



Current status and outlook in the application of microalgae in biodiesel production and environmental protection

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Microalgae have been currently recognized as a group of the most potential feedstocks for biodiesel production due to high productivity potential, efficient biosynthesis of lipids, and less competition with food production. Moreover, utilization of microalgae with environmental purposes (CO₂ fixation, NO_x, and wastewater treatment) and biorefinery has been reported. However, there are still challenges that need to be addressed to ensure stable large-scale production with positive net energy balance. This review gives an overview of the current status of the application of microalgae in biodiesel production and environmental protection. The practical problems not only facing the microalgae biodiesel production but also associated with microalgae application for environmental pollution control, in particular biological fixation of greenhouse gas (CO₂ and NO_x) and wastewater treatment are described in detail. Notably, the synergistic combination of various applications (e.g., food, medicine, wastewater treatment, and flue gas treatment) with biodiesel production could enhance the sustainability and economics of the algal biodiesel production system.

Keywords: microalgae, biodiesel, CO₂ capture, biological DeNO_x, wastewater treatment

INTRODUCTION

Current trends in energy supply and use are simply unsustainable – economically, socially, and environmentally (International Energy Agency, 2013a). Fossil fuels are at the core of energy demand in the transport and electricity generation sectors and account for most – over 80% – of global total primary energy supply (TPES) (International Energy Agency, 2013b). After the combustion of fossil fuels, fossil fuel flue gas can contain hundreds of different compounds, such as CO₂, nitrogen oxides (NO_x), sulfur oxides (SO_x), H₂O, O₂, N₂, unburned carbohydrates (C_xH_y), CO, heavy metals, halogen acids, and particulate matter (PM) (Simoneit et al., 2000; Van den Hende et al., 2012). Growing energy demand for fossil fuels comes from worldwide economic growth and development resulting in the sharp accumulation in CO₂ and NO_x emissions, responsible for the majority of greenhouse gas (GHG) emissions worldwide, which bring some irreversible changes to the climate system including global warming. Without decisive action, energy-related GHG emissions will be more than double by 2050 (International Energy Agency, 2013c). This issue has led to the expansion of research and development on alternative energy with renewability and sustainability.

In recent years, renewable energy sources (e.g., hydro, bioenergy, solar, wind, so on) have received lots of attention and have been used by different nations to reduce consumption of fossil fuels and GHG emissions. Based on the report from International Energy Agency in 2013, bioenergy (biomass and biofuels) currently account for 10% of TEPS compared with hydro energy 2.3% and others 1% (geothermal, solar, wind, heat). Compared with other forms of renewable energy, biofuels allow energy to be chemically stored, and also be used in existing engines and

transportation infrastructures after blending to various degrees with petroleum diesel (Miao and Wu, 2006b). Based on these evidences, biofuels especially biodiesel will play a more crucial role as an alternative renewable energy in the near future to further diversify the global energy sources and gradually replace the fossil fuels.

The feedstock selection for biodiesel production has evolved from the edible vegetable oils to the non-edible oils, and now focuses on microalgae (Amaro et al., 2011). Microalgae are microscopic photosynthetic organisms that distribute in a wide range of environmental conditions, not only aquatic but also terrestrial. It has been reported that using microalgae for biodiesel production has lots of advantages (Ginzburg, 1993; Dote et al., 1994; Hu et al., 2008; Wang et al., 2008). From inherent characteristics of the alga cell, they have much higher growth rate than other biodiesel feedstock. Many microalgae species have high lipids content or can be induced to accumulate substantial quantities of lipids. Under natural growth conditions, phototrophic microalgae require primarily three components to produce biomass, i.e., water, CO₂, and sunlight (Nigam and Singh, 2011). They absorb sunlight, and assimilate carbon dioxide from the air and nutrients from the aquatic habitats (Singh et al., 2011b). They can live in a variety of environmental conditions (freshwater and marine commonly), thus requiring much less land area. Moreover, they can remove CO₂ and NO_x from industrial flue gases by biofixation and be applied to treat wastewater by removal of NH₄⁺, NO₃⁻, and PO₄³⁻. In addition, algae biodiesel contains no sulfur and performs as well as petroleum diesel, while reducing emissions of PM, CO, hydrocarbons, and SO_x.

Microalgae are currently being pursued as the superior raw material for biodiesel production, more and more researches have been reported for all stages of microalgae biodiesel process chain. Biofuel derived from microalgae is encouraged by policy measures and its production is growing all over the world. However, biofuels cannot compete economically in market due to its higher expense in comparison with fossil fuels. One promising way to make algal biofuel production more cost-effective is to couple environmental pollution control, including biological fixation of GHG (CO₂ and NO_x) and wastewater treatment. The objective of this review is to give an overview of the current status of microalgae use for biodiesel production, covering upstream (strain selection, cultivation, biomass harvesting, and drying) to downstream processes (lipids extraction, biodiesel conversion techniques). Then, several environmental benefits (e.g., CO₂ sequestration, NO_x, and wastewater treatment) from microalgae and its combination with biodiesel production are presented. It is expected to provide a useful reference for revealing the potential and prospect of microalgae biofuel industry, and propelling the cost-effective and sustainable microalgae biodiesel industrialization.

THE OVERALL MICROALGAE BIODIESEL PROCESS CHAIN OPERATION

Recent investigations have indicated that microalgae biodiesel could be used as alternative fuel in conventional diesel engine; by this way, exhaust emission values can be improved. Tuccar and Aydin (2013) identify availability of microalgae biodiesel in diesel engines. Based on observation for fuel properties of pure microalgae biodiesel and its blends with diesel fuel, microalgae biodiesel satisfies European Biodiesel Standards (EN 14214) except its low cetane number. However, its low cetane number can be compensated by mixing microalgae biodiesel with diesel fuel. There are also decreases in CO and NO_x emission when using microalgae biodiesel. Microalgal biodiesel production mainly comprises six steps including strain selection, cultivation, biomass harvesting, drying, lipids extraction, and transesterification. Recently, renewable fuels can be produced through the refining of a liquid biocrude produced through the thermochemical conversion of biomass, either through hydrothermal liquefaction (HTL) or pyrolysis (Neveux et al., 2014; Xu et al., 2014).

STRAIN SELECTION

Microalgae are prokaryotic or eukaryotic photosynthetic microorganisms that are incredibly diverse in natural environment (typically in freshwater and marine systems). It has been estimated that about 200,000–800,000 algal species exist, of which only about 35,000 species have been described (Ebenezer et al., 2012). Note that microalgae strain selected, as the first and important step of algae-based biodiesel industry (Scott et al., 2010), determine the corresponding nutrients and cultivation unit available for their growth. During the past decades, lots of research effort have been focused on extensive collections of microalgae strains optimal for biodiesel production.

Microalgae used as biodiesel feedstock should ideally show, as mainly selective criteria, high biomass productivities and efficient biosynthesis of lipids. Fast growth promotes high biomass productivity, which consequently increases yield per harvest volume in a

certain period (productivity) and decreases cost (El-Sheekh et al., 2013). The lipid content varies among strains. Since biodiesel is produced by the transesterification of lipids (mainly triglycerides) with methanol (or other alcohol), high lipid content is one of the desirable features for using microalgae as biodiesel feedstock (Xu and Hu, 2013). High specific growth rate depends on cell proliferation and it does not reflect the microalgae specific capacity for producing and storing lipids (Nascimento et al., 2013). Generally, high oil-producing microalgae species often couple with reduced growth rate. For instance, *Chlamydomonas* sp. showed the lower special growth rate ($\mu = 0.3$) than *Chlamydocapsa bacillus* ($\mu = 0.75$), but the lipid content was higher (15.07 versus 13.52) (Nascimento et al., 2013). Species selection should be made according to the desired products. Lipid productivity, that is the mass of oil produced per unit volume of the microalgal culture per day, depends on the algal growth rate and the oil content of the biomass (Chisti, 2007). Thus, volumetric lipid productivity of the microalga, not the single parameter (lipid content or growth rate), can be the mainly selective criterion for biodiesel production. Biomass productivities, lipids content, and productivities of some reported representatives, freshwater and marine microalgae species for biodiesel production, were summarized in **Table 1**. For example, some potential microalgae reported for biodiesel production were *Chlorella*, *Scenedesmus*, and *Botryococcus braunii*, which possesses oil levels between 20 and 50%, along with favorable productivities (as shown in **Table 1**) (Bogen et al., 2013; Nascimento et al., 2013; Wang et al., 2013) and *Chlorella* appears in particular to be a good option for biodiesel production (Mata et al., 2010). Additionally, the marine microalgae (e.g., *Dunaliella* and *Nannochloropsis*) are more prone to mass product, a high salinity prevents extensive contamination, while allowing sea water to be directly used instead of depleting fresh water resources.

Furthermore, one of the important characteristics of microalgae strains for biodiesel production is the fatty acids' profiles of

Table 1 | Biomass productivities, lipids content, and productivities of different microalgae strains (Mata et al., 2010; Bogen et al., 2013; Nascimento et al., 2013; Wang et al., 2013).

Microalgae species	Biomass productivity (g/L/day)	Lipid content (% dry weight biomass)	Lipid productivity (mg/L/day)
<i>Ankistrodesmus falcatus</i>	0.34	16.49	56.07
<i>Ankistrodesmus fusiformis</i>	0.24	20.66	49.58
<i>Botryococcus braunii</i>	0.25	44.97	112.43
<i>Botryococcus terribilis</i>	0.20	49.00	98.00
<i>Chlamydomonas reinhardtii</i>	0.24	22.10	
<i>Chlamydomonas</i> sp.	0.24	15.07	36.17
<i>Chlorella vulgaris</i>	0.73	28.07	204.91
<i>Dunaliella tertiolecta</i>	0.098–0.12	16.7–71.0	
<i>Monoraphidium contortum</i>	0.307	22.20	
<i>Nannochloropsis</i> sp.	0.17–1.43	12.0–53.0	37.6–90.0
<i>Scenedesmus obliquus</i>	0.16	16.73	26.77
<i>Tribonema minus</i>	0.170	50.23	

Marine microalgae strains are indicated by bold text.

the microalgae cell. The total lipids composition of microalgal biomass is composed of a large variety of chemical fractions, ranging from triacylglycerols (TAGs) to sterols (Pruvost et al., 2009), not all of which are equally well suitable for biodiesel production (Bogen et al., 2013). Lipids containing TAGs and fatty acids (saturated and monounsaturated fatty acids or their derivatives) are preferred compounds because they can be easily converted into biodiesel via transesterification (Chisti, 2007). Special attention should be taken to the polyunsaturated fatty acids with four or more double bonds (C20:4, C20:5), which are susceptible to oxidation during storage and this reduces the acceptability as biodiesel (Wang et al., 2013). Free fatty acids (FFAs) can be used as a feedstock for biodiesel production using an acidic catalytic system. The fatty acids' compositions of oleaginous microalgae (range from C14:0 to C20:0) were summarized in **Table 2**. The fatty acids' compositions of microalgae species most reported as promising biodiesel feedstock are composed by high proportion of palmitic acid (16:0), palmitoleic acid (16:1), stearic acid (18:0), and oleic acid (18:1) (as shown in **Table 2**) (Bogen et al., 2013; Nascimento et al., 2013; Wang et al., 2013). In addition, many reports described that both lipids content and fatty acids profile are affected by cultivation conditions and vary during different growth phases. Especially, nitrogen deficiency and salt stress could stimulate the accumulation of neutral lipids in *Chlorella* due to the modification of the metabolic pathways related to lipids accumulation (Zhang et al., 2013; Chen et al., 2014). Neutral lipids production was strongly induced in *Monoraphidium contortum* by nitrogen deficient conditions, and the fatty acids profile shifted towards a pronounced increase of C18:1 and C16:0 fatty acids (Bogen et al., 2013). The TAGs content of the neutral lipids fraction of *Nannochloropsis* sp. F&M-M24 was increased from below 5% under nutrient sufficiency to about 95% under nitrogen starvation (Biondi et al., 2013).

Besides the above three factors, the ideal algal strains for biofuel production should also have characteristics as follows: (1) easy to biomass harvest and further process, (2) weak response to environmental disturbances such as temperature, and (3) accessible to metabolic engineering strategies (Chisti, 2007; Bogen et al., 2013). When the wild species available in formal collections are

not feasible for commercial production of biodiesel, the application of genetic methods in microalgae to develop organisms optimized for high productivity and energy value can be taken into account. However, the progress in the genetic engineering of algae was extremely slow until recently. Currently, methods successfully used for transformation have been fulfilled for the diatom *Phaeodactylum*, the green alga *Chlamydomonas*, and the cyanobacteria *Synechococcus* and *Synechocystis* (Pulz and Gross, 2004). All of these makes it impossible to develop a number of transgenic algal strains toward the synthesis of preferred products (Singh et al., 2012). Moreover, these promising advances should be viewed with caution because transgenic algae potentially pose a considerable threat to the ecosystem and thus will most likely be banned from outdoor cultivation systems and otherwise be under strict regulation (Pulz and Gross, 2004).

CULTIVATION

Cultivation conditions

In general, there are four major types of cultivation conditions for microalgae: photoautotrophic, heterotrophic, mixotrophic, and photoheterotrophic (Chojnacka, 2004). Under photoautotrophic cultivation, microalgal cells can trap light energy as the energy source and assimilate CO₂ as the carbon source (Yang et al., 2000). Heterotrophic utilize organic compounds (e.g., glucose, acetate, glycerol) as both energy and carbon source in total darkness, whereas light is required to use organic compounds as carbon source for photoheterotrophic process. Mixotrophy is broadly defined as a growth regime in which CO₂ and organic carbon are simultaneously assimilated, both respiratory and photosynthetic metabolism operating concurrently (Lee, 2007). It was reported that only photoautotrophic cultivation is technically and economically feasible to culture microalgae in commercial scale, typically at outdoor environment where sunlight is abundant and free (Lam and Lee, 2012). Carbon constitutes half of the weight of the biomass, it is necessary to supply large amounts of carbon in the form of carbon dioxide due to its low price. Moreover, photoautotrophic microalgae are able to capture CO₂ from flue gases, an added advantage to the culture system. However, photoautotrophic method has its limitation especially in countries where

Table 2 | Fatty acid profiles (% of total fatty acids) of different microalgae strains under regular phototrophic growth conditions (Bogen et al., 2013; Nascimento et al., 2013; Wang et al., 2013).

Microalgae strains	Fatty acid composition (% of total fatty acids)										
	C14:0	C15:0	C16:0	C16:1	C16:2	C17:0	C18:0	C18:1	C18:2	C18:3	C20:0
<i>Ankistrodesmus falcatus</i>	1.07		30.23	0.47		0.48	2.72	24.79	2.00	26.86	
<i>Ankistrodesmus fusiformis</i>	2.02		26.95	0.25		0.20	2.10	19.61	12.23	26.50	
<i>Botryococcus braunii</i>	0.73		7.17				1.59	77.88	5.16	5.34	
<i>Botryococcus terribilis</i>			35.22				3.12	39.74	5.02	7.22	
<i>Chlamydomonas reinhardtii</i>			14.30	4.10	11.40		2.40	26.30	17.60	10.40	
<i>Chlamydomonas</i> sp.	1.61		50.77	0.28		1.81	11.54	13.77	3.93	2.76	
<i>Chlorella vulgaris</i>	0.63		40.31	3.16		0.51	8.01	29.89	8.54	1.57	
<i>Monoraphidium contortum</i>			24.30	3.40	3.20		3.80	38.50	8.00	5.00	1.50
<i>Scenedesmus obliquus</i>	1.06		52.07				7.48	21.46	4.60	2.83	
<i>Tribonema minus</i>	6.85	0.56	28.35	50.65	1.55		1.02	2.96	0.71		

sunlight intensity is not always suitable for photoautotrophic culture available throughout the year. The introduction of sufficient natural or artificial light to allow massive growth and dense populations is the main objective and a limiting factor of the cultivation (Perez-García et al., 2011)

Alternatively, heterotrophic cultivation provides an immediate solution to this problem as some of the microalgae strains can grow under dark environment. Relatively high lipids yield and biomass productivity can be attained through heterotrophic cultivation mode. Heterotrophically cultivated *Chlorella protothecoides* has been shown to accumulate as much as 55% of its dry weight as oil, compared to only 14% in cells grown photoautotrophically (Miao and Wu, 2006b). This phenomenon indicates that heterotrophic culture provides an absolutely good opportunity for large-scale production by using conventional fermentation bioreactor. Nevertheless, this mode of cultivation possesses a number of drawbacks that need to be resolved: (1) the limited number of available heterotrophic algal species, (2) potential contamination by bacteria, (3) inhibition of growth by soluble organic substrates at low concentrations, and (4) the inability to produce some light-induced products, such as pigments (Chen, 1996). The former three problems can be resolved by screening microalgae strains, which have strong pollution tolerance and are able to grow in heterotrophic condition and adapt to a wide range of organic carbon. The limited light-induced products can be overcome through the following two ways: (1) Mixotrophic cultivation. For mixotrophic culture, microalgae are live either photoautotrophic or heterotrophic pathway depending on the concentration of organic compounds and light intensity available. Heterotrophic metabolism can obtain higher biomass but light-induced products are very low, whereas adequate lighting is conducive to the synthesis of light-induced product. Therefore, the artificial control of photoautotrophic and heterotrophic ratio (Ogbonna et al., 2002), such as modulating light intensity, increasing CO₂ supply, and reducing the addition rate of organic carbon to obtain higher biomass and light-induced products. (2) The two-step culture methods, namely, the first heterotrophic or mixotrophic cultivation is to obtain higher biomass, and then transferred to autotrophic conditions, where light induced to produce required metabolite products.

Bioreactors

Open systems (e.g., raceway ponds) and closed photobioreactors (PBRs) are two major types of microalgae culture systems. The shallow raceway pond, in which the suspension is mixed with a paddle wheel, is the most widely used among the prevailing microalgae culturing devices, because it is relatively easy and cheap to construct and operate (Chaumont, 1993; Doucha and Lívanský, 2006). Currently, over 90% of world microalgae biomass production is realized in large raceway ponds. However, the low biomass productivity at field level is its fatal drawback considering the competition of land for traditional crops when commercial developments of microalgae biofuel are promoted (Chen et al., 2013). They are strongly limited by contamination (by other algae, bacteria), the degree of which depends on climatic conditions (for example, it is very difficult to maintain an open algal culture in the tropics during the rainy season).

Because of the aforementioned drawbacks of open cultures, there are many different PBR designs, which are proposed for biofuel production. To develop an efficient PBR, it is necessary to consider various factors such as suitable construction materials, hydrodynamics, efficient mixing, mass and light transfer, heating/cooling, CO₂ supply and oxygen removal, etc. These factors also highly correlate to each other for the productivity of PBR (Yoo et al., 2013). For example, the tubular PBRs possess two regions, light-illuminated surface and dark region inside of reactor. For efficient light transfer to microalgal cells, mixing is required to circulate cells between dark and bright region of PBR in addition to optimization of light path length and shape of PBR. Note that, apply this PBRs system to indoor and outdoor conditions for long-term cultivation and evaluate its performance. PBRs appear in different common configuration: tubular reactors, flat plate reactors, and column reactors (bubble or airlift columns). The main advantages and limitation of open ponds and PBRs are summarized in **Table 3** (Singh et al., 2011b; Pires et al., 2012; Arbib et al., 2013). Compared to open ponds, PBRs have several advantages (Moazami et al., 2012; Arbib et al., 2013): (1) PBRs are closed to the atmosphere and protect the cultivated alga to some extent (note that by being closed, PBR are less prone, but not immune, to contamination); (2) growth parameters (e.g., temperature) can be better controlled; (3) due to a higher surface-to-volume (S/V) ratio, PBRs allow to reach higher volumetric productivities and cell concentrations; (4) closed systems eliminate or strongly reduce evaporation; (5) since PBRs have not been engineered to the extent of other bioreactors in commercial use, there are rooms for improvement. Although many different PBR designs have been proposed for biofuel production, few of them have been tested at pilot scale, none developed at the (large) scale necessary for a complete and correct evaluation because of high cost and reduced scalability. Thus, the main issues that impact on the reactor's performance (i.e., suitable construction materials, efficient mixing, heating/cooling, CO₂ supply and oxygen removal), although explored at pilot level, still await evaluation at real scale (Borowitzka and Moheimani, 2013). There is a dichotomy, need to be solved in future, between PBRs and open systems as the possible culture solution to be employed in large-scale microalgal biomass production.

BIOMASS HARVESTING

After cultivation, the microalgae biomass have to be separated from its growth medium and recovered for downstream processing. However, the algae grow in dilute suspension (<0.5 kg/m³ dry biomass) and the negative surface charge results in dispersed stable algal suspensions (Sanyano et al., 2013). Most of the oleaginous microalgae species are small-size single-cell microorganism (cell dimension < 30 μm) such as *Scenedesmus* sp. and *Chlorella* sp. (Phukan et al., 2011; Wang et al., 2013). Microalgae harvesting remain a major hurdle to industrial scale processing, and it was estimated to account for 20–30% of the total biomass production cost (Molina Grima et al., 2003). Thus, microalgae harvesting is still an active area for research, being possible to develop an appropriate and economical harvesting system.

When considering commercial-scale processes for dewatering and recovering algal biomass for further downstream processes, a traditional harvesting method may involve up to two steps:

Table 3 | Advantages and limitations of open ponds and PBRs (Singh et al., 2011c; Pires et al., 2012; Arbib et al., 2013).

Production system	Advantages	Limitations
Raceway pond	Easy to construct Relatively cheap Easy maintenance Good for mass cultivation	High evaporative losses High diffusion of CO ₂ to the atmosphere Poor light utilization Requirement of large areas of land Microbial and native algae contamination Poor biomass productivity
Tubular photobioreactor	Relatively high biomass productivities Suitable for outdoor mass culture Less prone to contamination	High initial investment costs Toxic accumulation of O ₂ fouling overheating
Flat plate photobioreactor	Relatively high biomass productivities Large illumination surface area Smaller light path Less prone to contamination Lower power consumption Small accumulation of dissolved oxygen	Scale-up require many support materials and large areas of land Difficult temperature control
Column photobioreactor (airlift and bubble column bioreactor)	High mass transfer capacity Easy to sterilize Low fouling Reduced photoinhibition/photo-oxidation Good mixing with low shear stress	Small illumination surface area High energy usage Construction require sophisticated Materials Decrease of illumination surface area upon scale-up

(1) bulk harvesting (known as primary harvesting) – to separate microalgae from suspension, such as sedimentation, flocculation, and flotation; (2) thickening (known as secondary dewatering) – to concentrate the microalgae slurry after bulk harvesting, such as centrifugation and filtration (Grima et al., 2003; Lam and Lee, 2012; Sharma et al., 2013). An optimal harvesting technique should be independent of the cultured species, consume little energy and few chemicals and not damage the valuable products in the extraction process. Here, we summarize the advantages and disadvantages of conventional techniques (Table 4) (Chen et al., 2011a; Salim et al., 2011; Milledge and Heaven, 2013; Ahmad et al., 2014a). The centrifugation process, whereby solid–liquid separation is driven by a much greater force (gravity) to promote accelerated settling of microalgae cells, can be used for almost all types of microalgae reliably and without difficulty (Pires et al., 2012). However, centrifugal recovery is only feasible if the targeted metabolite is a high-value product because the process is highly energy intensive. In addition, using this technique at a large scale is problematic because of high power consumption, which increases production costs.

Filtration is a physical separation process by filter (membrane), which is characterized by their efficiency, reliability, and safety for the solid–liquid separation. Membrane filtration removes completely debris and microalgae cells from the culture medium, realizing water recycling. Different membrane materials [e.g., polyvinylidene fluoride (PVDF), polyvinyl chloride (PVC), polysulfone membrane (PS)] (De Baerdemaeker et al., 2013; Sun et al., 2013a) and membrane pore size [microfiltration, ultrafiltration (UF)] were tested for algae harvesting, and PVDF was the superior

polymer, and UF showed better fouling resistance (De Baerdemaeker et al., 2013). However, fouling limits the widespread use of membrane separation technology due to the reduction of permeation flux during the separation process. The methods such as optimization of operating conditions, physical and chemical cleaning, new membrane development, or modification of existing membranes have applied to reduce the impact of fouling. Two types of thickening methods (without prior bulk harvesting) – centrifugation and filtration – are too costly and energy intensive for large-scale biofuels production. In this regard, the bulk harvesting technologies as pre-concentration step may play an important role in reducing the energy consumption during the thickening process of microalgae slurry (Uduman et al., 2010).

Flocculation is commonly performed before secondary dewatering processes for reducing the cost of harvesting microalgae. Flocculation can be achieved by various methods and addition of chemicals known as flocculants to counter the surface charge on the algae is widely applied. The inorganic coagulants are usually in the form of aluminum and ferric salts, such as aluminum sulfate, ferric sulfate, and ferric chloride (Grima et al., 2003). Sanyano et al. (2013) reported that the optimum flocculation of marine *Chlorella* sp. was achieved at ferric chloride dosage 143 mg/L, pH 8.1, and settling time 40 min. Harvesting efficiency of *Nannochloris oculata* was 96% when using 0.0016 ng of AlCl₃/cell at pH 5.3 (Garzon-Sanabria et al., 2012). Inorganic flocculants require acidic or alkaline pH for optimal microalgae flocculation. Recently, organoclay as a novel flocculant for rapid and efficient harvesting of microalgae was demonstrated. Lee et al. (2013b) synthesized cationic charged aluminum- and magnesium-backboned organoclays,

Table 4 | Comparison of microalgal harvesting and drying methods (Ahmad et al., 2014a; Salim et al., 2011; Chen et al., 2011a; Milledge and Heaven, 2013).

	Technique	Advantages	Disadvantages
Harvesting	Centrifugation	High harvesting efficiency Suitable for most algae types Rapid separation process Easy to operate	High capital and operational costs Cell damage Difficult bulk harvest
	Filtration	Water and nutrition reuse Wide variety of filter and membrane types available	Fouling Slow process Suitable for large algal cells
	Sedimentation	Low power consumption Low requirement for skilled operators Useful as a pre-concentration step	Algal species specific Slow sedimentation rates Low cell recovery
	Chemical flocculation	Wide range of flocculants available Ease of use	Chemical contamination Removal of flocculants Highly sensitive to pH level Flocculants may be algal species specific
	Flotation	Prone to harvest in mass culture	Algal species specific
Drying	Solar drying	Low capital costs Do not require fossil fuel energy	Slow process Require large areas of land Weather dependent
	Spray drying	Rapid drying process High drying efficiency Direct drying into powder	High capital and operational costs cause significant deterioration of microalgal pigments
	Drum drying	Rapid drying process Relatively low capital costs	Low efficiency
	Freeze drying	Operating under vacuum, protecting some substances easily oxidized (such as lipid) high cell recovery	High capital and operational costs

harvesting efficiencies of oleaginous *Chlorella* sp. are almost 100% at concentrations above 0.6 g/L while maintaining a neutral pH. However, the referred inorganic multivalent metal salts are toxic and expensive when commercial-scale culture of microalgae. The non-toxic organic polymer, such as polyacrylamide copolymers, chitosan, and cationic starch (Oh et al., 2001; Vandamme et al., 2010), have been intensively investigated for large-scale applications, but they are not economical for microalgae due to its higher price. The flocculant residuals in both algal biomass and harvested water are not only negative for later processing but also disadvantages for culture medium recycling.

Some studies reported that flocculation of microalgae can occur spontaneously without the need for chemicals, referred to as autoflocculation, which can be induced by increasing the medium pH (Beuckels et al., 2013). Harvesting efficiency higher than 90% of *Chaetoceros calcitrans* was achieved by adjusting the culture pH to 10.2 using either NaOH or KOH (Harith et al., 2009). However, autoflocculation can only be applied for the limited strains. The electrolytic method also has the potential to separate algae without the addition of chemicals. However, the high power that needs to be input are not easy to scale-up and results in a temperature increase that may damage the system (Kim et al., 2012).

Flotation can capture particles with a diameter of less than 500 nm by collision between a bubble and a particle and the

subsequent adhesion of the bubble and the particle (Chen et al., 2011a). Based on bubble sizes used in the flotation process, the applications can be divided into dissolved air flotation (DAF), dispersed flotation, and electrolytic flotation. 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 strains. The flotation activity of *Spirulina platensis* without flocculant showed a maximum value of 80% after 3 h of settling (Kim et al., 2005). Microalgae stain *Tribonema minus*, which were cultivated for 21 days in 40-L glass panel, were harvested by DAF without any flocculants in laboratory, the recovery efficiency reach up to 95.57% (Wang et al., 2013).

DRYING

The percentage of water contained in algal paste after secondary dewatering should not exceed 50% before oil extraction (Kumar et al., 2010), and extensive drying of microalgae biomass is required for biofuels production as the presence of water interferes with the extraction and/or conversion of algal lipids to biodiesel. There are some common methods for drying microalgae after secondary dewatering: spray drying, drum drying, freeze drying, and solar drying (Richmond, 2008). Solar drying is considered the best and economical method to dry wet microalgae paste. However, solar drying is not feasible in temperate countries where

sunlight at certain time of the year is limited (Sharma et al., 2013). And it requires large land areas for large-scale operations. The other drying methods are not economical and lead to a negative energy balance in producing microalgae biodiesel because of heavy dependency on fossil fuels. The advantages and disadvantages of conventional techniques in drying were summarized in **Table 4**. Thus, it is urgently needed to develop new technologies or approaches to ensure the sustainability of microalgae biofuel industry.

LIPIDS EXTRACTION

For biodiesel production, lipids and fatty acids have to be extracted from the microalgae biomass. Lipids' extraction efficiency is directly related to the overall process efficiency in biodiesel production. Therefore, extraction process is very important before transesterification of lipids takes place. It is necessary to develop efficient and cheaper extraction processes to reach industrial biodiesel production at appropriate costs. The extraction of microalgae lipids is usually performed using chemical solvent such as hexane Soxhlet extraction (Soxhlet, 1879) and mixed methanol–chloroform (2:1 v/v) (Bligh–Dyer method) (Bligh and Dyer, 1959). Hexane Soxhlet extraction is generally used to capture high-quality lipids such as triglycerides and fatty acids, which are easily esterified into biodiesel (Demirbas, 2008; Kanda et al., 2013). The Bligh–Dyer method is useful for extracting oily substances because a wide range of oily components can be isolated from microalgae. These two processes are effective to extract microalgae lipids, but the extraction efficiency is highly dependent on microalgae strains. Chemical solvent has high selectivity and solubility toward lipids and therefore, even inter-lipids can be extracted out through diffusion across microalgae cell wall. Nevertheless, the disadvantages of using chemical solvent are mostly related to their high toxicity toward human and surrounding environment. In addition, it is not sustainable to use *n*-hexane and chloroform since both solvents are conventionally derived from non-renewable fossil fuels.

One alternative to the use of organic solvent is to carry out extractions using the subcritical or supercritical lipids. Subcritical extraction used elevated pressures to keep the solvent as liquid state when the temperature reached above the boiling point, which greatly improved extraction efficiency. Considering ethanol is relatively cheap and safety than hexane and easy recycling, subcritical ethanol extraction was performed in total lipids extraction from microalgae paste. Chen et al. (2011b) applied subcritical ethanol extract lipids from wet microalgae paste of *Nannochloropsis* sp., the optimum extraction conditions were 10% moisture, 40:1 ratio (the solvent to microalgae phase ratio), 135°C, 1.5 MPa, and 50 min, which could produce 90.21% recovery rate of the total lipids. The recovery rate of the total lipid of *T. minus* was 72.82% when the solvent ethanol (95%, v/v) to microalgae (dry weight) ratio (v/w) was 20:1 at 105°C for 100 min (Wang et al., 2013). By using subcritical ethanol extraction, the drying process is not needed, which thus could avoid the energy cost. Note that the effects of various operational parameters including moisture content, solvent (volume) to microalgae (dry weight) ratio, extraction temperature, pressure, and time will be needed to investigate to improve extraction performance of total lipids. Several supercritical fluids mainly

supercritical-CO₂ have received the most interest and applied to microalgae lipids extraction for biodiesel production recently (Mendes et al., 1994; Andrich et al., 2006; Gouveia et al., 2007; Tang et al., 2011; Mouahid et al., 2013). Supercritical-CO₂ extraction is non-toxic and provide non-oxidizing environment to avoid degradation of extracts. Moreover, no separation step is needed since CO₂ is gaseous at ambient pressure (Mouahid et al., 2013). However, high cost of supercritical-CO₂ extraction prevents its development. Hence, a complete analysis is urgently required to compare the feasibility of supercritical lipids and chemical solvent extraction in industrial scale, typically in terms of energy efficiency and cost-effectiveness.

TRANSESTERIFICATION

The most accessible technology reported for biodiesel production currently is transesterification reaction in which triglycerides (lipids compounds) are reacted with short chain alcohol (e.g., methanol or ethanol) in the presence of catalyst and the final reaction products are known as biodiesel [fatty acid methyl esters (FAMES)] and glycerol (by-product) (Lam et al., 2010). The most commonly used catalysts for microalgae lipids transesterification are discussed in the next subsections.

Homogeneous alkaline and acid catalysts

Homogeneous alkaline catalysts (e.g., KOH and NaOH) have been the most commonly used route for biodiesel production as it catalyzes the reaction at low temperature and atmospheric pressure. In addition, high conversion yield can be achieved in short time (minutes), being the most economical way to catalyze the transesterification reaction (Meher et al., 2006; Sharif Hossain et al., 2008). However, formation of soaps in the presence of the FFAs (>1%) and water (>0.06%) will lead to lower biodiesel yield and increase the difficulty to separate biodiesel from co-product (Hidalgo et al., 2013). Therefore, acid catalysts have been proposed to overcome the limitations of high FFAs content as the catalysts are not sensitive toward FFAs level in oil. The commonly used acid catalysts in the transesterification process are H₂SO₄ and HCl. Besides, the acid catalysts can promote esterification (FFAs are converted to alkyl ester) and transesterification simultaneously. Velasquez-Orta et al. (2013) reported that lipids conversion of *Chlorella* sp. was obtained using sulfuric acid as catalyst, and a FAME yield of 92 ± 2% was obtained at a catalyst:lipids molar ratio of 0.35:1.

Based on currently available technologies, combination of both acid and base catalysts (two-step reaction) has been proposed to produce biodiesel from lipids with a high FFAs content (Canakci, 2007; Francisco et al., 2010). Microalgae lipids is initially subjected to acid pre-treatment so that its FFAs level is reduced to less than 1% weight, followed by the second transesterification step performed by using an alkaline catalyst. In a study, biodiesel production from *T. minus* oil was followed by two-step catalytic conversion, the conversion rate of TAGs reached 96.52% and the biodiesel product from *T. minus* conformed to Chinese National Standards (Wang et al., 2013). Nevertheless, high concentrations of the base catalysts are necessary to neutralize the acid catalysts from the first transesterification step, increasing the operational costs.

Heterogeneous catalysts

Heterogeneous catalysis (e.g., CaO, MgO, mixed CaO–Al₂O₃, etc.) has also been developed for biodiesel production. Heterogeneous solid catalysts have several advantages as it is non-corrosive and can be recycled. Furthermore, the catalysts offer facile product separation through filtration and therefore minimize product contamination and number of water washing cycle. However, the use of heterogeneous catalysts can result in low biodiesel conversion yields in comparison with homogeneous catalysts, which may be due to the fact that homogeneous catalysts can dissolve in the bulk liquid while heterogeneous catalysts do not dissolve in the bulk liquid producing mass transfer limitations (Liu et al., 2007; Lam et al., 2010)

Lipases

Recently, enzymatic transesterification using lipases has become more attractive for biodiesel production, as it allows the use of mild reaction conditions, avoids the generation of wastewater, and eases the recovery of high purity glycerol, which could solve downstream processing problems. Lipases can be used to transform lipids with high FFAs amount, consequently the catalytic effectiveness is not affected by FFAs and water in the reactors, which is a limitation for homogeneous alkaline catalysis. However, the high cost of enzyme remains a barrier for its industrial applications. In order to decrease the cost of the process, the enzyme could be immobilized in a suitable carrier and reused. Lipases do not require excessive energy expenditures, and it facilitates its recovery and reuse if the catalyst is immobilized (Arias-Penaranda et al., 2013). In relation to microalgae biodiesel production, there are only a few reports related to transesterification with lipases. The immobilized Novozym 435 has been investigated widely and is reported to exhibit the best performance among different lipases sources (Hernandez-Martin and Otero, 2008; Talukder et al., 2009). Moreover, converting *Scenedesmus incrasatulus* lipids to FAMES by Novozym 435 has come true and a higher FAMES yield can be obtained in a shorter reaction time (Arias-Penaranda et al., 2013).

In situ transesterification

Traditional process to produce biodiesel mainly consists of two separate steps: lipids extraction followed by transesterification. An alternative to the conventional process is the *in situ* transesterification (or direct transesterification) in which the lipids extraction and transesterification carried out in one step was extensively exploration. Thus, *in situ* transesterification, which is directly contacted with chemical solvent in the presence of catalysts to biodiesel, shows promise of both simplifying and reducing the cost of producing biodiesel from microalgae biomass (Lam and Lee, 2012). Chemical solvent plays two significant roles in this process: (1) as a solvent to extract lipids out from microalgae biomass and (2) as a reactant in transesterification reaction. **Table 5** shows a summary of different studies on direct transesterification for biodiesel production from microalgae biomass.

According to the recent report (Sathish et al., 2014), the presence of moisture at greater than 20% by mass in algal biomass significantly decreased biodiesel recovery when using *in situ* transesterification. Consequently, the algal biomass need to be dried prior to the *in situ* transesterification procedure due to the

inhibitory nature of water on the reaction. Three explanations have been commonly presented to describe this inhibitory effect (Sathish et al., 2014): (1) the formation of FAMES is a reversible reaction, therefore, water can hydrolyze biodiesel back to methanol and FFAs, (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 catalysts can be deactivated due to water competing for available protons in the reaction. In recent years, the *in situ* transesterification process was developed to directly convert wet oil-bearing microalgae biomass into biodiesel. Dang-Thuan et al. (2013) reported that *Chlorella vulgaris* ESP-31 biomass (water content of 86–91%, oil content of 14–63%) was pretreated by sonication to disrupt the cell walls and then directly mixed with methanol and solvent to carry out the enzymatic transesterification and achieve over 90% biodiesel conversion.

OTHER APPLICATIONS AND BY-PRODUCTS FROM MICROALGAE

ENVIRONMENTAL APPLICATIONS

CO₂ biofixation from flue gases by microalgae cultivation

Addressing global warming concern is one of the key global challenges and CO₂ acts as a principal GHG effect to contribute the global warming. About 75% of the total anthropogenic CO₂ emissions were derived from fossil fuel burning (Nakanishi et al., 2014). To date, several technologies used to capture CO₂ are physical and chemical absorption, cryogenic distillation, and membranes separation (Abu-Khader, 2006). The capture CO₂ is then transported and stored in geological formations. However, these procedures should only be considered as short-term solutions because they are energy consuming and the capture CO₂ need to be disposed of. Additionally, the CO₂ biofixation by microalgae has drawn much attention as an environmentally friendly CO₂ mitigation strategy. Carbon is a key resource for successful microalgae production as it is the main element of microalgae (36–65% of the dry matter). The diffusion of CO₂ from the atmosphere into microalgae culture is insufficient to support the rapid growth of microalgae (growth velocity less than 5% of its potential capacity) (Benemann, 1993). Therefore, microalgae culture by using the flue gases can reduce GHG emissions, while addressing the microalgae culture carbon supply and leading the production of biomass energy through photosynthesis.

Besides CO₂, flue gases contain many different compounds such as H₂O, O₂, N₂, nitrogen oxides (NO_x), sulfur oxides (SO_x), unburned carbohydrates (C_xH_y), CO, heavy metals, halogen acids, and PM. Several chemical compounds (SO_x, heavy metals, etc.) have shown to be toxic to some microalgae (Lee et al., 2000), but well tolerated by others. Yang et al. (2004) reported low concentrations of bisulfite (0–1 mmol/L) had no adverse influence on the growth of *B. braunii*, but bisulfite was toxic at high concentrations (>1 mmol/L). However, *Scenedesmus dimorphus* grew well (3.20 g/L) with simulated flue gas (15% CO₂, 400 ppm SO₂, 300 ppm NO, balance N₂) (Jiang et al., 2013). To better engineer flue gas-fed microalgal cultures, the effects of all flue gas compounds, tolerance of various microalgae to flue gas compounds, and the interaction of flue gas compounds and microalgae need to be assessed (de-Bashan and Bashan, 2010; Van den Hende et al., 2012).

Table 5 | Comparison of different catalysts for direct transesterification of microalgae biomass.

	Catalysts	Reaction conditions			Microalgae	Alkyl esters (%)	Reference
		Alcohol:oil (mol/mol)	T (°C)	Time (h)			
Acid catalysts	H ₂ SO ₄	56:1	30	4	<i>Chlorella protothecoides</i>	80	Miao and Wu (2006a)
	HCl	56:1	–	2	<i>Chlorella</i> sp.	74	Ahmad et al., 2014b
	HCl	82:1	65	6.4	<i>Scenedesmus obliquus</i>	91	Mandal et al. (2013)
Alkaline catalysts	KOH	12:1	65	0.5	<i>Tribonema minus</i>	96.5	Wang et al. (2013)
	KOH	12:1	65	0.5	<i>Scenedesmus</i> sp.	91.6	Chen et al. (2012)
	NaOH	6:1	65	0.85	<i>Chlorella protothecoides</i>	98.76	Makareviciene et al. (2014)
Heterogeneous catalysts	CaO/Al ₂ O ₃	30:1	50	4	<i>Nannochloropsis oculata</i>	97.5	Umdu et al. (2009)
Lipases	Novozyme 435	6:1	50	24	<i>Scenedesmus incrasatulus</i> CLHE-Si01	71.7	Arias-Penaranda et al. (2013)
	Novozyme 435	–	60	6	<i>Chlorella</i> sp. KR-1	75.5	Lee et al. (2013a)
	Novozyme	8:1	50	6	<i>Oedogonium</i> sp.	92	Haq et al. (2014)

Utilization of CO₂ by microalgae for its growth takes place by two stages: absorption of CO₂ from flue gases by mass transfer and CO₂ fixation by photosynthesis. Note that the ability of CO₂ uptake by microalgae varies dramatically among algal species. It is necessary to select suitable algal candidates to fix CO₂ from actual flue gases under a large collection of algal cultures although it is very time-consuming and technical challenging. Microalgae species reported for biological carbon fixation include *Chlorella* sp. (Doucha et al., 2005), *Scenedesmus* sp. (Liu et al., 2013), and *Dunaliella tertiolecta* (Farrelly et al., 2014). Lam and Lee (2012) developed a pilot-scale microalgal fixation CO₂ system to capture CO₂ from actual flue gas and *Scenedesmus obliquus* was able to tolerate high concentration of CO₂ up to 12% (v/v) with optimal removal efficiency of 67%. In addition, *B. braunii* is appropriate for cultivation with flue gas and lipid productivity reach 20.65 mg/L/day (Yoo et al., 2010). Liu et al. (2013) described a high throughput screening method to rapidly identify microalgae strains that can tolerate high CO₂ condition or flue gases from a large pool of culture collection species.

CO₂ mass transfer is a key factor in cultivating microalgae due to low solubility of CO₂ in water. The CO₂ solubility in water is generally very low, i.e., 1650 ppm at 25°C in pure water. Current methods for CO₂ feeding to algae cultures rely on the sparging of CO₂ directly from flue gases or pure CO₂. However, CO₂ feeding through sparging limits the amount of CO₂ that can be absorbed in the liquid leading to losses to the atmosphere (low carbon utilization efficiency) because of the poor CO₂ solubility in water (Soletto et al., 2008). Also, this system requires a considerable amount of energy for effective contacting of the CO₂ with the liquid phase and achieving high enough CO₂ concentrations in the liquid, resulting in its limitation during scale-up (Schipper et al., 2013).

Absorption of CO₂ by chemical methods based on the use of absorption liquids can be used to stabilize the CO₂ as carbonate and then is fed to algae. One such method is CO₂ capture in

alkaline solution as absorption liquid (Iancu et al., 2012). Because the carbon is introduced into the algae culture in a soluble form, this directly improves the efficiency of CO₂ uptake by the algae compared to direct injection of CO₂. Absorption liquids such as potassium carbonate are cheap and also reduce the energy requirement needed for the sparging of CO₂ into algae cultures (Schipper et al., 2013). However, it is necessary to select algae strains that should be able to grow under alkaline conditions. Hsueh et al. (2007) reported that the algae from alkaline hot spring could grow well over pH 11.5 and 50°C. They enhance the CO₂ mass transfer by fivefold with a high-performance alkaline absorber such as a packed tower and regenerating the alkaline solution with algal photosynthesis.

The structures of PBRs have an important effect on CO₂ fixation efficiency because they affect CO₂ dissolution efficiency and CO₂ utilization efficiency by microalgae (Li et al., 2013). Open-culture systems normally cannot use supplied CO₂ effectively, which easily escaped from the culture media due to the shallow depths and poor CO₂ mass transfer efficiency, and CO₂ fixation efficiency ranged from 10 to 30% (Weissman et al., 1988). Carbon fixation would be increased only when the CO₂ residence time was increased in bioreactors (Cheng et al., 2006). Compared with open-culture systems, closed PBRs could reduce CO₂ losses because they could prolong CO₂ retention time and improve mass transfer efficiency. Ketheesan and Nirmalakhandan (2012) designed an airlift-driven raceway reactor for microalgal cultivation with the maximum CO₂ utilization efficiency of 33%. In order to enhance CO₂ mass transfer rate as well as alleviate dissolved oxygen (DO) accumulation, a novel gas sparger of bubble tank was adopted in a PBR and the maximum CO₂ removal efficiency reached 94% (Chai and Zhao, 2012). Fan et al. (2008) also designed a membrane-sparged helical tubular PBR for carbon dioxide biofixation by *C. vulgaris*. Thus, microalgae CO₂ fixation with closed PBRs was a better choice (Li et al., 2013).

A practical operation in which the flue gas from coke oven of steel plant directly supplied the carbon source for biomass production of the microalgal strain has been demonstrated (Chiu et al., 2011). An isolated thermal- and CO₂-tolerant strain, *Chlorella* sp. MTF-7, was isolated by chemical mutagenesis. The flue gas, which contained approximately 25% CO₂, 4% O₂, 80 ppm NO, and 90 ppm SO₂, generated from coke oven of a steel plant was introduced into the microalgal cultures in a double-set PBR system by suction pump and air was supplied by an air pump. *Chlorella* sp. MTF-7 could be cultured with intermittent flue gas aeration controlled by a gas-switching cycle operation. For intermittent flue gas aeration, the flue gas was supplied in 30-min intervals every hour for 9 h during the day; a gas-switching cycle was performed with a flue gas inlet load for 30 min followed by an air inlet load for 30 min. The intermittent flue gas aeration strategy and high initial density (0.5 g/L) of *Chlorella* sp. overcome the environmental stress induced by high flue gases (CO₂, NO, SO₂) aeration and enhance microalgal growth. Besides, average efficiency of CO₂ removal from the flue gas could reach 60%, and NO and SO₂ removal efficiency was maintained at approximately 70 and 50%, respectively.

Removal of NO_x by microalgae cultivation

Besides CO₂, flue gases contain different NO_x species restricted by legislation and needed to remove in an additional gas treatment step such as chemical reduction and adsorption. NO is the major NO_x species present, comprising about 90–95% (Fritz and Pitchon, 1997). As nitrogen is one of the most important nutrients for algal production, a point of interest is that the NO_x can serve as a nitrogen source for microalgae cultivation and can be metabolized by microalgae compared to the requirement of expensive catalysts or adsorbents (Cant and Liu, 2000). Thus, biological denox (bio-denox) method by microalgae may be a method worthy of promotion for flue gases treatment to reduce NO_x emissions and merits further studies.

NO_x removal efficiencies varied among microalgae strains. Some microalgal species such as *Chlorella* sp., *Scenedesmus* sp. (Jin et al., 2008; Santiago et al., 2010), and *D. tertiolecta* (Nagase et al., 1998, 2001) had shown the possibility to bio-remove NO_x. Nagase et al. (1997) reported that an NO removal yield of approximately 65% by using the unicellular microalga *D. tertiolecta* when a model flue gas [100 ppm (v/v) NO and 15% (v/v) CO₂ in N₂] at a flow rate of 150 mL/min (0.0375 vvm) was supplied to the algal culture at an initial cell density of 0.7 g/L cell dry weight. In addition, when a gas mixture containing 300 ppm NO was treated through the *Scenedesmus* culture containing 5 mM Fe(II)EDTA, a constant level of 80–85% NO removal was achieved for a prolonged period. For microalgal NO-elimination, NO gas is first dissolved in the aqueous phase, after which it is oxidized to nitrite and nitrate coupled with dissolved oxygen and then assimilated by microalgal cell. Some researches have reported that the rate-limiting step in reactor system for NO-elimination is the dissolution of NO into the algae culture (Matsumoto et al., 1997; Niu and Leung, 2010). Attempts have been reported that enhance the solubility of NO gas by using efficient complexing agent like metal-chelated EDTA (Jin et al., 2008) or optimizing the reactor system structure (Nagase et al., 1998, 2001).

Wastewater treatment by microalgae cultivation

In the pilot scale of microalgae culture, the use of huge amounts of potable water is a serious problem, which would compete with land crops and human activities. Large amounts of nitrogen and phosphorus, which can be recovered from wastewater, are also required, but their cost in pure chemical form is high and their sustainability is low (Lardon et al., 2009). Consequently, microalgae production using wastewater as the nutrient source is a potential method, which offers added environmental advantages. The production of microalgae is effective in removing nitrogen, phosphorus, and toxic metals from a wide variety of wastewaters, producing cleaner effluents with high concentrations of dissolved oxygen (Gomez et al., 2013). However, it can not be ignored that there are a large number of endogenous bacteria in a real wastewater system affecting the growth of microalgae.

Cabanelas et al. (2013) used *C. vulgaris* for nitrogen and phosphorus removal from municipal wastewater with the highest removal rates of 9.8 (N) and 3.0 (P) mg/L/day. Other microalgae widely used for nutrient removal from different wastewater streams are *Chlorella* sp. (Gonzales et al., 1997; Cabanelas et al., 2013), *Scenedesmus* sp. (Martinez et al., 2000), and *B. braunii* (Álvarez-Díaz et al., 2013). Due to the complex characteristics of wastewater, the tests of growing algae in wastewater are mostly at laboratory scale. Pilot-scale algae cultivation continues to face many issues including contamination, inconsistent wastewater components, and unstable biomass production (Cai et al., 2013).

Integrated environmental pollution control and biodiesel production by microalgae cultivation

The capital and operating costs of algae-based biodiesel are still higher than petrol diesel, which encumber the development of microalgae biodiesel technology being commercial scale (Singh and Olsen, 2011; Singh et al., 2011a). Recently, some research results suggest that dual-use microalgae cultivation for environmental pollution control (especially wastewater treatment) coupling with biofuel generation is an attractive option in terms of reducing the energy cost, CO₂ emissions, and nutrient and freshwater resource costs (Sun et al., 2013b). Zhou et al. (2012) developed an effective hetero-autotrophic mode for improved wastewater nutrient removal, wastewater recycling, and enhanced algal lipid accumulation with *Auxenochlorella protothecoides* UMN280. The maximal biomass concentration and lipids content reached 1.16 g/L and 33.22% dry weight (DW). And the nutrient removal efficiencies for total phosphorus, ammonia, and nitrogen at the end of the two-stage cultivation were 98.48, 100, and 90.60%, respectively. Similar levels of total lipid content (38.72% DW) have been observed in *Chlorella pyrenoidosa* grown in wastewater from fermentation effluent in Riboflavin process. The study of Li et al. (2011) showed that by the end of a 14-day batch culture *Chlorella* sp. could remove ammonia, total nitrogen, total phosphorus, and chemical oxygen demand (COD) in raw centrate, a highly concentrated municipal wastewater stream generated from activated sludge thickening process, by 93.9, 89.1, 80.9, and 90.8%, respectively. The FAME content was 11.04% of dry biomass, providing a biodiesel yield of 0.12 g-biodiesel/L-algae culture solution. Therefore, further exploration is needed for improved wastewater treatment and

cost-effective microalgae-based biodiesel feedstock production (Zhou et al., 2012).

MICROALGAE FINE BIOLOGICAL REAGENT AND BIOACTIVE SUBSTANCES

Besides biodiesel, various high-value chemical compounds such as pigments, antioxidants, β -carotenes, polysaccharides, vitamins, and biomass can be extracted from microalgae, and they are largely used as bulk commodities in different industrial sectors (e.g., pharmaceuticals, cosmetics, nutraceuticals, functional foods) (Mata et al., 2010). β -carotene, a vitamin A precursor in health food was the first high-value product commercially produced from *Dunaliella bardawil*. The biomass of microalgae as sun-dried or spray-dried powder or in compressed form as pastilles, which can be sold mostly in the human health food market is the predominant product in microalgal biotechnology. The final product of biomass production was also used both in aquaculture and animal husbandry as animal nutrition (Pulz and Gross, 2004). In addition, marine algae are considered as valuable sources of structurally diverse bioactive compounds such as hydrocolloids alginate, agar, and carrageenan. Ngo and Kim (2013) reported that marine algae are rich in sulfated polysaccharides (SPs) such as carrageenans in red algae (*Rhodophyta*), fucoidans in brown algae (*Phaeophyta*), and ulvans in green algae (*Chlorophyta*). These SPs exhibit many health beneficial nutraceutical effects such as antioxidant, anti-allergic, anti-human immunodeficiency virus, anticancer, and anticoagulant activities (Borowitzka, 2013; Lin, 2013).

FUTURE PERSPECTIVE

Nowadays, rapid depletion of fossil fuels and rising GHG emissions has made microalgae as a biofuel source even more compelling. Moreover, lots of researches have demonstrated that microalgae biodiesel is technically feasible. Nevertheless, there is no current industrial production of algae biodiesel in the world. The higher capital and operating costs of microalgae farming compared to conventional agriculture, the non-sufficiently positive energy balance (after accounting for energy requirements for water, mixing, CO₂ and nutrient supply, biomass harvesting and processing), and the not yet established sustainability still prevent the development of microalgae biodiesel technology being commercial scale (Zittelli et al., 2013).

The cost for microalgal biodiesel production may be balanced in the future by the following: first, cost of the raw materials (generally nitrogen and phosphorus sources, enriched CO₂ supply, water) needs to reduce. Biodiesel production from microalgae can be more environmentally sustainable, cost-effective, and profitable if combined with processes such as wastewater and flue gas treatments. In other words, freshwater replace with wastewater, and flue gases were used as a carbon and inorganic nutrient source for culturing microalgae. One option is to locate algal ponds or PBRs near nutrient-rich wastewater streams, or by using feed sources such as anaerobic digester waste effluents. In order to achieve improved wastewater treatment and low-cost algal biomass as biofuel feedstock, an effective hetero-autotrophic mode was developed by Zhou et al. (2012). The culture process was separated into two independent parts. In the first stage, a heterotrophic-dominated mode using organic carbon in “Centrate” (a stream, generated

through dewatering of sludge from primary and secondary settling) was for maximal cell density and lipid accumulation. The algae were harvested by self-sedimentation, and the residual wastewater was reused as medium for cultivation of the same strain. Then, an autotrophic dominated stage where CO₂-rich flue gas during combustion could be sequestered by sparging into an algae PBR can be to further remove nutrients in the recycled wastewater. This strategy creates a process of combining wastewater treatment and CO₂ fixation, while using the energy stored within the waste algal biomass could help to run the production process. Recent studies have shown that it is technically feasible to utilize CO₂ from flue gases as carbon source to culture microalgae. However, latest research on NO_x and SO_x uptake by microalgae has been limited to a few publications. It is still unclear that acidic gases (CO₂, NO_x, and SO_x) in the flue gas uptake pathways by microalgae. In this regard, genetic engineering will have an important role in enhancing the overall life cycle of microalgae that are used as biofuels feedstock. Genetic modified microalgae that have the capability to grow under high concentration of CO₂, able to tolerate other components in flue gases or wastewaters, and could produce high lipid content within their cells may be created. Advancement in this research area is urgently required to bring a significant breakthrough in producing greener and sustainable microalgal biofuels.

Second, continuous technical breakthrough will be required to improve efficiency. For the downstream processes, energy consumption is a big concern in microalga biomass harvesting, and lipid extraction. When considering chemical, physical, and biological harvesting methods, each method has its advantages and limits. Developing hybrid techniques, which make use of all harvesting categories, may be a viable option that is worth exploring. The comparison also highlights the fact that none of the harvesting methods are cost-effective when considering cultivation of microalgae solely for biodiesel production. Hence, it is a necessity to fine by-products that has a higher market value. In addition, direct transesterification and wet lipid extraction/transesterification, which are important innovations in the algae lipid extraction and biodiesel conversion arena, are yet to be discovered to develop the optimum utilization of algal biomass for the commercial production of biofuel.

Finally, the bio-refining of microalgae is to increase the economic benefit for microalgal biodiesel production. The applications of algal biorefinery could possibly range from direct usage of biomass for food to high value-added products such as antioxidants and β -carotenes, and ecological applications such as wastewater and flue gas treatments. Glycerol, one of the main by-products of the transesterification process, can serve as a carbon feedstock for heterotrophic algal growth. Grown on crude glycerol, *C. protothecoides* has delivered biomass and lipid yields similar to growth on glucose (Chen and Walker, 2011; Georgianna and Mayfield, 2012). The cost of microalgal biodiesel could be substantially reduced when coupled with any of the biorefinery-based production strategies.

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