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The potential of microalgae in biodiesel production

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ABSTRACT

In recent years, biodiesel production has grabbed significant attention due to the awareness of fossil fuel exhaustion. Microalgae become interested feedstock candidate of biodiesel production as they have rapid growth rate and high oil content compared to crops. Efforts have been made to increase microalgae productivity and oil content. To investigate the potential of microalgae for biodiesel production, it is essential to knowledge if the microalgae oil is qualified as possible feedstock oil. Moreover, what would be the energy and environmental effect of the production? And whether the production cost would be reasonable? This paper compared the properties of microalgae oil with traditional biodiesel production oils (vegetable oils); the properties of the biodiesel produced from microalgae and vegetable oils; reviewed the net energy ratio (energy output to energy input), GHG emissions, and economic analysis of the process of biodiesel production from microalgae; as well as discussed the factors which would influent the energy, environment, and cost of the process.

1. Introduction

Microalgae have gained extensive interest in current age due to its rapid growth rate and vigorous vitality. They have been utilized as sources of many products including chemicals (vitamins, pigments, antioxidants), oils (omega-3 fatty acids), protein, animal feed (for larval bivalves), and biomass for the production of ethanol and methane [1–4]. Microalgae capable of accumulating high oil content were studied as the alternative of vegetable oils for biodiesel production [5–7]. *Chlorella zofingiensis*, *Chlorella protothecoids*, and *Schizochytrium limacinum* were well-known oil producer as they could accumulate more than 50% oil of the dry body weight [8–11]. The significant advantages of microalgae over agricultural crops are the rapid growth rate and no arable land requirement [6,12]. In addition, carbon sequestration and burning clean (of microalgae biodiesel) are also the attracting aspects of utilization microalgae for producing biodiesel [13–16].

Microalgae as feedstock of biodiesel production have been extensively reviewed [10,13,17–22]. It was explained what were microalgae, why they could be employed, what were the advantages of using microalgae for biodiesel, what types of microalgae (heterotrophic and autotrophic) could be utilized, what was the process (from strain isolation to biodiesel formed), and what were the factors to impact on the process. Any technology entering market from research stage requires feasibility analysis which refers to cost affordability and environmental benefit. To the best of our knowledge, these aspects have been given very little interest. This study reviewed the life cycle assessment (LCA) and techno-economic evaluation of microalgae for biodiesel production and discussed the cause of the difference of the study results.

2. Feasibility of microalgae to biodiesel

2.1. Characteristics of microalgae oil

Generally speaking, it is important that feedstock should have high lipid content, large productivity, and affordable price. However, physical and chemical properties of the feedstock oil are rather essential in biodiesel production as they influent the quality and yield of biodiesel. The properties include fatty acid composition, free fatty acid content, water content, phosphorus content, sulfur content, and saponification value.

2.1.1. Fatty acid composition

The main fractions of feedstock oils or fats are triglycerides (varying from 90% to 98% according to the oil or fat sources) [23,24]. Triglycerides are composed of one glycerol [$C_3H_5(OH)_3$] and three fatty acids (R–COOH) as the major reactive groups, which suggests that fatty acids affect the oil and fat characteristic most. In general, fatty acids include unsaturated (with double bonds) namely mono-unsaturated (one

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double bond,Cn:1) and polyunsaturated (more than one double bonds, Cn:2,3), and saturated (no double bond, Cn:0) fatty acids. The fatty acid composition plays significantly important role in biodiesel qualities as it determines the viscosity, oxidation stability, cetane number (CN) (indicator of ignition quality), cold flow property, flash point, calorific value (also called heat content or energy density), and density of biodiesel. Viscosity indicates the fuel features of spray, mixture formation, and combustion process. High viscosity can cause early injection and increase combustion chamber temperature. Normally, viscosity increases with the increase in the chain length and fatty acid saturation level, while better oxidation stability requires high level of fatty acid saturation [25–27]. CN increases as the increase in chain length and saturation degree of fatty acid [27,28]. Cold flow properties also depend on the saturation level of the feedstock oil in which the higher the saturation level is, the poorer the cold flow property is [29,30]. The flash point will be low when the chain length is short; greater saturation degree gives higher calorific value; and polyunsaturation level seems to be proportion to the density [31].

2.1.2. Free fatty acid (FFA) content

FFA can be described as R-COOH. Alkaline catalytic trans-esterification is the most common industrial biodiesel production route. The presence of FFA in the oil/fat can lead to the increase in the use of catalyst, and complicate the phase separation and product neutralization due to the soap formation (Eq. (1)). In order to avoid soap formation, normally, acid catalytic trans-esterification or acid pretreated alkaline catalytic trans-esterification has to be performed when FFA content is greater than 0.5% (wt/wt) [32–34].

$$RCOOH + KOH/NaOH \rightarrow RCOOK/Na(soap) + H_2O \tag{1}$$

where R represents fatty acid chains.

2.1.3. Water content

Water can cause triglyceride hydrolyzing to FFA, and hence result in soap formation [35,36]. Moreover, the presence of water could also cause emulsions. Therefore, when water content is greater than 0.05% (w/w), water removing step is required [35].

2.1.4. Phosphorus and sulfur content

Phosphorus can damage catalytic converters used in emissions control systems of the vehicles [37]; therefore, phosphorus content in feedstock oil, which will finally transferred to biodiesel, should be controlled to protect the systems. Similarly, sulfur presence can choke catalytic converter up and harm the emission control systems of vehicles. In fact, sulfur content of the current commercial biodiesel is nearly zero. It is the reason that normally in order to decrease the sulfur content in petrodiesel, biodiesel is used to blend with the petrodiesel [35].

2.1.5. Saponification value (SV)

An Index of the average size and weight of fatty acids. Fatty acid methyl esters with carbon chain length from 12 to 20 are considered as biodiesel. The saponification value indicates the chain length of triglycerides. Shorter chain length leads to higher SV [38].

By comparing the feedstock properties, microalgae oils have similar properties as plant seed oils and animal fats (Table 1). Comparing the property of biodiesel produced from microalgae oil, plant seed oil and animal fat (Table 2), it showed that microalgae were potential replacement of crops and animals.

2.2. Life cycle assessment of biodiesel production from microalgae

2.2.1. Energy ratio

Life cycle assessment has been extensively involved in evaluating the energetic and environmental benefits of biofuel production. The assessment normally starts from building-up process, defining boundaries, fixing parameters, and finally calculating energy ratio and greenhouse gas – GHG emissions. The process of microalgae-based biodiesel production majorly includes microalgae cultivation and harvesting, lipid extraction, and trans-esterification. The cultivation can be in open ponds (OP), photo-bioreactor (PBR), or closed fermenting system. The LCA depends on the process selection and the assumptions. Some of the LCA studied on microalgae to biodiesel have been summarized in Table 3.

In most of the LCA studied (Table 3), the process included microalgae cultivation, microalgae harvesting, lipid extraction, and trans-esterification, and few considered the biodiesel distribution part as well. In fact, the distribution parts have little effect on the net energy ratio: NER (energy produced/energy consumed) as it took up only around 0.6% of the total energy input [39–41]. Similarly, it has almost no impact on GHG emission. The parameters including cultivation mode (open ponds, photo-bioreactor, and fermenter), microalgae yield, lipid content in the microalgae, dewater technology (filtration, centrifugation), drying method (solar, steam), lipid extraction efficiency, and trans-esterification efficiency, utilized in LCA have great impact on the studies as well. Lipid extraction and trans-esterification are mature technologies, and generally the efficiencies were assumed to be 90% and 95%, respectively [42,43]. Thus, these two parts are not the great contributors to cause the difference of LCA results.

Open ponds and photo-bioreactor are the most applied system. The two systems can be fed with flue gas which is the power plant waste and rich in carbon dioxide. It is a solution of carbon sequestration and obtaining free carbon source for autotrophic microalgae cultivation system. The advantages of photo-bioreactors are high productivity, small land area requirement, low risk of contamination, avoiding water loss, and less depending on the climate compared to open pond cultivation. The major problem of the system is the high capital and operating cost [44]. Additionally, oxygen is produced during cultivation which can inhibit the growth of microalgae. In photo-bioreactor system, oxygen concentration builds up while cultivation, and thus can cause the low yield of biomass. Unlike photo-bioreactor, the oxygen produced can be spread to atmosphere during the mixing (paddle wheels and CO_2 bubbling) in open pond cultivation system. In fact, the main advantage of pond system is the low energy consumption and cost requirement. The weaknesses of the process are high contamination level, large amount of water loss, climatic dependence (annul average temperature > 15 °C), low biomass concentration which requires large dewatering energy input, and large land demand [45].

Even though, open pond system has its limitation, it is still commercially utilized in nutrient production for animals as it is cost affordable. Study has reported that open pond system (NER = 8.34) had higher net energy ratio than photo-bioreactors (NER = 4.51) to produce the equal amount of microalgae biomass [44]. The calculation was based on that the productivity of open pond and photo-bioreactor were 11 $g/m^2/d$ and 27 $g/m^2/d$, respectively. It indicates that open pond is not compatible with photo-bioreactor on the productivity but it still provides higher energy gain than photo-bioreactor. In the similar cultivation system, obviously, higher the microalgae yield and lipid content provided higher energy gain and GHG emission reduction [46]. 10% increase of lipid content could bring the NER from less than 1 to greater than 1 with other parameters being kept constant [47].

After cultivation, dewatering is normally followed to concentrate the biomass. The dewatering technologies currently applied are flocculation, centrifugation, screening, filtration, floatation, and settling [48,49]. The performance of each dewatering technology had been summarized by Uduman et al. [49]. It revealed that flocculation, centrifugation, filtration, and flocculation followed by flotation were stable and efficient, but centrifugation (8 $kW\,h/m^3$) and flocculation followed by flotation (10–20 $kW\,h/m^3$) required large energy input [50,51]. Filtration (natural or pressured) -screening (0.4–0.88 $kW\,h/m^3$) dewatering technology had shown similar performance as centrifugation but consumed much less energy [52]. In the LCA studies, mostly

Table 1
The biodiesel production feedstock properties.

Feedstock	Saturation level (%)	FFA (%)	WC (%)	PC (ppm)	SC (ppm)	SV (mg KOH/g)	Ref.
Soybean oil	15.34	0.07	0.029	3.7	0.8	195.3	[32,35]
Sunflower oil	9.34	0.04	0.02	< 0.1	0.1	193.14	[25,35]
Palm oil	47.3	0.54	0.049	7.3	1.0	208.62	[35,117]
Canola oil	4.34	0.34	0.085	17.9	5.7	189.80	[25,35]
Corn oil	14	12.22	0.153	< 0.1	10.5	183.06	[25,35,117]
Peanut oil	16	< 2	< 0.5	NA	10	191.50	[117–119]
Cottonseed oil	30.6–29	9.8	0.05	0.5	10	194	[25,120,121]
Coconut oil	68.7	0.07	0.027	2.0	2.7	267.56	[35,117]
Jatropha curcas oil	27.1	1.17	0.073	322.9	3.5	200.80	[35,122]
Poultry fat	29.69	1.7	0.065	209.3	27.2	188.08	[35,123]
Microalgae	12–21	0.45–1.75	0.014–0.021	286.2–339.7	15.4–28.1	160.6–185.82	[35,124]

centrifugation or its combination with screening, flocculation, or filtration were generally employed in order to guarantee the high performance [39,53–56]. The energy input could be reduced when filtration (natural or pressured) -screening is employed in LAC studies.

Drying has to be performed to further eliminate water when the selected extraction method is greatly impacted by the water content of the biomass. Solar drying is the most cost- and energy- economic mode; however, it has large requirement on the land and time, and high dependence on the climate. Thermal drier powered by natural gas or electricity was generally adopted in LCA studies as it is commercially utilized [39,46]. Certainly, the energy input is elevated in thermal drying system comparing to solar drying system.

Microalgae oil extraction is still in lab-scale and no confirmed technology has been practically employed in large scale lipid extraction from microalgae. Organic solvent (hexane) is commercially being utilized in soybean oil extraction. Thus, it is generally chosen to be the technology of lipid extraction from microalgae in LCA studies. However, the microalgae cells are different from vegetable seed cells. The lab study of microalgae lipid extraction is currently performed by using chloroform and methanol [57–59]. It indicates that the efficiency of hexane lipid extraction from microalgae is still questioning and requires evidence to support. There was also report on utilizing bead mill for lipid separation from cells [60]. The mechanism of lipid extraction with bead milling is that: cells are disrupted during bead milling, and thereafter lipid releases from cells and finally flows to the top as it doesn't dissolve in water and has low density than water. In fact, the method should be verified as study has found that solo cell disruption wouldn't lead to the lipid separation [61,62]. A reliable and practical lipid extraction method is highly demanded.

Trans-esterification is the reaction to synthesize biodiesel from lipid and methanol/ethanol with acid or alkaline catalysis. It is practically applied in industrial biodiesel production and has stable conversion efficiency (above 95%). Most of the LCA analysis assumed the efficiency was 95% [54,63]. Energy input calculation of trans-esterification step is considered simple because there are large amount available industrial reports of vegetable oil to biodiesel, which can be directly used for microalgae oil to biodiesel. There is a by-product glycerol in the trans-esterification process. It is generally assigned as a credit and normally the credit is around 2 MJ for every one kilogram biodiesel produced [43,64].

Apart from lipid, there is residual biomass of microalgae which can be allocated as an energy credits. The energy value of the residual

Table 2
The properties of biodiesel produced from different raw materials.

Biodiesel, diesel	Properties							Ref.
	Acid value (mg KOH/g)	Cold filter plugging point (°C)	Density 15 °C (kg/m^3)	Iodine value (g I^2/100 g)	Kinematic viscosity 40 °C (mm^2 /s)	Oxidation stability 110 °C (h)	CN	
Diesel	< 0.005	−6	0.85	1.35	1.3–4.1	17.3	40–55	[125,126]
ASTM D6751[a]	≤ 0.5	NM	NM	NM	1.9–6.0	3.0	≥ 47	[126,127]
EN 14214[b]	≤ 0.5	NM	0.86–0.90	≤ 120	3.5–5.0	6.0	≥ 51	[126,128]
From soybean oil	< 0.5	−4.4	0.89	2.98	4.1	16	50.9	[129,130]
From canola oil	0.13		0.88	66	4.63	44.9	55	[130]
From chicken fat	0.32	3	0.88	76	5.85	NM	NM	[131]
From beef tallow	0.21	10	0.87	45	5.40	8.1–41.0	54.3–64.8	[130,131]
From pork lard	0.20	5	0.87	76	4.96	72	63.6	[130,131]
From microalgae (*Chlorella protothecoides*)	0.29	−13	0.88	112.2	4.43	4.52	NM	[126]
From microalgae (*Nannochloropsis oculata)*	NM	−4.8	0.88	81	4.2	95.7	55.0	[132]
From microalgae (*Phaeodactylum tricornutum)*	NM	−7.8	0.89	114	3.74	NM	47.3	[132]
From microalgae (*Scenedesmus dimorphus)*	NM	−4.6	0.91	184	4.63	5.6	32.9	[132]
From microalgae *(Chlorella protothecoides)*	NM	−0.99	NM	111.75	NM	NM	54.57	[133]
From microalgae *(Chlorella emersonii)*	NM	3.55	NM	114.18	NM	NM	54.24	[133]
From microalgae *(Chlorella salina)*	NM	2.58	NM	117.92	NM	NM	49.93	[133]
From microalgae *(Chlorella vulgaris)*	NM	4.60	NM	135.26	NM	NM	44.00	[133]

NM = not mentioned.
[a] Standards of American Society of Testing and Materials for biodiesel.
[b] European Committee for Standardization for biodiesel.

Table 3
The LCA of biodiesel production from microalgae.

Process	Capacity (ha)	Lipid content (/dry weight)	Biomass productivity	NER (energy produced/consumed)	GHG emissions (g CO_2/MJ energy produced)	Ref.
Cultivation (*Nannochloropsis salina* in PBR) → harvesting → extraction →trans-esterification →transportation & distribution	260	50%	25 g/m^2/d	1.08	−75	[39]
Cultivation (*Nannochloropsis* sp. PBR and OP) → harvesting → extraction → trans-esterification	0.0008	25%	PBR: 25 g/m^2/d OP: 25 g/m^2/d	0.22	NA	[43]
Cultivation (OP) → harvesting → extraction → trans-esterification	400	40%	30 g/m^2/d	NA	−23	[65]
Cultivation (*Dunaliella*in PBR) → harvesting → extraction → trans-esterification	NA	45%	30 g/m^2/d	NA	+15	[64]
Cultivation (*Nannochloropsis* sp. in PBR) → harvesting → extraction → trans-esterification	NA	30.2%	16 g/m^2/d	0.12	NA	[60]
Cultivation (OP and PBR) → harvesting → extraction → trans-esterification	100	20–50%	20–30 g/m^2/d	1.09–1.69	+10–18.7	[46]
Cultivation (OP) → harvesting → extraction → trans-esterification	400	25%	26 g/m^2/d	2.03	−20	[56]
Cultivation (OP) → harvesting → extraction → trans-esterification	2.5	35%	15 g/m^2/d	1.28	+90	[134]
Cultivation (*Chlorella* in OP) → harvesting → extraction → trans-esterification → biodiesel final use	NA	25%	25 g/m^2/d	1.15	+120	[40]
Cultivation (OP) → harvesting → extraction → trans-esterification	10	50%	3 g/m^2/d	0.73	+180	[55]
Cultivation (*Nannochloropsis occulata* in OP) → harvesting → extraction → trans-esterification	80	46%	20 g/m^2/d	0.93	+55.6	[54]
Cultivation (*Nanochloropsis salina* in OP) → harvesting → extraction → trans-esterification	NA	24.3%	NA	0.82	−16.2	[47]
Cultivation (*Scenedesmus obliquus* in OP) → harvesting → extraction → trans-esterification → biodiesel final use	NA	30%	30 g/m^2/d	1.21	+110	[41]
Cultivation (*Chlorella vulgaris* in PBR and OP) → harvesting → extraction → trans-esterification	NA	35%	11 g/m^2/d	1.61	−54.1	[63]

GHG emission: "−" means reduction; "+" means production.
NA: not available.
PBR = photo-bioreactor.
OP = open ponds.

biomass is determined by its final application. Biogas production with the residue was the main stream of LCA studies [40,46,60,65]. It was also reported to produce ethanol from the residue [56]. But the energy credits taken for biogas and ethanol were similar, which was around 11 MJ for per kilogram biodiesel produced.

It is clear that trans-esterification step and energy credits from residual biomass and glycerol were not responsible for the large variation of NER in the studies (Table 3). As hexane extraction is the common selected lipid extraction method in LCA studies, extraction step also has stable energy input requirement. Hence, the LAC results are more sensitive to the selection on the mode of cultivation and the technology of dewatering, the assumption of biomass productivity, and lipid content than other aspects. Plant scale seemed no significant impact on NER which increased around 10 times with 1000 times scale increasing [55]. However, in some extend it revealed that large scales were more energy efficient than small scales [66]. Study showed that the system became energy gain process (NER $\geq$ 1) when plant was scale up to 100 ha [55]. However, it requires a huge cost investment. It would be difficult to put in practice before it is proved to be a profitable project.

The energy input fraction of each part (microalgae cultivation, harvesting, lipid extraction, trans-esterification, biodiesel transportation) out of the total energy input of the process has great variation according to the chosen individual process. Cultivation and trans-esterification processes took 30–50% and 5–10% of the total energy input, respectively. The biggest differences of LAC studies are dewatering and lipid extraction parts. When drying was performed in dewatering part, the energy input of dewatering part (30–60% of the total energy input) became high but that of lipid extraction (around 5% of the total energy input) was low [56,60]. When wet biomass with a dry biomass weight of 15–30% w/w was directly used for lipid extraction, the energy input of dewatering part (1–10% of the total energy input) was low but that of lipid extraction (30–80% of the total energy input) became high [43,47,60]. It is due to that lipid extraction from biomass with high moisture requires higher concentration solvent comparing to the biomass with low moisture in the same weight of dry biomass basis. Consequently, it needs large volume extraction reactor which would require more energy to heat it to required temperature (50–60 °C) and to maintain the temperature. Additionally, distillation energy input would become high due to the large amount solvent required to be recovered. Thus, the total energy input of the extraction process turns to be high.

In terms of NER, biodiesel production from microalgae (NER < 2.5) is not comparable with the production of fossil diesel which normally has NER around 5 [39,64,65,67]. However, biodiesel production from microalgae is still important as the issue is not only the energy gain but also the depletion of fossil fuel that our generation encounters.

2.2.2. Greenhouse gas emissions

Carbon dioxide is substrate of autotrophic microalgae. It implies that microalgae sequestrate carbons while growing. However, GHGs are emitted from the utilization of fuels and powers in the process of biodiesel production from microalgae. It can be GHG emission production or reduction process according to the difference of the GHG emitted from and sequestrated during the processes (cultivation, harvesting, lipid extraction, trans-esterification). It is affected by the fuel used and power generation source (coal, hydro). In general, autotrophic microalgae to biodiesel doesn't necessarily reduce GHG emissions as the carbon sequestrated during cultivation would finally return to atmosphere by burning biodiesel and biogas/ethanol generated from the residual biomass. But it would be GHG emission reduction process comparing with fossil fuel burning when the total GHG emitted from the utilization of fuels and power in per unit of biodiesel produced from microalgae is smaller than that emitted from the amount of fossil fuel which has the equal energy effect on the vehicle. In addition, when considering microalgae cultivation to biodiesel continuous, which means that it is a cycle (cultivation-extraction-transesterification-utilization-cultivation), CO_2 emitted from biofuel burning would be captured again in the following cultivation. Hence, it would be a GHG emission reduction process.

2.3. Economic analysis of microalgae to biodiesel

Studies have revealed that feedstock took up to 70% of the cost of biodiesel production from vegetable oils and animal fats [12,68,69]. Microalgae oil can be considered as alternative feedstock when its production cost is equal or lower than the traditional feedstock vegetable oils and animal fats. The microalgae oil production includes their cultivation, harvesting, and lipid extraction. Since not only comparing with traditional biodiesel but also with petro-diesel, the step of trans-esterification and biodiesel distribution sometimes included in the estimation processes. Aspen plus is the most popular software employed in the estimation, but also some studies have developed their own model to calculate [70–72]. Unlike NER analysis in which the major influence of the calculation was only from the parameters (cultivation mode, biomass productivity, lipid content, dewatering method, lipid extraction method and efficiency, trans-esterification efficiency), economic assessment would be also greatly impacted by the price of land, labor, power, fuel, chemicals, and tax, which have large variation from region to region. It explains that why there was great diversity of the results of cost estimation (Table 4).

Cultivation modes used are generally ponds or photo-bioreactors. Photo-bioreactors require a large investment on the construction which would come down to impact on the depreciation. The lifetime of the plant was generally considered to be 10 years which was taken to calculate the depreciation [73,74]. It was found that the depreciation took 60–80% of the unit biodiesel production cost in photo-bioreactors system after breaking down the unit production cost to raw materials, labor, utilities, taxes, depreciation, and lab/QA/QC, but the depreciation was only 15% of the unit biodiesel production cost in ponds system [48,70,75]. The reports have revealed that unit biodiesel cost of photo-bioreactor was 2–10 folds of that of ponds with the same biodiesel production capacity [46,48,70,75,76]. It suggested that open pond was more realistic than photo-bioreactors in terms of cost concern. The plant scale had shown large impact on the cost as well [74]. The cost reduced to 6.3 US $ from 8.1 US $ when the annual biodiesel production increased to 100,000 ton from 10,000 ton. It suggests that large scale is more cost profitable than small scale plant with other parameter constant.

The cost of dewatering process was from the utilization of energy (nature gas, electricity, coal, or fuel), which involved to the utilities cost of the plant. When considering that flue gas can be obtained from nearby power plants, then the heat from flue gas would be used for drying biomass, but the results showed no significant change on biodiesel production cost by recovery flue gas heat comparing with the one without [77,78]. The selection of dewatering technology (3.06 US $/L biodiesel for centrifuge, 3.02 US $/L biodiesel for pressure filtration, and 2.92 US $/L biodiesel for bed drying) was found to have very slight impact on the biodiesel production cost [46].

In extraction, the cost was from the utilities as well as solvent lost (considered in chemical cost) during the process. The utilities and chemical cost were affected by the regions. The price of these items had to be obtained according to the plant location and plant operation years.

Trans-esterification was a well-established technology and it took 10–15% of the unit biodiesel production cost [46,70,79]. It has been reported that the feedstock lipid price was critical impact factor of the trans-esterification step, while methanol price had small influence on the biodiesel production cost [80]. It indicates that the factors impacting lipid production cost (biomass productivity and lipid content) would have profound effect on biodiesel production cost.

The biomass productivity selected normally laid between 20 and 40 $g/m^2/d$ for ponds and 1–2 $kg/m^3/d$ for photo-bioreactors [48,76].

Table 4
Cost of biodiesel production from microalgae.

Process	Conditions	Lipid cost (US $/gal)	Biodiesel cost (US $/gal)	Ref.
OP cultivation; dewatering; lipid extraction from dry biomass; trans-esterification	Capacity: 500 ha; Biomass yield: 8.8 kg/m^2/d; Lipid content: 40% w/w dry biomass;	–	13.66	[75]
PBR cultivation; dewatering; lipid extraction from dry biomass; trans-esterification	Capacity: 500 ha; Biomass yield: 14.5 kg/m^2/d; Lipid content: 40% w/w dry biomass;	–	85.36	[75]
OP cultivation; dewatering; lipid extraction from dry biomass; trans-esterification	Capacity: 1950 ha; Biomass yield: 25 g/m^2/d; Lipid content: 25% w/w dry biomass;	8.52	9.84	[70]
PBR cultivation; dewatering; lipid extraction from dry biomass; trans-esterification	Capacity: 1950 ha; Biomass yield: 1.25 kg/m^3/d; Lipid content: 25% w/w dry biomass;	18.10	20.53	[70]
OP cultivation; dewatering; lipid extraction from dry biomass; trans-esterification	Capacity: 405 ha; Biomass yield: 15.38 kg/m^2/d; Lipid content: 35% w/w dry biomass;	–	3.11	[135]
OP cultivation; dewatering; lipid extraction from dry biomass; trans-esterification	Capacity: 333 ha; Biomass yield: 25 g/m^2/d; Lipid content: 35% w/w dry biomass;	9.69	11.36	[46]
PBR cultivation; dewatering; lipid extraction from dry biomass; trans-esterification	Capacity: 333 ha; Biomass yield: 1.25 kg/m^3/d; Lipid content: 35% w/w dry biomass;	18.03	19.00	[46]
OP cultivation; dewatering; lipid extraction from dry biomass; trans-esterification	Capacity: 1618 ha; Biomass yield: 25 g/m^2/d; Lipid content: 25% w/w dry biomass;	Average:13.49	Average:34.50	[76]
PBR cultivation; dewatering; lipid extraction from dry biomass; trans-esterification	Capacity: 1618 ha; Biomass yield: 1.25 kg/m^3/d; Lipid content: 25% w/w dry biomass;	Average:14.18	Average:35.83	[76]
PBR cultivation; dewatering; lipid extraction from dry biomass; trans-esterification; distribution	Capacity: 10,000 ton biodiesel/y; Biomass yield: 1.7 kg/m^3/d; Lipid content: 40% w/w dry biomass;	–	3.41	[71]
OP cultivation; dewatering; one-stage biodiesel synthesis (combined extraction and trans-esterification)	Biomass yield: 30–60 g/m^2/d; Lipid content: 50% w/w dry biomass;	–	1.59–3.68	[136]
OP cultivation; dewatering; lipid extraction from dry biomass; trans-esterification	Capacity: 40,000,000 ton biodiesel/y; Biomass yield: 30 g/m^2/d; Lipid content: 15% w/w dry biomass;	–	17.07	[78]
OP cultivation; dewatering; lipid extraction from dry biomass; trans-esterification; distribution	Capacity: 1 ha; Biomass yield: 30 g/m^2/d; Lipid content: 30% w/w dry biomass;	10.76	–	[41]
PBR cultivation; dewatering; lipid extraction from dry biomass; trans-esterification	Capacity: 100,000 ton biodiesel/y; Biomass yield: 3.2 kg/m^2/d; Lipid content: 30% w/w dry biomass;	–	7.75	[74]
OP cultivation; dewatering; lipid extraction from dry biomass; trans-esterification	Capacity: 170,550 ton biodiesel/y; Biomass yield: 50 kg/m^2/d; Lipid content: 46% w/w dry biomass;	–	3.85	[73]
CP cultivation; dewatering; lipid extraction from dry biomass; trans-esterification	Capacity: 100 ha; Biomass yield: 39.2 g/m^2/d; Lipid content: 40% w/w dry biomass;	18.35;	21.11	[79]
OP cultivation; dewatering; lipid extraction from dry biomass; trans-esterification	Capacity: 40 m^3; Biomass yield: 17.9 g/m^2/d; Lipid content: 30% w/w dry biomass;	–	13.31	[91]
OP cultivation; dewatering; lipid extraction from dry biomass; trans-esterification	Capacity: 1746 m^3; Lipid content: 25% w/w dry biomass;	–	5.18	[98]
PBR cultivation; dewatering; lipid extraction from dry biomass; trans-esterification; distribution	Capacity: 0.15 kg/m^3/day; Lipid content: 30% w/w dry biomass;	13.10–13.95		[137]

(continued on next page)

Table 4 (*continued*)

Process	Conditions	Lipid cost (US $/gal)	Biodiesel cost (US $/gal)	Ref.
OP cultivation; dewatering; lipid extraction from dry biomass; trans-esterification	Capacity: 1 ha 200 kg biomass/d; Lipid content: 30% w/w dry biomass;		4.65	[138]
OP cultivation; dewatering; lipid extraction from dry biomass; trans-esterification	Capacity: 20 g/m^2/day; Lipid content: 30% w/w dry biomass;		6.27	[139]
OP cultivation; dewatering; lipid extraction from dry biomass; trans-esterification	Capacity: 23.76 ton algae/yr; Lipid content: 65% w/w dry biomass;	–	2.52	[140]
OP cultivation; dewatering; lipid extraction from dry biomass; trans-esterification	Capacity: 23.55 g/m^2/day;	–	4.48–13.12	[92]

OP = open ponds.
PBR-photo bioreactors.

The microalgae productivity is critical in cost estimation as there will be great difference on the output in the same capital investment with different biomass productivity. For instance, the microalgae biomass yield will be doubled when their productivity increased from 20 to 40 $g/m^2/d$, which indicates the final products (biodiesel and residual biomass) would be doubled as well. With equal amount of capital investment, the high production of biodiesel and residual biomass would certainly reduce the cost. The cost reduction was from 30% to 50% with every 50% productivity increasing [41,74,78].

The lipid content of microalgae was normally assumed to be 25–50% w/w dry biomass, which was seemed to have great difference. However, the study showed that the cost reduction (3.1 US $ to 2.8 US $/ L biodiesel) was very small even though the lipid content increased double (from 25% to 50% w/w dry biomass) when the residual biomass (after lipid extraction) was allocated a selling value [41]. But the great difference in the cost (from 9.9 US $/L biodiesel for 25% lipid content to 4.5 US $/L biodiesel for 50% lipid content) was observed when microalgae oil was the only product given value [41,70]. Similar results were obtained by other researchers as well [74,77]. That giving microalgae oil value but not residual biomass, was normally not taken as real situation as there was value of the residual biomass. Residual biomass could be sold as animal feed, or produce ethanol, biogas, or hydrogen [17,42,46]. The final application method (for biogas, hydrogen, or ethanol) of residual biomass had almost no impact on the cost [46]. Thus, it indicates that lipid content should not be significant impact parameter of cost estimation as long as the microalgae residua are granted a value. However, for the same lipid content, the content of ash in the lipid extracted from algae had large impact on the cost as it indicated that the available lipid (lipid content minus ash content) could be converted to biodiesel [77,78].

The effect of land, labor, utilities, water, and raw materials on the cost of biodiesel production is highly depending on the regions. Apparently, plant constructed in rural area would be cheaper than that constructed in urban area in the same region. The land cost also varies from one country to another and the value used in the estimation normally was referred from the local price. Similarly, labor cost is country depending. In US, Canada, UK, Russia, French, Australia, South Korea, China, Japan, and South Africa, the basic labor cost per hour was around 7.25 US $, 9.5 US $, 11.84 US $, 1.04 US $, 12.22US $, 17.39 US $, 4.63 US $, 1.19US $, 8.48 US $, 0.3–1.5 US $, respectively [81]. It suggested that labor cost effect on biodiesel production cost in Australia would be more profound than that in South Africa countries. Price of energy (power, natural gas, fuels) is also region depending, and hence their impacts on the biodiesel production cost also vary from one to another region [46,79].

Water loss (around 0.3 cm/d) due to evaporation is remarkable in ponds cultivation [70]. In addition, remaining water becomes wastewater after cultivation and requires treatment. Thus, normally, it is assumed that the water would be recycled for cultivation. So the cost from water utilization would be the amount to replace the water loss. Studies have revealed that using municipal or industrial wastewater instead of sea/fresh water would reduce the biodiesel production cost as wastewater contained nitrogen and phosphorous which were the nutrients required by microalgae. Nutrient cost was responsible to 1–10% of the biodiesel production cost [41,46]. Wastewater had similar nitrogen to phosphorous ratio as microalgae growth demanded [46]. It suggests that using wastewater (cost free) could reduce the production cost by supplying cheap water source and some nutrients (nitrogen and phosphorous) as well as treating the wastewater.

Carbon dioxide is the basic substrate required by microalgae cultivated with ponds and photo-bioreactors. Its requirement is calculated by the microalgae formula ($CO_{0.48}H_{1.83}N_{0.11}P_{0.01}$) and the capture efficiency of CO_2 by microalgae [46]. When it is assumed that the flue gas is available in nearby industrial facilities, the CO_2 cost will be free. But the transportation of the CO_2 from the industry to microalgae cultivation system would have to be added to the capital cost. If CO_2 has a price, it would involve to the raw material cost. But studies showed that the effect from CO_2 price (0–80 US $/metric ton CO_2) on biodiesel production cost was insignificant [77,78].

There is a by-product glycerol in biodiesel production through trans-esterification. Around 0.1 kg glycerol is generated in per kg biodiesel produced. Glycerol is normally assigned as credit of the production cost. As the glycerol is in fact a mixture of glycerol, catalyst, water, methanol, and residual lipids, it is called crude glycerol. Crude glycerol price was around 0.1 $ per kg in 2012 [82]. As it had low price, it wouldn't be a great impact factor of cost estimation [73]. Apart from by-product, waste (mainly in harvesting and extraction process) is generated and the cost required to treat the waste has to be added to biodiesel production cost. The waste treatment cost is highly associated with the extraction method (wet or dry biomass) [78]. When the extraction was performed from dry biomass, the waste treatment cost was less than 1% of the biodiesel production cost, but it would go up to 20% when the extraction was with wet biomass [78]. It indicates that extraction with dry biomass is more favorable in terms of cost consideration.

The current price of commercial biodiesel and petro-diesel is 3.03 US $/gal and 2.46 US $/gal, respectively [83]. From studies on cost estimation, microalgae biodiesel was far beyond affordable (Table 4). Some countries have the policy to give subsidies for biodiesel production, which was around 1 US $ to 1.5 US $/gal [72,73]. Even through, biodiesel production from microalgae was still not comparable with vegetable biodiesel and petro-diesel. By breaking down the total cost, cultivation and harvesting, extraction, trans-esterification, purification, and distributions were responsible to 50–65%, 15–25%, 10–15%, 2–3%, and 2–3% of the total cost [46,70,72,79].

As discussed above, the production cost was greatly affected by cultivation mode, biomass productivity, the purity of the lipid extracted, plant scale, and the capital investment (land, equipment, and constructions). To reduce the cost to an acceptable level, improvement has to be made by developing low cost equipment, high efficiency dewatering and extraction technology, isolating high productivity microalgae strain with high lipid content, finding cheap replacement of raw materials, and studying the technology of combination of extraction and trans-esterification, which is also called in-situ trans-esterification.

3. Large scale biodiesel production from microalgae

The world first microalgae pilot plant was photo-bioreactors built on the rooftop of MIT for food propose [84]. A cost estimation report based on the system showed that it would require 1.2 million US $ per hectare cultivation (soybean cultivation 750 US $/ha) [85]. To reduce the cost, raceway open ponds system was constructed in a wastewater treatment plant of Concord, in 1960s [85,86]. Since after, many algae plants (ponds or photo-bioreactors) have been built in US, South East Asia, and Europe countries. These plants were for the production of food additives, edible oils, and vitamins.

Studies of biofuel (biodiesel, hydrogen, ethanol, and syngas) production from microalgae have also been extensively reported, but they were in lab or pilot scales. In 2008, PetroSun delivered information of their commercial algae plant for biodiesel production which had a capacity of producing 4.4 million gallons algae oil per year with a land area of 1 100 acres located in Rio Hondo, Texas [87]. In 2010, US navy invested 12 million US $ to Dynamic Fuels for biofuel production from microalgae. The cost of the biodiesel was 424 $/gal in the first year (2010), and then it dropped to 27 US $/gal in the following year [88]. It is still too high to be affordable but the good sign is the dramatic decrease of the cost within a year. Besides US, European countries have also joined in biodiesel production from microalgae. There was a report of microalgae cultivation pilot plant with 11.5 million EUR capacity, which would be developed in Cadiz of Spain [89]. The current situation of the project has not been stated. Thereafter, another report was released on industrial scale microalgae cultivation for biodiesel production from Brazil in 2012 [90]. A plant producing 1.2 million L fuel per year was planned to start from late of 2013 in north-eastern Brazilian state of Pernambuco. However, no further news has been delivered to flow afterwards. In fact, there are many reports on the plans to build microalgae biodiesel production plants; however, no successful case (cost comparable with petro-diesel and vegetable biodiesel) has been known so far. But it is still worth to invest to the studies on microalgae biodiesel production as fossil fuels is getting depletion and the price of vegetable oils gradually increasing.

4. Strategies for reducing the cost of biodiesel production from microalgae

Microalgae oil is a promising feedstock of biodiesel production; however, the high cost has hindered the development of biodiesel production from the oil. How to reduce the cost but not impacting on the biodiesel productivity and quality needs to be further studied. In fact, to bring the process of microalgae for biodiesel production into reality, four strategies: 1) microalgae cultivation in rural area; 2) simultaneous microalgae oil production with other valuable product production such as protein and vitamins; 3) combination of microalgae cultivation with fish, tortoise and shrimp cultivation; 4) simultaneous microalgae cultivation with other treatments, can be employed. It would reduce the cost of biodiesel production from microalgae.

4.1. Microalgae cultivation in rural areas

As discussed in Section 2.3, the cost is highly depending on the plant location as land cost varies from one place to another. Compared to the cities, land price of rural area is much cheaper. For instance, the land price in Shenzhen of China is around 2000–8000 US $/m^2 but it was 50–200 US $/m^2 in rural area. According to Superpro Designer (a software to estimate the cost of a bioprocess), the land cost highly impacts on the capital investment of the plant, which is around 15% of the total investment. Some researchers have proposed to production microalgae biodiesel in desert area where the land cost was low [91]. It was reported that the capital expense (land, construction, instrumentation, etc.) took up to 68% of the total biodiesel production cost. As the production plant was built in desert area, hence, the cost from the land utilization was less than 4% [91]. Many reports have stated that land occupation had great influence in the cost of biodiesel production and production of microalgae biodiesel in non-fertile and rural areas could increase the potential of microalgae for biodiesel production in practice [77,92,93].

Production of microalgae biodiesel in rural area could reduce the cost from the land utilization. However, many other factors including transportation (materials and equipment), power, fuel, etc. have to be taken into consideration as the cost from these items could be high in rural area than that in cities. So far, no specific studies have been conducted to evaluate and compare the cost difference of the biodiesel production from microalgae in different locations when the production capacities are fixed. Relative work is required in order to reveal the feasibility of the biodiesel production from microalgae in rural areas.

4.2. Microalgae oil production with other valuable product production

Generally, after extracting microbial oil from microalgae, the residual biomass was considered to be dumped as waste in the economic analysis [76,92]. In fact, apart from microbial oil, there are also other substances such as protein, pigments, vitamins, astaxanthin, PHB and anti-oxidants in the microalgae cells [94–97]. The production of microbial oil integrating with other valuable products production from microalgae could be a feasible way of reducing the biodiesel production cost from microalgae. It was reported that the biodiesel production cost reduced to 13.73 US $/L from 17. 26 US $/L when protein was obtained from the microalgae apart from the microbial oil (for biodiesel production) [95]. Other researchers have also reported similar trend [98]. With consideration of co-production of astaxanthin and PHB, the cost of biodiesel production from microalgae was reduced to 0.54 US $/L from 3.90 US $/L [96]. Bielsa et al. has proposed a process for simultaneous production of biodiesel and exopolysaccharides (EPS) from microalgae and predicted that the cost of biodiesel production could be reduced due to the production of EPS [99].

In addition to the co-product production in biodiesel production from microalgae, other products such as biogas and ethanol could be produced from the residual biomass obtained after microbial oil production [100–102]. After extracting the lipid from microalgae, there are abundant carbohydrates in the cell debris, which can be converted to ethanol after fermentation or biogas after anaerobic digestion. Studies have revealed that the 1 kg microalgae biomass could generate 71 g biodiesel and 446 L biogas [103]. The economic analysis showed that the cost of biodiesel production was reduced by 35% when

Biodiesel production was integrated with biogas production (solo biodiesel production: 72 US $/L biodiesel, both biodiesel and biogas production: 47 US $/L biodiesel) [42].

Overall, the production of microalgae oil along with other valuable product production is a promising way to bring down the cost of biodiesel production from microalgae. However, quality of the co-products should be evaluated if the extraction of these products is after the lipid extraction. In addition, the content of the co-product in the microalgae is also important factor to determine the necessity of processing to obtain the product.

4.3. Microalgae oil production with fish, tortoise and shrimp cultivation

As discussed in Section 2.3, utilization of open pond to cultivate microalgae for biodiesel production is far cheaper compared to that of the photo-bioreactor. Open pond has similar characteristics as lakes which is suitable for the growth of fish, tortoise and shrimp. It has been revealed that microalgae could grow in the shrimp cultivation ponds and enhanced the shrimp productivity; additionally, the presence of microalgae in the system has improved the water quality [104]. Similarly as shrimp, fish and tortoise could co-cultivate with microalgae. However, there is also possibility of that fish, shrimp, and tortoise could consume microalgae as food, and hence reduce the productivity of microalgae [105,106]. In addition, microalgae could secrete toxic compounds and hence effect on the fish, fish, shrimp, and tortoise [107].

So far, no study has been performed to investigate the biodiesel production from microalgae along with the cultivation of fish, shrimp, and tortoise. Moreover, no cost assessment has been reported on this issue as well. It is a highly interesting area to explore.

4.4. Microalgae oil production with other treatments

4.4.1. Microalgae oil production and wastewater treatment

Many studies have conducted to cultivate oleaginous microalgae in wastewater for biodiesel production [108,109] . Studies have revealed that the microalgae could remove the contaminants from wastewater as well as accumulate lipid in the cell body. Rinna et al. reported that the removal of phosphorus and nitrogen were 100% and 65%, respectively, when the wastewater was used to cultivate microalgae *Botryococcus braunii* [110]. *Botryococcus* sp. was used to treat domestic wastewater and obtained lipid accumulation up to 61.7% w/w dry cell as well as nitrogen removal of 64.5%, phosphorus removal of 89.8%, and total organic carbon removal of 67.9%, respectively [111]. Gutiérrez-Alfaro et al. [111] have also obtained great co-operation between wastewater treatment and microalgae cultivation.

In addition to municipal wastewater, microalgae could also grow in industrial wastewater to achieve contaminant removal as well as produce valuable products. Mohd Udaiyappan have reviewed the studies on microalgae to treat industrial wastewater including palm oil mill effluent, rubber mill wastewater, starch wastewater, textile wastewater, and heavy metal wastewater [109]. Fazal et al. have discussed the potential of textile wastewater as water and nutrient source for microalgae cultivation for biodiesel production [108]. The COD removal efficiency reached 75% when the textile wastewater was used to cultivate *Chlorella* sp. which accumulated 20% lipid in cell dry weight [112]. Researchers have also achieved the removal of endocrine disrupter compounds including 4-tert-octylphenol (OP), technical-nonylphenol (t-NP), 4-nonylphenol (4-NP) and bisphenol-A (BPA) from wastewater with microalgae [113]. Similar results have been also reported by others [114].

Studies have revealed that biodiesel production and wastewater contaminant removal could be simultaneously achieved. Producing biodiesel in this way would efficiently reduce the cost of biodiesel production from microalgae as it would not require extra land and nutrient addition for cultivation; additional, accomplish wastewater treatment. So far, the studies are mainly in research stage. Application in practice is not reported yet, and relative work is required.

4.4.2. Microalgae oil production and biogas upgrading

Biogas generated from anaerobic digestion is a mixture of methane, carbon dioxide, nitrogen gas, hydrogen gas, hydrogen sulphide and so on. Generally, carbon dioxide content in biogas could reach 50%, and hence, biogas upgrading is normally performed to concentrate the methanol concentration by mainly removing carbon dioxide. Autotrophic microalgae growth requires carbon dioxide. It suggests that the microalgae can capture carbon dioxide from biogas and then the methane content in biogas could be increased when the biogas is fed to microalgae culture. It was reported that methane content in biogas was remarkably increased from 60% to 92% with *Scenedesmus* sp. lipid content of 27.6% w/w cell dry weight [115]. Srinuanpan et al. [116] used the strategy of stepwise-increasing of CO_2 supply to *Scenedesmus* sp. and obtained higher lipid content (34.1% w/w cell dry weight) and methane content in the biogas (> 98%) [116].

The studies provide an insight of cooperation of biodiesel production and biogas upgrading. As the quality of biogas is highly increased without other treatment except passing through the oleaginous microalgae culture, there can be credits applying to the biodiesel production from microalgae. Hence, it would indirectly reduce the cost of biodiesel production from microalgae.

Microalgae cultivation in rural area, simultaneous microalgae oil production with other valuable product production , combination of microalgae cultivation with fish, tortoise and shrimp cultivation, and simultaneous microalgae cultivation with other treatments could be efficient ways to reduce the microalgae biodiesel production cost. However, there are still a lot of unknown aspects such as: 1) the impact of microalgae derived toxic compounds on fish, tortoise and shrimp when co-cultivation is performed; 2) the impact of the toxic compounds in wastewater on microalgae growth and lipid accumulation, effect on the final products (biodiesel); 3) the cost to extract the valuable product from the microalgae (is it worth to do so?); 4) the construction or operation difficulties that will meet if the plant is built in rural area. These have to be found out and cost analysis is highly demanded.

5. Conclusions

Biodiesel production from microbial oil is significantly important when there are problems on utilization of petrodiesel (depleting) and vegetable based biodiesel (price increasing and food competing). From LCA and economic analysis studies, it was found that there was still distance to make biodiesel production from microalgae commercialized. But it is encouraging that governments and companies have provide large investments to develop novel technologies which trend to reduce the cost and energy consumption of biodiesel production from microalgae. Research has been undertaken to make it into reality. From this point of view, there is great possibility of that biodiesel will be commercially produced from microalgae in further.

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