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Microalgae biodiesel: Current status and future needs for engine performance and emissions

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ABSTRACT

Microalgae feedstock is recognised as one of the most promising resources for producing triglycerides which is subsequently converted to biodiesel. However, the large-scale technology required to generate biodiesel from microalgae is still in its early stages of development. Microalgae research to date may be placed into four broad categories: (i) growth, (ii) harvesting, (iii) oil extraction and (iv) fuel properties for engine performance and emissions. More than 1000 manuscripts have been published on the first category with progressively less on subsequent groups. Finally, effects of microalgae methyl esters on engine performance have only been reported in 9 scientific articles. This review will place extraction techniques and engine performance of microalgae biodiesel in the context of the preceding two categories and examine the practical problems associated with fuel properties, engine performance and emissions. Considering energy consumption, toxicity, and time, many of the extraction techniques used in the laboratory show moderate potential for commercial scale. An important finding is that variation of conditions in the first three categories can have a significant effect on biofuel quality which can cause fuel properties to be out of standard and/or adversely affect engine performance and emissions.

1. Introduction

With worldwide concerns over both petroleum prices and climate change, researchers around the world have been dedicated to finding renewable energy sources. Currently, fossil fuels provide a large proportion of the global energy demand. Biofuels, such as biodiesel and ethanol, are therefore being developed as alternative fuels. Biodiesel from vegetable oils and animal fats only make up approximately 0.3% of the current demand for transport fuels [1].

Biodiesel can be produced from renewable sources such as vegetable oils, animal fats and recycled cooking oils [2]. However, vegetable oil feedstocks are in high demand as food sources that increase their price and challenge their potential as large-scale fuel resources.

Biofuels can be classified as first generation biofuel (FGB), second generation biofuel (SGB) and third generation biofuel (TGB) based on their feedstock or production technologies. First generation biofuels are mainly sourced from food crops such as sugar cane, corn, starch and vegetable oils or animal fats [3]. FGBs produce from food crops are limited in their ability to achieve sustainability targets for petroleum diesel substitution, environmental benefit and economic growth because of competition with their alternative uses as food products.

SGBs are generally classified as being from non-edible feedstock such as wheat straw, wood and solid waste. The SGBs can avoid many of problems faced by FGBs by producing biofuels from agricultural and forest residues instead of food stocks. However, lack of available source materials in many countries may limit the potential for large-scale petroleum replacement [3].

In contrast, biodiesel from non-edible and non-agricultural sources make up the TGBs with microalgae considered to be one of the best options for biodiesel production because many of them show potential for high oil yields and ability to grow on non-arable land [3,4]. Microalgae may also be the only renewable source with the capacity to meet the world's transport fuel needs [5]. This is due to high microalgae productivities and oil yield/fatty acid content compared to other oil/fatty acid-based feedstock; potentially no competition with food production; cultivation potential on non-arable and marginal land; and the production of both biodiesel and higher value co-products [1]. It has been estimated that microalgae biodiesel production could potentially replace petroleum diesel entirely [5].

However, the technology required to generate biodiesel from microalgae at large-scale is still in its early stages of development. There is considerable research effort concerning the growth of micro-

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algae; but a significantly lower number of research studies focus on large-scale oil extraction and on how to create biodiesel from microalgae biomass and evaluation of microalgae biodiesel in engines. This study will focus on:

1. Current developments on large scale microalgae growth, harvesting and their limitations and potential solution.
2. Limitation on large commercial scale oil extraction and potential solutions
3. Scarcity of literature on microalgae biodiesel performance in regular automotive engine

And will be explained based on available literature on microalgae biodiesel.

2. Emergence of microalgae research

2.1. Potential of biodiesel production from microalgae

Since the 1950s, microalgae have been cultivated commercially for many different products like proteins, cattle feed and pharmaceuticals [6]. Throughout the 1980s, the US Department of Energy (DOE) and the Solar Energy Research Institute (SERI) initiated the Aquatic Species Program and constrained investigations exclusively to the analysis of microalgae. The goal was to develop biofuels from microalgae at prices competitive to those of fossil fuels [7,8]. However, initial optimistic cost and performance projections were not met, and, as future petroleum cost were expected to decline due to unlimited supplies of cheap fossil fuel and remain low, the microalgae biodiesel project was closed in 1995.

In recent year's microalgae have gained renewed significant attention as a biodiesel feedstock for several reasons including the absence of competition with food crops for land and economic and social development potential in rural communities [8,9]. While it is not expected that all current fuel use will be replaced with microalgae biodiesel, conceptual replacement calculations can be instructive. Projections based on the fuel consumption rate of 2007 predicts 0.53 billion cubic metres of biodiesel would need to be produced annually to replace all the transportation fuel used in the United States [5] and 0.4 billion cubic metres for Europe [10]. If all biofuel is supplied through microalgae with a productivity of 400,000 liters per hectare, 9.25 million hectare of land area would be needed for the European market [10]. Corn, soybean and canola oil require unrealistic percentages of existing area for cultivation for total fossil fuel replacement. Palm oil is the highest oil-producing crop (5950 L/ha/year). However, palm oil would still require one quarter of the existing global cropping land for cultivation to meet half of the transport fuel requirements. As for microalgae containing 30% and 70% oil content, the percentage of total cropping area required for total fossil fuel replacement would be 2.5% and 1.1%, respectively. These values are 10 times lower than the area requirements for palm oil cultivation providing good reason to evaluate microalgae cultivation for large-scale fuel production [5].

2.2. Classification of microalgae

Autotrophic microalgae can be grown in freshwater, brackish, marine and hyper-saline water over a range of temperatures [11,12]. The size of microalgae, depending on the species, can vary from 1 to 100 (μm) [9,13]. It is estimated that around 30,000 to 40,000 microalgae species have been identified and classified in groups [6,14,15]: microalgae in commercial use belong to five phyla [16] as shown in Table 1.

2.3. Lipid classification and content

Lipids are mainly classified into two categories, storage lipids (non-polar lipids) and structural lipids (polar lipids)[17–21]. Both forms of lipids can be transesterified to produce biodiesel [22–24]. Storage lipids are in the form of triacyl-glycerol (TAG) with the fatty acids being saturated and unsaturated. Structural lipids consist mainly of glycosylglycerides enriched in the chloroplast membrane that makes up about 5–20% of their dry biomass. There are also significant amounts of phosphoglycerides, phosphatidyl ethanolamine (PE) and phosphatidyl glycerol(PG) present as polar lipids in the mitochondria, the plasma membrane, the endomembrane system consisting of the endoplasmic reticulum, the Golgi apparatus and endosomes/lysosomes and the nuclear envelope [17,25].

TAGs are stored as potential sources of metabolic energy through catabolism [26]. In general, TAGs are mostly synthesized in the light, stored in cytosolic lipid bodies and then reutilized for polar lipid synthesis in the dark [26]. Some oil-rich species such as *Crypthecodiniumcohni* have the capacity to build up high levels of long-chain polyunsaturated fatty acids (PUFA) eicosapentaenoic acid C20:5 (EPA), docosahexaenoic acid C22:6 (DHA) and docosapentaenoic acid C22:5 DPAas TAG [17,27]. PUFA-rich TAG may donate specific acyl groups to mono-galactosyldiacyl glycerol (MGDG) and other polar lipids to enable rapid adaptive membrane reorganization [28]. Table 2 lists different species of microalgae and reported total lipid and fatty acid contents including percentage of total poly unsaturated fatty acid content [5,14,15,29].

3. Microalgae biodiesel research assessment

A substantial amount of research has focussed on growth of microalgae at laboratory- and pilot-scale. However, downstream processes (oil extraction, biodiesel conversion and evaluation of engine performance) for biodiesel production from microalgae still remain very limited commencing only to large-scale for research and not to commercial scales. Section 4 details present limitations of downstream processes for large-scale biodiesel production from microalgae. Table 3 summarises the current knowledge on microalgae biodiesel and their engine performance.

Table 3 summarised extraction, biodiesel conversion, fuel property and engine performance of some different species of microalgae. As evident, to date there is limited research on fuel property analyses and very limited data on engine performance mainly due to the lack of sufficient microalgae production for the high volume-low value markets.

4. Challenges in microalgae biodiesel production

4.1. Microalgae culture and harvesting

Microalgae provide various advantages for biodiesel production compared to traditional crops [5]. Some have been considered for commercial-scale cultivation for cattle feed [57], but very few for cultivation for large-volume-low value markets such as fuel, as there are economic challenges that are yet to overcome [58]:

Identification of an ‘all-rounder’ species that is suitable across a wide range of factors including environmental tolerance, high growth rate, high lipid content, and easy harvesting and extraction [58].

The biochemical composition of lipids is another barrier compared to other traditional feedstock in relation to biofuel properties. Depending on species, microalgae oil can be quite rich in polyunsaturated fatty acids with more than four double bonds [12]. Examples include eicosapentaenoic acid (EPA, C20:5n-3;five double bonds),docosapentaenoic acid (DPA n-6) and docosahexaenoic acid (DHA,C22:6n-3; six double bonds) [59]. The European biodiesel standard EN 14214 placed restrictions on FAME contents with ≥4 double bonds to a maximum of $1\,g100\,g^{-1}$ FAME. This biodiesel standard also limits the linolenic acid methyl ester (C18:3) content to $12\,g100\,g^{-1}$ FAME [5]. Due to higher concentrations of polyunsatu-

Table 1
Microalgae genera in commercial use.

Phyla	Growing condition	Structure	Potential
1. Chlorophyta	Marine, freshwater and terrestrial environment	Unicellular, Multicellular	Commercial lipid and hydrocarbon production
2. Dinophyta	Mainly marine	Unicellular	Good source of docosahexaenoic acid (DHA)
3. Stramenopiles			
Eustigmatophyceae	Marine freshwater and terrestrial environment.	Unicellular	Used in live aquaculture feed.
Bacillariophyceae	Marine and freshwater	Mainly unicellular, few multicellular	Used as aquaculture feed
Labyrinthulomycetes	Mostly marine		Commercial interest for pigment and fatty acids
4. Haptophyta	Mainly marine, some freshwater	Unicellular, colonial	Used as feed microalgae in aquaculture
5. Rhodophyta	Mainly marine, very few freshwater species	Mostly multicellular,	Carbohydrate and sugar

rated fatty acids, microalgae biodiesel has a higher iodine value, which is restricted to a maximum of 120 g $I_2$100 g^{-1} fat in the EN14214.

Economic harvesting (recovery) of biomass from microalgae suspension cultures is problematic with regards to volume requirements for biodiesel production, contributing upto 50% of the final production cost [58,60]. It is also reported that the energy requirement to produce microalgae derived biodiesel is higher than the energy contained in biofuel [61].

Globally, very few companies target large-scale production of microalgae for biofuel, limiting the quantity of biomass available at university research for biodiesel production.

4.2. Oil extraction from microalgae

Most of the structural lipids in microalgae are naturally long chain with more than two double bonds which negatively impact on fuel quality such as lower cetane number, lower combustibility and higher iodine values. Therefore, extraction methods for biodiesel production should be able to bias extraction aiming at enriching the extraction of neutral lipids (TAGs) from the cellular matrix and minimizing theco-extraction of less desirable structural lipids (phospholipids and glycolipids). Some common approaches to extract lipid from microalgae include:

a. Mechanical disruption
b. Ultrasonic-assisted extraction
c. Solvent extraction
d. Thermo-chemical liquefaction
e. Supercritical fluid extraction

Conventionally for mechanical disruption and solvent extraction, wet biomass requires further downstream processing such as thickening, dewatering and drying prior to extraction. These processes are energy-intensive and costly, so alternative extraction methods able to process wet biomass have gained increasing attention [62]. Techniques that extract oil from wet algal biomass include thermo-chemical liquefaction and supercritical fluid extraction.

4.2.1. Mechanical disruption

Mechanical disruption of algal cells aims to damage cell walls to provide access to the intracellular content [63]. Disruption methods avoid use of chemicals and contamination of the left over biomass, leaving the extracted biomass for further product development (e.g. high protein animal feed etc.). Common methods include bead milling, homogenisation, and mechanical pressing [63,64].

Bead milling occurs as a result of agitation of small glass beads inside a vessel rotating at high speeds causing cell breakage through shear stress [65]. Conversely, homogenisation forces biomass through an orifice, and produces a rapid pressure change and high shear stress. Mechanical pressing extracts oil by crushing the cell walls using a press. The amount of disruption caused to the cells is affected by the size, strength and shape of the microalgal cells [63,64]. However, some microalgae species such as *Chlorococcum*, *Botryococcus* sp, *Chlorella vulgaris* and *Senedesmus* sp. resist shear stress and crushing, therefore this process is inefficient for oil extraction from microalgae [66,67].

4.2.2. Ultrasonic-assisted extraction

Ultrasonic-assisted extraction is the process of applying sound energy to agitate the sample and disrupt the cell walls and membranes of the algae cells, causing them to release their cellular contents [21]. The release of the cellular content is enhanced by the use of solvents. Typically in ultrasonic-assisted extraction, a centrifuge is used to separate the residual algae biomass from the solvent and extracted lipid at the end of the process [14].

Table 2
Total lipid and fatty acid content of some common microalgae species [5,14,15,29].

Groups	Species	Total lipid content (%DWB)	Total fatty acid (mg $g^{-1}$of DWB)	PUFA (% FAME)	Reference
Cyanobacteria	*Spirulina platensis*	7.2	60.2	2.11	[30–32]
Chlorophyta	*Chlorella minutissima*	57	94–113.5	59.73	[33]
	Chlorella protothecoides	14–57	–	62.8	[15]
	Chlorella sorokiniana	19–22	14–31.1% of lipid	62–71	[34]
	Chlorella sp.	10–48	17–19	49–68.2	[35]
	Chlorella vulgaris	5–58	24.94	34.4	[36]
	*Ankistrodesmus*sp.	24–31	39	68.3	[35]
	Dunaliellasalina	6–25	34	78	[35]
	Dunaliellaprimolecta	23	411.5	38	[37]
	Chlamydomonas	–	89–649	35–54	[38]
Dinoflagellate	*Crypthecodiniumcohnii*	20–51	82–102	37–57	[39]
Bacillariophyceae	*Skeletonemasp*	15.9	13	25.1	[40,41]
	Phaeodactylumtricornutum	21.7	187.3	17.8	[42]
Stramenopiles (Eustigmatophyceae)	*Nannochloropsis oculata.*	22–29	267.1	9.5	[42]
Cryptophyta	*Rhodomonassalina*	5.4	20	77.6	[41]
Haptophyta	*Isochrysissp.*	7–33	218.5	12.3	[43,44]
Rhodophyta	*Porphyridiumcruentum*	–	35.4	54	[45]

Table 3
Summary of microalgae biodiesel research including extraction, fuel property and engine performance.

Class	Species	Grow	Lipid extraction	Biodiesel conversion	Biodiesel properties							Engine Test	
					Density g cm^{-3}	Viscosity cSt	HHV M Jkg^{-1}	CN	Flash point °C	Acid value mgKOH g^{-1} oil	Sulphur content mg kg^{-1}	Per	Emi
Cyanobacteria	*Spirulina platensis* [46,47]		LS (Hexane)	TE	0.864	5.66	45.6	70.0[1]	189	0.75	0.0	Yes	Yes
Chlorophyta	*Chlorella protothecoides* [48–50]	Fresh water	**IS**(Soxhlet)	TE	0.886	4.47	Yes	48.3[1]	165	0.29	0.01	Yes	Yes
	Ankistrodesmusbraunii [51]	Fresh water	**BS**(US/Soxhlet)	TE	0.869	4.19	40.72		144			Yes	Yes
	Chlorella vulgaris [52]	Fresh water	LS(Hexane)	TE	0.867	5.76			149		0.0002	Yes	Yes
	Chlorella sp. [53]	Fresh water	**BS**(Soxhlet)	TE	0.883	4.73	39.5		179	0.37	0.0081	Yes	Yes
	Chlorella sp. [54]	Marine	LS	Yes									
	Chlorella pyrenoidosa [47]	Fresh water	LS (Hexane)	TE	0.872	5.82	40.8			0.40			
	Tetraselmis sp. [35]	Marine	LS (Chloroform)	DETE									
	Nephroselmis sp. [35]	Marine	LS	TE									
	Dunaliellatertiolecta [54]	Marine	LS	Yes									
	Dunaliellamaritima [54]	Marine	LS	Yes									
	Dunaliellasalina [54]	Marine	LS	Yes									
Dinoflagellate	*Crypthecodiniumcohnii* [55]	Marine	**PS**(Hexane)	TE	0.912	5.06	39.86	46.5	165	0.14	0.0075	Yes	Yes
	Gymnodiniumkowalevskii [54]	Marine	**PS**	Yes									
Bacillariophyceae	*Chaetoceros gracilis* [56]		**PS**	DETE	0.885	3.4	39.51	51[1]				Yes	Yes
	Skeletonemacostatum [35]	Marine	LS										
	Skeletonemasp [35]	Marine	LS										
	Amphora coffeaformis [35]	Marine	LS										
	Chaetoceros sp. [35]	Marine	LS										
	Fragilariapinnata [35]	Fresh water	LS										
	Nitzschiafrustulum [35]		LS										
	Nitzschia sp. [35]		LS										
	Phaeodactylumtricornutum [42,54]	Marine	LS	Yes									
	Skeletonemacostatum [54]	Marine	LS	Yes									
	Chaetocerosmuelleri [54]	Marine	LS	Yes									
	Chaetocerosconstrictus [54]	Marine	LS	Yes									
Stramenopiles (Eustigmatophyceae)	*Nannochloropsis oculata* [54]	Marine	LS	Yes									
Cryptophyta	*Cryptomonassp* [35]	Marine	LS	TE									
	Rhodomonas sp. [35]		LS	TE									
	Chroomonassalina [54]		LS	Yes									
Haptophyta,	*Isochrysis* sp. [35]	Marine	LS	TE									
Rhodophyta,	*Rhodosorus* sp. [35]	Marine	LS	TE									
	Porphyridiumcruentum [54]	Marine	LS	Yes									

In this review article the extraction scale is consider as follow: **LS**(Laboratory-Scale): when less than 100 g of biomass is used in laboratory; **BS** (Bench-Scale): when more than 100 g and less than a kilogram of biomass used in laboratory; **PS** (Pilot-Scale): when more than one kg of biomass is used to produce enough fuel for an engine test and **IS** (Industrial-Scale): when produced biodiesel is sold commercially;**TE**: Transesterification; **DETE**: Direct extraction transesterification; **Per**: performance; **Emi**: Emission; **Yes**: experiment carried out* estimated from the fatty acid profile.

[1] indicated the data presented was calculated/estimated from the fatty acid profile

The main benefits of using an ultrasonic extraction process are the ability to increase the yield of algae crude oil and reduce the time of the extraction process with moderate cost [14]. Another benefit is the fact that the biomass does not require drying before extraction, if the hexane-centrifugation method is used but using centrifugation for separation will add extra cost.

4.2.3. Solvent extraction

Solvent extraction makes use of specific chemicals to extract the lipids and separate them from the crude biomass. Possible solvents for extraction include benzene, iso-propanol, ethanol and hexane or ethanol-hexane mixtures [14,68,69]. The most widely used solvent however, is hexane due to its lower cost, ready availability, density and boiling point [63,64,70].

Solvent extraction of lipids from microalgae biomass is a process where extracted lipids are dissolved in the solvent and form a phase separate to the aqueous phase containing water-soluble cell components and cell debris [63]. This is due to lipids being highly soluble in the organic solvents used in this process [63]. Extraction efficiencies are enhanced when the solvent can penetrate algal cells and has polarity similar to that of the crude lipids being extracted. Non-polar solvents typically extract non-polar lipids, whereas extraction of polar lipids gradually increases with the degree of polarity of the solvent. As such, the choice of solvent polarity influences and can minimise the co-extraction of non-lipid contaminants (protein and carbohydrates) [22], but often at the expense of polar lipids, which can be also beneficial, if the majority of PUFAs are located in this fraction. Higher lipid yields can be achieved by either disrupting cells before adding the solvent or using combination of solvent such as hexane (non-polar), methanol (polar) and water [63,64]. Contamination is a major obstacle when using organic solvents as pigments are extracted as well. Extracted pigment further complicate crude oil purity since some of them are highly non-polar and are not miscible with water [71].

An experiment carried out by Halim et al. [66] examined the lipid yields of *Chlorococcum* sp. extracted with hexane and hexane/isopropanol. For the hexane extraction, dried and wet paste biomass was used. Wet algal paste yielded33% fewer lipids than the use of dried biomass. The combination of n-hexane and isopropanol produced a three-fold increase in lipid yield when compared to hexane extraction from dried biomass. This was due to the algal cell walls preventing direct contact between the non-polar solvent hexane and the cell membrane reducing the effectiveness of lipid extraction. The use of alcohol (polar solvent) can disrupt the membrane-based lipid-protein interactions by forming hydrogen bonds with the polar lipids [63]. This allows hexane to extract a larger amount of lipids, and therefore, hexane: alcohol extraction is seen as the most suitable method for industrial-scale production. A techno-economic analysis of conventional solvent extraction and biodiesel production from microalgae by Klein-Marcuschamer et al was carried out considering capital investment, project life time, nutrient cost for growing microalgae, operating cost, consumable and labour cost [72]. Results from their analysis shows, capital investment specially harvesting equipment (almost 70% of total capital investment cost) for microalgae is the single most expensive part of the process whereas labour charges are minimum [72].

Solvent extraction at higher temperature and pressure known as accelerated solvent extraction (ASE),has also been investigated and found to be very efficient with maximal final lipid recovery of 90.21% of total lipids [73].ASE can also be used with wet biomass (sample appearance as a liquid) by adding a drying agent (diatomaceous earth-(DE)) up to 5:2 sample to DE ratio [74], reducing sample pretreatment costs and preparation time compared to conventional hexane extraction. However, due to the higher pressures, ASE generally extracts a higher amount of PUFAs, which is undesirable for biodiesel quality. This problem can minimised through optimisation of the operating temperature and moisture levels of the biomass [75].

4.2.4. Supercritical fluid extraction

Supercritical fluid extraction (SFE) is a process that occurs when a fluid is in a state resembling both a liquid and a gas as the temperature and pressure rise above the critical point [63,65,76]. This situation enhances the solvating power and increases diffusivity to produce faster extractions, yields and separation [63,76]. The most commonly researched solvent is carbon dioxide (CO_2), given its moderately low critical temperature (31.1 °C) and pressure (72.9 atm). Added advantages of using CO_2 include chemical inertness, low toxicity, relative pricing, availability and its ability to be handled in large quantities [63,65]. Supercritical CO_2 has a low polarity, and as a result is less efficient in extracting compounds with moderate to high polarity. To increase solvent polarity and thus lipid extraction, modifiers (co-solvents) such as ethanol are used in combination with CO_2[76]. One possible disadvantage is the presence of moisture in the biomass that acts as an additional layer over the cells and decreases the diffusion efficiency of CO_2[63].

In SFE, after extraction is completed, the temperature and pressure are returned to atmospheric conditions, and the CO_2at room temperature, is separated from the final product as a gas [63,65]. Supercritical fluid extraction is being more widely investigated because it does not leave harmful solvent residues, has a faster extraction time than mechanical disruption and solvent extraction, and is used for thermally sensitive products [63]. In-addition, use of co-solvents can enhance the selectivity of extraction of certain compounds in the extract [73]. However, the pressure vessel installation cost and unfavourable energy requirements, as well as CO_2-demand limit the scalability of supercritical fluid extraction at present [63,73].

4.2.5. Thermo-chemical liquefaction

Unlike pressing or chemical extraction, microalgae slurries containing 5–20% mass fraction of biomass can be liquefied in sub-critical conditions, converting wet biomass to bio-crude oil [23,68]and reducing the need for drying of the biomass. Only 12% of energy is required to achieve desired biomass concentrations compared to complete dewatering [77]. One of the most promising liquefaction processes for microalgae biomass is hydrothermal liquefaction (HTL) which utilizes water-based slurries at medium temperature (300–350 °C) and sufficient pressure (20 MPa) to maintain the water in the liquid phase [77–79].

The main benefit of HTL is that water is used as a reaction medium [78]. It is estimated that production cost of $2.80 per liter can be achieved with algae oil assuming biomass contain 30% oil by weight and without considering oil to biodiesel conversion, transportation and marketing cost [5]. This assumes the recovery process contributes 50% cost of total recovered oil cost.HTL converts the biomass to bio-crude which can be upgraded to aviation quality fuel. However, high amount of nitrogen content in bio-crude complicate the refining process and can lower the biofuel quality. A two stage HTL process with a mild stage I (< 200 °C) and severe stage II (250–300 °C) has been proposed by Jazrawi et al [80] to reduce nitrogen content by upto 50% in bio-crude and improve biofuel quality. This two stage HTL process can be implemented to extract high value products such as omega-3 before converting to bio-crude which may prove beneficial for overall production economics [68]. However, HTL has not yet been adequately developed despite the fact it has been identified as “the most promising path to sustainable biocrude production” due to its high energy efficiency, using only 10–15% of the energy of the feedstock biomass [81].

4.2.6. Discussion

The majority of harvesting and extraction processes for microalgal biomass are still in the early stages of development, with information generally restricted to journal articles relating to university experiments. An optimum lipid extraction process at commercial-scale will be a trade-off between key factors including extraction efficiency, time

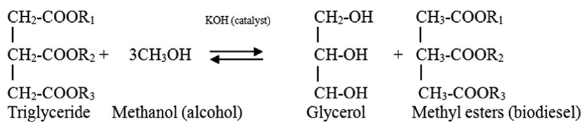

Fig. 3.1. The transesterification reaction [5].

taken, reactivity with lipids, capital cost, operating cost (including energy consumption), process safety and waste generation.

The hydrothermal liquefaction of wet biomass to bio-crude seems promising for scaling up to industrial scale [78]. A detailed HTL process of microalgae including bio-crude yield, carbon, nitrogen and hydrogen content in bio-crude and upgraded biocrude is presented where bio-crude quality can also be improved by vacuum distillation (normally used in petroleum refinery) without using a catalyst [78].

4.3. Biodiesel conversion

Vegetable oils and fats, and more specifically, microalgae oils, are 10–17 times more viscous than petroleum diesel [82]. Researchers began to reconsider using vegetable oil during the energy crisis of the 1970s, and developed a simple method called transesterification, for converting vegetable oil into a suitable diesel fuel with an appropriate viscosity, where an alcohol and a catalyst are added to the oil to initiate the reaction [83].The transesterification reaction is shown in Fig. 3.1 where blending alcohol with vegetable oil and adding a catalyst initiate the reaction and convert the oil to ester (methyl- or ethyl esters, depending on the alcohol used) and glycerol. These esters are called biodiesel [24,83]. However, pre-esterification is required to rid vegetable oils of free fatty acids, which will form soap in transesterification process, if left in the mix [69].

The transesterification process was developed several decades ago and is considered highly efficient and techno-economic analysis of the process shows that harvesting and dewatering of biomass contribute the most expense to the facility, which is the biggest hurdle for commercializing the process [72]. Recent experimentation and development has been carried out combining the McGyan (continuous catalyst process)and the supercritical processes [84]. A combined process of enclosed flat plane photo-bioreactor and direct transesterification with super critical methanol found with lower water consumption and decrease greenhouse gas emission by 86% compare to base individual technology [85]. In the McGyan process, supercritical methanol and porous titania microspheres in a fixed bed reactor are used to catalyse the simultaneous esterification and transesterification for free fatty acid and triacyl-glycerides, respectively [84].

5. Standard biodiesel properties

The rise in consumption of biodiesel has led to the need for standardising the quality requirements for alkyl ester-based fuels. The United States and European Union are at the forefront of the fuel regulation industry with internationally recognised standards ASTM D6751 and EN14214 used throughout the world. For pure biodiesel (B100) and blends of petroleum diesel with varying concentrations of biodiesel, the standards below can be employed [86,87]. Following this trend, the Australian government has established a biodiesel standard titled, “Fuel Standard (Biodiesel) Determination 2003” [88]. Using the EN and ASTM as starting points, the fuel properties have been determined for the Australian climate (Table 4).

Fatty acid profiles play a significant role in determining the physical and chemical properties of biodiesel. Researchers have made efforts to predict important fuel properties from fatty acid profiles. For example, some researcher use FAME composition to calculate cetane number whereas other researchers used iodine and saponification values [90,91] . The Smittenberg relation was used to estimate the density of saturated methyl esters at 20 °C and 40 °C [92] and an empirical correlation of saturated and unsaturated FAME was proposed for estimating viscosity [93].

6. Fatty acid composition of microalgae methyl ester and fuel properties

A systematic analysis of the fatty acid methyl ester (FAME) composition and comparative fuel properties is crucial for species selection for biodiesel production. The most common fatty acids from microalgae are Palmitic (hexadecanoic - C16:0), Stearic (octadecanoic - C18:0), Oleic (octadecenoic - C18:1), Lenoleic (octadecadienoic - C18:2) and Linolenic (octadecatrienoic - C18:3) acids [94]. Most algae have only small amounts of eicosapentaenoic acid (EPA) (C20:5) and docosahexaenoic acid (DHA) (C22:6), if present at all, however, depending on cultivation condition a substantial amount of polyunsaturated fatty acids (PUFAs) can be accumulate in some species of particular genera [44]. In general, diatoms and eustigmatophytes makes appreciable amounts of EPA, while dinoflagellates and haptophytes typically produce both EPA and DHA [95]. Schenk et al. [96] recommended a good quality biodiesel should have 5:4:1 fatty acid ratio of C16:1, C18:1 and C14:0. The composition of fatty acids in microalgae biodiesel has direct influence of their biodiesel property including cetane number, iodine value, oxidation stability and cold filter plugging point.

6.1. Cetane number

Cetane number (CN) is one of the most significant indicators of fuel combustion ability [97]. The CN relates to the autoignition quality and CN decreases with decreasing chain length and increased branching or higher unsaturation of the fatty acid chain [98]. The ASTM D-6751 standard specifies the minimum allowable CN as 47, whereas EN 14214 specifies a higher value of 51. A lower CN indicates longer ignition times causing engine knocking and incomplete combustion; increasing exhaust pollutants [99]. Saturated esters levels are advantageous to meet cetane number requirements but produce biodiesel with poor cold-flow properties [100]. Unsaturated, especially polyunsaturated fatty esters improve the cold-flow properties because of the lower melting point, which is desirable but also lowers the cetane number and oxidation stability which is an undesirable quality for fuel [100].

6.2. Density

Density is a measure of the mass per unit volume of a substance. In terms of engine performance, fuels with greater densities have the capability to provide more energy per litre than fuels with lower densities as injector pumps meter fuel to the engine volumetrically. The higher the density, the greater the amount of energy supplied. Biodiesel has a higher density than petroleum diesel and can potentially provide more power but at the cost of fuel consumption [101]. Relationships between the fatty acid composition and density of biodiesel have been identified in various studies [92,99]. Tests have shown that density increases with an increasing degree of unsaturation.

6.3. Kinematic viscosity

The kinematic viscosities of extracted microalgae oilsare high and require conversion into biodiesel to reduce the level of viscosity to a level similar that of diesel fuel. To ensure adequate supply to injector biodiesel must have an appropriate kinematic viscosity [102]. Kinematic viscosity limits are set to 1.9–6.0 $mm^2\,s^{-1}$ and 3.5–5.0 $mm^2\,s^{-1}$ as per ASTM 6751-02 and EN 14214 for 100% biodiesel. A higher viscosity affects the fuel atomisation and can lead to deposits forming inside the engine. It is also well known that inverse proportionality to temperature also affects the Cold Filter Plugging Point (CFPP) for engine operation at low temperatures. Furthermore,

Table 4
Biodiesel standards and test methods.

Fuel properties	Units	Europe (EN 14214) [86]	USA (ASTM 6751-12) [89]	Australia [88]	Test method
Density @15 °C	kg/m^3	860–900	Report	860–890	ASTM D1298
Viscosity @4 °C	mm^2/s	3.5–5.0	1.9–6.0	3.5–5.0	ASTM D445/ENISO 3104
Distillation T90	°C	n/a	360	360 max	ASTM D1160
Flash point	°C	120 min	130 min	120 min	ASTM D93
Flash point (close cup)	°C	–	93 min	–	ASTM D93
Sulphur	mg/kg	10.0 max	15 max	10.0 max	ASTM D5453
10% carbon residue	%mass	0.30 max	n/a	0.30 max	ASTM D4530
100% carbon residue	%mass	n/a	0.050 max	n/a	–
Sulphated ash	%mass	0.02 max	0.020 max	0.02 max	ASTM D874
Water and sediment	%vol	0.05 max	0.05 max	0.05 max	ASTM D2709
Total contamination	mg kg	24 max	n/a	24.0 max	EN 12662
Cu strip corrosion	3 h@50 °C	calss 1 max	No. 3 max	calss 1 max	ASTM D130/EN ISO 2160
Oxidation stability	h@ 110 °C	6.0 min	3 min	6.0 min	EN 14112/prEN 15751
Cetane number	–	51.0 min	47 min	51.0 min	ASTM D613/ASTM D6890
Linolenic acid (C18:3)	%mass	12.0 max	n/a	n/a	–
Polyunsaturated ≥4	mg/kg	1 max	n/a	n/a	–
Acid value	mg KOH/g	0.50 max	0.50 max	0.80 max	ASTM D664
Methanol	%mass	0.20 max	0.2 max	0.20 max	EN 14110
Ester content	%mass	96.5 min	n/a	96.5 min	EN 14103
Monoglyceride	%mass	0.80 max	n/a	n/a	–
Diglyceride	%mass	0.20 max	n/a	n/a	–
Triglyceride	%mass	0.20 max	n/a	n/a	–
Free glycerol	%mass	0.020 max	0.020 max	0.020 max	ASTM D6584
Total glycerol	%mass	0.25 max	0.240 max	0.250 max	ASTM D6584
Iodine number	gI_2/100 g	120 max	n/a	n/a	–
Phosphorus	mg/kg	10.0 max	10 max	10 max	EN 14107
Group I (Na+K)	mg/kg	5.0 max	5 max	5 max	EN 14538
Group II ((Ca+Mg)	mg/kg	5.0 max	5 max	5 max	EN 14538
Cold soak filterability	Seconds	n/a	360 max	n/a	Annex A1 to D6751–08
Cloud point (Summer/winter)	°C	report on request	Report on request	Report on request	ASTM D2500
CFPP	°C	≤5/≤−20	Report on request	Report on request	ASTM D4539

viscosity is directly proportional to the chain length of fatty acids but is inversely proportional to the amount of double bonds [99]. Biodiesel standard EN 14214 has limitation for the maximum amount of 4 double bond content to 1% of total fatty acids. Microalgae species naturally contain higher amounts of PUFA compared to other seeds oils. Therefore, selecting a microalgae species for biodiesel production should consider species producing lower amounts of PUFAs.

6.4. Higher heating value

One of the most important properties of fuel is its energy content, which is quantified by the higher heating value (HHV), also known as the heat of combustion. The HHV is determined by the amount of heat released during complete combustion of a unit quantity of fuel under standard atmospheric conditions (101 kPa, 25 °C) [103]. Typically, HHV of gasoline and regular diesel is around 46 and 43 MJ/kg, respectively and biodiesel is 10% lower than petroleum diesel [104]. Since unsaturated hydrocarbons are rare in crude oil, it is expected that the HHV for diesel is higher than biodiesel [104]. An increase in chain length and degree of saturation in the fatty acid composition also increases the HHV for microalgae biodiesel whereas 10–12% oxygen content in it reduces the HHV [104,105]. Therefore, microalgae species with higher amounts of long chain saturated fatty acids would be ideal for biodiesel production with better HHV.

6.5. Oxidation stability

Oxidation stability is one of the crucial fuel properties for storage time and distribution of any liquid fuel in large-scale production. A Rancimat test is undertaken to quantify the time it takes for fuel degradation producing volatile acids. If the "induction" time is short, the sample is said to be unstable. Therefore the ASTM D-6751 and EN14214 have set the minimum threshold of three and six hours, respectively. The oxidation stability of microalgae biodiesel depends on the chemical structure of the fatty acid methyl esters, especially degree of unsaturation and the presence of air, heat, light, traces of metal, antioxidants and peroxides [106]. The presence of double bonds in fatty acid chains and their position determine the rates of oxidation of the compound. It is reported that, Palmitic (C16:0) and Oleic (C18:1) acid in microalgae biodiesel have a positive effect on oxidation stability, whereas Linoleic (C18:2) and Linolenic acid (C18:3) have an adverse effect [107]. Therefore, the EN14214 specifies a limit of ≤12% mass for linolenic acid content in biodiesel.

6.6. Cold filter plugging point

Another critical fuel property is the cold filter plug point (CFPP), which is directly depends on the amount of unsaturated fatty acids in the fuel. CFPP is the lowest temperature, expressed in degrees Celsius (°C), at which a given volume of fuel still passes through a standardized filter and limits have been set to≤5/≤−20 °C in the EN 14214 for summer and winter respectively [86]. The higher the amounts of unsaturated fatty acids or low concentration of saturated fatty acids, lower the temperature range for CFPP [107,108]. In general microalgae biofuel contains higher amounts of unsaturated fatty acids which are desirable for CFPP, but this adversely affects the Iodine value for which limits of 120 gI_2/100 g biodiesel have been set in the EN14214. Therefore, an optimum ratio of saturated and unsaturated fatty acids in microalgae biodiesel should be determined so that quality complies biodiesel standards.

6.7. Biodiesel mandates around the world

Biodiesel can be used in a conventional diesel engine blended with petroleum diesel in any ratio [109]. There are an increasing number of literature reports supporting the performance of biodiesel in conventional diesel engines [53,63,110–122]. Results from the use of biodiesel show a substantial reduction in emissions of unburned hydrocar-

Table 5
Biodiesel blend mandates in different country.

Country	Current biodiesel/ethanol blend mandates (2014)	Future target
Argentina	B10/E5	–
Brazil	B5/E25	B6/E27.5
Canada (British Columbia)	RD4/E5	RD10/E10 (2020)
Canada (Alberta)	RD2/E5	–
Canada (Saskatchewan)	RD2/E7.5	–
Canada (Manitoba)	RD2/E8.5	–
Canada (Ontario)	RD2/E5	RD4 (2017)
USA (Minnesota)	B10	B20 (2018)
France	B6	B7
UK	E4.75	B10/E10 (2020)
Australia (NSW)	B2/E6	–
Australia (QLD)	–	E5(2017)/E10 (2020) Proposed
China	E10	B10 (2020)
India	B5/E5	B10/E10 (2017)
Malaysia	B5	B10
South Korea	B2	B3 (2018)

bons (HC), carbon monoxide (CO) particulate matter (PM) and sulphur oxides [63,110]. Many countries even implemented legislation and mandates for the use of biodiesel summarised in Table 5 [123].

7. Engine performance and exhaust emission for microalgae biodiesel

Typically, diesel engine performance parameters refer to engine power, torque, brake specific fuel consumption (BSFC) and brake thermal efficiency (BTE). Rodríguez et al [124] has summarised number of engine test result with microalgae biodiesel and their blends. It is commonly argued that biodiesel slightly reduces the power output and torque compared to petroleum diesel due to its lower calorific value [125–128]. Using 100% waste frying oil methyl esters, Utlu and Kocak [125] reported reductions in power and torque of 4.5% and 4.3%, respectively compared to petroleum diesel. However, higher density of biodiesel causes an increased amount of fuel injected into the combustion chamber, which could lead to an increase in power but poor atomisation due to higher viscosity can reduce the combustibility of fuel and reduce power [129–131]. Furthermore, higher lubricity of biofuel will reduce frictional loss and consequently recover engine power and torque [132]. Biodiesel from microalgae is reported to have higher viscosity, density and lower calorific values compared to other biodiesel [55]. It is reported that the species used in this experiment had very high amount 68% of long chain PUFA which are responsible for increased viscosity, density and lower combustibility (Cetane number). It is also reported that the blend of this high viscus microalgae biodiesel with petroleum diesel shows improve the quality and reduced emission such as unburn hydrocarbon. However, the brake specific fuel consumption (BSFC) of microalgae biodiesel 20% blend with petroleum diesel increased 5% compared to petroleum diesel and reduced the indicated mean effective pressure (IMEP) by 3.5% [55]. Therefore, biodiesel with a high long chain fatty acid profile is undesirable and selecting microalgae species would have a greater impact in fuel quality and ultimately in engine performance.

There are several investigations on the effect of microalgae biodiesel on exhaust emissions including CO, HC, NO_x and PM (Particulate Matter) [48,52,53,55,56,114,133–135]. Except for NO_x, a significant reduction in almost all gaseous emissions when using microalgae biodiesel blends with petroleum diesel compared to 100% petroleum diesel has been reported in most studies. Biodiesel contains 10–12% oxygen by mass, while petroleum diesel is almost void of oxygen. Oxygen content of biodiesels has been suggested to enable more complete combustion to occur resulting in reduced gaseous emissions. For the same reason, NO_x emissions are believed to be higher for microalgae biodiesel due to the more complete combustion and higher combustion temperatures; although some studies reported reduction of NO_x when used with biodiesel as shown in Table 6 [56,114]. These seeming contradictions can be explained by variation of microalgae species, chemical composition, biodiesel properties, and feedstock source and engines types used [136,137]. Higher levels of unsaturation and longer chain lengths increases NO_x emissions while saturated fatty acids and shorter chain length reduce the NO_x emissions [138]. Monyem and Gerpen linked oxidation state of biodiesel to reductions in CO and HC by approximately 15% and 21%, respectively compared to petroleum diesel [129]. There is a similar trend found for microalgae biodiesel for CO and HC emission in Table 6. Published engine tests with microalgae fatty acid methyl esters are rare, with the summary of the studies outlined in Table 6.

The general trend with microalgae biodiesel found with lower CO and unburn HC emission but increasing NOx emission. However, some research found with decreasing NOx emission with microalgae biodiesel [56,141]. Miocroalge oil (10%) blend with ethanol (10%) and petroleum diesel (80%) MEO20 tested in a single cylinder engine results upto 13.85% NOx reduction [142]. The NOx emission is mainly caused due to higher amount of long chain unsaturated fatty acids and its directly depends on species. The species used in those researches with lower NOx emission had lower percentage of long chain poly

Table 6
Engine performance and emission tests with microalgae methyl ester.

Used fuel	No. of cylinder (volume)	Algae oil blend	NO_x	HC	CO	PM	Ref
Microalgae methyl ester (produced in lab, Aleksandras Stulginskis University)	3 (3.3 L)	B30	↑	(15%)↓	(10%) ↓	–	[53]
Microalgae methyl ester (produced in lab, Delhi Technological Univ)	1	B20	(38%)↑	(31%)↓	(20%)↓	–	[52]
Microalgae methyl ester (Sourced from Soley institute, Turkey)	3	B20	–	–	(5.7%)↓	–	[48]
Microalgae methyl ester (Sourced from Soley institute, Turkey)	4 (3.9 L)	B20	(14%) ↑	–	(12%)↓	–	[49]
Microalgae methyl ester (produced in lab, UAE University)	1 (0.51 L)		–	–	–	–	[134]
Microalgae methyl ester (produced in lab, Utah State University, USA)	2 (0.48 L)		(24%)↓	(30%)↓	(17%)↓	–	[56]
Simulated microalgae oil methyl ester, (Colorado State University)	4 (2.4 L)	B100	(10%)↓	↑↓	(22.8%)↓	↑↓	[114]
Microalgae oil methyl ester from *Crypthecodiniumcohnii* (Produce in pilot-scale at QUT)	4 (2.0 L)	B50/10	14/0%↑	-/64%↓	–	(90/62%) ↓	[55,135]
Microalgae oil methyl ester from *Chlorella vulgaris* (Anna Uni.India)	1(0.51 L)	B40/60	c	≤1%↓	≤1%↓	–	[139]
Microalgae oil methyl ester from *Chlorella protothecoides* (VNIT, Nagpur, India)	1(0.51)	B100	↓	4%↓	2%↓		[140]
Microalgae oil methyl ester from *Chlorella protothecoides* (USQ, Australia)	1(0.219 L)	B100	7,4%↓	–	69.4%↑	–	[141]
Microalgae oil from *Chlorella protothecoides* and ethanol with petroleum diesel (USQ, Australia)	1(0.219 L)	B20	13.9%↓	18.8%↓	16.7%↓	–	[142]
Microalgae oil methyl ester from *Chlorella vulgaris* and *Chlorella sorokiniana* (Kun Shan University, Taiwan)	4(2.8 L)	B2	2%↑	50%↓	≤1%↓	22%↓	[143,144]

Increase ↑; decrease ↓; Ref: reference.

unsaturated fatty acids and causes reduction of NOx emission.

8. Conclusion

The paper has discussed the possible species selection process, difficulties of commercial harvesting biofuel production and overall microalgae biodiesel property and their performance. There are number of issues arises after careful analysis of current status of microalgae research and future need :

- A sensible selection of microalgae species would be necessary to ease the downstream process.A selection process based on the chemical composition of the extracts in conjunction with empirical fuel quality parameters calculated from the chemical profile would allow for species selection before commencing costly field set ups and trials.
- High value products such as omega-3 and omega-6separation from extracts would improve the biodiesel quality and provide a cushion for commercially viable biodiesel production from microalgae.
- High cost extraction processes are posing the biggest hurdle for microalgae biodiesel production for commercialisation. However, new technologies such as HTL and supercritical fluid extraction/ transesterification will potentially take the challenge.
- As engine performance and emissions are related to the fuel compositions and the test engine configurations,carefully designed comparable engine tests are required to verify effects of fuel quality in comparison to other biodiesels and regular petroleum diesel.
- Engine tests for microalgal biodiesels comparative with regular diesel are hampered by availability of biomass and limitation of large-scale microalgae biodiesel production.
- Nonetheless, the very limited number of engine tests conducted with microalgae biodiesel show impressive performance with regards to engine emission, i.e. reduction of hydrocarbon(HC) and PM.

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