater treatment procedures.

The present chapter aims to review the developments made and success stories reported in different aspects of algal cultivation and harvesting/extraction within the framework of integrated biofuel production/wastewater treatment systems. Furthermore, the application of microalgae in integrated systems, i.e., microalgae-driven wastewater treatment and algal-based carbon biofixation with simultaneous biofuels production, has been brought into attention.

13.2 Algae Cultivation Systems

13.2.1 Suspended Culture

The most common large-scale algae production systems are based on suspended culture. In these cultures, including open ponds and closed reactors, single cells and small groups of cells are maintained in liquid medium. This medium requires agitation and gas exchange.

Table 13.3 Potentials for application of wastewater generated in major cities around the world as a source of nutrients for algal cultivation

City	Population	Petroleum Consumption ($m^3\ yr^{-1}$)	Wastewater generation ($m^3 d^{-1}$)	Annual algal oil production potential from wastewater (m^3)	Max. potential of wastewater-driven algal oil to replace petroleum demand of city (%)	References
A large US city as model	10,000,000	35,770,000	3,780,000	425,000	1.07	Chisti (2013)
Toronto	5,132,794	19,015,718	1,940,196	218143.745	1.03	Present study[a]
Tehran	8,293,140	10,473,406	3,134,806	352458.45	1.67	Present study
New York	8,405,837	28,840,426	3,177,406	357248.0725	1.11	Present study
Beijing	21,150,000	87,233,175	7,994,700	898,875	9.29	Present study
Paris	2,273,305	3,592,844	859,309	96615.4625	2.42	Present study
Sydney	4,840,628	12,774,175	1,829,757	205726.26	1.45	Present study
Moscow	11,500,000	14,817,175	4,347,000	488m750	2.97	Present study
Tokyo	13,350,000	26,995,035	5,064,300	569398.8	1.97	Present study
Berlin	3,562,166	6,019,882	1,346,498	151392.05	2.26	Present study

[a] These values were calculated according to Chisti (2013) and are reported for the first time in the present work

Table 13.4 Pros and cons of microalgal-based wastewater treatment systems in comparison with the conventional wastewater treatment procedures

Wastewater treatment	Type	Advantages	Disadvantages	References
Algae-based systems	HRAP[a]	– Simple and cost effective	– Algal biomass harvesting is difficult – Risk of contamination is high – Control on algal species is low – Unapplicable water footprint	Park et al. (2011)
	Immobilized	– Algae harvesting is facilitated and economical – Possibility of nutrient removal as well as other pollutants such as heavy metals and industrial pollutants	– Phosphate-removal efficiency is dependent on elevated pH of the wastewater – It is always accompanied with enhance removal of nutrients	de-Bashan and Bashan (2010)
	Attached algal system	– Biomass harvesting is facilitated – Improved water quality	– There is no consensus on the best method of growing and harvesting algal biofilms	Christenson and Sims (2011)
Conventional methods	Chemical	– Low energy requirement	– Cost of treatment is higher than those of the other methods (physical and biological)	Gupta et al. (2012)
	Physical	– Possibility of volatile and semi-volatile organic compounds removal – Possibility of removal of coarse solids	– Energy requirements are high	https://www.teicrete.gr
	Biological	– Cost effective – Possibility of controlling the amount of aeration to avoid excessive dissolved oxygen – Improve efficiency of aeration system	– BOD removal by biological treatment requires higher energy than BOD removal by primary treatment	Mittal (2011)

[a]HRAP: High rate algal pond

13.2.1.1 Open Ponds

Open ponds can be categorized into natural waters (lakes, lagoons, and ponds) and artificial ponds. The most commonly used systems for algae cultivation include circular ponds and raceway ponds. Algae cultivation in open system has some disadvantages, such as the difficulties in controlling contamination, culture environment conditions, poor light utilization by the cells, and requirement for large areas of land, while biomass harvesting is costly as well (Carvalho et al. 2006). Circular ponds are generally round, simple, and mixed with a rotating circular arm fixed in the pond center (Lee and Lee 2001). Raceway ponds are shallow ponds and are used for commercial microalgal production, usually lined with plastic, with a 15–20 cm depth in which water and nutrients circulate around a racetrack with a rotating paddle wheel (Brennan and Owende 2010). High-rae algal ponds (HRAP)s are raceway-type ponds and have 0.2–1 m depth, paddle wheel-mixed, and provide improved wastewater treatment. They are efficient and cost-effective upgrades for treating municipal, industrial, and agricultural wastewater (Park et al. 2011; Craggs et al. 2012). These ponds are in fact a combination of algal reactor and amplified oxidation ponds.

13.2.1.2 Closed Reactors

Closed reactors are expensive to build. However, compared with open ponds, they are much easier to control contamination and environmental conditions. Closed reactors require chemical sterilizers to effectively sterilize. In such reactors, cost of harvesting is less than open ponds and the obtained biomass concentration is higher than open ponds (Lee and Lee 2001; Scott et al. 2010). There are four key requirements for algal growth in reactors. The photosynthetic activity of microalgae depends on light; therefore, light is one of the restrictive factors in the algae culture; if light is too low, growth of microalgae will be slow and their photosynthesis will decline. Conversely, if it is too high, photoinhibition and oxidative damage would occur (Kumar et al. 2010a, b, c). Another key parameter is temperature, and too low and too high values would result in slow growth and cell death, respectively. Fluctuations in temperature can lead to significant decreases in productivity, while the optimal growth temperature for microalgae is often in the range of 20–30 °C (Chisti 2008). Mixing is also an important parameter in microalgal cultivation that improves gas exchange, keeps cells in suspension, distributes the nutrients, and decreases photoinhibition on the surface (Ugwu et al. 2008). Mixing in photobioreactors (PBR) is provided by pumping or aeration through a variety of gas transferring systems. Finally, nutrients are also instrumental in achieving am efficient cultivation system. Low nutrient availability leads to growth inhibition, while high concentration may exert toxic effects. Essential elements for algal growth include nitrogen (N), phosphorus (P), and, in some cases, silicon (Chisti 2007).

Closed reactors can be categorized into flat-plate reactors and tubular reactors. Flat-plate reactors are vertical reactors made up of narrow panels with 10-mm glass plates that are pasted together. Tubular reactors are another type of closed reactors that can be categorized into: horizontal tubular, vertical airlift, and helical tubular. The only type of closed systems used on large scales is tubular reactors (Chisti 2007). The control of temperature and pH in tubular photobioreactors is better than that in open ponds. In comparison with open ponds, tubular photobioreactors can generally provide a better protection against culture contamination, less evaporative loss, better mixing, and higher cell densities (Mata et al. 2010). Horizontal tubular systems are composed of thin-diameter tubing lying or stacked horizontally, while vertical tubular systems are composed of vertical tubes that can be easily erased and kept sterile. This type of reactor is suitably set and manufactured at low cost (Ugwu et al. 2008). Helical tubular systems are constructed of tubing coiled around a circular framework, and hence, angle to sunlight is reduced; subsequently, the

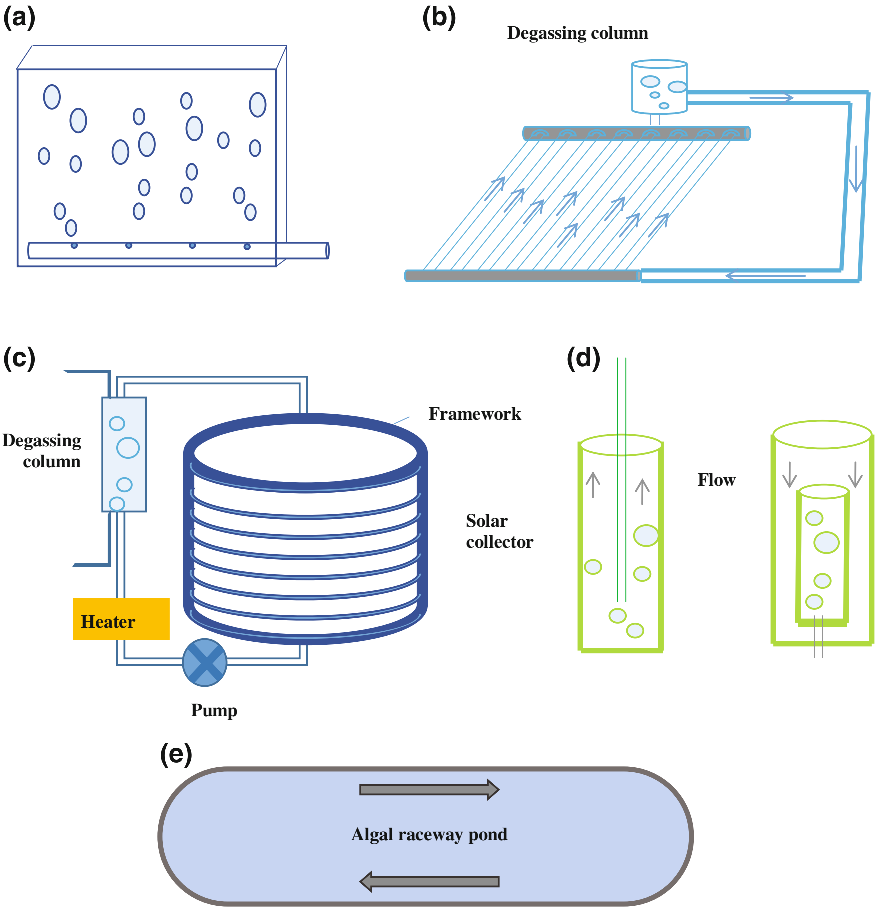

Fig. 13.1 Schematic diagrams for closed reactors: **a** flat plate, **b** horizontal tubular, **c** helical tubular, **d** vertical airlift, and **e** algal raceway pond (Chisti 2007; Xu et al. 2009; Mata et al. 2010; Park et al. 2012)

Table 13.5 Comparison of open and closed culture systems for microalgae[a]

Culture systems for microalgae	Open systems (Ponds)	Closed systems (PBRs)	
		Tubular photobioreactor	Flat-plate photobioreactor
Contamination control	Difficult	Easy	Easy
Species control	Difficult	Easy	Easy
Weather dependence	High light intensity, temperature, rainfall	Medium light intensity, cooling required	Medium light intensity, cooling required
Biomass productivity	Poor biomass productivity	Good biomass productivities	High biomass productivities
Sterility	None	Easy to sterilize	Easy to sterilize
Mixing	Poor mixing	Good mixing	Good mixing
Space required	Large area of land required	Requires large land space	Requires large surface area
Operation costs	Open systems $\ll$ closed systems	Expensive compared to open ponds	Expensive compared to open ponds
Illumination surface area	Light only effectively penetrates 2′–3″ in ponds	Large illumination surface area	Large illumination surface area
Temperature control	Difficult temperature control	More uniform temperature	Difficult temperature control
Evaporation of growth medium	High	Low	Low
Scalability	High	Medium	Difficult
Gas transfer control	Low	High	High
O_2 inhibition	Usually low enough because of continuous	High (O_2 must be removed to prevent photosynthesis inhibition)	High (O_2 must be removed to prevent photosynthesis inhibition)
Maintenance	Easy	Hard	Hard

[a] *Sources* Mata et al. (2010), Brennan and Owende (2010), Christenson and Sims (2011) and Singh et al. (2016)

required land area is reduced (Morita et al. 2001). A comparison of open and closed culture systems for microalgae is shown in Table 13.5 (Pluz 2001; Brennan and Owende 2010; Ugwu et al. 2008). An efficient hybrid system of photobioreactor and open ponds was also suggested by Narala and co-authors (2016). Schematic diagrams for closed reactors and open pond are shown in Fig. 13.1.

13.2.2 Immobilized Algal Culture

Harvesting microalgae is a major challenge in suspended culture at large-scale algae production system. Using immobilized cultures (attached algal processes) could play a major role in overcoming this major challenge to production, i.e., harvesting of microalgae (Hoffmann 1998). In addition to that, immobilization offers a number of other advantages over free-cell systems as well, including less space requirements, easier handling, higher resistance to unfavorable environmental conditions, and the possibility of using higher cell densities in the process as well as reusing the biomass for product generation (Mallick 2002; de-Bashan and Bashan 2010; Christenson and Sims 2011; Eroglu et al. 2015). It should also be mentioned that immobilized cells have been reported to possess higher biosorption capacity and bioactivity (Mallick 2002). These collectively mark immobilized algal cultivation systems as cost-effective processes for scale-up processing. Among the various immobilization processes, the most common ones are discussed herein.

13.2.2.1 Matrix-Immobilized Microalgae

In this method, microalgal cells are immobilized or entrapped in a 3D matrix made of natural (such as agar, cellulose, alginate) or synthetic (such as polyacrylamide, polyurethane, polyvinyl) polymers. de-Bashan and Bashan (2010) argued that the latter is comparatively more stable in wastewater samples, while natural polymers such as alginate are advantageous in terms of their higher nutrient/product diffusion rates and their eco-friendly features (de-Bashan and Bashan 2010). In spite of the promising aspects of matrix immobilization of algal cells, this method is still limited to laboratory scale for the cost of the immobilization matrix is yet to be further decreased in order to be economically justified (Chevalier et al. 2000).

13.2.2.2 Algal Biofilms

The main advantage of algal cultivation systems designed based on algal biofilm is the facilitated harvesting of algal cells by scraping. As mentioned earlier, expensive harvesting systems used in suspended cultures, e.g., flocculation and centrifugation, generally jeopardize the economic viability of these systems, and therefore, algal biofilms when become economically available could assist with overcoming this shortcoming.

13.3 Algal Cultivation Optimization

In order to achieve an economically viable biodiesel production system from microalgal biomass, optimization of algal cultivation in terms of algal biomass and lipid content is of prominent importance. Numerous attempts of different nature have been made in order to achieve the above-mentioned goals. For instance, Bohutskyi et al. (2014) introduced an innovative, mixed trophic state process based on *Auxenochlorella protothecoides* grown phototrophically, to obtain high lipid content for generating algal biodiesel. They argued that simultaneous nitrogen deprivation and glucose supplementation during the heterotrophic stage could boost total lipid content by over threefolds. They also proposed to couple biodiesel production with anaerobic digestion in order to produce biogas from the remaining biomass after oil extraction and stated that the overall energy output of the coupled process could be increased by up to 40% (Bohutskyi et al. 2014).

In a different study, various nutritional modes, including glucose supplementation, were investigated with an aim to enhance biomass and lipid productivity in different microalgal strains. They reported that lipid productivity ranged from 2 to 13% under photoautotrophic conditions, 1.7–32% under mixotrophic conditions, and 0.9–20% under heterotrophic conditions. While under heterotrophic conditions where glucose supplementation was practiced, polyunsaturated fatty acids (PUFA) fraction of the oil was decreased by around 2–4-folds depending on the microalgae strain under investigation. On the other hand, saturated fatty acid (SFA) fraction was also negatively impacted by glucose supplementation. Oils rich in SFA containing low PUFA are ideal feedstock for achieving high oxidative stability in biodiesel, and therefore, glucose supplementation could be serve this purpose well (Ratha et al. 2013).

Another strategy proposed by Duong et al. (2015) was to target both algal lipid and protein simultaneously to improve the economic viability of algal biodiesel production. More specifically, they tried to isolate algal strains meeting three criteria of fast growth, high lipid content, and protein-rich biomass, while that last could be used for animal feed (Duong et al. 2015). Converti et al. (2009) explored the effects of temperature concentration on lipid content in *Nannochloropsis oculata* and *Chlorella vulgaris*. They argued that variations in the investigated factor strongly impacted lipid content. For instance, a temperature boost from 20 to 25 °C increased lipid content by 100%, while an opposite was observed for *C. vulgaris* when the temperature was increased from 25 to 30 °C (Converti et al. 2009).

Nitrogen concentration in the cultivation media is also an important parameter. It is well documented that nitrogen deprivation could result in increased lipid content but could also negatively affect algal growth. Therefore, a trade-off should be observed to achieve the highest lipid productivity. In-depth understanding of the relationships between cell nitrogen content, growth, and cell composition is essential in order to be able to identify an optimal nitrogen content required for most favorable lipid productivity in batch or continuous cultivation modes (Griffiths et al. 2014). It should be highlighted that nitrogen deprivation could also improve the fatty acid profile of algal oils leading to more favorable biodiesel properties (Griffiths et al. 2014).

Nitrogen-to-phosphorus (N/P) ratio could also have a crucial effect on the biomass growth (Alketife et al. 2017). Xin et al. (2010) showed that this ratio is significantly effective on biomass yield and lipid accumulation of a freshwater microalga *Scenedesmus* sp. LX1. They claimed that under nitrogen (2.5 mg L^{-1}) or phosphorus (0.1 mg L^{-1}) limitations, the microalgae under investigation could accumulate lipids to as high as 30 and 53% of its algal biomass, respectively and lipid productivity was not enhanced reportedly. Similar observations were made by Kalla and Khan (2016) who also studied the effect of decreasing nitrogen and phosphorus concentrations on growth, biomass, and lipid content of *C. vulgaris*. They argued that significant decreases were recorded in growth and by decreasing nitrogen and phosphorus concentrations in the medium from (1.5–0.0 g/l) and (0.04–0.0 g/l), respectively. On the contrary, lipid accumulation was enhanced under the phosphorus and nitrogen limitations.

Different ions could also impact algal growth and lipid production significantly. For instance, Huang et al. (2014) investigated the effects of ferric ion concentrations on three species of microalgae (*Tetraselmis subcordiformis*, *Nannochloropsis oculata,* and *Pavlova viridis*). They concluded that growth, lipid content, as well as the fatty acid profiles of the studied microalgae varied in response to changes in ferric ion concentrations and that an optimum ferric ion concentration can improve the properties of respective algal biodiesels.

In a different study performed in high-glycerol content media, the effect of calcium and magnesium ions supplementation was studied using two fast-growing algal strains of *Aurantiochytrium* sp. DBTIOC-18 and *Schizochytrium* sp. DBTIOC-1 for biomass and lipid production (Singh et al. 2016). It was revealed that increasing both calcium and magnesium ions' concentration promoted glycerol utilization and resulted in a significant boost in biomass and lipid production. Such findings highlight the importance of calcium and magnesium ions' concentrations in preventing substrate inhibition under high nutrient concentrations, especially carbon sources to achieve high biomass and lipid yields (Singh et al. 2016).

Sulfate ions are also effective on growth of microalgae. In a recent study, Lv et al. (2017) strived to look into the responses of the self-flocculating microalga *Chlorococcum* sp. GD to different sulfate concentrations in a synthetic municipal wastewater. Their results showed that the microalgal cells grew better in the synthetic municipal wastewaters containing 18, 45, 77, 136, and 271 mg/L SO_4^{2-} than in the control wastewater without SO_4^{2-}. They argued that sulfate deprivation led to significant decreases in antioxidative enzymes and photosynthetic activities and that these in turn significantly weakened the growth and self-flocculation properties of the algal cells (Lv et al. 2017).

pH is also important for the microalgal growth and the accumulation of intracellular lipids. This was confirmed by the findings of Sakarika and Kornaros (2016) who investigated the impacts of various pH values on *C. vulgaris* cultivation. They also argued that the fatty acid composition of the algal cultures was not impacted by pH variations. Illumination, i.e., length of photoperiod and light intensity, could also result in changes in algal growth and lipid content and have to be optimized (Wahidin et al. 2013). Overall, producing high amounts of lipids while maintaining a high algal growth rate is critical for an economic algal biodiesel production simply because high algal biomass productivity would lead to high yield per harvest volume and high lipid content would decrease the cost of extraction per unit product (Tan and Lee 2016). On such basis and since high lipid content and high biomass growth rate basically contradict each other, efforts have been being made to construct algal strains capable of producing high amounts of lipids without sacrificing growth through genetic and metabolic engineering (Talebi et al. 2015).

13.4 Harvesting and Drying of Microalgae

Harvesting in general constitutes a major fraction (20–30%) of the costs associated with microalgal production (Ndikubwimana et al. 2016). Two-step separation, i.e., thickening followed by dewatering, is usually practiced to decrease the cost of the final product. The concentration of the algal cells is increased to approx. 2–7 and 15–25% (TSS basis) through the two stages, respectively. There are several methods for harvesting algae including (Christenson and Sims 2011): (1) filtration—algae can be filtered out by passing through membranes; in this method, recovery rate is high and lower energy inputs are involved, but dewatering might be required; (2) centrifugation—a mechanical method for harvesting microalgae which does not involve contamination with chemicals and, like filtration, the rate of recovery is high; (3) flocculation, a method for separating algae using chemicals that lead to

Table 13.6 List of some microorganisms used for bioflocculation of microalgae[a]

Microorganism	Type	Bioflocculated microalgae
Bacillus licheniformis	*Bacteria*	*Desmodesmus* sp.
Pseudomonas stutzeri and Bacillus cereus		*Pleurochrysis carterae*
Paenibacillus sp.		*Chlorella vulgaris*
Paenibacillus polymyxa		*Scenedesmus* sp.
Bacillus subtilis		*Chlorella vulgaris*
Bacillus sp.		*Nannochloropsis oceanica* sp.
Ankistrodesmus falcatus	*Fungi*	*Chlorella vulgaris*
Scenedesmus obliquus		*Chlorella vulgaris*
Tetraselmis suecica		*Nannochloropsis oleabundans*
Skeletonema sp.		*Nannochloropsis* sp.
Tetraselmis suecica	*Microalgae*	*Chlorella* sp. *and Nannochloropsis* sp.

[a] Al Hattab et al. 2015, Powell and Hill (2013), and Kawaroe et al. (2016)

aggregation of algal cells. In this method, destruction of algal cells is less than in centrifugation and low energy is required; (4) floatation is a separation method in which algae are floated into the surface using bubbling, often used in combination with flocculation for wastewater treatment. No disturbance is made to the cells, and low-energy requirement is also considered as an advantage of this system; (5) ultrasonic separation in which sound waves cause the cells to agglomerate.

Choice of harvesting methods depends on the characteristics of microalgal strain/consortium, while the type and value of the end product are also of importance (Barros et al. 2015). Among different methods, bioflocculation, i.e., the use of microorganisms for the recovery of microalgae biomass, has been most widely used as it is accompanied with significantly less dewatering cost which is economically critical for their full-scale application (Ndikubwimana et al. 2016). A list of microorganisms used in bioflocculation is tabulated in Table 13.6 (Al Hattab et al. 2015; Kawaroe et al. 2016). These microorganisms when added to an algal culture lead to the settlement of the algal cells by adhering and consequent weight increase (Al Hattab et al. 2015). For instance, Ndikubwimana et al. (2014) claimed 98% removal efficiency when they use *Bacillus licheniformis* as bioflocculant for harvesting *Desmodesmus* sp. culture. In a different study, Zhang and Hu (2012) employed a co-culture of *Chlorella vulgaris* and filamentous fungi and successfully extracted the oil for biodiesel production.

In general, both algal oil extraction and its conversion into biodiesel are strongly negatively affected by the presence of water and, therefore, algal biomass should be effectively dried prior to the transesterification reaction (Kumar et al. 2010a, b, c). As a result, different drying methods are usually employed after secondary dewatering (Richmond 2008). Solar drying is the most economically viable drying method especially in places where abundant sunlight is available throughout the year (Sharma et al. 2013). On the contrary, drying methods which are dependent on fossil-oriented energy carriers for their operation, e.g., spray drying and drum drying, are economically and environmentally justified for microalgae biodiesel production (Zhang et al. 2014).

13.5 Lipid Extraction

Microalgal lipids are divided into nonpolar (hydrocarbons, waxes, eicosanoids, fatty acids, and acylglycerols) and polar (phospholipids and glycolipids). There are several methods for cell disruption and extracting microalgal lipids such as mechanical (expeller press), physical (decompression, microwave, freeze-drying, and thermolysis), chemical (organic solvent, chelating agent, supercritical CO_2, detergent, and antibiotics), and enzymatic (lytic, autolysis, and phage) (Kumar et al. 2015).

Mechanical extraction methods offer a number of advantages over the other methods including less dependency on the type of microalgae species to be processed and no contamination of the extracted lipid (Ramesh 2013). Nevertheless, higher energy requirements are considered as a drawback of mechanical extraction methods. This is ascribed to the fact that heat is generated during mechanical extraction of lipids and, in order to prevent damages to the lipids, cooling needs to be performed whose energy and equipment costs negatively impact the overall economics of the process (Lee et al. 2012). Moreover, for a successful implementation of mechanical extraction methods, low-moisture-content algal biomass is required and, therefore, a drying stage needs to be included which could also considerably increase the overall extraction costs. It should also be noted that the amount of pressure employed during mechanical extraction is of critical importance. More specifically, increasing pressure to an optimal level could improve the extraction efficiency, while above-optimal pressure values could negatively affect the process leading to decreased lipid recovery and increased heat generation (Ramesh 2013). Expeller press is one of the simplest mechanical techniques for extracting various oil feedstocks including algae. Nevertheless, its major technical drawback is the presence of pigments along with oil. This method also requires huge amounts of energy, and its efficiency rate is low to moderate.

Among the physical extraction methods, microwave-assisted extraction has attracted a great deal of attention due to its effectiveness in disrupting algal cell walls, being non-toxic, and the possibility of reusing the media after extraction (Lee et al. 2010; Halim et al. 2012; Hattab and Ghaly 2015). Nevertheless, the high costs associated with its maintenance still limit its large-scale application. Freeze-drying and autoclave techniques are also classified among physical extraction methods. However, both these methods suffer from drawbacks such as high costs and long processing times (Hattab and Ghaly 2015).

Cell disruption and consequently extraction of lipids can be also achieved by using a large variety of chemical compounds including antibiotics, chelating agents, chaotropes, detergents, solvents, hypochlorites, acids, and alkali, through different mechanisms though (Günerken et al. 2015). For instance, basic compounds disrupt the cell membranes through saponification of the membrane lipids, while acidic compounds exert their disruptive properties through poration of the cell membrane/wall (Halim et al. 2012; Günerken et al. 2015). In general, lipid extraction from algal biomass is currently carried out using organic solvents such as chloroform, methanol, water, chloroform/methanol (1:2 v/v), chloroform/methanol/water (1:2:0.8 v/v/v), hexane, isopropanol, hexane/isopropanol (3:2 v/v), and ethanol (Zhang et al. 2014). It should be mentioned that organic solvent-based extraction is time- and labor-demanding and, more importantly, it is most efficient for lipid extraction from some algal strains, while it is not reportedly applicable for all algal strains (Ranjith Kumar et al. 2015).

Extracting oil from algal cells is generally limited due to the presence of algal cell wall (Johnson and Wen 2009). Therefore, the use of enzymes such as cellulase, neutral protease, alkaline protease, papain, and lysozyme has been practiced to facilitate cell disruption (Taher et al. 2014; Hattab and Ghaly 2015). Compared to mechanical and chemical methods, enzymatic extraction of algal lipids is very efficient and rapid while causing no corrosion as is the case when chemical

Table 13.7 Different processes used for converting algal biomass to various types of biofuels[a]

Conversion process			Final product	Advantages and limitations
Biochemical conversion		Photobiological hydrogen production	Hydrogen	–
		Fermentation	Bioethanol, acetone, bioethanol	Co-products can be utilized, conversion of sugar to bioethanol possible, long processing time required, biomass has to be preprocessed to be converted to sugars
		Anaerobic digestion	Methane, hydrogen	
Thermochemical conversion	Dry feedstock	Gasification	Syngas	–
		Pyrolysis	Bio-oil–charcoal–syngas	High bio-oil yields possible(up to 57.5% *w/w* for fast and flash pyrolysis, high-energy content required to dry feedstock
	Wet feedstock	Liquefaction	Bio-oil	Algal wet slurry can be used, energy (and cost) reduction, high yields possible (up to 60% *w/w*), reactors are complex and expensive
		Direct combustion	Power generation	–
Chemical reaction		Transesterification	Biodiesel	Enhanced physical properties of renewable fuels, biodiesel has a current market that simplifies commercialization, limited to conversion of lipids and does not utilize carbohydrate and protein fractions of feedstock

[a] Tsukahara and Sawayama (2005) and Vardon et al. (2012)

extraction methods are used. However, the application of enzyme-based methods is limited owing to the high cost of enzymes.

13.6 Microalgae Biofuel Production Pathways

After oil extraction from microalgae for biodiesel production, the remaining biomass can be converted into different types of biofuels, i.e., biohydrogen (Fedorov et al. 2005; Kapdan and Kargi 2006), biomethane (Sialve et al. 2009), and bioethanol (Dexter and Fu 2009) (Table 13.7).

13.6.1 Biochemical Conversion of Algal Biomass

Technologies for biochemical conversion of algal biomass include anaerobic digestion (or biomethanation) and fermentation. More specifically, in biochemical conversion, carbohydrates are digested into sugars using bacteria, microorganisms, and enzymes, which are then transformed into gaseous or liquid fuels, such as biogas (biomethane and biohydrogen) and bioethanol (Zamalloa et al. 2012). For instance, Batista et al. (2015) converted the biomass of an algal consortium (*Chlorella vulgaris, Scenedesmus obliquus*) grown on wastewater into biohydrogen through dark fermentation by an *Enterobacter aerogenes* strain. The highest biohydrogen production yield achieved was 56.8 mL H_2/gVS.

13.6.2 Thermochemical Conversion of Algal Biomass

Thermochemical conversion involves the use of heat to convert algal biomass into gaseous or liquid fuels. Thermochemical conversion can be classified according to the primary desired product (solid, liquid, gas) and the water content of the feedstock (dry or wet).

13.6.2.1 Biocrude Oil Production by Hydrothermal Liquefaction (HTL) of Wet Algal Biomass

The thermochemical conversion of wet algal biomass (75–98% moisture) into biocrude oil in the presence of a solvent at 200–350 °C temperatures and 5–25 MPa pressure to maintain water in the liquid state is called hydrothermal liquefaction (HTL) (Biller et al. 2011). In HTL, biomass is broken down into shorter carbon chains that have a higher energy density (Brennan and Owende 2010). Oxygen, sulfur, and water contents are very low in crude HTL oil. HTL oil recovers more than 70% of the feedstock carbon content. The product is a heavy oil or tarry material, which is called biocrude oil (Biller et al. 2011). The size of biomass particles, residence time, solvent media type, and hydrogen donor solvents are effective for the bio-oil yield and the product quality (Akhtar and Amin 2011). The basic reaction mechanisms involve: (a) depolymerization of the biomass, (b) decomposition of biomass monomers, and (c) recombination of reactive fragments (Toor et al. 2011).

13.6.2.2 Biofuel Production by Pyrolysis of Algal Biomass

Pyrolysis is one of the subclasses of thermochemical conversion in which dry algal biomass is decomposed in the absence of oxygen (or any halogen) and converted into biofuels such as bio-oil–charcoal–syngas. This conversion occurs in the temperature range of 401.85–701.85 °C and 0.1–0.5 MPa pressure (Demirbas 2006). On the basis of operation conditions, pyrolysis process is classified into: (1) slow pyrolysis with operation temperature of 286.75–676.85 °C (Bridgwater 2003), (2) fast pyrolysis with operation temperature of 577–977 °C under inert atmospheric conditions (Mohan et al. 2006), and (3) flash pyrolysis with operation temperature of 777–1027 °C (Balat et al. 2009).

13.6.2.3 Syngas *Production* Through Gasification of Microalgal Biomass

Syngas (a combination of hydrogen, carbon monoxide, and carbon dioxide) is usually produced through the gasification of different carbonous materials including algal biomass (Brown et al. 2010). Gasification process is in fact a partial oxidation process that converts dry algal biomass for instance into a mixture of gases. Gasification is classified into low temperature gasification (700–1000 °C) and high temperature gasification (1200–1600 °C) (McKendry 2002). Yield of syngas depends on various factors including microalgal biomass quality, the equipment (gasifier) used, as well as process parameters (e.g., temperature and catalysis used). In a study, Raheem et al. (2015a, b) reported that syngas yield increases from 28 to 57% by increasing temperature from 552 to 952 °C. The generated syngas could eventually be used for hydrogen production, liquid biofuels production, synthetic natural gas (SNG) production, etc. (Mondal et al. 2011).

13.6.3 Chemical Reaction

13.6.3.1 Biodiesel Production by Transesterification of Algal Oil

Biodiesel, also known as methyl or ethyl esters of long-chain fatty acids, is an alternative to mineral diesel fuel produced from vegetable oils, animal fats, and algal oil mainly through the transesterification reaction with an alcohol (methanol and/or ethanol) and in the presence of a catalyst (mostly NaOH or KOH). The main advantages of biodiesel as fuel include widespread availability, renewability, clean-burning features compared with mineral diesel, and lower sulfur and aromatic contents (Demirbas 2007). These are numerous reports confirming that biodiesel lowers exhaust emissions from diesel engines (Hayyan et al. 2010), i.e., particulate matter (PM) (Kolesárová et al. 2011), unburned hydrocarbons (HC), and carbon monoxide (CO). On the contrary, there is no consensus on the impact of biodiesel on nitrogen oxide (NO_x) emission as there are reports claiming increases in NO_x due to the oxygen content of biodiesel (Sharma et al. 2008). There are four methods for biodiesel production and utilization, direct use and raw oils blending, microemulsions, pyrolysis, and transesterification. As mentioned earlier, the last procedure is most commonly used (Demirbas 2003). Through transesterification, biodiesel and its co-product, i.e., glycerin, is produced in several stages. Afterward, the excess methanol is recovered from the methyl esters through evaporation, and the final biodiesel is eventually washed with water, neutralized, and dried (Xu et al. 2006). Since fossil oil is derived from spores and planktonic algae that were under high pressure and temperature over millions of years, the chemical properties of microalgal lipids and the consequent biodiesel are also very similar to those of mineral diesel (Demirbas and Demirbas 2011).

Transesterification reaction can be acid/base/enzyme catalyzed. Alkaline catalysts include NaOH, NaO^-, KOH, and KO^{-1}, while acid catalysts include HCL and H_2SO_4. Enzymatic catalysts such as lipases that are able to catalyze the transesterification of triglycerides effectively in either aqueous or nonaqueous systems are more environmentally friendly than the other two groups as they result in no wastewater and the produced glycerin needs minimal purification (Fukuda et al. 2001). In another word, the weak points of transesterification reaction by alkaline catalysts are difficult recovery of glycerol, the need for alkaline wastewater treatment, free fatty acid and water interference with the reaction, energy intensity, and the necessity of removing the catalyst from the product (Meher et al. 2006). Some properties of diesel, biodiesel from various oil feedstocks, and microalgae biodiesel are shown in Table 13.8 (Kiss et al. 2007; Huang et al. 2010; Veillette et al. 2012).

13.7 Applications of Microalgae in Integrated Systems

Integration of algal biodiesel production with other activities such as wastewater treatment with an aim to enhance the economic viability of the whole process could be regarded as an efficient strategy to overcome most of the challenges faced. For instance, and as mentioned earlier, wastewater resources are rich in nutrients, such as nitrogen and phosphorus, and could be served microalgal growth as cultivation medium. In fact, using wastewater for microalgal biofuel production not only can reduce freshwater footprint and the cost of these fuels (Clarens et al. 2010) but also could offer new algal-based wastewater treatment systems (Table 13.5). It is worth mentioning that non-fuel products such as fertilizers, chemicals, pharmaceutics, dyes, paints, and animal feeds could also be obtained from microalgaes grown on wastewater (Bhatt et al. 2014).

Table 13.8 Properties of diesel and biodiesel produced from various oil feedstocks*

Property	Diesel	Biodiesel							
		Average biodiesel	Soybean	Jatropha	Rapeseed	Crambe	Corn	Microalgae	*Nannochloropsis oculata*
Fuel composition	C10–C21 HC[a]	C12–C22 FAME[b]	–	–	–	–		–	–
Degree of unsaturation (DU)	–	–	143.70	120.17	123.20	43.60	87	–	53.20
Saponification value (SV)	–	–	202.26	198.94	196.73	72.60	165.26	–	186.67
Iodine value (IV)	–	–	136.84	108.78	111.17	44.00	78.67	–	50.79
Oxidation stability (OS) (h)	–	–	4.56	5.52	6.52	11.52	184.2	–	93.31
Heating value (MJ L^{-1})	36–38	32–36	39.6	39.04[c]	39.3[c]	11.54[c]	28.7[c]	35.40	34[c]
Kinetic viscosity, $mm^2\ s^{-1}$ (at 40 °C)	1.9–3.8	2.8–5.7	1.29	1.31	1.33	0.5	0.84	3.87–5.2	1.12
Density (kg L^{-1})	0.838	0.84–0.90	0.89	0.87	0.87	0.32	0.65	0.864	0.76
Cetane number	40–55	45–70	42.5	49.26	49.3	111.58	61.63	39–54	64.11
Specific gravity, (15.5 °C)	0.81–0.86	0.86–0.89	–	–	–	–	–	0.864	–
Boiling point (°C)	188–343	182–338	–	–	–	–	–	–	–
Flash point (°C)	60–80	100–170	–	–	–	–	–	115	–
Cold filter plugging point (°C)	−3(max −6.7)	Summer max. 0	−1.3	−2.43	−11.29	−0.11	−10	−11	−4.79
Pour point (°C)	−35 to −15	−15 to 10	−5.79	−4.87	−10.81	−11.10	−7.67	–	6.15
Sulfur (wt%)	0.01–0.04	0.0000–0.0024	0.004	0.001	–	–	–	0.0069	–
Stoichiometric air/fuel ratio (AFR)	15	13.8	–	–	–	–	–	–	–
Acid value (mg KOH g^{-1})	Max 0.5	Max 0.5	0.21	0.21	0.98	0.33	–	0.374	–
Lubricity (25 °C)	0.509–0.283	0.114	–	–	–	–	–	–	–
H/C ratio	1.81	–	–	–	–	–	–	1.81	–

[a] HC—hydrocarbons

[b] FAME—fatty acid methyl esters

[c] MJ/Kg

**Sources* Kiss et al. (2007), Huang et al. (2010), Veillette et al. (2012), Uthman and Saka (2013), Oliveira and Silva (2013), Islam et al. (2013), http://www.chempro.in, and http://www.brteam.ir/biodieselanalyzer

Beside biofuel production, biofixation of carbon could also be the secondary objective of algal-based biofuel production systems. This is increasingly important given the criticality of climate change and the very recent international call for immediate action to address this crisis to the level that even the leader of the Catholic Church Pope Francis raised the issue during his visit to the USA in September 2015 (The Gurdian 2015).

The following sections summarize the efforts made during the last several years in order for integrating algal biofuel production systems with wastewater treatment and carbon biofixation.

13.7.1 Algal Biofuel Production and Wastewater Treatment

A major requirement of an efficient wastewater treatment is obviously the need to remove high concentrations of nutrients, in particular N and P. As mentioned earlier, microalgae are capable of uptaking such nutrients as well as heavy metals and organic pollutants from wastewater and producing biomass. Thus, it offers great promises for the treatment of various municipal, agricultural, and industrial wastewaters (Feng et al. 2011; Zhu et al. 2013). However, there are many reports indicating that most of the microalgal species with high lipid contents do not adapt well to grow in wastewater (Xin et al. 2010). Contrary to these reports, there are also a number of success stories through which efficient integration of algal biofuels production and wastewater treatment has been accomplished (Zhou et al. 2011a, b, 2013; de Alva et al. 2013; Hena et al. 2015). For instance, Zhou et al. (2011a, b) claimed that five species of microalgae isolated from Minnesota wastewaters including *Chlorella* sp., *Heynigia* sp., *Hindakia* sp., *Micractinium* sp., and *Scenedesmus* sp. showed high growth rate (0.455–0.498 d^{-1}) and lipid productivities (74.5–77.8 mg L^{-1} d^{-1}) on municipal wastewater.

In a more recent investigation, de Alva et al. (2013) also cultivated *Scenedesmus acutus* in pretreated municipal wastewater with a dual focus on biomass productivity and lipid accumulation. They argued that *S. acutus* could successfully remove nutrients from the wastewater and that they achieved 249.4 mg L^{-1} biodiesel from the referred algal oil. It should be pointed out that in a series of experimental surveys, Chinnasamy et al. (2010a, b), Kong et al. (2010), and Zhou et al. (2011a, b) revealed that municipal wastewater was a better option compared with industrial wastewater for algal biomass and lipid production. A comparison of biomass productivity, lipid content, and lipid productivity of different microalgae species grown on various wastewater is tabulated in Table 13.9. The following sections present the integration of biofuels production with algal-based treatment of different types of wastewaters, namely high N/P content wastewater, high heavy metal-content wastewater, and high organic-content wastewater (PAHs aromatic hydrocarbons and polychlorinated biphenyls (PCBs)).

Zhu et al. (2013) proposed *Chlorella zofingiensis* cultivation on piggery wastewater with a dual purpose of wastewater treatment and biodiesel production.

Table 13.9 Comparison of biomass and lipid productivities in microalgae grown in various wastewater conditions

Microalgae species	Wastewater	Biomass (DW*) productivity (mg L^{-1} d^{-1})	Lipid content (% DW)	Lipid productivity (mg L^{-1} d^{-1})	References
Auxenochlorella protothecoides	Municipal centrate	268.80	28.90	77.7	Zhou et al. (2011a, b)
B. braunii	Industrial (carpet mill)	34.00	13.20	4.50	Chinnasamy et al. (2010a, b)
Chlamydomonas reinhardtii (biocoil-grown)	Municipal centrate	2000	25.25	500	Kong et al. (2010)
Chlamydomonas mexicana	Piggery wastewater	Not available	33[a]	0.31[a]	Abou-Shanab et al. (2013)
Chlorella sp.	Agricultural (dairy)	2.6 gm^{-2} d^{-1}	9	230 mg m^{-2} d^{-1}	Johnson and Wen (2010)
Chlorella sp.	Municipal centrate	231.40	33.53	77.50	Zhou et al. (2011a, b)
Chlorella sp.	Municipal centrate	241.70	30.91	74.70	Zhou et al. (2011a, b)
Chlorella saccharophila	Industrial (carpet mill)	23.00	18.10	4.20	Chinnasamy et al. (2010a, b)
Chlorella sp.	Agricultural (digested dairy manure, 20 × dilution)	81.4[b]	13.6[c]	11[c]	Wang et al. (2010)
C. pyrenoidosa	Piggery wastewater	–	–	6.3	Wang et al. (2012)
Mix of *Chlorella* sp., *Micractinium* sp., *Actinastrum* sp.	Agricultural (dairy wastewater, 25% dilution)	59[d]	29	17	Woertz et al. (2009)
Mix of *Chlorella* sp., *Micractinium* sp., *Actinastrum* sp.	Municipal (primary treated + CO_2)	270[e]	9	24.4	Woertz et al. (2009)

(continued)

Table 13.9 (continued)

Microalgae species	Wastewater	Biomass (DW*) productivity (mg L^{-1} d^{-1})	Lipid content (% DW)	Lipid productivity (mg L^{-1} d^{-1})	References
Dunaliella tertiolecta	Industrial (carpet mill)	28	15.20	4.30	Chinnasamy et al. (2010a, b)
Pleurochrysis carterae	Industrial (carpet mill)	33	12	4	Chinnasamy et al. (2010a, b)
Scenedesmus obliquus	Municipal sewage	26^{f}	31.4^{g}	8^{g}	Krishna et al. (2012)
Scenedesmus sp.	Municipal centrate	247.50	30.90	74.50	Zhou et al. (2011a, b)
Scenedesmus acutus	Municipal	79.9	–	280 mg L^{-1}	de Alva et al. (2013)
Botryococcus braunii	Agricultural	700	–	69	Krishna et al. (2012)
Hindakia sp.	Municipal centrate	275	28.30	77.80	Zhou et al. (2011a, b)

*DW—dry weight

[a] Estimated from biomass value of 1000 mg L^{-1} after 40 d

[b] Estimated from biomass value of 1.71 g L^{-1} after 21 d

[c] Fatty acid content and productivity determined rather than total lipid

[d] Estimated from lipid productivity and lipid content value

[e] Estimated from biomass value of 812 mg L^{-1} after 3 d

[f] Estimated from biomass value of 197 mg L^{-1} after 31 d

[g] Fatty acid content and productivity determined rather than total lipid

In a different investigation, Maity et al. (2014) investigated the integration of biofuel and bioelectricity production with wastewater treatment using one species of microalgae, i.e., *Leptolyngbya* sp. JPMTW1 (KF977831). They argued that only after 7 d of cultivation, biomass production, rate of biomass production, lipid production, and rate of lipid production stood at 3300 mg L^{-1}, 471.42 mg L^{-1} day^{-1}, 1068.383 mg g^{-1} dry wt. biomass, 152.62 mg g^{-1} dry biomass/day, respectively. The also reported that over the same period, electrical conductivity (EC), chemical oxygen demand (COD), and total dissolved solid (TDS) decreased from 982 to 854 (mS/cm), 255 to 112 mg L^{-1}, and 490–427 mg L^{-1}, respectively. Overall, their findings were indicative of the possibility of the production of biofuel, bioelectricity, and wastewater treatment by *Leptolyngbya* sp. JPMTW1. In another study, Chen et al. (2014) produced biocrude

Table 13.10 Nutrient removal efficiency of microalgal species

Microalgal species	Wastewater type	Nitrogen (%)	Phosphate (%)	COD removal (%)	References
Chlorella Mexicana	Piggery	62	28%	–	Abou-Shanab et al. (2013)
Chlorella vulgaris	Textile	44.4–45.1	33.1–33.3	38.3–62.3	Lim et al. (2010)
Chlorella vulgaris	Municipal	55–88	12–100	–	Khan and Yoshida (2008), Ruiz-Marin et al. (2010)
Chlorella kessleri	Artificial medium	8–19[a]	8–20[b]	–	Cai et al (2013)
Chlorella sp.	Municipal centrate	89.1	80.9	90.8	Li et al. (2011)
Chlorella sp.	Dairy manure	75.7–82.5	62.5–74.7	27.4–38.4	Wang et al. (2010)
Chlorella pyrenoidosa	Industrial	87–89	70	–	Hongyang et al. (2011)
Chlorella minutissima	Primary- and tertiary-treated	70–80	60–70	–	Malla et al. (2015)
Chlamydomonas reinhardtii	Artificial medium	12–83	13–14	–	Kong et al. (2010)
Chlamydomonas polypyrenoideum	Dairy	74–90	70	–	Kothari et al. (2012)
Scenedesmus obliquus	Municipal	79–100[a]	47–98	–	Cai et al. (2013)
Scenedesmus acutus	Municipal	66	94	–	de Alva et al. (2013)
Euglena sp.	Sewage treatment plant	93	66	–	Mahapatra et al. (2013)

[a] Nitrate, nitrite
[b] Total orthophosphates

oils from a mixed-culture algal biomass harvested from a functioning wastewater treatment system as well.

13.7.1.1 High N/P Content Wastewater

Nitrogen is a critical nutrient required for algal growth, and the application of nitrogen starvation for enhancing algal cell lipid content is well documented (Brennan and Owende 2010). Likewise, another key factor in algal energy metabolism is phosphorus which is found in a variety of biological substances, such as nucleic acids, lipid, proteins, and intermediates of carbohydrate metabolism. All eukaryotic algae require inorganic nitrogen, while some algal species are capable of using both inorganic and organic phosphorus (Liang 2013). In recent years, investigations into the ability of microalgae to simultaneously grow on wastewater streams and remove nutrients have revealed many microalgae species with high protectional for N and P removal from wastewaters (Table 13.10). For instance, Cai et al. (2013) achieved an N removal efficiency of 79–100% by *S. obliquus* from municipal wastewater. Earlier in the year 2010, Lim et al. made an attempt to treat textile wastewater medium using *C. vulgaris* and reportedly managed to remove N and P by 45 and 33%, respectively. A wide range of N (55–88%) and P (12–100%) removal has been reported when municipal wastewater was used as the waste stream (Khan and Yoshida 2008; Ruiz-Marin et al. 2010; Li et al. 2011). Mixed municipal and industrial wastewater was used by Gentili (2014) to produce *Selenastrum minutum* algal biomass and lipid, while effective wastewater treatment was also targeted. Their results showed that ammonium and phosphate contents were decreased from 96 to 99% and 91 to 99%, respectively, while the highest biomass and lipids yields (dry matter basis) reaching 37%.

Lu et al. (2015) used meat processing wastewater for the cultivation of the microalgae *Chlorella* sp. (UM6151) aiming at simultaneous biomass production, wastewater treatment, and nutrient removal. They implemented an innovative cultivation approach based on wastewater mixing to supply nutrients and improve biomass yield at economic rates. They claimed that algal biomass yield (0.675–1.538 g/L) achieved using mixed wastewater was much higher than those obtained using individual wastewater and synthetic medium. Moreover, they achieved improved ammonia nitrogen removal efficiencies (68.75–90.38%) and total nitrogen removal efficiencies (30.06–50.94%). Interestingly, by using wastewater mixing, algal protein content was also enhanced reaching as high as 60.87–68.65%.

In an effort, Abou-Shanab et al. (2013) strived to integrate biofuel production and the treatment of piggery wastewater (TN: 56 ± 2 and TP:13.5 ± 0.6 mg/L). They reported that six microalgal species including *Ourococcus multisporus*, *Nitzschia cf. pusilla*, *Chlamydomonas mexicana*, *S. obliquus*, *Chlorella vulgaris*, and *Micractinium reisseri* were capable of efficiently treating wastewater and producing high oil content for biodiesel production. Among the studied species, *C. Mexicana* was proven to have the highest removal rates, i.e., N (62%), phosphorus (28%), and inorganic carbon (29%). Hence and due to the higher lipid productivity and lipid content (0.31 ± 0.03 g/L and 33 ± 3%, respectively), compared with the other species, the authors suggested that *C. mexicana* could be a suitable candidate for integrated biodiesel production and wastewater treatment.

In a study, Min et al. (2014) suggested an efficient method, i.e., a pilot-scale stacked-tray bioreactor to increase nutrient removal rate from piggery wastewater coupled with biofuel production. Through their proposed cultivation system, algal biomass productivity (based on TSS) was enhanced from 19.15 to 23.19 g m^{-2} day^{-1} and they achieved lipid contents ranging between 1.77 and 3.55%. Wang et al. (2012) looked into the impact of dilution on algal biodiesel production and nutrient removal from high N/P content wastewater. In their study, primary piggery wastewater was used as the waste stream and was treated by mixotrophic cultivation of *Chlorella pyrenoidosa.* They stated that there was a positive linear correlation between algal biomass productivity and the initial COD values ranging from 250 to 1000 mg L^{-1}. The maximal lipid productivity 6.3 mg L^{-1} day^{-1} was recorded with an initial COD of 1000 mg L^{-1}, while nutrients such as ammonium were removed efficiently at rates as high as >90% in all diluted samples. Can et al. (2015) also explored the potential of microalgae *Spirulina platensis* for biofuel and biochemical production coupled with domestic wastewater treatment. Similar to Wang et al. (2012), in their experimental approach, wastewater was also diluted with distilled water to achieve different concentrations of 100, 75, 50, and 25%. Their findings were in line with those of Wang et al. (2012), revealing that the highest biomass yield was recorded when the wastewater without dilution (100%) was used. In terms of lipid production, however, the maximal value was measured in 25% wastewater. Therefore, a trade-off should be observed in order to maximize lipid productivity.

In a different study, Malla et al. (2015) also studied the potential of *Chlorella minutissima* for biodiesel production coupled with wastewater treatment. Their results indicated that after 12 d of the experiment, *C. minutissima* removed about 90–98% TDS, 70–80% N, 60–70% P, and 45–50% K from the high N/P content wastewater. They also converted the algal lipid extracted to biodiesel as part of the integrated system. Hena et al. (2015) investigated the potentials of a consortium of native microalgae species grown on a dairy farm treated wastewater for biodiesel production. The claimed that biomass production and lipid content of the consortium were 153.54 t ha^{-1} $year^{-1}$ and 16.89%, respectively, and that 72.70% of the algal lipid obtained could be converted into biodiesel.

13.7.1.2 High Heavy Metal Content Wastewaters

Heavy metals mainly include transition metals, metalloids, lanthanides, and actinides. These metals have a highly specified gravity and are toxic to a level that even at low concentrations represents a significant environmental concern (Bhargava et al. 2012). Various methods have been investigated for heavy metal removal, which are tabulated in Table 13.11 (Fu et al. 2011). Among these methods, algae have been proposed as ideal candidates for heavy metal removal from various

Table 13.11 Heavy metal wastewater treatment techniques (Fu and Wang 2011)

Technique	Conventional processes	Material used in the process	Removed ions
Chemical precipitation	Hydroxide precipitation	$Ca(OH)_2$, NaOH	Zn^{2+}, Cr^{3+}, Pb^{2+}, Hg^{2+}, Cu^{2+}
	Sulfide precipitation	Iron sulfide (FeS)	Pb^{2+}, Cu^{2+}, Cd^{2+}
	Heavy metal chelating precipitation	1,3-benzenediamidoethanethiol, hexahydrotriazine dithiocarbamate (HTDC), ethyl xanthate	Hg^{2+}, Cu^{2+}
Ion exchange	–	Clinoptilolite	Pb^{2+}, Ni^{2+}, Zn^{2+}
Adsorption	Activated carbon adsorbents	–	Pb^{2+}, Cu^{2+}
	Low-cost adsorbents	Chemically modified plant wastes, agricultural waste material, industrial by-products such as lignin, natural substances	Pb^{2+}, Ni^{2+}, Cd^{2+}
	Carbon nanotube absorbents	(1) Single-walled CNTs (SWCNTs)	Pb^{2+}, Ni^{2+}, Cd^{2+}, Cu^{2+}
		(2) Multi-walled CNTs (MWCNTs)	
	Bioadsorbents	Non-living such as potato peels, sawdust, coffee husks as well as living such as algal biomass and microbial biomass	Pb^{2+}, Cd^{2+}, Cu^{2+}
Membrane filtration	Ultrafiltration	Micellar-enhanced ultrafiltration (MEUF) and polymer-enhanced ultrafiltration (PEUF)	Pb^{2+}, ASO^{4-}, Cd^{2+}, Zn^{2+}, Cr(III), Cr(VI), Cu^{2+}, Cr^{3+}, Ni^{2+}
	Reverse osmosis	–	Zn^{2+}, As, Cu^{2+}, Ni^{2+}
	Nanofiltration	NF90 andN30F	Cr(VI), Cu^{2+}
	Electrodialysis	–	Pb^{2+}, Cr(III)
Coagulation and flocculation	–	Polyferric sulfate (PFS), polyacrylamide (PAM)	Ni^{2+}, Cu^{2+}, Pb^{2+}, Zn^{2+}
Electrochemical treatment	–	–	Zn^{2+}, Ag^{+}, Cu^{2+}, Ni^{2+}
Flotation	–	–	Cd^{2+}, Pb^{2+}, Cu^{2+}

wastewaters through either uptake or accumulation of Hg, Cd, Zn, Au, Ag, Co, Mn, Cs, Ni, Fe, Cu, and Cr from their environment (Chekroun and Baghour 2013). In fact, algae produce polypeptides called chelating agents capable of binding to heavy metals. Apart from that, large surface area of algal cells is also effective in removing heavy metals (Kumar et al. 2015). More specifically, metal absorption by microalgae occurs at two stages: first, at the surface of algal cells through very quick physical adsorption or ion exchange. The second stage, also called

Table 13.12 Heavy metal removal by microalgae from different wastewater source

Microalgae species	Wastewater type	Metal studied	Removal efficiency or accumulation	References
Scenedesmus sp.		Cu, Ni		Kumar et al. (2015)
Chlorella vulgaris	Synthetic wastewater	Cr	43.3 mg g^{-1} biomass	Xie et al. (2014)
Spirulina maxima and Chlorella vulgaris	Secondary effluent	Cu	81.7%	Chen et al. (2014)
Pavlova lutheri, Tetraselmis chuii, Nannochloropsis, and *Chaetoceros muelleri*	Municipal wastewater	Leachate		Richards and Mullins (2013)
Scenedesmus quadricauda	Synthetic wastewater	Pb	82%	Mirghaffari et al. (2015)
Phaeodactylum tricornutum	Seawater enriched	Hg	2229 mg g^{-1} biomass	Deng and Lu (2013)
Chlorella vulgaris, Spirulina maxima and *Synechocystis sp.*	Wastewater treatment plant discharge	Cu, Zn		Chan et al. (2013)
Scenedesmus bijuga, Oscillatoria quadripunctulata	Sewage wastewater and petrochemical effluents	Cu, Co, Zn, Pb		Ajayan et al. (2011)
Dictyosphaerium chlorelloides	Leather tanning, tincture wood preservatives, and the electroplating industry wastewater	Cr (III)		Pereira et al. (2010)

chemisorption, takes place at a slower rate intracellularly and is driven by metabolic processes involving active binding groups (Zhou et al. 2012).

Richards and Mullins (2013) studied algal-based bioremediation of municipal leachate by using a consortium of four marine microalgae species, i.e., *Pavlova lutheri, Tetraselmis chuii*, *Nannochloropsis*, and *Chaetoceros muelleri* while also targeting enhanced lipid production. Their results revealed that algal-based bioremediation was a feasible method for simultaneous treatment of waste streams and lipid production. Yang et al. (2015) proposed an integration of heavy metal wastewater utilization and biofuel production as an alternative solution to address energy shortage and environmental concerns. They claimed that *Chlorella minutissima* UTEX 2341 had strong resistance to cadmium, copper, manganese, and zinc ions under heterotrophic culture condition and could efficiently remove these heavy metals through intracellular accumulation and extracellular immobilization. Moreover, lipid accumulation was not negatively affected by heavy metals. Heavy metal removal by some species of microalgae from various wastewater sources is depicted in Table 13.12.

Table 13.13 Degradation of organic pollutants by algal species

Microalgae species	Organic pollutant	References
Monoraphidium braunii	Bisphenol	Gattullo et al. (2012)
Chlamydomonas reinhardtii	Herbicide (fluroxypyr)	Zhang and Hu (2012)
Pediastrum tetras *Ankistrodesmus fusiformis* *Amphora coffeaeformis*	Herbicide (mesotrione)	Valiente Moro et al. (2012)
Scenedesmus quadricauda	Herbicide (isoproturon)	Dosnon-Olette et al. (2010)
Scenedesmus obliquus GH2	Crude oil degradation	Tang et al. (2011)
Scenedesmus obliquus	Nonylphenol, octylphenol	Zhu et al. (2013)
Skeletonema costatum	Phenanthrene, fluoranthene	Hong et al. (2008)
Selenastrum capricornutum	Benzene, toluene, chlorobenzene, 1,2-dichlorobenzene, nitrobenzene, naphthalene, 2,6-dinitrotoluene, phenanthrene, di-n-butylphthalate, pyrene	Lei et al. (2007), Gavrilescu (2010)
Nitzschia sp.	Phenanthrene, fluoranthene	Hong et al. (2008)
Chlorella sp. *Scenedesmus obliquus* *Stichococcus sp.*	Phenol	Zhang and Hu (2012)
Chlorella vulgaris	Atrazine	Dosnon-Olette et al. (2010)
Chlorella fusca var. *vacuolata*	2,4-Dichlorophenol	Zhang and Hu (2012)
Chattonella subsalsa *Chattonella marina* var. *marina* *Chattonella marina* var. *ovata*	PCB (Aroclor 1242)	Niestroy et al. (2014)

13.7.1.3 High Organic-Content Wastewater

Organic pollutants are chemical substances that persist in an environment through industrial discharges and agricultural usages. They are also resistant to environmental degradation through chemical, biological, and photolytic processes and have harmful effects on human health. Among these organic pollutants, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) are highly persistent compounds and, if introduced into the food chain, they have been proven to be carcinogenic (Gilden et al. 2010). Microalgae are capable of decomposing different kinds of organic pollutants including phenolics, pesticides, as well as PAHs and PCBs. *Ankistrodesmus braunii*, *Scenedesmus quadricauda, Ochromonas danica,* and *Monoraphidium braunni* are examples of microalgae species that can biodegrade phenolic and biophenolic compounds (Mukherjee et al. 2013; Gattullo et al. 2012). Ali et al. (2012) introduced microalgae such as *chlorella vulgaris* as a low-cost adsorbent for removing organic pollutants from wastewaters. Attempts for degradation of organic pollutants by some species of microalgae are summarized in Table 13.13.

- *PAHs aromatic hydrocarbons*

  PAHs and polyaromatic hydrocarbons are ubiquitous environmental pollutants which are found in petroleum and fossil fuels, or are formed during the incomplete combustion of these energy carriers (Chekroun et al. 2014). These are neutral and nonpolar hydrocarbons that are composed of two or more benzene rings or pentacyclic molecules. Certain types of PAHs including benzo [a] anthracene, chrysene, benzo [b] fluoranthene, benzo [a] pyrene, and benzo [ghi] perylene are potentially carcinogenic for human beings and, due to their carcinogenic and mutagenic characteristics, are dangerous air pollutants (Gariazzo et al. 2015). Some types of the PAHs such as fluoranthene (Fla), pyrene (Pyr), benz[a]anthracene (BaA), chrysene (Chr), benzo[a]pyrene (BaP), benzo[k]fluoranthene (BkF), and dibenzo[a,h] anthracene (DA)] have half-lives of about 1000–3000 h in aquatic environments (Luo et al. 2014). Absorption, chemical degradation, photolysis, and volatilization and microbial degradation are significant methods for PAH removal. Nevertheless, the major process of removing PAH contamination in the environment is microbial degradation and algae are no exception (Ukiwe et al. 2013).

  Microalgae release biosurfactants that could further enhance phenanthrene degradation. Moreover, microalgae are able to produce the O_2 required by acclimatized bacteria to biodegrade hazardous pollutants such as polycyclic aromatic hydrocarbons, phenolics, and organic solvents (Chekroun et al. 2014). For example, some kinds of marine algae such as cyanobacteria, *Oscillatoria,* and *Agmenellum* spp. are known to degrade naphthalene through pathways that are similar to fungus (Haritash and Kaushik 2009; Barrios et al. 2011). The capability of *S. obliquus* and *Nitzschia linearis* in removing n-alkanes and PAHs has also been reported (Subashchandrabose et al. 2013).
- *Polychlorinated biphenyls* (*PCBs*)

  PCBs; organic chemical compounds of chlorine attached to ‘biphenyl, are a class of the worst persistent organic pollutants (POPs) (Gauthier et al. 2014). Due to their characteristics such as high toxicity, carcinogenicity, and slow biodegradation, exposure to PCBs can cause neurological disorders, reproductive toxicity, endocrine disruption, cancer, and even at extremely low concentrations (Pandelova et al. 2010). There are several technologies for PCB remediation, including biological treatment (phytoremediation, aerobic biodegradation, anaerobic dechlorination), physical methods, thermal treatment, and chemical treatment (Gomes et al. 2013). Bioaccumulation of PCBs by algae has attracted a great deal of attention (Chekroun et al. 2014), while its integration with biodiesel production is also of interest (Usher et al. 2014). The efficiency of algal-based remediation of PCBs could be influenced by water quality, chlorination, phytoplankton composition, the structure of the PCBs, and the algal cell wall (Zhao et al. 2014).

13.7.2 Biofixation of Carbon and Biofuel Production Systems

Microalgae use inorganic carbon for growth, while they can also fix CO_2 from industrial exhaust gases (Shilton et al. 2008). Utilization of microalgae for biofixation of carbon has numerous advantages as follows: (1) Microalgae have much higher CO_2 fixation abilities compared with other crops, since they have a higher growth rate (Chisti 2007; Li et al. 2008), and (2) microalgae are able to convert CO_2 into chemical energy through photosynthesis, which can then be converted into biofuels (Demirbas et al. 2004). Therefore, combination of wastewater treatment, biofuel production, and biofixation of CO_2 and GHG may provide a very promising alternative to climate change mitigation strategies.

For instance, CO_2 fixation rate ($g/m^3/h$) by *Chlorella vulgaris* has been reported at 80–260 (Cheng et al. 2006). Yoo et al. (2010) studied three species of microalgae, *Botryococcus braunii, Chlorella vulgaris*, and *Scenedesmus* sp., cultivated with ambient air containing 10% CO_2 and flue gas. Their results showed that the biomass and lipid productivity in flue gas condition rose by 1.9-fold (39.44 mg L^{-1} d^{-1}) and 3.7-folds (20.65 mg L^{-1} d^{-1}), for *Scenedesmus* sp and *B. braunii,* respectively. Moreover, they suggested that *B. braunii* was suitable for biodiesel production, due to its high lipid content, whereas *Scenedesmus* sp. was suitable for mitigating CO_2 as a result of high biomass productivity. In another study by Tang et al. (2011), two species of microalgae, *S. obliquus* and *Chlorella pyrenoidosa,* were explored as suitable species for mitigating CO_2 in the flue gases and biodiesel production.

CO_2 removal efficiency (%) by *Euglena gracilis, Porphyridium* sp., *S. platensis* has also been recorded at 3.1, 3–18, 38.3–60, respectively (Chae et al. 2006; Shibata et al. 2004; Kumar et al. 2010a, b, c). Nayak et al. (2013) also demonstrated biomass productivity and CO_2 biofixation of three strains of *Scenedesmus* sp. in the presence of different NaOH concentrations in algae cultivation media. They stated that under their experimental conditions, the algal lipids were mainly composed of C16/C18 fatty acids and were favorable for biodiesel production.

Exogenous CO_2 concentration could also impact algal biomass yield, nutrients removal rate, as well as biodiesel production potentials. For instance, in a study, Li et al. (2011) looked into the effects of environmental factors including exogenous CO_2 concentration on wastewater nutrient removal and biodiesel production using 14 strains of microalgae belonging to the genus of *Chlorella*, *Haematococcus*, *Scenedesmus*, *Chlamydomonas*, and *Chloroccum* cultivated. The results of this study proved that the environmental factors had effects on the yields of algal biomass and lipid accumulation which could consequently result in significantly different biodiesel production potentials. Among the algal strains investigated, *Chlorella kessleri* and *Chlorella protothecoides* represented the highest biomass accumulation of 2.01, 1.31 g/L, respectively. Overall, biomass accumulation, biodiesel production rate, and the removal rates of nitrogen and COD were increased by higher light intensity and exogenous CO_2 concentration as well as longer lighting period, while higher phosphorus removal rates were achieved in lower exogenous CO_2 concentrations.

13.8 Conclusions and Future Prospects

Widespread utilization of fossil fuels is among the major causes of GHG emissions and the resultant tragic environmental consequences such as global warming. Biofuels such as biodiesel produced from algae could be regarded as a promising solution to turn this scenario around. However and in spite of these attractive features of algal fuels, current technologies are yet to be further improved to lead to economically justified production of these alternative fuels. Accordingly, it seems that the integration of algal fuels production with wastewater treatment and/or carbon biofixation could potentially serve as cost-effective and eco-friendly platform to achieve the above-mentioned goals.

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