Review

A Review: Microalgae and Their Applications in CO_2 Capture and Renewable Energy

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ABSTRACT

Fossil fuels, which are recognized as unsustainable sources of energy, are continuously consumed and decreased with increasing fuel demands. Microalgae have great potential as renewable fuel sources because they possess rapid growth rate and the ability to store high-quality lipids and carbohydrates inside their cells for biofuel production. Microalgae can be cultivated on opened or closed systems and require nutrients and CO_2 that may be supplied from wastewater and fossil fuel combustion. In addition, CO_2 capture via photosynthesis to directly fix carbon into microalgae has also attracted the attention of researchers. The conversion of CO_2 into chemical and fuel (energy) products without pollution via this approach is a promising way to not only reduce CO_2 emissions but also generate more economic value. The harvested microalgal biomass can be converted into biofuel products, such as biohydrogen, biodiesel, biomethanol, bioethanol, biobutanol and biohydrocarbons. Thus, microalgal cultivation can contribute to CO_2 fixation and can be a source of biofuels. This article reviews the literature on microalgae that were cultivated using captured CO_2, technologies related to the production of biofuels from microalgae and the possible commercialization of microalgae-based biofuels to demonstrate the potential of microalgae. In this respect, a number of relevant topics are addressed: the nature of microalgae (e.g., species and composition); CO_2 capture via microalgae; the techniques for microalgal cultivation, harvesting and pretreatment; and the techniques for lipid extraction and biofuel production. The strategies for biofuel commercialization are proposed as well.

Keywords: Microalgae; Pretreatment; Extraction; CO_2 capture; Biofuels.

INTRODUCTION

Currently, global energy exigency is increasing greatly with the increasing world population and improving quality of life. In general, fossil fuels have been the major source of energy. Nevertheless, decreasing fossil fuels with increasing fuel demand is unavoidable. To replace fossil fuels, various biomass feedstocks, including both terrestrial plants and aquatic algae, have been discovered to generate renewable fuels (Bahadar and Khan, 2013). Aquatic microalgae are ideal for producing liquid fuels because their rapid growth, high biomass yields, product variety and simple harvest from ponds or closed bioreactor systems allow them to be potentially consumed as sustainable environmentally friendly carbon-neutral fuel sources (Gao *et al.*, 2012; Sing *et al.*, 2013).

Microalgae are microscopic organisms that typically grow suspended in water and are driven by the same photosynthetic process as that of higher plants (Hanelt *et al.*, 2007).

Microalgae can comprise bacteria (cyanobacteria), diatoms (e.g., Chromalveolata), other protists (e.g., Chromista), and unicellular plants (e.g., Chlorophyta) (Bahadar and Khan, 2013). However, unlike higher plants, microalgae do not require a vascular system for nutrient transport, as every cell is photoautotrophic with directly absorbing nutrients. Microalgal cells are sunlight-driven cell factories that can convert carbon dioxide (CO_2) into raw materials for producing biofuels (e.g., biohydrogen, biodiesel, and bioethanol), animal food chemical feedstocks and high-value bioactive compounds (e.g., Docosahexaenoic acid (DHA)) (Spolaore *et al.*, 2006; Milledge, 2011; Razzak *et al.*, 2013). In particular, the ability of these cells to absorb CO_2 suggests microalgae cultivation as an attractive alternative for CO_2 sequestration that can be applied to fossil fuel power plant gas effluents to facilitate the reduction of greenhouse gas emissions (Yun *et al.*, 1997).

CO_2 fixation via microalgae is a potential and promising method for CO_2 capture and storage (Masakazu and Masahiro, 1997; Naoto and Masahiro, 1997; Razzak *et al.*, 2013; Zhao and Su, 2014). CO_2 fixation and storage via microalgae are essentially photosynthesis, which can transform water and CO_2 to organic compounds without extra energy addition or consumption and without secondary pollution. Compared to other carbon capture and storage

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(CCS) methods, CO_2 fixation via microalgae has many benefits, such as a high photosynthesis rate (e.g., 6.9×10^4 cells/mL/h (Suali and Sarbatly, 2012)), a rapid growth rate (0.7–3.2 day^{-1} (Maeda *et al.*, 1995; Ryu *et al.*, 2009)), good environmental adaptability and low cost of operation. As a special advantage, biomass from microalgae for energy consumption is provided after CO_2 capture. The performance of CO_2 fixation via microalgae and biomass production depends on the cultivation conditions (e.g., temperature, light, pH, and nutrient availability), species of microalgae, CO_2 concentration and toxic pollutants in the flue gas (Zhao and Su, 2014).

The high CO_2 and nutrient requirements of microalgae can be supplied by flue gases and waste water from other industrial processes, providing ecological advantages while lowering the cost of biomass production. Microalgae can be used as bioremediation agents to remove inorganic nutrients from wastewaters (cost of wastewater treatment about 0.15–6.0 USD/m^3 (Fu *et al.*, 2008; Yuan *et al.*, 2010)) and to improve water quality due to their high capacity for nutrient uptake (Razzak *et al.*, 2013). Microalgae that grow in the bioremediation of waste are further processed into a wide spectrum of fuel products, including (1) hydrogen (H_2) via direct and indirect biophotolysis, (2) biodiesel through transesterification, (3) biomethane via anaerobic digestion, (4) bioethanol and biobutanol via fermentation, (5) bio-oil via thermochemical conversion and (6) green diesel and gasoline through direct catalytic hydrothermal liquefaction (Demirbas, 2009a, b; Damartzis and Zabaniotou, 2011; Nigam and Singh, 2011; Huang and Tan, 2014). An integration of microalgal cultivation with CO_2 utilization and microalgal application as biofuel is presented in Fig. 1. Microalgae grow by consuming nutrients that are dissolved in water or wastewater and CO_2 from ambient air or the exhaust gases from combustion process under solar energy. Here, the microalgae biomass is obtained, and O_2 is released into the atmosphere. Finally, the biomass is converted into energy and food supplementation for humans and animals through a process including extraction (e.g., organic solvent extraction, supercritical fluid (SCF) extraction, etc.), fermentation, transesterification, etc. Therefore, the cultivation of microalgae provides three important benefits, including (1) CO_2 capture from a fossil fuel-based power plant, (2) wastewater treatment and (3) renewable energy sources (Razzak *et al.*, 2013).

Recently, studies are being developed to establish processes and technologies for application of microalgae at the industrial scale. However, the transition from pilot-scale to industrial-scale operations often exposes microalgae cells to hostile circumstances, resulting in reduction of product yields. The recovery of microalgae from highly dilute suspensions requires steps to lyse cells and reduce the extract yield. Therefore, the integration of the best microalgae cells and bioprocessing engineering methods to ensure economic and environmental feasibility and to minimize the number of full-scale tails is still challenging. Conditions for technically and economically viable biofuel resources should be competitive or cost less than petroleum fuels, have low to no additional land use, enable air quality improvement (e.g., CO_2 sequestration), and have minimal water use (Bahadar and Khan, 2013). Production costs associated with microalgae-based biofuels are the major barrier in the commercialization of biofuel, but fuels from microalgae are still promising in the view of competition with petroleum fuels.

This article reviews the literature on microalgae that were cultivated using captured CO_2, technologies related to the production of biofuels from microalgae and the idea of the commercialization of microalgae-based biofuels to demonstrate the potential of microalgae. In this respect, a number of relevant topics are addressed: the nature of microalgae (e.g., species and composition); CO_2 capture via microalgae; the techniques for microalgae cultivation, harvesting and pretreatment; and the techniques for lipid extraction and biofuel production. The strategies for biofuel commercialization are proposed as well.
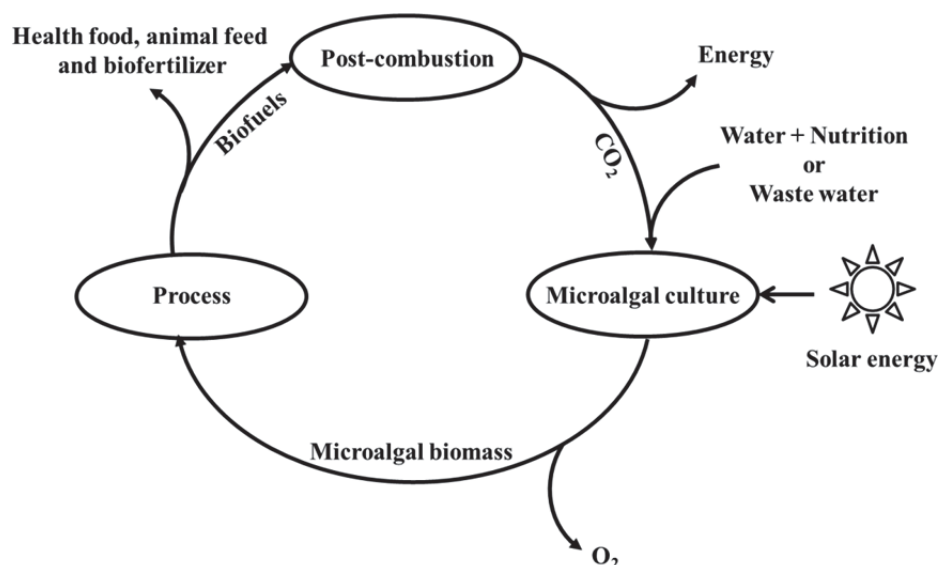

Fig. 1. An integration of microalgae cultivation with CO_2 utilization and their applications as biofuels.

MICROALGAE SPECIES AND THEIR COMPOSITIONS

Microalgae are made up of eukaryotic cells. Microalgae cells consist of cell wall, plasmatic membrane, cytoplasm, nucleus and organelles, such as mitochrondria, lysosomes and golgi (Taher *et al.*, 2011). Microalgae also have plastids, the bodies with chlorophyll that carry out photosynthesis. However, various strains of microalgae have different combinations of chlorophyll molecules - some have only Chlorophyll A, some A and B, while other strain, A and C (Um and Kim, 2009). The biomass of microalgae contains three main components: proteins, carbohydrates and lipids. The biomass composition of various algae is shown in Table 1. To achieve the maximum benefits from microalgae cultivation, it is essential to pay attention to the selection of suitable species. Microalgae cultivation is composed of a single specific strain that is precisely selected for producing the desired product and the most beneficial outcome of the cultivation process. The cultivation conditions, including (1) water media with adequate pH and temperature, (2) necessary contained nutrients and (3) CO_2 dosed in a controlled manner in the presence of sunlight, are also required for microalgae cultivation. The nitrogen source (e.g., ammonia and nitrates), other minerals and vitamins are the nutrients that must be provided sufficiently to ensure the proper growth of microalgae.

CULTIVATION TECHNIQUES

A wide range of microalgae cultivation techniques has been reported in the literature (Wang *et al.*, 2008; Suali and Sarbatly, 2012; Bahadar and Khan, 2013; Zhao and Su, 2014). There are many types of microalgae cultivation techniques depending on (1) the investment cost, (2) the desired products, (3) the source of nutrients and (4) CO_2 capture. The cultivation systems are categorized into open and closed systems. The open systems are outdoor facilities consisting of ponds, lagoons, deep channels, shallow circulating units and others. In contrast, the closed systems are vessels or tubes with walls that are made of transparent materials and that are located in outdoors under sunlight irradiation or artificial irradiation (Razzak *et al.*, 2013).

Open System

Until now, open ponds have been used for large-scale microalgae cultivation considering their simple construction and easy operation. The cultivation systems can be classified as (1) natural waters (e.g., lakes, lagoons, ponds, etc.) and (2) artificial water systems (e.g., artificial pond, tanks, and container). The shapes, sizes and types (agitated, inclined, etc.) of open systems depend on the applications. There are various types of ponds, including unstirred, raceway and circular ponds. Unstirred ponds (Fig. 2(a)) are the most economical due to their simple management and construction. Commercial unstirred ponds are built in natural water ponds of less than half a meter in depth. Unstirred ponds are commercially used for some microalgae species, such as *Dunaliella salina* (Borowitzka and Borowitzka, 1990). However, this type of pond is very limited in its applications, given that microalgae are not able to grow under frequently poor growth conditions and competitive growth with contaminating protozoa, bacteria and viruses (Chaumont, 1993).

Raceway ponds (or stirred paddle wheel open ponds) (Fig. 2(b)) are the most famous open system in current use. Raceway ponds are usually shallow, between 15 and 25 cm in depth. These ponds are normally constructed as either a single channel or groups of channels that are built by connecting individual raceway ponds. The productivity of the biomass

Table 1. Compositions of microalgae based on dry matter (Um and Kim, 2009; Sydney *et al.* 2010; Singh *et al.*, 2012).

| Microalgae specie	Protein (%)	Carbohydrate (%)	Lipid (%)
Anabaena cylindrical	43–56	25–30	4–7
Aphanizomenon flos-aquae	62	23	3
Arthrospira maxima	60–71	13–16	6–7
Botryococus braunii	8–17	8–20	21
Chlamydomonas rheinhardii	48	17	21
Chlorella pyrenoidosa	57	26	2
Chlorella vulgaris	51–58	12–17	14–22
Dunaliella bioculata	49	4	8
Dunaliella salina	57	32	6
Euglena gracilis	39–61	14–18	14–20
Isochrysis sp.	31–51	11–14	20–22
Neochloris oleoabundans	20–60	20–60	35–54
Porphyridium cruentum	28–39	40–57	9–14
Prymnesium parvum	28–45	25–33	22–38
Scenedesmus obliquus	50–56	10–17	12–14
Spirogyra sp.	6–20	33–64	11–21
Spirulina maxima	60–71	13–16	6–7
Spirulina platensis	46–63	8–14	4–9
Synechococcus sp.	63	15	11
Tetraselmis maculata	52	15	3
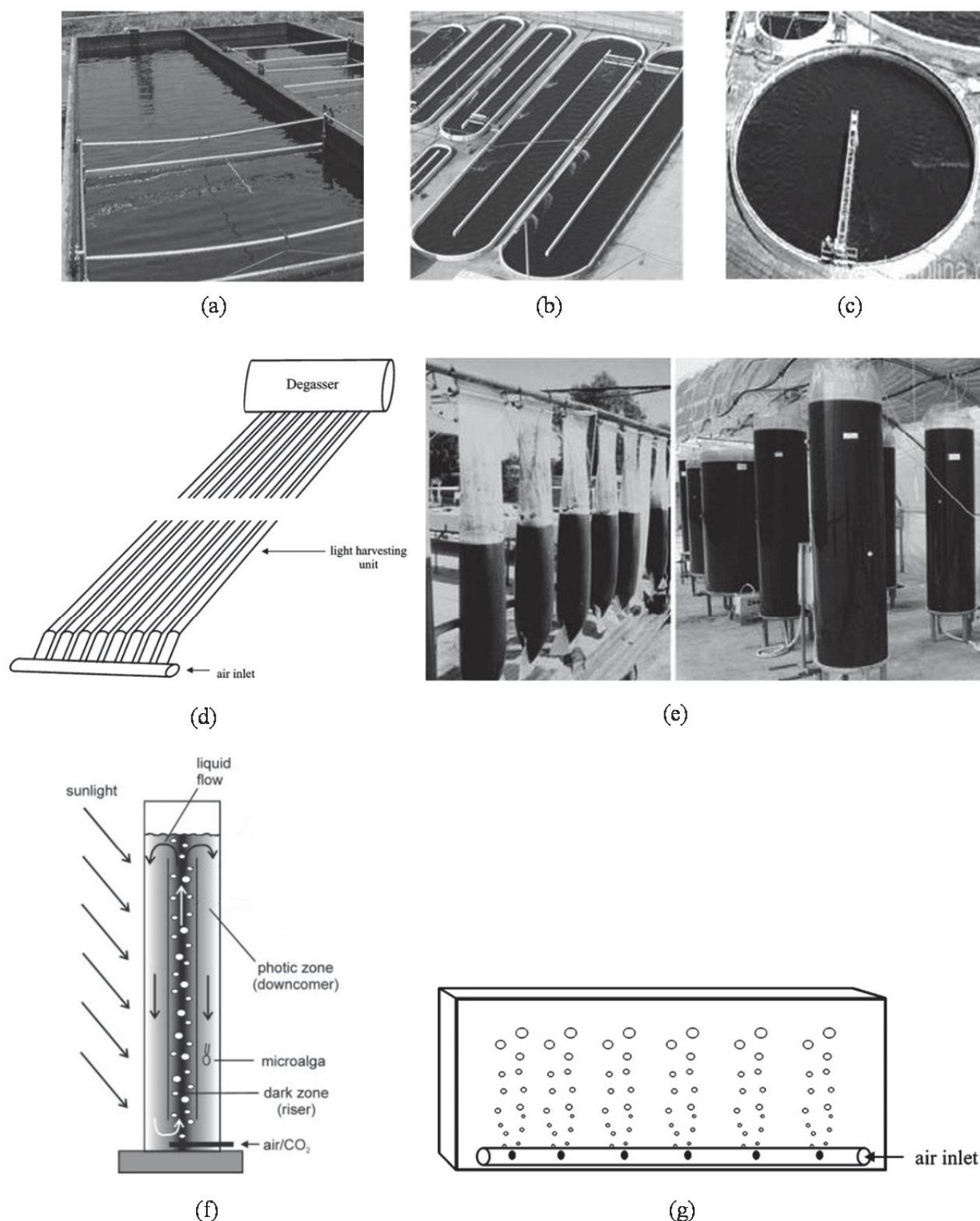
Fig. 2. Cultivation systems: (a) unstirred pond, (b) raceway pond, (c) circular pond (Chen *et al.*, 2009), (d) tubular photobioreactor (Carvalho *et al.*, 2006), (e) plastic bag photobioreactor (Richmond, 2008), (f) air-lift loop reactor (Barbosa *et al.*, 2003) and (g) flat plate photobioreactor (Carvalho *et al.*, 2006).

in the raceway pond is 60–100 mg dry weight/L/day (Razzak *et al.*, 2013). Raceway ponds are mostly used for the commercial culturing of four species of microalgae:

Chlorella sp., *Spirulina platensis*, *Haematococcus* sp. and *Dunaliella salina* (Moheimani and Borowitzka, 2006). The circulation of the cultivation media in the raceway pond is

induced by paddle. The optimal circulation velocity is required for water flow without the deposition of sedimentation or the aggregation of cells (Brindley *et al.*, 2002). However, problems with solid deposition in stagnant areas are difficult to overcome.

Circular ponds (central pivot) (Fig. 2(c)) are the oldest large-scale algae-cultivation open ponds. The depth of these ponds is approximately 25–30 cm. Microalgae are usually grown in concrete circular ponds of up to 45 m in diameter with agitation by rotating paddles. A 20-to-30-cm-thick layer of inorganic nutrient solution with algae is exposed to sunlight and CO_2 bubble under the continuous movement of paddle wheels (Lee, 2001).

Although there are many advantages of the open systems, as described above, their limitations are as follows: (1) poor light consumption by cells, (2) evaporative losses, (3) diffusion limitation of CO_2 from the atmosphere, (4) large land area requirement and (5) easy contamination by unwanted algae, mold, and bacteria (Razzak *et al.*, 2013). These limitations can be overcome using translucent plastic covers or greenhouses over the open ponds. However, this proposal cannot solve the contamination issues; in addition, the high capital cost, maintenance and overheating make open ponds that are covered by translucent plastics impractical due to the large land area of the pond.

Closed System

Microalgae can be grown in a closed system under controlled conditions, such as light utilization, area required and percentage of carbon dioxide. The closed system can address some of the problems that are associated with open systems. Photobioreactors are a type of closed pond system that are used for microalgae cultivation, as they can reduce contamination risk from unwanted algae, mold, and bacteria; control temperature; minimize water evaporation; and remove carbon dioxide losses. However, it should be noted that although photobioreactors significantly reduce the growth of competitive algal weeds, they cannot completely eliminate the growth of contaminants. Two disadvantages of this system are that it is difficult to construct and operate and is costly. The various designs of photobioreactors include flat plate, tubular, etc. (Borowitzka, 2007).

Tubular photobioreactors (Fig. 2(d)) are made with transparent materials and are placed in outdoor facilities under sunlight irradiation. Gas exchange vessels to supply CO_2, air and nutrients and to remove O_2 are connected to the main reactor (Richmond, 2004; Chisti, 2007). For the design of these cultivation vessels, a large surface area per unit volume is required to maximize the exposure of microalgae to sunlight. The tube sizes are generally less than 10 cm in diameter to maintain sunlight permeability. In a typical tubular microalgae cultivation system, the medium is circulated through the tubes, where it is exposed to sunlight for photosynthesis. The medium is circulated back to a reservoir using a mechanical or an airlift pump. The pump also helps to maintain a highly turbulent flow within the reactor to prevent the flocculation of microalgal biomass (Chisti, 2007). A fraction of microalgae is usually harvested after it circulates through the solar collector tube, permitting

the continuous operation of the system. However, tubular photobioreactors are still studied on the laboratory scale but are not practical. In some photobioreactors, the tubes are a coiled spiral, forming helical-tubular photobioreactors. These types of reactors are suitable for the cultivation of microalgal species in the presence of sunlight. An artificial light sometime replaces natural light to enhance microalgae growth. However, the use of artificial light adds to the investment costs, leading to the helical-tubular photobioreactors only being adequate for the manufacture of high-value added products (Morita *et al.*, 2001; Briassoulisa *et al.*, 2010).

Microalgae can be cultivated in transparent polyethylene bags, called plastic bag photobioreactors (Fig. 2(e)). These bags are hung or placed in a cage under sunlight irradiation, while the microalgae are mixed with air in the bottom of the bags (Razzak *et al.*, 2013; Xia *et al.,* 2013). Transparent polyethylene sleeves that are sealed at the bottom in a conical shape are used to prevent cell settling.

Airlift photobioreactors (Fig. 2(f)) are simple and cost-effective reactors for the mass cultivation of various types of microalgae. Acrylic glass is used as a material to construct airlift photobioreactors because it is inexpensive and available. There are two zones in airlift photobioreactors, including dark (called rinser) and irradiated zones. The airlift photobioreactors are believed to meet the desired criteria for the new generation photobioreactors of high light penetration and biomass production, ease of maintenance, and minimal contamination (Barbosa *et al.*, 2003). However, airlift photobioreactors are sometimes difficult to scale-up given their complex flow pattern (Mirón *et al.*, 2000). Vertical bubble columns and airlift cylinders can attain a substantially increased radial movement of fluid, with a high cycling of medium between the irradiated and dark zones. The advantages of these units include (1) high mass transfer, (2) good mixing with low shear stress, (3) low energy consumption, (4) relatively easy operation under sterile conditions, (5) good for the immobilization of algae on moving particles and (6) less photo inhibition and oxidation. However, the limitations of these units include (1) high manufacturing and maintenance costs, (2) smaller irradiation per unit surface area, (3) more sophisticated construction materials, (4) higher shear stress on microalgal cultivations, and (5) larger number of units needed to construct a commercial plant (Razzak *et al.*, 2013).

Flat plate photobioreactors (Fig. 2(g)) are very effective for the biomass cultivation of microalgae. These photobioreactors provide a high surface area to volume ratio for illumination and have a convenient modular design for scale-up (Barbosa *et al.*, 2005). The biomass productivity of microalgae rapidly increases with the mixing rate, which can provide a proper amount of CO_2 to the cultivation while removing excess oxygen and increasing the flashing effect. Flat plate photobioreactors are suitable for outdoor and indoor cultivations, are good for algae immobilization, and are relatively cheap and easy to clean (Ugwu *et al.*, 2008). Vertical flat plates can be accommodated in 1000–2000 L volume capacity units that are successfully operated for a long time period. Therefore, these are fully scalable

Table 2. Cultivation of microalgae species in closed photobioreactor systems.

Type of photobioreactor	Strain	Capacity (L)	Biomass concentration	Advantage	Disadvantage	Reference
Tubular	*Phaeodactylum tricornutum*	200	1.19 g/L	1. Very effective light use, 2. Excellent temperature control, 3. Reasonable scale up.	Fouling with some growth along walls.	Acien Fernández *et al.*, 2001.
	Phaeodactylum tricornutum	75	1.38 g/L			Hall *et al.*, 2003.
	Porphyridium cruentum	6000	35 g/m²/d			Sakai *et al.*, 1995.
	Spirulina sp.	10000	25 g/m³/d			Torzillo *et al.*, 1986.
	Spirulina platesnsis	5.5	0.62 g/L			Huntley and Redalje, 2007.
	Spirulina platensis	65	32.5 g/m²/d			Negoro *et al.*, 1993.
	Spirulina sp.	100	0.01 g/L/g			Chiu *et al.*, 2008.
Airlift	*Botryococcus branuii*	3	2.31 g/m³/d	1. Good light use, 2. High temperature control, 3. High mass transfer coefficient	1. Low hydrodynamic stress on algae, 2. Difficult to scale up.	Avila *et al.*, 2001.
	Chaetoceros sp.	170	0.80 g/L			Krichnavaruk *et al.*, 2007.
	Chlorella sp.	n/a	109–264			James and Al-Khars, 1990.
	Chlorella vulgaris	2	0.28–0.89 g/L/d			Hanagata *et al.*, 1992.
	Haematococcus pluvialis	3	4.09 g/L			Kaewpintong *et al.*, 2007.
	Nannochloropsis sp.	n/a	32.5–95.3 g/m³/d			James and Al-Khars, 1990.
Bubble column	*Aphanothece microscopica*	3	0.77 g/L/d	1. Scalable; homogeneous culture environment, 2. Low cooling requirement, 3. Effective light use.	Increased shear stress by pumps limiting biomass productivity.	Jacob-Lopes *et al.*, 2009.
	Chaetoceceros sp.	170	3.31 g/L			Krichnavaruk *et al.*, 2007.
	Chlorella vulgaris	1.8	1.41 g/L			de Morais and Costa, 2007b.
	Cyanobium sp.	1.8	0.071 g/L			Henrard *et al.*, 2011.
	Phaeodactylum sp.	1.9	n/a			Brindley *et al.*, 2002.
	Isochrysis aff. Galbana	5	n/a			Mandalm and Palsson, 1998.
	Monodus sp.	64	0.03–0.20 g/L			Bosma *et al.*, 2007.
	Monoraphidium sp.	4.5	23 g/m³/d			Miyamoto *et al.*, 1988.
	Scenedesmus obliquus	1.8	2.12 g/L			de Morais and Costa, 2007b.
	Spirulina sp.	3.5	4.13 g/L			de Morais and Costa, 2007b.
Flat plate	*Chlorella vulgaris*	1.5–3.0	0.027–0.045 g/L/h	1. Excellent light use and temperature control, 2. Low cooling requirement, 3. High gas transfer coefficient.	Difficult to scale up.	Satoh *et al.*, 2001.
	Dunaliella sp.	3.4	1.5 g/L			Barbosa *et al.*, 2005.
	Dunaliella tertiolecta	30	3.42 g/d			Chang and Yang, 2003.
	Nannochloropsis sp.	200	0.225 g/L			Richmond and Wu, 2001.
	Nannochloropsis sp.	440	0.27 g/L			Richmond and Wu, 2001.
	Phaeodactylum sp.	5	1.38 g/L			Meiser *et al.*, 2004.
Plastic bag	*Tetraselmis* sp.	50	20–30 g/m³/d	-	-	Trotta, 1981.

photobioreactor units (Richmond, 2004). The ideal strain, advantages and disadvantages of each photobioreactor that has been used to cultivate microalgae are summarized in Table 2.

CO₂ CAPTURE VIA MICROALGAE

Biotechnological techniques for CO_2 fixation are conducted to reduce CO_2 emission. These processes are based on the use of reactors to generate photosynthetic reactions in which microalgae are used as biocatalysts in a series of biochemical reactions that are responsible for the conversion of CO_2 into photosynthetic metabolic products (Jacob-Lopes *et al.*, 2010). Microalgal biomass contains approximately 50% carbon by dry weight (Mirón *et al.*, 2003). This carbon is typically obtained from carbon dioxide; roughly, 100 tons of microalgal biomass fixes 183 tons of CO_2 (Huang and Tan, 2014). Microalgae are capable of using three different inorganic carbon assimilation pathways: (1) direct carbon dioxide assimilation via the plasmatic membrane; (2) the use of bicarbonate by inducing the enzyme carbonic anhydrase, which converts HCO_3^- to CO_2; and (3) the direct transport of bicarbonate via the plasmatic membrane. However, there are a few differences in inorganic carbon assimilation pathways for different microalgae as demonstrated in Table 3.

CO_2 must be fed continuously during daylight hours. The control of CO_2 feeding can be evaluated by pH measurements to minimize the loss of CO_2. As a result, CO_2 fixation using microalgae can reduce the CO_2 emission from power plants, which has the positive environmental impact (Inoue *et al.*, 1995; Yun *et al.*, 1997; Abu-Khader, 2006; Brennan and Owende, 2010).

Photosynthesis

A promising pathway to capture CO_2 in the form of microalgal biomass is provided by biological processes via photosynthesis. In microalgae, photosynthesis releases oxygen and can be called "oxygenic photosynthesis". CO_2 is converted into lipids and other hydrocarbons in this process, explaining the designation of "CO_2 fixation process". In oxygenic photosynthesis, water is the electron donor, and oxygen is released after hydrolysis. The equation for photosynthesis can be written as follows:

$$H_2O + CO_2 + Photons\ (light) \rightarrow [CH_2O]_n + O_2 \qquad (1)$$
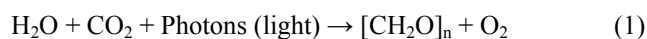
In this reaction, the standard free energy for the synthesis of glucose is 2,870 kJ/mol (Zhao and Su, 2014). The overall reaction can be divided into two pathways: (1) light-dependent reaction and (2) dark or light-independent reaction.

Table 3. Pathway for inorganic carbon assimilation for microalgae (+, present; -, absent; n/a, not available).

Species	CO₂ (pathway (1))	carbonic anhydrase (pathway (2))	HCO₃⁻ (pathway (3))	Reference
Chlamydomonas reinhardtii	+	+	+	Sültemeyer *et al.*, 1989.
Dunaliella terteolecta	+	+	+	Amoroso *et al.*, 1998.
Scenedesmus obliquus	+	+	+	Palmqvist *et al.*, 1994.
Chlorella saccharophila	+	+	+	Rotatore and Colman, 1991.
Chlorella ellipsoidea	+	-	+	Rotatore and Colman, 1991.
Chlorella kesslerii	+	-	+	Bozzo *et al.*, 2000.
Navicula pelliculosa	+	-	+	Rotatore and Colman, 1992.
Phaeodactylum tricornutum	+	+	+	Colman and Rotatore, 1995.
Cyclotella sp.	+	+	+	Rotatore *et al.*, 1995.
Ditylum brightwellii	+	-	+	Korb *et al.*, 1997.
Skeletonema costatum	+	-	+	Korb *et al.*, 1997.
Chaetoceros calcitrans	+	-	+	Korb *et al.*, 1997.
Thalassiosira punctigera	+	-	n/a	Elzenga *et al.*, 2000.
Thalassiosira pseudonanna	n/a	-	+	Elzenga *et al.*, 2000.
Porphyridium cruentum	+	+	+	Colman and Gehl, 1983.
Emiliania huxleyi	+	+	n/a	Elzenga *et al.*, 2000.
Dicrateria inornata	+	+	+	Colman *et al.*, 2002.
Isochrysis galbana	+	+	+	Colman *et al.*, 2002.
Phaeocystis globosa	+	+	n/a	Elzenga *et al.*, 2000.
Vischeria stellata	+	-	+	Huertas *et al.*, 2002.
Eremosphaera viridis	+	-	-	Rotatore *et al.*, 1992.
Nannochloris atomus	+	-	-	Huertas *et al.*, 2000a.
Nannochloris maculata	+	-	-	Huertas *et al.*, 2000a.
Amphidinium carterae	+	-	-	Colman *et al.*, 2002.
Heterocapsa oceanica	+	-	-	Colman *et al.*, 2002.
Nannochloropsis gaditana	-	-	+	Huertas *et al.*, 2000b.
Nannochloropsis oculata	-	-	+	Huertas *et al.*, 2000b.
Monodus subterraneus	+	-	-	Huertas *et al.*, 2002.

The light-dependent reaction involves both photochemical and redox reaction steps. The overall equation for light reaction is as follows (Mukherjee and Moroney, 2011; Razzak *et al.*, 2013):

$$2H_2O + 2NADP^+ + 3ADP + 3P + light \rightarrow 2NADPH + 2H^+ + 3ATP + O_2 \quad (2)$$
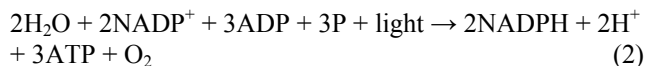
where ADP, P and NADP are adenosine diphosphate, phosphate and nicotinamide adenine dinucleotide phosphate. Light energy is used to synthesize ATP (adenosine triphosphate) and NADPH (nicotinamide adenine dinucleotide phosphate) which are energy storage molecules.

In the light-independent reaction or dark reaction, the enzyme ribulose bisphosphate carboxylase/oxygenase (RuBisCO) captures CO_2 from the atmosphere. This process requires the newly formed NADPH, called the Calvin-Benson Cycle (Peter *et al.*, 2010). To be more specific, carbon fixation produces an intermediate product that is then converted into the final carbohydrate products. The carbon skeletons that are produced by photosynthesis are then used in a variety of ensuing processes, forming other organic compounds. For example, cellulose is the precursor for lipid and amino acid biosynthesis or is a fuel for respiration. The overall equation for the light-independent reaction is as follows (Razzak *et al.*, 2013):

$$3CO_2 + 9ATP + 6NADPH + 6H^+ \rightarrow C_3H_6O_3\text{-phosphate} + 9ADP + 8P + 6NADP^+ + 3H_2O \quad (3)$$
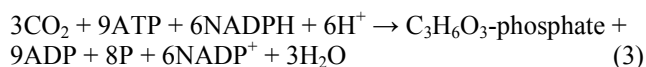
The fixation or reduction of CO_2 takes place by combining CO_2 with a five-carbon sugar, ribulose 1,5-bisphosphate (Ru5BP), generating two molecules of a three-carbon compound, glycerate 3-phosphate (GP), also called 3-phosphoglycerate (PGA). In the presence of ATP and NADPH (from light-dependent stages), GP is reduced to glyceraldehyde 3-phosphate (G3P). This product is also referred to as 3-phosphoglyceraldehyde (PGAL) or even as triose phosphate. Most of the G3P that is produced is used to regenerate Ru5BP so that the process can continue. Of the six molecules of triose phosphates, one is not "recycled" and often condenses to form hexose phosphate, yielding sucrose, starch and cellulose. The sugars that are produced during carbon metabolism yield carbon skeletons that can be used for other metabolic reactions, such as the production of amino acids and lipids (Razzak *et al.*, 2013).

CO2 Source

Microalgae use CO_2 as a carbon source. These microorganisms cannot grow without a CO_2 supply. An insufficient CO_2 supply is often the limiting factor of productivity. Thus, reducing the atmospheric CO_2 by microalgal photosynthesis is considered safe and advantageous for the human ecosystem (Mukherjee and Moroney, 2011).

The growth performance of some microalgae, e.g., *Chlorella* sp., can be negatively affected by CO_2 at a higher than 5% (v/v) concentration (Silva and Pirt, 1984; Lee and Tay, 1991; Cheng *et al.*, 2006). However, some microalgae

can grow under a flue gas CO_2 concentration level (10–15%), but the carbon fixation and biomass production rates are less than that under lower CO_2 concentration. Very few microalgal species are able to tolerate extremely high CO_2 levels of up to 70% (e.g., *Chlorella* sp. KR-1 and *Chlorella* sp. ZY-1) and 100% (e.g., *Chlorella* sp. T-1). The optimal CO_2 concentration for most microalgal species is usually recommended to be 0.038–10%, for example, the maximum biomass production was observed at 2.5% CO_2 for microalgae *Chlorella* sp. (Chiu *et al.*, 2008) and at 6% for *Scenedesmus obliquus* and *Chlorella kessleri* (de Morais and Costa, 2007b).

Toxic Pollutants in Combustion Flue Gas
SO2

The presence of SO_2 strongly inhibits microalgae growth. When the SO_2 concentration exceeds 100 ppm, it is almost impossible to grow most microalgae (Hauck *et al.*, 1996). Some microalgal species are able to grow with difficultly under conditions of high SO_2 concentration; however, they have a longer lag phase than in the absence of SO_2. When the SO_2 concentration increases, the inhibition effect is enhanced, resulting in a sharp reduction in carbon fixation and biomass production, for instance, even *chlorella* sp. KR-1, which is considered a high-performance microalgal species, could not survive under the conditions of 150 ppm SO_2 with 15% CO_2 (Lee *et al.*, 2000). SO_2 may not directly inhibit the microalgal growth (Matsumoto *et al.*, 1997). The inhibition effects of SO_2 on microalgae growth may be attributed to the increased acidity. The H^+ release that is generated by the hydrolysis of SO_2 results in an increase in the acidity of the cultivation medium (Du *et al.*, 2010). When the pH is less than 3.0, the microalgae cells are killed (Maeda *et al.*, 1995). However, if the pH of the cultivation medium is artificially maintained constant by the neutralization method, the microalgal growth characteristic is approximately the same as that without SO_2 (Zhao and Su, 2014). These results seem to indirectly demonstrate that the effect of SO_2 on microalgae is transmitted through the pH value in the batch strategy; however, this situation is not seen in all occasions. Other investigations have indicated that the effect of SO_2 on microalgae is related not only to the pH value but also to SO_4^{2-} and HSO_4^- obtained from the hydrolysis of SO_2. SO_4^{2-} and HSO_4^- are also inhibition factors of microalgal growth (Chiu *et al.*, 2011).

NOx

In flue gas, the NO_x emission level varies from several hundred to several thousand ppm with more than 90–95% NO and 5–10% NO_2. After the flue gas de-NO_x process, NO is still at the level of 50–200 ppm. It is difficult for NO to directly impact the growth of microalgae via pH in the cultivation medium. The NO concentration usually has a two-sided influence on the growth of microalgae. An extremely low concentration of NO may even be absorbed by the cultivation medium and transformed into NO_2^- as the source of nitrogen nutrition for microalgae when using inorganic forms (Zhao and Su, 2014). However, this positive influence is quite limited: the increased NO concentration results in at least a decreased growth rate of microalgae for

most microalgal species; NO at higher than 300 ppm may decrease microalgae growth (Chaumont, 1993)

Typically, SO_x and NO_x in flue gas can be treated separately by flue gas desulfurization (FGD) and selective catalytic reduction (SCR) processes, respectively, or simultaneously by the combined treatment systems before the treated gas stream enters a microalgae reactor. The FGD commonly uses $CaCO_3$ to absorb SO_2 to form $CaSO_4$. SCR uses ammonia or urea and a catalyst such as titanium oxide, silico-alumino-phosphate, zeolite, Al_2O_3, etc. to decompose NO_x into N_2 and H_2O (Jin *et al.*, 2005; Hende *et al.*, 2012). The examples of combined treatment systems are the DeSoNox or SNOX processes, where a catalytic reduction of NO_x is combined with a catalytic oxidation of SO_2 (Trozzi *et al.*, 2010).

CO₂ Fixation

Microalgae can eventually produce some CO_2 overnight, as occurs with other plants; however, the net CO_2 uptake is still positive. The CO_2 fixation rate is directly related to the light utilization efficiency and the cell density of microalgae. Microalgal CO_2 fixation involves photoautotrophic growth in which anthropogenically derived CO_2 may be used as a carbon source. Therefore, biomass measurements and growth rate evaluations are critical in assessing the potential of a microalgal cultivation system for direct CO_2 removal (Costa *et al.*, 2004; Cheng *et al.*, 2006). The CO_2 removal efficiency in a photobioreactor with microalgal cultivation can be determined as the difference in the CO_2 concentration of the incoming and outgoing effluents. The removal efficiency (%) can be determined using the following formula (Chiu *et al.*, 2009):

$$Efficiency\,(\%) = \frac{Influent\ of\ CO_2 - Effluent\ of\ CO_2}{Influent\ of\ CO_2} \times 100$$

(4)

The efficiency of CO_2 removal or fixation in a closed cultivation system depends on the (1) microalgal species, (2) CO_2 concentration, (3) photobioreactor design and (4) operating conditions (de Morais and Costa, 2007a; Chiu *et al.*, 2009). *Chlorella vulgaris* possesses a maximum CO_2 removal efficiency of 55.3% at 0.15% CO_2 in a membrane photobioreactor, and *Spirulina* sp. and *Scenedesmus obliquus* possess a maximum CO_2 removal efficiency of 27–38% and 7–13%, respectively, in a three serial tubular photobioreactor (Cheng *et al.*, 2006), while their CO_2 fixation efficiencies were reduced to 7–17% and 4–9% under 12% CO_2 aeration (de Morais and Costa, 2007). In the other words, the CO_2 removal efficiency and fixation depend on the species of microalgae due to the physiological conditions of microalgae, such as the potential for cell growth and CO_2 metabolism.

The CO_2 fixation rate could be determined from the carbon content of the microalgal cell (Yun *et al.*, 1997). The fixation rate was calculated as follows:

$$R_{CO_2} = C_C \times \mu_L \times \left(\frac{M_{CO_2}}{M_C}\right)$$

(5)

where R_{CO_2} and μ_L are the fixation rate (g CO_2/m³ h) and the volumetric growth rate (g dry weight/m³ h), respectively, while M_{CO_2} and M_C are the molecular weights of CO_2 and elemental C, respectively. C_C is the average carbon content as measured by elemental analysis. The microalgal growth rate is determined in the linear growth regime.

Due to climatic, land and water restrictions, there are challenges to collecting and utilizing microalgae directly on site. However, the economics of microalgae utilization can be increased using a two-stage process. In such a process, the CO_2 from a power plant or other source is first scrubbed (e.g., amine scrubber) and concentrated with a conventional process (Rochelle, 2009; Yu *et al.*, 2012). The concentrated CO_2 is then transported to a suitable site for microalgae production. This process can be compared to the economics of other conventional CO_2 processes where CO_2 capture involves a separation process followed by transportation and finally disposal in deep oceans and/or depletion in gas wells (Razzak *et al.*, 2013). One should consider that some microalgae species are tolerant to relatively high temperatures (close to and greater than 30°C). These types of microalgae can be cultivated in conjunction with the use of high-temperature flue gases from industrial neighbor sites (Wang *et al.*, 2008). These thermo-tolerant strains may also simplify species control, as the optimum growth temperature of most microalgal species is in the range of 20–30°C. Several unicellular microalgae strains, for example, *Chlorella* sp., grow at temperatures of up to 42°C, and their tolerance to both high temperatures and a high CO_2 content makes them potentially appropriate microbial cells for photobioreactors that are involved in CO_2 capture for flue gases (Wang *et al.*, 2008). Table 4 presents the microalgae strains that have been studied for CO_2 fixation.

Improvement and Application of the CO₂ Fixation Process

Until now, CO_2 fixation by microalgae, biomass production and energy consumption have experienced great progress in both laboratory-scale scientific research and pilot-scale applications (Chisti, 2010; Chi *et al.*, 2011; Chisti and Yan, 2011). Process improvement as a middle measurement is necessary to more efficiently guarantee CO_2 fixation and biomass production. Whether in laboratory-scale research or pilot-scale application, the performance of CO_2 fixation and biomass production highly depend on the process conditions and parameters. For laboratory-scale research, the microalgae selection, cultivation and promotion to obtain high performance of microalgae species may be important factors. The evaluations of microalgae performance under drastic process and environmental factors, such as high CO_2 concentration, high temperature and toxic pollutants in flue gas, are also required. For pilot-scale application, microalgal cultivation is mainly affected by the cultivation temperature, light exposure and hydrodynamic conditions. Open cultivation is more influenced by outdoor temperature, light intensity (day and night) and season (adversely influences microalgal growth) compared to closed cultivation (Zhao and Su, 2014).

For closed systems, the improvements of the process parameters for CO_2 fixation and biomass production are

Table 4. Cultivation compositions (CO_2, pH, amount of N and P sources) and CO_2 fixation of microalgae strain.

Strain	CO_2 (%)	Temperature (°C)	pH	N (mg/L)	P (mg/L)	Biomass productivity (g/L d)	CO_2 fixation rate (g/L d)	Reference
Aphanothece microscopica	15	35	8.0	24.71	7.13	0.800	1.5	Jacob-Lopes *et al.*, 2009.
Botryococcus braunii	15	30	8.3	-	-	1.1	>1.0	Murakami and Ikenouchi, 1997.
Chlorococcum littorale	40	30	5.5	1250	1250	-	1.0	Iwasaki *et al.*, 1998.
Chlorella kessleri	18	30	6.4	34.65	17.10	0.087	0.163	de Morais and Costa 2007b.
Chlorella valgaris	15	27	7.0	125.4	-	0.360	0.624	Yun *et al.* 1997.
	Air	25	-	1250	1250	0.040	0.075	Scragg *et al.* 2002.
	Air	25	-	1250	1250	0.024	0.045	Scragg *et al.* 2002.
	10	22	6.0	2.25	13.36	0.278	0.522	Stephenson *et al.*, 2010.
Chlorella sp.	40	42	9.4	-	-	-	1.0	Sakai *et al.* 1995.
	5	26	-	46.33	4.76	0.207	0.389	Chiu *et al.*, 2008.
	20	18	8.2	12.35	1.29	0.271	0.510	Zhao and Su, 2014.
Chlorella sp. AG10002	5	20	7.2	17.65	0.29	0.335	1.446	Ryu *et al.*, 2009.
Chlorella sp. WT	25	25	-	37.06	3.88	0.200	0.376	Chiu *et al.*, 2011.
Chlorella sp. MTF-7	25	25	-	37.06	3.88	0.358	0.674	Chiu *et al.*, 2011.
Dunaliella tertiolecta	3	27	-	1000	535	0.170	0.313	Kishimoto *et al.* 1994.
Haematococcus pluvialis	16–34	20	-	-	-	0.076	0.143	Huntley and Redalje 2007.
Scenedesmus obliquus	Air	17	-	51.66	4.46	0.009	0.016	Gomez-Villa *et al.* 2005.
	Air	30	-	51.66	4.46	0.016	0.031	Gomez-Villa *et al.* 2005.
	12	30	7.0	173.27	284.93	0.140	0.26	de Morais and Costa 2007b.
Spirulina sp.	12	30	7.0	173.27	284.93	0.220	0.413	de Morais and Costa 2007b.

mainly physicochemical parameters, including light exposure, nutrition conditions and hydrodynamic parameters, such as mixing and mass transfer rates by increasing the gas-liquid contact area and retention time, even though the economy nd practical use are in contention for their scale-up (Mirón *et al.*, 1999). CO_2 fixation and biomass production can be improved using a high aeration rate to achieve turbulence mixing, a high CO_2 mass transfer rate and a high removal rate of excess oxygen in the cultivation medium (Sierra *et al.*, 2008). Nevertheless, high stress to microalgal cells and high operating costs are still important challenges (Zhao and Su, 2014).

As a result, CO_2 fixation through microalgae and biomass production in practical use are influenced by various factors, such as microalgal species and physicochemical and hydrodynamic conditions. To maximize the CO_2 fixation and biomass production performance, the synergistic effect and optimization process parameters are of concern and still need to be solved when using microalgae for CO_2 fixation and mass production on industrial scales.

HARVESTING OF THE MICROALGAE BIOMASS

Harvesting is an expensive and problematic segment of the industrial production of microalgae biomass due to the low cell density of microalgae, being typically in the range of 0.3–0.5 g/L, with exceptional cases reaching 5 g/L. However, the optimal requirement for the industrial scale is a cell sludge containing at least 300-400 g/L. Thus, the effluent microalgal suspension needs to be concentrated at least 100 times, which is an energy-intensive process (Wang *et al.*, 2008).

After microalgae cultivation has reached the stationary phase, it is separated from water and the biomass recovered for downstream processes. However, the microalgae-harvesting process possesses a challenging task because microalgae have a micron size (1–20 μm) and are suspended in liquid. Currently, there are several methods to harvest microalgae, including (1) bulk harvesting to separate microalgae from suspension, such as natural gravity sedimentation, flocculation and floatation, leading to a concentration factor of 100–800 times, and (2) thickening to concentrate the microalgae slurry after bulk harvesting, such as centrifugation and filtration, leading to a concentration factor of 30 times. Flocculation and flotation are widely used for the bulk harvesting. The flocculation mechanism is to neutralize or reduce the negative charge on the microalgal cellular surface to aggregate cells in suspension, which can be collected by adding flocculants such as multivalent cations and cationic polymers (Molina *et al.*, 2003). Flocculation can be performed by adjusting the pH of the cultivation to between 10 and 10.6 using NaOH to neutralize the negative charges on the cell surface, and the non-ionic polymer Magnafloc LT-25 is added. The ensuring flocculate is harvested and neutralized to generate a final concentration factor of between 200- and 800-fold. Here, significant breakthroughs in developing polymeric flocculants are urgently required through intensive research to further strengthen the potential use of flocculation in the harvesting process (Lam and Lee, 2012). Flotation depends

on trapping cells by dispersed micro-air bubbles without the addition of any chemical reagents. The flotation can capture particles with a diameter of less than 500 mm by collision between a bubble and a particle and the subsequent adhesion of the bubble and the particle. The flotation process where microalgae float to the surface of medium is prone to harvest in microalgae mass culture, and has been used for specific strain, such as *Spirulina platensis* (Zhang *et al.*, 2014). The obtained sludge is very clean. However, the floating method is still challenging at a large scale (Wang *et al.*, 2008). The Centrifugation is an efficient but energy-intensive method (Molina *et al.*, 2003). The efficiency of centrifugation depends on the settling characteristics of the cells, the residence time of the cell slurry, and the settling depth. The settling depth can be kept small by the design of the centrifuge, and the resident time of the slurry can be controlled by the flow rate. The centrifugal recovery of the biomass is feasible for high-value products because it can process large volumes relatively rapidly, and the biomass remains fully contained during recovery (Heasman *et al.*, 2000). Filtration, which operates under pressure or vacuum, is the preferred method for harvesting relatively large filamentous microalgae; however, the small cells of microalgae are not suitable, while membrane microfiltration and ultrafiltration are possible alternatives. However, current large-scale microalgal biomass production facilities do not generally apply membrane filtration because of its cost, to which membrane replacement and pumping are the major contributors (Hung and Liu, 2006). Moreover, microfiltration is more cost-effective than centrifugation (Molina *et al.*, 2003). As a strategy to save biomass-harvesting costs, easy-to-harvest microalgal strains for CO_2 fixation and biomass production should be selected (Wang *et al.*, 2008). The comparison of harvesting methods is demonstrated in Table 5.

BIOFUEL PRODUCTION BASED ON MICROALGAE

Pretreatment
Drying of Microalgae Biomass
The extensive drying of microalgae biomass is required for biofuel production, as the presence of water will inhibit several downstream processes, such as lipid extraction and transesterification. Nevertheless, the drying step is energy-intensive, which adds to the cost complexity of the overall production process. The microalgal slurry moisture content must be reduced to at least 10% by drying and dehydration. There are many types of drying technologies, such as drum drying, oven drying, freeze drying, and spray drying, that have been used with microalgae. The selection of the best drying method depends on the required operation scale and desired product value. Sun drying is an old and cheap drying method that can be performed easily by exposure to solar radiation. However, solar drying is not feasible, taking a long drying time and requiring a large drying area due to limited sunlight and uncertain sunlight time (Taher *et al.*, 2011; Lam and Lee, 2012). Moreover, sun drying does not have any sterilization effect, unlike oven or drum drying. In contrast, spray drying can be used for high-value

Table 5. Comparison of microalgal harvesting and drying methods (Taher *et al.*, 2011; Zhang *et al.*, 2014).

	Method	Advantage	Disadvantage
Harvesting	Flocculation	1) Wide range of flocculants available 2) Ease of use	1) Chemical contamination 2) Removal of flocculants 3) Highly sensitive to pH level
	Floatation	1) Prone to harvest in mass culture	1) Challenging at a large scale
	Sedimentation	1) Low power consumption 2) Low requirement for skilled operators 3) Useful as a pre-concentration step	1) Slow sedimentation rates 2) Low cell recovery
	Centrifugation	1) High harvesting efficiency 2) Rapid separation process 3) Easy to operate	1) High capital and operational costs 2) Cell damage 3) Difficult bulk harvest
	Filtration	1) Water and nutrient reuse 2) Wide variety of filter and membrane types available	1) Fouling 2) Slow process 3) Suitable for large microalgal cell
Drying	Sun drying	1) Cheap (no running cost, low capital cost)	1) Difficult 2) Slow 3) Weather dependent 4) Large area requirement 5) Easy contamination
	Spray drying	1) Fast 2) Continuous 3) Efficient	1) Cost intensive 2) Species deterioration (i.e., pigments)
	Drum drying	1) Fast 2) Efficient 3) High temperature sterilization	1) Cost intensive 2) Species deterioration
	Oven drying	1) Fast 2) Efficient 3) High temperature sterilization 4) Batch or continuous	1) Cost intensive 2) Species deterioration
	Freeze drying	1) Gentle 2) Low species deterioration	1) Slow process 2) Cost intensive

products but has the disadvantages of being expensive and possibly significantly deteriorating the algae. Among the drying technologies that can be applied to microalgal concentrate, freeze drying is favored for its mild operating onditions. Thermal drying, although commonly used in laboratory practice, is not recommended because it degrades thermodegradable lipids, results in the evaporative loss of volatile lipids, and yields powder of a nonuniform particulate size (Pourmortazavi and Hajimirsadeghi, 2007). Moreover, freeze drying has the advantage of breaking up species cells and turning them into fine powder, making homogenization unnecessary (Ahlgren and Merino, 1991). However, freeze drying is a slow process and requires a very high capital investment. Table 5 summarizes the advantages and disadvantages of each drying technique.

Particular Size Reduction

Once dried, microalgal biomass forms powder or agglomeration that can be milled into different particulate sizes. Reducing the particulate size of microalgal powder prior to lipid extraction generally enhances lipid recovery because it increases the interfacial surface area that is available for biomass-solvent contact and shortens the diffusion pathway of the extraction solvent. However, the exceedingly small particulate size of the microalgal powder

may lead to a higher tendency of lipid re-adsorption and fluid channeling effects in the extraction vessel (for supercritical carbon dioxide ($scCO_2$) extraction) and in homogeneous lipid extraction (Pourmortazavi and Hajimirsadeghi, 2007). Pourmortazavi and Hajimirsadeghi (2007) also conducted a study on the $scCO_2$ extraction of oil from biomass and verified that smaller biomass resulted in higher oil recoveries.

Cell Disruption Technique

The cell disruption method aims to destruct the cell wall of microalgae to release the intracellular components and to enhance the efficiency of the extraction. Cell wall disruption methods can be categorized into biological, chemical and physical methods.

1) Biological Method
Enzymatic Disruption

Enzymes can be applied in oil extraction from microalgae, as they can mediate the hydrolysis of cell walls, enabling the release of their content. For cell wall degradation, cellulases are the most frequently applied enzymes (Mercer and Armenta 2011; Gonçalves *et al.*, 2013). Despite being expensive, enzymes offer several advantages over other cell wall disruption methods. Enzymes present a higher degradation selectivity than physical methods. Furthermore,

microalgal cell walls are more recalcitrant than those of other microorganisms, being very resistant to degradation. Thus, the use of physical methods requires higher energy amounts (Gonçalves *et al.*, 2013). Chen *et al.* (2013) studied bacteria of *Flammeovirga yaeyamensis* in co-cultivation with microalgae of *Chlorella vulgaris* ESP-31. The cell wall of the microalgae was hydrolyzed by hydrolytic enzymes (i.e., amylases, cellulases and xylanase) that were produced by the bacteria, increasing the microalgae oil extraction efficiency by nearly 100%.

2) Chemical Method
Ozonation

Ozone is an effective oxidizer of organic compounds and an effective disinfecting agent that is capable of deactivating a variety of organisms. Ozonation has been widely used in water treatment for disinfection and for the oxidative removal of contaminants (Huang *et al.*, 2014). Cell rupture by ozonation is due to the oxidative reaction of ozone, which compromises the cell membrane's ability to regulate the permeability of substances, eventually leading to the outflow of the cytoplasm (Erden *et al.*, 2010; Yang *et al.*, 2013). Recently, the pressure-assisted ozonation (PO) technique was developed, which is capable of solubilizing cellular materials at high efficiency via cell wall disruption (Cheng *et al.*, 2012). Huang *et al.* (2014) examined the performance of ultrasonication, conventional bubbling ozonation (CBO) and PO as cell disruption techniques to obtain microalgae lipids from *Chlorella vulgaris* BG11. The highest lipid yield was obtained using PO, whereas the lowest yield was obtained using CBO. Using CBO and PO involving ozone, saturated fatty acids (C16:0 and C18:0) were predominant. Thus, PO is an effective cell disruption method for biodiesel production with a high yield of lipids and saturated hydrocarbon products.

Hydrogen Peroxide

Alkaline hydrogen peroxide (H_2O_2) treatment is recognized to decrystallize cellulose and has widely been used for bleaching high-lignin wood pulps. The role of H_2O_2 is to provide highly reactive radicals. When the pH is above 6, H_2O_2 is readily decomposed to active radicals such as hydroxyl radical ($^{\bullet}OH$) and superoxide anion radicals (O_2^-). These active radicals can degrade and oxidize cell wall of biomass (Gould, 1985; Phan and Tan, 2014). *Chlorella vulgaris* was pretreated by aqueous solution of H_2O_2 and H_2O_2 with $FeSO_4$ (Fenton's reactant) to disrupt cell wall prior to lipid extraction (Steriti *et al.*, 2014). The use of H_2O_2 allowed increasing the extraction lipid yield from 6.9 to 9.2%, while that with $FeSO_4$ led to a corresponding increase of lipid yield to 17.4%. According to the Fenton's reaction, the reaction between H_2O_2 and Fe^{2+} ion (from $FeSO_4$) can produce hydroxyl radical ($^{\bullet}OH$) which in turn may attack and degrade the organic compounds constituting the cell wall. Probably such a reaction occurs preferentially in specific zones of the cell wall constituted by organic compounds that are easily oxidized by $^{\bullet}OH$ radicals, leading to the disruption of cell wall and the release of the intracellular material, including lipids (Wu *et al.*, 2010).

Once transferred in the liquid bulk, even lipids, especially unsaturated lipids, might be attacked by hydroxyl radical generating degradation products such as lipid peroxide (González *et al.*, 2012), leading to positive effect for upgrading fatty acid methyl ester composition in biodiesel.

Acid Treatment

The acids that are commonly applied in the cell disruption step are hydrochloric acid and sulfuric acid, which are everyday industrial chemicals carrying minimal toxicity in their applied concentrations. The acid treatment is preferable, as it provides high efficiency in converting polysaccharide-containing materials. In the case of microalgal biomass, complex carbohydrates are entrapped in the cell wall; thus, cell disruption through acid hydrolysis is preferred. Harun and Danquaha (2011) explored the influence of acid exposure as a microalgal pretreatment strategy for bioethanol production. Using a 1% sulfuric acid solution at 140°C for 30 min as a pretreatment to hydrolyze the cell wall of *Chlorococcum humicola* enhanced the bioethanol yield from 16% to 52% (g bioethanol/g microalgae) due to the release and conversion of the carbohydrates that were entrapped in the microalgae cell wall into simple sugars prior to the fermentation process. Recently, acid-catalyzed hot water was used to treat wet microalgae of *Chlorella vulgaris* before lipid extraction for the production of biodiesel. The use of hot water weakened or disrupted the crystal structure of cellulose and acid (i.e., sulfuric acid), aimed to improve the efficiency of hot water through acid hydrolysis. The extracted-lipid yield was enhanced through pretreatment by acid-catalyzed hot water (Park *et al.*, 2014).

3) Physical Method
Electrical Method

The application of electrotechnologies, such as pulsed electric field (PEF) and high voltage electric discharges, were promising for intracellular extraction from bio-suspensions (Lebovka *et al.*, 2011). PEF seems to be a potential alternative for oil extraction from microalgae. This technique applies brief pulses of a strong electric field to cells in the range of nanoseconds to microseconds, inducing the non-thermal permeabilization of the cell membrane and improving mass transport across the cell membranes. Permeabilization depends on the field strength and pulse number (Guderjan *et al.*, 2005; Taher *et al.*, 2011). Under sufficient conditions, irreversible damage of the membrane is reached, and the hardness of the cell is lost; thus, PEF can lead to the complete disruption of cells into fragments. Moreover, PEF requires less time and energy than other applied methods (Guderjan *et al.*, 2005), and its use as a pretreatment for organic solvent extraction requires fewer organic solvents (usually presenting high toxicity) than the conventional organic solvent extraction (Guderjan *et al.*, 2007). Nevertheless, few studies have applied PEF to pretreat microalgae. Grimi *et al.* (2014) investigated the intracellular extraction from the microalgae *Nannochloropsis* sp. disrupted by PEF, which permitted the selective extraction of water-soluble ionic components and microelements, low-molecular-weight organic compounds (amino acids) and water-soluble proteins.

However, this electrically based disruption technique was ineffective for the extraction of pigments, which required subsequent application of such methods as ultrasonication and high-pressure homogenization, but its power consumption was always noticeably smaller.

Ultrasonication

Another method that can be used to promote the cell wall disruption of microalgal cells is the application of ultrasonication. In ultrasonic-assisted pretreatment, cavitation emerges in the solvent. When cavitation bubbles suddenly burst, the extreme high pressure and temperature destroy the adjacent tissue. In this case, the cell walls are damaged by the repeated bursting of cavitation bubbles (Adam *et al.*, 2012). The application of this ultrasound-assisted method to microalgal biomass can improve extraction efficiencies by reducing the extraction times and increasing the oil recovery yields. The experiments that were performed by Cravotto *et al.* (2008) with the microalgae *Crypthecodinium cohnii* showed cell disruption after applying ultrasonic pretreatment prior to Soxhlet extraction with n-hexane, resulting in an increase in the oil extraction yields from 4.8 to 25.9% compared to Soxhlet extraction without any pretreatment. Additionally, Ranjan *et al.* (2010) compared the oil extraction yields from *Scenedesmus* sp. using the following methods: (1) Bligh and Dyer's method and (2) ultrasound-assisted extraction followed by Bligh and Dyer's method. The obtained results demonstrated that method (2) generated a greater yield of oil extraction than method (1) due to the disruption of the cell wall and the release of the cell components to the solvent after pretreatment.

Microwave

Microwave pretreatment is frequently used in biochemical extraction from plants. In the microwave process, the oscillation of polar species and ionic conduction causes the temperature to rapidly increase, thereby improving the extraction efficiency (Ganzler *et al.*, 1990). When microwaves irradiate the interior of a cell, the oscillation of polar substances causes rapid heating, moisture evaporation and increasing pressure, which lead to cell rupture followed by the release of the cell contents. Cravotto *et al.* (2008) applied organic solvent extraction with n-hexane and microwave-assisted solvent extraction (using the same solvent) to the microalgae *Crypthecodinium cohnii*, achieving oil recovery yields of 4.8 and 17.8%, respectively. Recently, Balasubramanian *et al.* (2011) promoted the cell wall disruption of *Scenedesmus obliquus* using the microwave-

assisted method for oil extraction. Oil analysis indicated that microwave-extracted oil contained higher percentages of unsaturated and essential fatty acids (indicating higher quality) than that extracted without pretreatment. This study also validated for the first time the efficiency of a continuous microwave system for the extraction of lipids from microalgae. Higher oil yields, faster extraction rates and superior oil quality demonstrated that this system is feasible for oil extraction. Ma *et al.* (2014) used dichloromethane to extract oil from microalgae that were pretreated with microwave and ultrasound. The microwave pretreatment was more effective than the ultrasonic pretreatment for cell disruption because the microwave process provided pressure change through the rapid heating of the microalgae cells, whereas in the ultrasonic process, this rupture was caused by a shock wave outside of the microalgae cells.

Mechanical Method

The mechanical method is the most traditional method of disruption. For example, bead milling is a common way to grind materials into fine powder by shear force, which is caused by the mechanical force. Zheng *et al.* (2011) used bead milling as a pretreatment for microalgae, obtaining an approximately 10% lipid yield. However, a greater lipid yield could be obtained with manual grinding in liquid nitrogen. A blender was also used to disrupt the cell of *Nannochloropsis oculata*. The results in Table 6 show that the blender has a relatively high efficiency in breaking the microalgae cell wall and is the most energy efficient method (McMillan *et al.*, 2013).

Laser

In an application of laser for cell disruption, it is suspected that the cell is disrupted by thermal effects similar to those associated with the thermolysis technique. It may be possible that cavitation bubbles induce shear damage, as well. McMillan *et al.* (2013) applied a laser to a microalgae slurry. The typical damage of perforated cells showed that lipid matter was released, and dried lipid matter was observed on the underside of the cover slip that was in contact with the sample and had risen to the top of the treated solution. Heavily deformed cells displaced lipid material, demonstrating that high temperatures were a major influence on disruption, even more so than bubble cavitation. Large air bubbles were also observed in the sample that encapsulated the lipid matter and pushed away disrupted cells and debris in their path. Cell disruption was achieved at 96.53% though laser pretreatment (Table 6).

Table 6. Merit for different treatments, defined as disruption per unit applied Joule/1000 mL per volume fraction of device (McMillan *et al.*, 2013).

Treatment method	Disruption (%)	Energy (J/1000 mL)	Sample volume (mL)	Volume of device (cm^3)	Fractional volume[a]
Microwave	94.9	74565	10	27500	0.00036
Ultrasonic	67.7	132	350	1050	0.33
Blender	93.0	540	80	320	0.25
Laser	96.5	16000	0.003	-	1

[a] Fractional volume is referred to a ratio of sample volume to volume of device.

Use of a laser is the most efficient disruption method, but only slightly better than the microwave. However, laser lysis was highly energy consuming and spatially limited to processing very small volumes at a time.

As above, there are many works focused on cell disruptions with biological, chemical and physical methods. However, only merits of physical method were reported (McMillan *et al.*, 2013). Thus, the merits of biological and chemical methods are suggested to be further investigated.

Extraction

Lipids are one of the main components of microalgae, depending on the species and growth conditions. Microalgae contain lipids at approximately 2–60% of the total cell dry weight. Lipid oils that are derived from microalgae are interesting because these oils contain fatty acids (mostly 16 carbons to 22 carbons in chain length) and triglyceride compounds that can be transesterified into biodiesel. Technology for the production of biodiesel from microalgae must have a high specificity for lipids to minimize contaminants, i.e., carbohydrates, proteins, etc. (Banerjee *et al.*, 2002). Purification technology should also favor the production of acylglycerols over other lipids, such as ketones, chlorophylls, sterols, polar lipids, and carotenes, which are not readily converted into biodiesel (Mojaat *et al.*, 2008). Moreover, technology should have low operating and capital costs, require little energy and time, be safe, and show no sign of reaction with lipids. Table 7 demonstrates the methods, including organic solvent extraction, Soxhlet solvent extraction, and supercritical fluid extraction, that have been used to extract lipids from microalgae.

Organic Solvent Extraction

Lipid-solvent systems are governed by the principle of like-dissolves-like in which lipids are extracted using non-polar organic solvents, such as chloroform or hexane (Geciova *et al.*, 2002). This extraction can be classified into five steps: (1) the microalgae are exposed to the solvents, which penetrate the cell membrane and enter the cytoplasm; (2) the solvent interacts with neutral lipids via van der Waal's forces to form a solvent-lipid complex; (3) this complex diffuses across the cell membrane, such that the neutral lipids enter the organic phase, while water and solvent-contaminant complexes (with carbohydrates or proteins) enter the aqueous phase; (4) the organic phase is further separated; and (5) crude lipids are transesterified to generate biodiesel (Lewis *et al.*, 2000; Halim *et al.*, 2012; Bahadar and Khan, 2013). However, the disadvantages of using chemical solvents are mostly related to their high toxicity to humans and the surrounding environment. Chemical solvents, such as n-hexane, methanol, and ethanol, and mixed polar/non-polar solvents, such as methanol/chloroform (2:1 v/v, Bligh and Dyer method) or isopropanol/hexane, are effective at extracting microalgae lipid, but the extraction efficiency is highly dependent on the microalgae strains. One study found that using isopropanol as a co-solvent (0.068 g of lipid/g of microalgal biomass) increased the lipid yield from *Chlorococum* sp. by up to 300% more than using pure hexane (0.015 g of lipid/g of microalgal biomass)

(Halim *et al.*, 2011). Another study reported that bead-beaten *Botryococcus braunii* was exposed to five different organic solvents; chloroform/methanol yielded the highest lipid content (0.29 g of lipid/g of microalgal biomass). Non-polar solvent n-hexane, although widely used to extract oil from various seed crops, is insufficient to extract microalgae lipids because microalgal lipids are composed of a high concentration of unsaturated fatty acids; thus, the selectivity of lipids toward the solvent is largely reduced (Ranjan *et al.*, 2010; Lam and Lee, 2012). n-Hexane, methanol and chloroform are highly toxic compounds that can cause safety and health hazards if proper precautionary steps are not taken. In contrast, ethanol emerged as a greener solvent because it has low toxicity level and can be generated from renewable sources. However, ethanol always generates a low extraction efficiency due to an azeotrope mixture (5% water), and the presence of water will reduce the extraction efficiency (Lam and Lee, 2012).

Organic solvent extraction is usually carried out in a non-continuous batch process, limiting lipid mass transfer equilibrium (Medina *et al.*, 1998). Soxhlet solvent extraction continuously evaporates and condenses the solvent, avoiding the lipid mass transfer limitation and reducing solvent consumption (Wang and Weller, 2006). Soxhlet lipid extraction is more effective than batch extraction (Halim *et al.*, 2011). However, the continuous distillation is an energy-consuming process. For extraction using organic solvents, diffusion is always the rate limiting factor in the overall mechanism. This factor becomes more serious in microalgae because the cell wall further resists solvent penetration into the inner cell. Thus, the cell disruption method (as describe above) can be used to increase solvent diffusion efficiency and results in the improvement of the microalgae lipid recovery rate. Although a higher microalgae lipid yield can be achieved after cell disruption, additional energy is required (Lam and Lee, 2012).

Direct Liquefaction

Oil can be directly obtained from dried or wet microalgae by liquefaction. The high moisture content of microalgae requires much energy to remove water. Liquefaction directly converts the biomass into oil via reaction with water and carbon monoxide/hydrogen in the presence of sodium carbonate. These processes require high temperatures and pressures. In the liquefaction process, biomass is decomposed first into small molecules. These small molecules are unstable and reactive and can repolymerize into oily compounds with a wide range of molecular weight distributions. Liquefaction can be attained directly or indirectly. Direct liquefaction involves rapid pyrolysis to produce liquid tars and oils and/or condensable organic vapors. Indirect liquefaction involves the use of catalysts to convert non-condensable gaseous products of pyrolysis or gasification into liquid products. Minowa *et al.* (1995) used direct liquefaction at 300°C and 10 MPa to produce oil with a yield of 78.4% from *Dunaliella tertiolecta* with a moisture content of 78.4%. For high-moisture *Botryococcus braunii* treated with or without a catalyst of 5% Na_2CO_3 at 300°C, more than 95% hydrocarbons was recovered (Bahadar and Khan, 2013).

Table 7. Methods used to extract lipids from microalgae.

Extraction method	Species	Solvent	Yield (wt%)	Time (min)	T (°C)	P (MPa)	Reference
Organic solvent	*Chaetoceros muelleri*	1-Butanol	94	60	70	-	Nagle and Lemke, 1990.
	Chlorococcum sp.	Isopropanol/hexane	6.8	450	80	-	Halim *et al.*, 2011.
		Hexane	1.5	450	80	-	Halim *et al.*, 2011.
	Phaeodactylum tricornutum	Ethanol	29	1440	25	-	Fajardo *et al.*, 2007.
Soxhlet	*Botryococcus braunii*	DBU/octanol	81	240	60	-	Samori *et al.*, 2010.
	Chlorella vulgaris	Hexane	1.8	140	70	-	Suarsini and Subandi, 2011.
	Chlorococcum sp.	Hexane	3.2	330	80	-	Halim *et al.*, 2011.
	Isochrysis sp.	Methanol/Chloroform	23.1	1080	105	-	Hernández *et al.*, 2014.
	Nannochloropsis gaditana	Methanol/Chloroform	17.7	1080	105	-	Hernández *et al.*, 2014.
	Scenedesmus almeriensis	Methanol/Chloroform	22.4	1080	105	-	Hernández *et al.*, 2014.
	Scenedesmus obliquus	Hexane	40.7	600	65	-	Balasubramanian *et al.*, 2011.
	Tetraselmis sp.	Methanol/Chloroform	18.1	1080	105	-	Hernández *et al.*, 2014.
Supercritical fluid	*Crypthecodinium cohnii*	CO_2	9	180	50	30	Couto *et al.*, 2010.
	Chlorococcum sp.	CO_2	5.8	80	60	10–50	Halim *et al.*, 2011.
	Isochrysis sp.	CO_2	14.7	90	45	30	Hernández *et al.*, 2014.
	Nannochloropsis gaditana	CO_2	12.9	90	45	30	Hernández *et al.*, 2014.
	Nannochloropsis sp.	CO_2	25	360	40	55	Andrich *et al.*, 2005.
	Scenedesmus almeriensis	CO_2	13.2	90	45	30	Hernández *et al.*, 2014.
	Scenedesmus sp.	CO_2	7.4	720	53	50	Taher *et al.*, 2014b.
	Spirulina platensis	CO_2	8.6	60	40	40	Sajilata *et al.*, 2008.
	Tetraselmis sp.	CO_2	14.8	90	45	30	Hernández *et al.*, 2014.
	Tetraselmis sp.	CO_2	10.9	720	40	15	Li *et al.*, 2014.

Supercritical Fluid Extraction

Supercritical carbon dioxide ($scCO_2$) can be used as a solvent for lipid extraction from microalgae. $scCO_2$ extraction is a green technology that promises to replace organic solvent extraction. The basic principal of this technology is achieving a supercritical phase that is beyond the critical point of the fluid, at which separation of the liquid and vapor phase disappears and a single homogeneous phase appears. $scCO_2$ extraction is very efficient for lipid extraction for several reasons: (1) the crude lipid products are solvent free, (2) the rapid penetration of $scCO_2$ into microalgae cells promotes a high lipid yield, (3) the properties of $scCO_2$ are tunable through the adjustment of the operating temperature and pressure, and (4) degumming is not required because $scCO_2$ is not able to solubilize polar phospholipids (Mendes *et al.*, 2003; Herrero *et al.*, 2006; Sahena *et al.*, 2009).

The applications of $scCO_2$ to extract microalgae lipids for biodiesel production have been explored recently. Lipid from wet paste *Chlorococum* sp. biomass was extracted using $scCO_2$ with a yield of 7.1% at a temperature of 60°C, a pressure of 30 MPa and an extraction time of 80 min. Moreover, the lipid yield obtained from the wet paste was greater than that obtained from the dried paste (5.8%), indicating that the energy consumption for the drying process can be reduced. The presence of water in the system acts as a natural polar co-solvent, facilitating the extraction of polar lipids and improving the total lipid yield (Halim *et al.*, 2011). However, $scCO_2$ extraction and organic solvent extraction were compared by extracting lipid from *Crypthecodinium cohnii*. The lipid yield attained from organic solvent extraction was approximately twice that from $scCO_2$ extraction, indicating that microalgae strains and cultivation conditions play a significant role in determining the appropriate lipid extraction methods (Couto *et al.*, 2010). Lipid extraction from *Chlorococcum* sp. using $scCO_2$ yielded 0.058 g of lipid/g of microalgae with a resident time of 80 min, while that using Soxhlet solvent extraction yielded 0.032 g of lipid/g of microalgae after 5.5 h (Halim *et al.*, 2011). One study showed that 90% of lipids was recovered from *Spirulina platensis* using $scCO_2$ at 70 MPa and 55°C for 15 min, while the same amount was obtained using Soxhlet hexane extraction for 6 h (Bahadar and Khan, 2013). Chen *et al.* (2013) used a continuous high-pressure CO_2 extraction process to extract lipid from microalgal biomass with an extraction yield of 90.56%. Recently, $scCO_2$ (pressure of 50 MPa at 50°C and a flow rate of 3 mL/min) was applied for lipid extraction from *Scenedesmus* sp. and compared with static n-hexane extraction (shaker at 50°C and 100 rpm, overnight) (Taher *et al.*, 2014a). The lipid extraction yield obtained from the former was 75% greater than that obtained from the latter. Taher *et al.* (2014b) also optimized the conditions for $scCO_2$ extraction. $scCO_2$ extraction was superior to other extraction techniques (Soxhlet, Bligh and Dyer lipid extraction, n-hexane, n-hexane/isopropanol). The best operating conditions were 53°C, 50 MPa and a flow rate of 1.9 g/min, producing a lipid extraction yield of 7.41%. Li *et al.* (2014) extracted lipid from *Tetraselmis* sp. (strain M8) using different extraction methods, including Bligh and Dyer lipid extraction,

organic solvent extraction, direct saponification and $scCO_2$ extraction. $scCO_2$ extraction technique (15 MPa, 40°C, 12 h for soaking and a flow rate of 5 mL/min for 30 min for flushing) resulted in the most effective extraction of microalgal lipids, especially for long-chain unsaturated fatty acids.

CO₂-Expanded Liquid Extraction

CO_2-expanded liquids (CXLs) have been applied in extraction, reaction and separation (Chen and Tan, 2007; Golmakani *et al.*, 2012; Yang *et al.*, 2012; Lin *et al.*, 2013; Wei *et al.*, 2013). CXLs can be continuously tuned from the neat organic solvent to $scCO_2$ through changing the CO_2 composition in liquid by adjusting operating pressure (Jessop and Subramaniam, 2007). CXLs have the benefit of requiring mild operating pressure and temperature, leading to the reduction of energy consumption hence the cost of the process (Sih *et al.*, 2008). Similar to supercritical fluids, CXLs have shown to improve mass transfer by decreasing interfacial tension, reduce viscosity and increasing diffusivity (Eckert *et al.*, 2007; Lin and Tan, 2008a, b; Herrero *et al.*, 2013). Recenty, the CO_2-expanded ethanol (CXE) has been applied for extraction of valuable bioactive from natural sources, since ethanol emerges as a greener solvent than other organic solvents. One work studied the extraction of gamma-linolenic acid from *Arthrospira platensis* (*Spirulina*) using CXE showed its extraction yield similar to pressurized extraction but greater than $scCO_2$ extraction (Golmakani *et al.*, 2012). CXE was also applied to extract polar antioxidant of astaxanthin from *Haematococcus pluvialis*. Temperature and ethanol content had significant influences on astaxanthin yield and antioxidant activity (Reyes *et al.*, 2014). Our group successfully applied CXE as the solvent to extract docosahexaenoic acid (DHA)-rich lipid from *Schizochytrium* sp. in continuous extraction process (Wang, 2014a). The lipid yield extracted via CXE in continuous extraction for 30 min was obtained approximately 87% of total lipid content determined by 48-h Soxhlet extraction, much better than ethanol and pressurized ethanol regarding yield and extraction time. The obtained results indicated that the CXE was appropriate for fast and efficient lipid extraction resulting from high diffusion, low viscosity and easy penetration into microalgae cell.
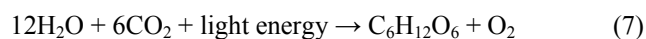
Biofuel Products
Biohydrogen

Renewable biohydrogen production is of increasing interest because fossil fuel supplies are being depleted. Hydrogen gas has excellent potential as a renewable energy source because it produces only water when combusted, unlike the carbon pollution of fossil fuels. Biohydrogen has become a viable source given the current energy demand and environmental issues. The main objective is to improve hydrogen yield to make this process more economically available. However, the low yield and rate of hydrogen production are barriers for the commercialization of biohydrogen production. Cheap raw materials, efficient production techniques, and pilot tests of photofermentation plants should make biohydrogen a commercially available

source of energy in the near future (Bahadar and Khan, 2013). Biohydrogen can be produced by photolyzing water using solar energy and hydrogenase and/or nitrogenase enzymes. Microalgae use solar energy to transfer electrons to NADPH and ferredoxin to generate hydrogen. Photolysis can be classified into several subcategories: direct photolysis involves splitting water into hydrogen and oxygen using sunlight energy as follows:

$$2H_2O + \text{light energy} \rightarrow 2H_2 + O_2 \qquad (6)$$
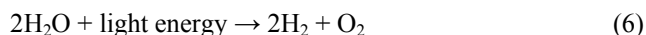
Hydrogen is produced in two steps. First, photosystem II adsorbs light and generates electrons that are transferred to ferredoxin using light energy, which is absorbed by the photosystem. Hydrogenase then accepts the electron from ferredoxin to generate hydrogen. Indirect photolysis is well suited for nitrogenase-based systems. The reaction is as follows:

$$12H_2O + 6CO_2 + \text{light energy} \rightarrow C_6H_{12}O_6 + O_2 \qquad (7)$$

$$C_6H_{12}O_6 + 12H_2O + \text{light energy} \rightarrow 12H_2 + 6CO_2 \qquad (8)$$

However, hydrogen production from a nitrogenase-based system is 1000-fold lower than that from a hydrogenase-based system (Bahadar and Khan, 2013). Nitrogenase is prohibited by oxygen; thus, oxygen must be removed from these systems. A CO_2 concentration between 4 and 18% is optimal, resulting in higher cell densities and more hydrogen production (Das and Veziroglu, 2008). *Chlamydomonas reinhardtii*, *Calothrix* sp., *Oscillatoria* sp., *Synechococcus* sp. and *Gloeobacter* sp. can produce hydrogen via both hydrogenase and nitrogenase (Abed *et al.*, 2009; Hallenbeck, 2009). Photosynthetic hydrogen production is the best for converting solar energy into hydrogen; therefore, the current focus of research is to increase the light-use efficiency and to design better reactors for hydrogen production (Bahadar and Khan, 2013).

Biodiesel

The extracted lipid from microalgae is further converted into biodiesel. Biodiesel is a promising renewable energy that does not require engine modification and reduces CO (by 50%) and CO_2 (by 78%) emissions (Chisti, 2007). A main problem of microalgae-based biofuels is their high viscosity. Such high-viscosity fuels are difficult to combust and leave deposits on the fuel injector of diesel engines. Thus, biodiesel is usually blended with conventional diesel. There are several techniques to overcome this problem, including pyrolysis, micro-emulsification, dilution and transesterification (Marchetti *et al.*, 2007). Among these techniques, transesterification offers the most promise for lowering the viscosity.

1) Transesterification

The chemistry of biodiesel synthesis is relatively simple and occurs via transesterification, in which a triglyceride (from the lipid) reacts with a mono-alcohol (most commonly ethanol or methanol) in the presence of a catalyst as follows:

$$(9)$$
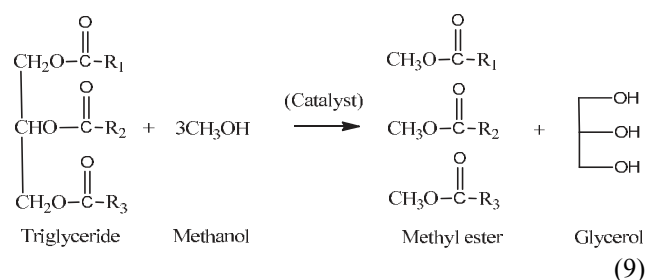
The reaction converts three moles of alcohol and one mole of triglyceride into one mole of glycerol and three moles of methyl esters. Although this reaction requires much alcohol, the methyl ester yield is greater than 98% (Chisti, 2007). A homogeneous base catalyst (e.g., KOH or NaOH) is usually used to accelerate the reaction. However, the base catalyst can react with free fatty acids in microalgae lipids to form soap, leading to a lower biodiesel yield and increasing the difficulty of separating biodiesel from glycerol. Acid catalysts (e.g., H_2SO_4) are alternative options because a catalyst is not sensitive to the free fatty acid level in oil; therefore, esterification (in which free fatty acid is converted into alkyl ester) and transesterification can occur simultaneously (Lam and Lee, 2012). However, base-catalyzed reactions are 4000 times faster than acid-catalyzed ones (Bahadar and Khan, 2013). Commercially, alkoxides of sodium and potassium are used at 1% per weight of lipid formed because they have a greater catalytic activity than simple alcohols. Base catalyst reactions are optimized at 60°C under atmospheric pressure for 90 min. At higher temperatures and pressures, although the reaction is faster, the operating cost is greater. The reaction generates two layers of excess methanol and oil. The biodiesel is then separated from contaminants, for instance, glycerol and solids, in a flask separator (Hossain *et al.*, 2008). The biodiesel may be then washed with water to remove contaminated free fatty acids, which cause saponification. Transesterification consumes 4.3 MJ/L biodiesel (Demirbas, 2007).

Heterogeneous catalysts (base or acid) have also been explored extensively for transesterification to produce biodiesel. Unlike homogeneous catalysts, the heterogeneous catalyst can be recycled, regenerated and reused for a subsequent transesterification reaction. Furthermore, this catalyst can be easily separated through filtration after the reaction is finished, which can minimize the product contamination and the number of water-washing cycles for purification. Until now, the application of a heterogeneous catalyst in microalgae biodiesel production is still restricted because it is a relatively new feedstock and is not commercially available in the market. Therefore, additional breakthrough findings are required to address the feasibility of heterogeneous catalysts in the microalgae biodiesel industry (Lam and Lee, 2012). CaO supported by Al_2O_3 was tested for very a short time in the transesterification of lipid from *Nannochloropsis oculata*. The obtained yield was 97.5% at a reaction temperature of 50°C, with a methanol-to-lipid ratio of 30:1, a catalyst loading of 2% and a reaction time of 4 h (Umdu *et al.*, 2009). Fu *et al.* (2013)

synthesized a sulfonated carbon-based solid acid catalyst through the in situ partial carbonization and sulfonation of microalgae residues with sulfuric acid. The obtained catalyst possessing a high acid density (5.3 mmol/g) was used in the transesterification of triolein with methanol for biodiesel production. The yield of methyl ester was 24% for a 12-h reaction time. *Chlorella vulgaris* ESP-31 containing 22.7% lipid was used as the oil source for biodiesel production via transesterification catalyzed by SrO/SiO_2. The solid catalyst SrO/SiO_2 worked well with water-removed, centrifuged *Chlorella vulgaris* ESP-31 and a biodiesel conversion of 80% (Tran *et al.*, 2013).

2) In Situ Transesterification

In situ transesterification is a simple process that permits extraction and transesterification to occur in one step, in which the lipid-bearing biomass is directly in contact with the chemical solvent in the presence of a catalyst. There are two roles of the chemical solvent, including (1) a solvent for the extraction of lipid from biomass and (2) a reactant for the transesterification reaction. The advantages of in situ transesterification over conventional transesterification include a minimized solvent separation step, a reduced processing time and, consequently, a reduced overall biodiesel production cost (Shuit *et al.*, 2010).

Many studies have investigated the in situ transesterification of microalgae. *Chlorella* sp. was in situ transesterified under a reaction temperature of 60°C, with methanol to lipid ratio of 315:1, a H_2SO_4 concentration of 0.04 mol and a reaction time of 4 h to produce a 90% biodiesel yield (Ehimen *et al.*, 2010). As a result, high methanol consumption was required. However, methanol consumption can be reduced by adding a co-solvent during in situ transesterification. *Chlorella pyrenoidosa* with an oil content of 56.2% was subjected to in situ transesterification, and a 95% biodiesel yield was attained with a hexane (co-solvent) to lipid molar ratio of 76:1, a methanol to lipid molar ratio of 165:1, a reaction temperature of 90°C, 0.5 M H_2SO_4 and a reaction time of 2 h (Miao *et al.*, 2011). Other solvents, such as toluene, chloroform and dichloromethane, were also suggested for use during in situ transesterification (Xu and Mi, 2011). However, using wet paste, microalgae biomass has an adverse effect on in situ transesterification; thus, the wet microalgae biomass must be initially dried to ensure efficient and optimum performance during in situ transesterification. Recently, the microalgae biomass with a water content greater than 31.7% caused the inhibition of in situ transesterification, leading to negligible biodiesel conversion (Ehimen *et al.*, 2010). Sathish *et al.* (2013) reported that microalgal biomass with a moisture content greater than 20% by mass led to statistically significant reductions in biodiesel discovery. Three explanations have been commonly presented to describe this inhibitory effect: (1) the formation of free fatty acid methyl esters is a reversible reaction, and therefore, water can hydrolyze biodiesel back to methanol and free fatty acids; (2) water contained within the biomass can shield lipids from the extracting solvent, preventing lipids from being brought into the reaction; and (3) the acid catalyst can be deactivated due to water competing for available protons in the reaction. To avoid adverse effects, water must be removed from the recovered methanol or ethanol before the methanol or ethanol is used for subsequent in situ transesterification; otherwise, the subsequent reaction might not proceed under optimum conditions due to the presence of water. Therefore, extensive biomass drying is also required prior to reaction to avoid the unrequired side reaction and to simplify the subsequent separation processes (Lam and Lee, 2012). Im *et al.* (2014) proposed the method for the in situ transesterification of wet *Nannochloropsis oceanica* (65% moisture content) using chloroform, methanol and sulfuric acid in a one-pot reaction. The addition of chloroform can improve the performance of transesterification with methanol by reducing the diffusion limit of the reactant to the liquid reaction phase. This study provided a yield of greater than 90% without the addition of extra energy inputs for drying microalgae prior to in situ transesterification. Thus, this method can reduce the production cost of biodiesel from microalgae.

### 3) Supercritical Fluid Transesterification

Supercritical methanol transesterification is now being tested for the production of biodiesel. In the supercritical state, depending on pressure and temperature, intermolecular hydrogen bonding in the methanol molecule will be significantly decreased. As a result, the polarity and dielectric constant of methanol are reduced, allowing it to act as a free monomer. Subsequently, methanol under supercritical conditions can solvate the non-polar triglycerides to form a single-phase lipid/methanol mixture and yield fatty acid methyl esters and diglycerides (Saka and Kusdiana, 2001). These advantages make the supercritical methanol process more beneficial than conventional transesterification methods, in which homogeneous and heterogeneous catalyst processes often face difficulties due to the free fatty acid content and water content in the feedstock, resulting in lower yields and requiring purification of the final product to meet the biodiesel standards (Tan *et al.*, 2011). Patil *et al.* (2011) used a one-step process for the direct liquefaction and conversion of wet microalgal *Nannochloropsis* sp. biomass containing approximately 90% water to biodiesel under supercritical methanol conditions. This one-step process enables the simultaneous extraction and transesterification of wet microalgal biomass. Optimal conditions for this process are reported as follows: wet algae to methanol (w/v) ratio of approximately 1:9 and a reaction temperature and a time of approximately 255°C and 25 min, respectively. A more than 84% yield of fatty acid methyl esters was obtained. Moreover, the direct conversion of microalgal *Nannochloropsis* sp. biomass into biodiesel using supercritical methanol compared with microwave-assisted transesterification methods was investigated by Patil *et al.* (2012). Wet algal biomass was used as feedstock in the supercritical methanol process, and dry algal biomass was used for the microwave-assisted transesterification. A higher yield of fatty acid methyl esters was obtained using the supercritical methanol process.

A concurrent extraction and transesterification of lipids can also be achieved under supercritical ethanol conditions. Similar to methanol, the dielectric constant of ethanol

decreases into the range of organic solvents with increasing temperature, and the exothermic hydrogen bonding in ethanol shifts toward the free monomer above critical conditions, permitting the extraction of lipids from wet microalgae and ethanol to perform the transesterification of triglycerides to fatty acid ethyl esters. Recently, supercritical ethanol was used for the simultaneous extraction and transesterification of lipids in algae to produce fatty acid ethyl esters (Reddy *et al.*, 2014). A maximum yield of approximately 67% of fatty acid ethyl esters was obtained at 265°C with 20 min of reaction time and a 1:9 dry algae to ethanol (w/v) ratio. Furthermore, the obtained fatty acid ethyl esters possessed high oxidative stability and a calorific value of 43 MJ/kg, comparable to that of regular diesel fuel. Significant energy savings were possible by eliminating the microalgae drying step through the simultaneous extraction and conversion of wet algae to biodiesel. Thus, these green conversion processes have the potential to provide an energy-efficient and economical route for the production of renewable biodiesel.

Biomethanol

Microalgae can be used to produce biomethanol as a renewable fuel. Methanol was synthesized theoretically by the following reaction:

$$C_mH_{2n} + mH_2O \rightarrow mCO + (m + n)H_2 \qquad (10)$$

$$CO + 2H_2 \rightarrow CH_3OH \qquad (11)$$
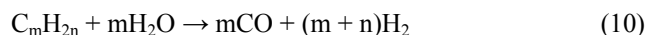
To convert hydrocarbons into methanol, the reforming of hydrocarbons in the syngas is carried out by a water-gas shift reaction, indicated by Eq. (10). Then, to achieve the reaction indicated by Eq. (11), the ratio of H_2/CO is adjusted to the ideal value of 2 by the next step of water-gas shift reaction indicated by Eq. (12):

$$CO + H_2O \leftrightarrow H_2 + CO_2 \qquad (12)$$

Spirulina sp. is converted to methanol by gasification (Hirano *et al.*, 1998). The *Spirulina* biomass was partially oxidized at temperatures of up to 1000°C to produce H_2, CO, CO_2 and hydrocarbon. The maximum yield was 0.64 g of methanol per gram of microalgal biomass at 1000°C. The carbon dioxide produced by the reaction was removed by absorption using alkanolamine.

Bioethanol

Generally, two methods are normally modified for the production of bioethanol from biomass. The first method is a biochemical process, i.e., fermentation, and the other method is a thermo-chemical process or gasification. The recent attempts at producing ethanol have focused on microalgae as a feedstock for the fermentation process. Microalgae are rich in carbohydrates (Table 1), which are entrapped within their cell wall; an economical physical pretreatment process, such as extrusion or mechanical shear, is required to break down the cell wall so that the carbohydrates can be released and used as carbon sources

for fermentation. Bacteria, yeast and fungi are used to ferment carbohydrates to produce ethanol under anaerobic conditions. The products include not only ethanol but also CO_2 and H_2O, as demonstrated in the following equation:

$$C_6H_{12}O_6 \rightarrow 2CH_3CH_2OH + 2CO_2 \qquad (13)$$
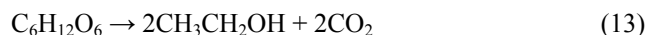
According to this equation, the stoichiometric yields are 0.51 kg of ethanol and 0.49 kg of CO_2 per kg of carbon sugar, i.e., glucose.

The most recent studies of bioethanol production by fermentation have been reported. *Chlorella vulgaris* and *Chlorococcum* sp. are widely used for bioethanol production because of their high starch contents. Harun *et al.* (2010) used *Saccharomyces bayanus* for the fermentation of *Chlorococcum* sp. with 3.83 g/L ethanol produced from 10 g/L lipid-extracted microalgae. *Chlorella vulgaris* cake was enzymatically hydrolyzed and fermented with *Saccharomyces cerevisiae* to produce the reducing sugars and ethanol. The yields obtained for reducing sugars and ethanol were 0.55 and 0.17 g/g of cake, respectively (Moncada *et al.*, 2013). *Schizochytrium* sp. was separated into sugars (mainly D-glucose and L-galactose), lipids and proteins. The separated sugars were then converted to ethanol by *Escherichia coli* KO11, resulting in 11.8 g of ethanol/L being produced from 25.7 g/L of glucose (Kim *et al.*, 2012).

The bioethanol fermentation from microalgae involves less intake of energy, and the process is much simpler compared to the biodiesel production system. In addition, the undesired CO_2 byproduct can be recycled as a carbon source to cultivate additional microalgae, resulting in the reduction of greenhouse gas emissions. However, the commercial production of bioethanol from microalgae is still being investigated (Singh and Gu, 2010; Bahadar and Khan, 2013).

Biobutanol

Biobutanol has been identified as a potential fuel from renewable sources. Comparing with ethanol, butanol provides several benefits as a biofuel such as higher energy contents, lower vapor pressure and lower hygroscopy. Moreover, it can be partially or totally blended with gasoline or diesels fuels (Ventura and Jahng, 2013). In general, a fermentation of sugars with *Clostridium* sp. can produce acetone, butanol and ethanol (ABE) in the ratio of 3:6:1 acetone:butanol:ethanol, which can be called ABE fermentation (Potts *et al.*, 2012). ABE fermentation is typically featured by anaerobic bacterial metabolisms, acidogenesis and solventogenesis. The acetic acid and butyric acid are the primary acid produced during acidogenesis. Clostridia species absorb these acids to produce ABE. In contrast to the ABE fermentation, Ramey (2006) proposed to accomplish the fermentation in two steps to improve yield, in which sugar was converted to butyric acid in the first step by bacterium such as *Clostridium tryobutyricum* in acidogenesis phase, and butyric acid to butanol in the second step by solventogenesis bacterium such as *Clostridium beijerinckii* (Du *et al.*, 2012; Ellis *et al.*, 2012; Potts *et al.*, 2012). However, in order to convert butyric acid to butanol, additional energy in the form of

glucose is required, adversely affecting the selection of two-step fermentation methods. Microalgae are carbohydrates-rich biomass which can be regarded as a source for butanol production. Ellis *et al.* (2012) used *Clostridium saccharoperbutylacetonium* N1-4 for ABE fermentation of acid- pretreated wastewater microalgae with 2.26 g/L butanol produced from 8.92 g/L total soluble sugar. Acid-pretreated microalga *Scenedesmus obliquus* was fermented by *Clostridium saccharoperbutylacetonium* to produce ABE. The obtained butanol yield was 1.91 g/L (Anthony *et al.*, 2013). An isolated *Chlorella vulgaris* was used in butanol fermentation with a *Clostridium acetobutylicum* via ABE fermentation. 3.37 g/L butanol was produced from 111 g of acid-pretreated biomass of *Chlorella vulgaris* (Wang *et al.*, 2014).

Bio-Oil and Biohydrocarbon

Hydrothermal liquefaction can be an alternative way to produce bio-oil from microalgae through the aqueous-conversion method, in which freshly harvested wet microalgae biomass are directly processed without drying. During hydrothermal liquefaction, water is heated to a sub-critical condition between 200 and 350°C under pressurization to reduce its dielectric constant. The dielectric constant can even drop to a value similar to that of ethanol; therefore, sub-critical water is able to solubilize a less polar compound (Duan and Savage, 2011; Kumar *et al.*, 2011). In other words, water at the sub-critical condition can serve as an effective solvent with significantly less corrosion than other chemical solvents. In an experiment, 43% yield of bio-oil was obtained by hydrothermal liquefaction of *Nannochloropsis* sp. (initial water content of 79%) at 350°C and 35 MPa for 60 min. Major bio-oil constituents included phenol and its alkylated derivatives, heterocyclic N-containing compounds, long-chain fatty acids, alkanes and alkenes, and derivatives of phytol and cholesterol. CO_2 was always the most abundant gas product. However, the bio-oil required deoxygenation and denitrogenation to N and O-containing compounds to upgrade the bio-oil (Brown *et al.*, 2010).

Alkanes were also produced by hydrogenation and decarbonylation of microalgae oil. The reaction was catalyzed by a Ni/ZrO_2 to produce ketone intermediates, which were then hydrogenated to aldehydes in the presence of Ni catalysts. The reaction was performed in an autoclave. Propane (3.6%) and methane (4.6%) were the main products in the vapor phase, which were formed by the hydrogenolysis of triglyceride and the methanation of CO/CO_2 with H_2, respectively (Fig. 3). The total liquid yield contained a 70% yield of n-heptadecane and a 75% yield of total liquid alkanes (Peng *et al.*, 2012a). The alkanes in the range of C15 to C18 were synthesized through hydrodeoxygenation of microalgae oil in the presence of 10% Ni on Hbeta zeolite (Ni/Hbeta, Si/Al = 180) in an autoclave at 260°C in the presence of hydrogen at 4 MPa. The obtained products yielded 78% liquid alkanes (60% C18, octadecane), 3.6% propane and 0.6% methane. A Ni/Hbeta catalyst increased the hydrogenation rate and produced propane and fatty acids from saturated triglyceride (Peng *et al.*, 2012b).

A methane fermentation technology can be applied to microalgae to produce methane. The gross stoichiometry of the methane fermentation from glucose as a substrate can be approximated by the following (Klass, 1984):

$$C_6H_{12}O_{6\,(aq)} \rightarrow CH_{4\,(g)} + 3CO_{2\,(g)} \tag{14}$$
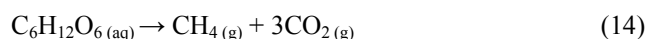
The methane from anaerobic digestion by anaerobic microorganisms can be used as fuel gas and be converted to generate electricity (Holm-Nielsen *et al.*, 2009). The residual biomass from anaerobic digestion is further processed to make fertilizers. Due to the absence of lignin and lower cellulose, microalgae exhibits good process stability and high conversion efficiency for anaerobic digestion (Singh and Gu, 2010). Frigon *et al.* (2013) screened microalgal strains for the production of methane, which was obtained from anaerobic digestion of microalgae. The strains *Scenedesmus* sp., *Isochrysis* sp. and *Scenedesmus dimorphus* displayed the best methane yield, with 410, 408 and 397 mL of CH_4/g of total volatile solid, respectively (Table 8). Thermal pretreatment using an autoclave at 120°C for 40 min
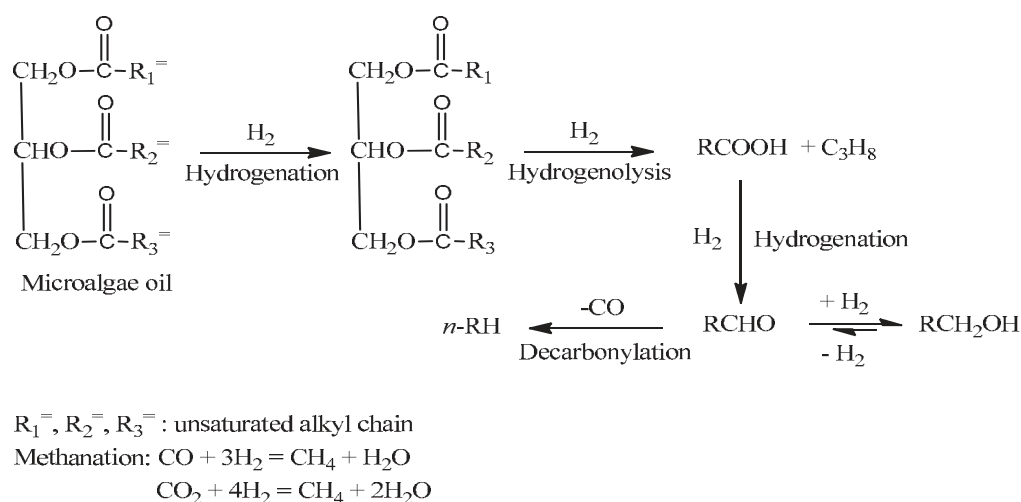

$R_1^=$, $R_2^=$, $R_3^=$: unsaturated alkyl chain
Methanation: $CO + 3H_2 = CH_4 + H_2O$
$CO_2 + 4H_2 = CH_4 + 2H_2O$

Fig. 3. Main reaction pathways for microalgae oil transformation to alkanes over Ni/ZrO_2 catalyst (Peng *et al.*, 2012a).

Table 8. The methane production from microalgae (Frigon *et al.*, 2013).

Strain	TVS[a] (g/kg)	Methane production (mL/g TVS)
Botryococus braunii	153	343
Botryococus braunii	240	370
Chlamydomonas debaryana	138	302
Chalamydomonas sp.	143	333
Chlorella sorokiniana	255	283
Chlorella sorokiniana	218	331
Chlorella vulgaris	200	361
Chlorella vulgaris	254	263
Chlorella sp.	233	309
Chlorella sp.	290	302
Isochrysis sp.	305	408
Micractinium sp.	215	360
Nannochloropsis gaditana	263	228
Neochloris oleoabundans	189	308
Porphyridium aeruginosa	184	352
Scenedesmus dimorphus	246	397
Scenedesmus sp.	234	258
Scenedesmus sp.	330	410
Scenedesmus sp.	210	306
Thalassiosira weissflogii	133	265

[a] TVS is referred to total volatile solid.

was conducted to treat *Chlorella vulgaris* and *Scenedesmus* sp. to enhance carbohydrate solubilization, resulting in the enhancement of anaerobic digestibility and 21–50% methane production compared with untreated microalgae (Mendez *et al.*, 2014). Alzate *et al.* (2014) reported that the anaerobic digestion of lipid-extracted *Nannochloropsis* sp. exhibited higher CH_4 production rates than its non-extracted counterpart. Moreover, thermal pretreatment supported a CH_4 productivity enhancement of 40% for the non-extracted *Nannochloropsis* sp. and 15% for the lipid-extracted *Nannochloropsis* sp. As a result, it appears that the pretreatment and lipid removal are important processes to enhance the yield of methane production.

As indicated above, microalgae are a promising source for biofuel production. They are also used for the generation of other products. The cost of producing microalgae-based biofuel can be compensated for by earnings received from other co-products of the microalgal biomass. The integrated production of microalgae-based biofuels can recover energy consumed in bioenergy production processes, meaning that the final products in the form of biodiesel, ethanol and methane can compensate for their energy input or loss (Zhu, 2014). As a result, a hybrid microalgae refinery is more profitable compare to only a product-based or energy-based biorefinery. The challenge lies in the identification of suitable species and in minimizing the costs of production (Singh and Gu, 2010). The use of microalgae presents numerous routes to the integration of raw materials, processes and products to create a hybrid biorefinery, as presented in Fig. 4.

STRATEGIES FOR COMMERCIALIZATION OF MICROALGAE-BASED BIOFUELS

There are five key strategies for successful microalgae

biofuel commercialization, including (1) faster, (2) fatter, (3) cheaper, (4) easier and (5) fractionation marketing approaches to help producers reduce costs and accelerate the commercialization of microalgae-based biofuels (Thurmond, 2009). First, faster is a primary strategy for most microalgae-based biofuel producers, who search for microalgae species possessing a high oil content and a quick growth rate for biofuel production. Microalgae with a high oil content, such as *Botryococcus braunii*, grow slowly and can be harvested only a few times a week, while algae with a lower oil content, such as *Dunaliella* sp. or *Nannochloropsis* sp. (20-40% oil content), will grow more quickly and can be harvested daily or a few times a day. For this reason, most algae research and development projects and pre-commercial projects consume algal strains with 20–40% oil content. Second, for fatter, microalgae producers are especially interested in using microalgal species with a high triglyceride oil content for biofuel production, resulting in a significant reduction of capital and operating costs and savings for systems twice their size using species with a lower oil ontent. As a result, signification innovation and improvement are the challenges for microalgae producers to lower costs to enter biofuel markets. Next, in view of economic processes for the commercialization of microalgae-based biofuel and ease of implementation, cheaper and easier processes are the next two important strategies. The estimated costs to produce microalgae oils and microalgae biodiesel range from $9 to $25 per gallon in ponds and from $15 to $40 in photobioreactors. Because several sub-sets of systems (i.e., harvesting, drying, pretreatment, extraction, etc.) are required, a reduction in the number of steps in microalgae-based biofuel production is necessary to provide easier, better and lower cost systems. A crucial economic challenge for microalgae producers is to discover low cost oil extraction
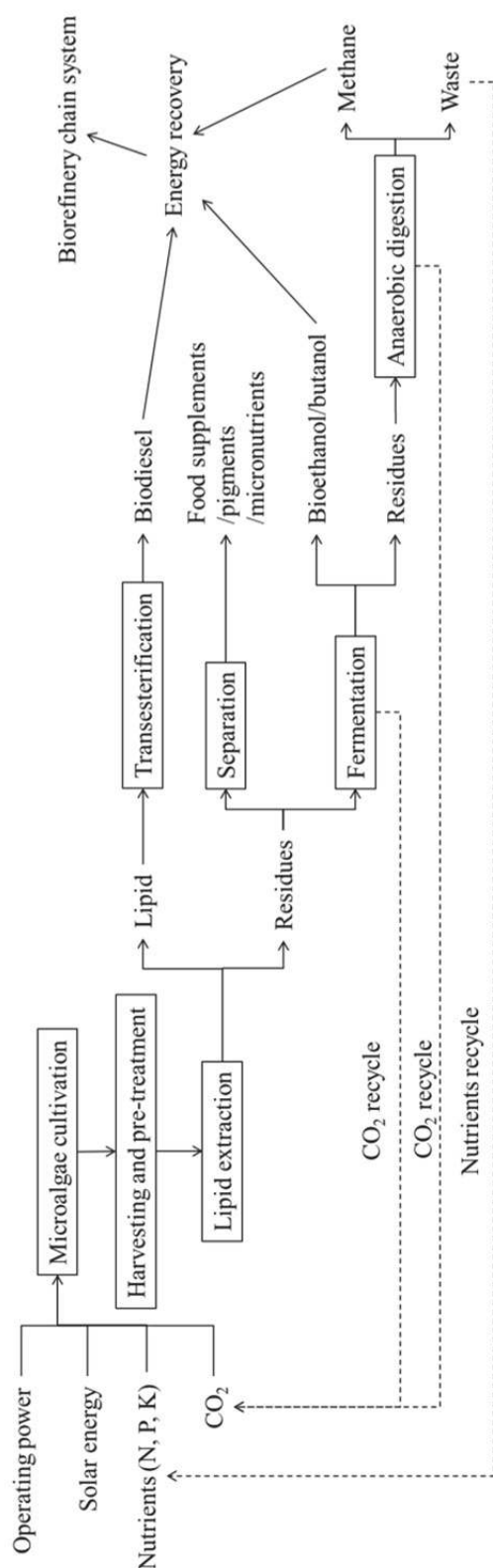
Fig. 4. Schematic flow of a microalgae hybrid biorefinery chain.

and harvesting methods. Extraction systems have estimates of up to $15 per gallon of oil produced, depending on the extraction method, which can be less than cost effective (Singh and Gu, 2010). For example, the combination of harvesting, pretreatment and extraction system technology into a single process can reduce system complexity and costs for microalgae producers. Finally, the co-production of some more valuable fractions and their marketing is also important to success. This biomass fraction of microalgae contains valuable proteins, chemicals or molecular compounds that can be used to produce animal feeds, green plastics, detergents, cleaners and biodegradable polymers and can be sold at a premium price over traditional petroleum-based products. These biomass co-product marketing strategies will be crucial to the success of biofuel producers. As a result, a hybrid biofuel refinery concept (Fig. 4) can be implemented profitably for microalgae-based biofuels. CO_2 and nutrients can be recycled for microalgae cultivation and thus help in carbon sequestration. The biofuel part and other valuable products can be co-generated to make the commercialization process a profitable venture.

Currently, a commercial-scale microalgae-based biofuel industry is still not available due to the high cost deriving from start-up and running operations, maintenance and management (Zhu and Ketola, 2012). However, this cost might be reduced if microalgal productivity and/or lipid concentration is substantial. This industry might become more attractive if substantial subsidies or tax breaks are made available. Thus, the future of microalgae-based biofuels looks bright and promising. Additionally, a certain amount of research and innovation is still demanded, which can include research and development programs focusing on solutions for future energy concerns and on making economically practical biofuels.

CONCLUSIONS

Microalgae have recently received growing attention due to their potential for CO_2 capture and utilization in renewable energy. The use of microalgae exhibits a number of advantages over the use of other plant feedstock, including (1) high photosynthetic conversion, (2) rapid production, (3) high capacity to produce a wide variety of biofuel feedstock, (4) high capability for environmental bioremediation, such as CO_2 fixation from the atmosphere/flue gas and water purification, and (5) non-competitiveness for land with crops and food. In addition, the net CO_2 emission is assumed to be essentially zero if CO_2 released from the microalgae-based biofuel can be recycled and reused for the cultivation of microalgae. As a result, these advantages and potentials make microalgae suitable candidates to solve CO_2 reduction and energy issues.

For the cultivation of microalgae, the cultivation conditions, including water media at the adequate pH and temperature, nutrients and CO_2 dosed in a controlled manner in the presence of sunlight, are a concern. The cultivation in closed photobioreactor systems is more promising than that in open ponds or raceways for meeting the need of biofuel industries.

Pretreatment, especially cell disruption, prior to solvent extraction is necessary to effectively recover the lipid. However, it should be noted that some of the cell disruption methods require a large energy input that may lead to a negative energy balance. For extraction, the selected technology should be efficient (both in terms of time and energy), non-reactive with the lipid, relatively cheap (both in terms of capital cost and operating cost) and safe. Notably, the lipid extraction yield is influenced by the techniques and operating conditions of cell disruption and extraction, which is also related to the microalgae strain. As a result, combination techniques, instead of a single method, are proposed by researchers.

The biofuels obtained from microalgae are not only biodiesel but also biohydrogen, biomethanol, bioethanol, biobutanol and biomethane. These products are conventionally produced using transesterification, fermentations, liquefaction, etc. A hybrid biofuel refinery concept, rather than an individually product-based or energy-based biorefinery, can be implemented profitably for microalgae-based biofuels through recycling of CO_2 and nutrients. The biofuel part and other valuable products can be co-generated to make the commercialization process a profitable venture.

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