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Algal biomass and diesel emulsions: An alternative approach for utilizing the energy content of microalgal biomass in diesel engines

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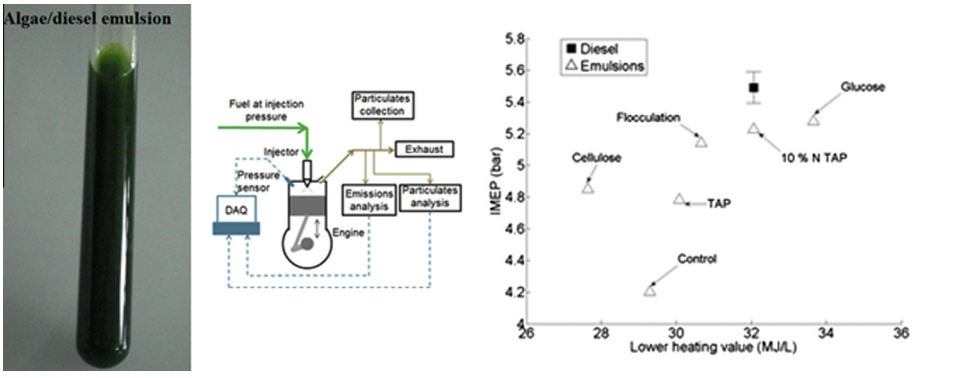
HIGHLIGHTS

- Diesel engine tests of algal slurry diesel emulsions with up to 6.6% wt/wt biomass.
- Novel surfactant package developed for preparation of stable emulsions.
- Engine work increased with algal biomass concentration and emulsion energy content.
- Algal slurry emulsions emitted lower exhaust NOx and PM than fossil diesel.
- Intact algae cells were found after high pressure fuel injection but not in exhaust PM.

GRAPHICAL ABSTRACT



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ABSTRACT

The use of algal biomass for the production of sustainable biofuels has attracted significant interest due to the fast reproduction rates and high lipid content of many microalgal species. However, existing methods of extracting algal cellular lipids are complex and expensive, with regards to both energy input and economic costs. This work explores an alternative method of utilizing the energy content of microalgae through the preparation of wet algal biomass slurry/fossil diesel emulsions containing up to 6.6% wt/wt algae biomass, using a specific surfactant combination, for direct injection diesel engine combustion of microalgae without prior biomass drying or lipid extraction.

A high lipid containing green microalgae, *Chlorella sorokiniana,* was used to produce algal biomass for the study. The preparation of wet algal slurry/diesel emulsions from algae grown under standard conditions, and also those under conditions intended to increase cellular lipid content or growth rates was investigated, and in all cases a surfactant pack of Span80, CTAB and butanol was found to produce a stable emulsion. A correlation between the engine work produced during combustion of the emulsions in a modern direct injection compression ignition and the lower heating value of the wet slurry emulsions was found, with no evidence of individual algae cells persisting to the engine exhaust. Engine exhaust emissions of nitrogen oxides (NOx) and particulate matter were lower for all of the wet algal slurry/diesel emulsions relative to a reference fossil diesel tested under similar conditions, while in the case of the emulsion prepared from algal biomass to which a flocculating agent had been added, emissions of carbon monoxide (CO) were found to increase significantly.

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Nomenclature

GHG	greenhouse gas emissions
FAME	fatty acid methyl ester
EU	European Union
IC	internal combustion
NOx	nitrogen oxides
PM	particulate matter
CO	carbon monoxide
CTAB	cetyltrimethylammonium bromide
SEM	scanning electron microscopy
CAD	crank angle degrees
THC	total hydrocarbons
IMEP	indicated mean effective pressure
BTDC	before top-dead-centre
N	nitrogen
SOI	start of injection
TDC	top-dead-centre
SOC	start of combustion
TAP	Tris Acetate Phosphate
γ	gamma

1. Introduction

Alternative liquid fuels from renewable sources will increasingly be required to displace those from fossil sources, with growing evidence and acceptance of the negative impacts of global climate change arising from the combustion of fossil fuels [1]. However, there is also increasing awareness that for alternative fuels to be truly sustainable, consideration must be given to the total greenhouse gas emissions (GHG) produced during the entire lifecycle of the fuel production to usage. Therefore, there is a need to develop new fuels that not only minimize combustion emissions of fossil bound CO_2 and other regulated pollutants, but also require a minimum of processing and energy consumption prior to use.

Since the early 1980's, increasing attention has been given to first vegetable oils, and then the transesterified products of vegetable oils, fatty acid methyl esters (commonly referred to as FAME or biodiesel) as substitutes for fossil diesel fuels [2,3]. Biodiesels from various sources are now commercially produced and mandatorily mixed with fossil diesels (for example to meet renewable energy commitments in EU member states [4]). At present, the most commonly used sources of vegetable oils for biodiesel production are food crops such as soybean, sunflower seed, rapeseed and corn [5]. However, the potential of biofuels produced from food crops is inhibited by a number of factors, including limited production capacity and a lack of suitable arable lands [6]. Microalgae are an alternative source of oils suitable for biodiesel production, and relative to other sources possess several unique properties such as: a fast growth rate, high lipid content, low nutrient requirement, adaptability to a wide range of environmental conditions and presenting no competition with human food supply [7].

Despite these advantages, there are however still many obstacles to achieving the successful industrial scale production of algal biofuels, with several elements of the process of algae cultivation to oil extraction either prohibitively cost intensive or vulnerable to external influences, for example environmental and operating conditions [6]. Minimizing the cost of algal biomass recovery from the cultivation media is key to reducing overall costs, with Chisti [8] suggesting that the cost of the recovery process could comprise 50% of the final cost of oil production from bioreactor cultivated microalgae. In particular, existing methods of extracting algal ellular lipids are known to be complex and expensive, with low extraction efficiencies attributable to the challenges of water removal and cell disruption [6,9], and various means of increasing the efficiency of these processes have been investigated [10–12]. These include the selection of high lipid yield mutant strains of microalgal species by Anandarajah et al. [13], who reported a 22% increase in total lipid productivity relative to parent wild type *Nannochloropsis* sp, and the development of high cell density cultivation methods for both increased algal biomass and lipid concentrations by Zheng et al. [14], who achieved an algal biomass concentration of 103.8 gL^{-1} and lipid concentration of 40.2 gL^{-1} (relative to typical algal biomass concentrations of 0.5–2.0 gL^{-1} when cultivated in open ponds [9,15]). Thermochemical approaches to the production of liquid fuels from micro-algae, such as pyrolysis and hydrothermal liquefaction, have also received significant interest in recent years and can utilize wet algal biomass as a feedstock [15–18]. However, there remains much potential in developing further alternative approaches to utilizing algal biomass as a sustainable fuel source for internal combustion (IC) engines, which circumvent the need for complete dewatering of the biomass or for cellular lipid extraction.

In addition to the displacement of fossil bound carbon emissions during combustion, a further possible advantage of all biofuels, including those from micro-algae, is that the composition of these fuels can also result in the reduction of other regulated engine exhaust pollutants, such as nitrogen oxides (NOx) and particulate matter (PM) emissions. Exhaust emission levels of NOx and PM from diesel engines when fueled with pure biodiesel, or biodiesel blends, relative to operation with fossil diesel fuel has received significant interest [19–25], with an impact of biodiesel molecular composition apparent on the emitted levels of both pollutants [26]. During combustion, rates of NOx formation, and PM production and oxidation, are strongly influenced by in-cylinder temperatures [27,28], which (in addition to engine operating conditions) are dictated by the duration of fuel ignition delay and the extent of the premixed burn fraction [29]. This therefore presents an opportunity to optimize the molecular composition of future fuels for reduced emissions levels, especially in the context of fuel production from photo-synthetic micro-organisms (such as micro-algae) which can be modified through means of metabolic engineering to produce a wide range of hydrocarbon and oxygenate molecules [30].

Modifying the composition of the in-cylinder charge in IC engines as a means of controlling combustion temperatures has a long history of use; for example in the early 20th century by introducing water into the air fuel mixture as a means of reducing engine knock in spark ignition combustion [31]. More recently, water and fossil diesel fuel emulsions have received significant attention as a potential route to simultaneous reductions in NOx and PM emissions while maintaining engine thermodynamic efficiencies [32,33]. However, due to the immiscibility of diesel fuel and water, the formation of stable and uniform emulsion systems requires the use of specific additives. These act as surfactants and modify the surface properties of micro-water droplets, reducing the interfacial tension between the water and hydrocarbons that comprise fossil diesel fuels [34]. To date, numerous surfactant technology related techniques have been developed to both improve the stability of water/diesel emulsions and increase the emulsion water content, with typical water/diesel emulsions containing between 10% and

20% (v/v) water [35]. Zhao et al. [36] explored various factors that impact on the maximum water content of stable emulsions and found that the combination of two surfactants to be superior to the use of either single surfactant in isolation. Furthermore, both the amount of surfactant, or alcohol co-surfactant, and the chemical structure of the surfactants utilized had a significant influence on the formation of a stable emulsion. An alternative approach has been the use of enzymes as an additional additive for the formation of stable water/diesel emulsions, with Lin et al. [37] investigating the potential of the natural organic enzyme-7F to assist the formation of water and soybean derived biodiesel emulsions.

However, an inevitable disadvantage of water/diesel emulsions is the reduction in the energy density of the fuel supplied to the engine, with water of zero calorific value displacing fossil diesel or biodiesel. Given the complexities and economic cost of recovering algal biomass, an alternative approach to utilizing the energy content of algal cellular lipids (and also that of the algal cellular carbohydrates, proteins, nucleic acids and other macromolecules) may be to form emulsions of wet algal slurry with fossil fuels (or biofuels), similar to emulsions of water and fossil diesel. Such an approach has not been widely considered previously and has the potential to drastically reduce the process energy input required for utilizing algal biomass as a fuel for IC engines, combined with the possible reduction in emission of exhaust pollutants that may be achieved with water and diesel emulsions, while offsetting the reduced calorific content of such blends.

Previous experimental investigations into the potential of algal slurry/diesel emulsions have been very limited, with Scragg et al. [38] investigating the use of an emulsion of *Chlorella vulgaris* with a rapeseed oil derived fatty acid ethyl ester in a direct injection diesel engine. The emulsion tested comprised of 80% v/v rapeseed ethyl ester, 20% v/v water, 0.5% v/v of the surfactant Triton X-100 and 2 g per litre of dry algal biomass. An increase in CO emissions and decrease in NOx emissions during testing of the emulsion was reported relative to a fossil diesel. More recently, Al-Iwayzy et al. [39] compared emulsions of water and a cottonseed biodiesel with an emulsion of water, cottonseed biodiesel and *C. vulgaris* biomass in a single cylinder air-cooled direct injection diesel engine. The algal biomass emulsion utilized consisted of 79.2% v/v of cottonseed biodiesel, 19.8% v/v water, 1% v/v surfactant and dry algal biomass at a concentration of 0.4 g per litre of emulsion. Relative to an emulsion without algal biomass, but of otherwise identical composition, the authors found no impact on engine emissions of the algal biomass.

This paper presents experimental investigations into the preparation and engine testing of algal biomass slurry/fossil diesel emulsions with a variety of algal biomass concentrations at levels significantly higher than those previously investigated. However, unlike a water/diesel emulsion, an algal biomass slurry/diesel emulsion is not a simple two-phase system, but is in fact significantly more complex, as the surface of an algal cell is a solid thick cell wall composed of complicated polysaccharides and glycoproteins. These membranes are difficult for surfactant chains to penetrate, and the algal cells are not easily deformed or broken into smaller structures relative to water droplets. Furthermore, due to the negatively charged cell surfaces, the most commonly used non-ionic surfactants for water/diesel emulsions are not suitable for algal biomass slurry/diesel emulsions. Therefore, a novel combination of a non-ionic surfactant, Span80, and a cationic surfactant, CTAB, was developed and used in the preparation of the algal biomass slurry/diesel emulsions, with *n*-butanol used as a co-surfactant to help improve the stability of the emulsion system. Combustion and emissions characterization of the algal biomass slurry/diesel emulsions was conducted in a single cylinder direct injection diesel engine, with scanning electron microscopy (SEM) used for investigation of the persistence of whole algal cells during the fuel injection and combustion processes.

2. Materials and methods

2.1. Preparation of algal slurry/diesel emulsions

2.1.1. Algal strain and cell cultivations

A wild type strain of the green alga *C. sorokiniana* (UTEX1230) was obtained from the Culture Collection of Algae at the University of Texas (Austin, Texas, USA). Tris Acetate Phosphate (TAP) medium was used to maintain and culture the alga [40]. A modified TAP medium containing only 10% nitrogen of that in normal TAP medium (i.e. 0.75 mM instead of 7.5 mM) was used for cell growth to encourage cellular lipid accumulation. TAP medium enriched by 10 g/L glucose was used to obtain lipid-rich algal cells and a higher biomass concentration. Algal cultures in liquid medium were maintained in 3 L Erlenmeyer flasks containing 1.5 L medium with cotton plugs at 25 ± 1 °C under continuous shaking (120 rpm). Continuous light (~65 μmol photons/[m^2 s]) was provided by cool white fluorescence lamps in the incubators. Small stock cultures with 25 mL medium were grown to mid-log phase by inoculation from colonies on TAP-2% agar plates and diluted 1 in 100 (v/v) as inoculum for larger cultures.

Samples of culture broth were taken regularly during the cultivation to monitor cell growth. Cell growth was determined by measuring the optical density at a wavelength of 750 nm (OD_{750nm}) using a UV/Vis spectrometer (Thermo Electron Co. UK) and by counting the cells of the culture broth using a haemocytometer and a microscope (Olympus, Japan). The final biomass concentration of the culture broth was measured as dry cell weight per litre following lyophilization [41]. Cultures were grown to a final biomass concentration of approximately 1 g/L before harvesting. Neutral lipid content was semi-quantitatively determined by staining with the lipophilic fluorescent dye, Nile red [42]. Nile red was added to the culture broth to a final concentration of 2 μg/mL and the fluorescence measured using a Perkin-Elmer LS-55 Luminescence Spectrometer (Perkin Elmer, USA), with the excitation wavelength set at 510 nm and the emission scanned between 520 and 800 nm.

2.1.2. Algal biomass harvesting

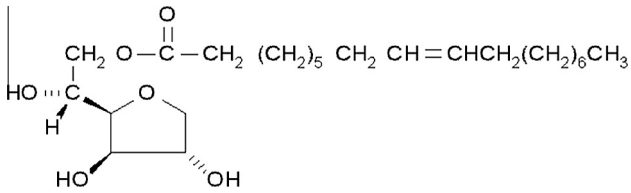
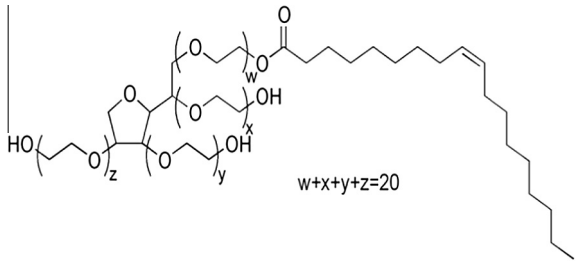
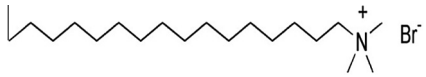
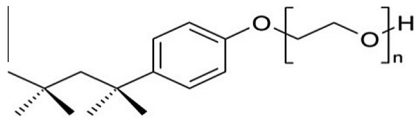
Algal biomass was harvested either by centrifugation, or flocculation followed by centrifugation. The preparation of algal biomass by centrifugation of large volume culture broth (~800 mL) alone was carried out with a Sorvall Evolution RC Superspeed Centrifuge (Thermo Scientific, UK) for 15 min at 2500 rpm under room temperature. Cell pellets were then re-suspended in a smaller volume (~20 mL) of distilled water and transferred into 20 mL centrifugation tubes. These algal suspensions were centrifuged again with a MSE Mistral 1000 centrifuge (Thermo Life Science, UK) for 10 min at 4500 rpm. The harvested algal cells were re-suspended once more in distilled water to 10 mL and centrifuged further with a Heraeus Megafuge 16R Centrifuge (Thermo Scientific, Germany), with the supernatant removed after centrifugation. The volume and weight of the wet slurry was measured, washed with distilled water and either dried by lyophilization before blending with diesel, or used directly for blending without drying.

The harvesting of algal biomass by flocculation and subsequent centrifugation of the culture broth of *C. sorokiniana,* using chitosan as the flocculant, was conducted in 1 L flocculation jars, in accordance with the optimal conditions obtained from our previous flocculation study [43]. After flocculation and sedimentation, clarified supernatants were removed, and the remaining floc suspensions were then concentrated further by centrifugation and blended with diesel in accordance with the method described for harvesting by centrifugation without flocculation.

2.1.3. Preparation of algal biomass slurry in diesel emulsions

For the preparation of the algal biomass/diesel emulsions, Span80 was obtained from Sigma–Aldrich Company Ltd. (Dorset,

Table 1
Properties of the surfactants tested for preparation of stable algae/diesel emulsions, where HLB refers to the hydrophilic-lipophilic balance of a surfactant, a measure of the degree of hydrophilic or lipophilic; W/O-water in oil emulsion, O/W-oil in water emulsion.

Chemical	Formula	Ionic property	HLB	Function
Span80		Non-ionic	4.3	Emulsifying agent W/O
Tween80		Non-ionic	15	Emulsifying agents O/W; Detergents
CTAB		Cationic	10	Emulsifying agent O/W
Triton X-100		Non-ionic	9.5	Emulsifying agent O/W; Wetting and spreading agents

UK), while CTAB was obtained from Fisher Scientific (Leicestershire, UK), and Tween80 was obtained from Koch-Light Laboratories Ltd (Suffolk, UK). Triton X-100, methanol, ethanol and butan-1-ol were all obtained from BDH Laboratory Supplies (UK). The chemical structures and properties of the surfactants and co-surfactants considered are listed in Table 1.

These surfactants were used either alone or combined with others to determine the optimum surfactant pack for formation of stable algae/diesel emulsions. Following the harvesting of cultures of *C. sorokiniana* in accordance with the methodology described in Section 2.1.2, the wet algal cells were washed with distilled water to reduce the presence of any remaining ions from the medium, and cell pellets were obtained after centrifugation. Two approaches were taken to the preparation of algal slurry diesel emulsion: in the first, dry algal cells obtained after freeze drying were grinded as finely as possible, and the required amount of dry biomass was weighed and added to a reference fossil diesel. In the second approach, a volume of wet algae slurry was measured and blended with a measured amount of diesel to form the targeted algal concentration. The systems prepared were agitated thoroughly for at least two minutes and then left to settle for approximately 10 min. The stability of the emulsion systems was then identified by visually observing the separation of the algal slurry phase and diesel phase.

2.2. Combustion and emissions characterization of algal slurry/diesel emulsions in a single cylinder diesel engine

2.2.1. Apparatus

All combustion experiments presented were conducted in a normally aspirated single cylinder direct injection diesel engine specially designed for combustion research. This consisted of components taken from a production 2.0 L 4 cylinder-turbo charged automotive diesel engine (Ford Duratorq 2.0 CD132 130PS) mounted onto a single cylinder crank case (Ricardo Hydra). The production cylinder head, injector, piston, connecting rod and cylinder liner were retained to preserve realistic combustion geometry. Preliminary experiments with algal slurry diesel emulsions resulted in the immediate failure of the conventional solenoid valve direct injectors used, and this was suspected to be attributable to the blocking of various control orifices within the injector by agglomerates of individual algae cells. The engine was instead equipped with a mechanically operated fuel delivery system, utilizing a six-hole injector (with each hole of diameter of 154 μm), which was specific to the design of engine head, opened at a fuel pressure of 275 bar and mechanically closed by a high strength compression spring. Test fuels, pressurized to the requisite injection pressures, were delivered to the injector by a single plunger fuel pump, driven by the engine crankshaft. The fuel pump was fed by a small sealed reservoir tank, with the fuel pre-pressurized for ensuring constant flow to the fuel pump by the application of laboratory compressed air at 2 bar to the tank headspace. The fuel pressure between the fuel pump and injector was measured with a piezoresistive pressure transducer (Gems Sensors 3100H22002TS). A schematic of the experimental apparatus is shown in Fig. 1, with further details of the engine and control apparatus given in Table 2.

The normally aspirated engine had a geometric compression ratio of 18.3:1, and for all the tests air was aspirated into the combustion chamber at atmospheric pressure and temperature. The engine cylinder gas pressure was measured and logged with a PC data acquisition system (National Instruments) at a resolution of 0.2 CAD using a piezoelectric pressure transducer (Kistler 6056AU38) and charge amplifier (Kistler 5011). The cylinder pressure was pegged at bottom-dead-center of every combustion cycle by the data acquisition system using a piezoresistive pressure transducer (Druck PTX 7517-3257) located in the intake manifold, 160 mm upstream of the inlet valves. Measurement of various control and experiment temperatures was undertaken with K-type thermocouples and logged with the PC data acquisition system.

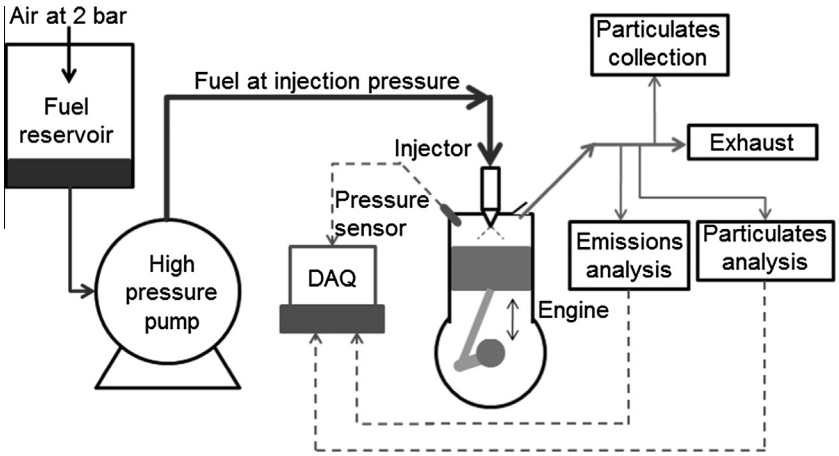


Fig. 1. Schematic showing engine testing experimental layout.

Table 2
Engine specification.

Engine head model	Ford Duratorq
Engine bottom end model	Ricardo Hydra
Number of cylinders	1
Cylinder bore	86 mm
Crankshaft stroke	86 mm
Swept volume	499.56 cc
Compression ratio	18.3: 1
Maximum cylinder pressure	150 bar
Peak motoring pressure at test conditions	45 bar
Piston design	Central ω – bowl in piston
Oil temperature	80 ± 2.5 °C
Water temperature	80 ± 2.5 °C
Fuel injection pump	Single – cam radial – piston pump (MICO LIC. BOSCH)
Injectors	6 – hole opening on pressure (DELPHI)
Shaft encoder	0.2 CAD resolution

In-cylinder pressure data was measured and logged for 100 consecutive combustion cycles, and during post-processing of this data the net apparent heat release rate was derived utilizing a one dimensional and one zone model assuming homogeneity and ideal gas behavior of the cylinder contents. The values of gamma used for the calculation were those suggested by Heywood [44], namely $\gamma = 1.35$ during the compression stroke prior to TDC and $\gamma = 1.28$ subsequent to TDC during the expansion stroke. While such an approach was considered sufficient for the analysis of heat release rates in this instance, much work has been undertaken in developing advanced multi-zone approaches for determining gross heat release rates which account for heat transfer during combustion [45,46].

Exhaust gas sampling occurred at 180 mm downstream of the exhaust valves to determine concentrations of gaseous species and also particulate size distribution. A gas analyzer system (Horiba MEXA 9100 HEGR), supplied with sample gas via heated lines, was used to measure the following: NOx concentrations by chemiluminescence (at an accuracy of ±0.1 ppm); CO and CO_2 concentrations with non-dispersive infrared (at an accuracy of ±0.1 ppm and ±0.1% vol/vol respectively); paramagnetic analysis to determine O_2 concentrations (at an accuracy of ±0.01% vol/vol); and levels of unburnt hydrocarbons were measured with a flame ionization detector (at an accuracy of ±1 ppm). Size and mass distributions of the sub-micron particles in the exhaust gas were determined by a differential mobility spectrometer (Cambustion DMS500). Sampling of exhaust gases for particulate measurements was made via a heated line, with a dilution cyclone located at the connection between the engine exhaust and heated line. Particulate samples were collected by partially diverting the exhaust gases through a 0.22 micron glass fiber filter 700 mm downstream of the exhaust valves.

2.2.2. Experimental conditions

All of the wet slurry diesel emulsions under consideration were tested at a constant engine speed of 1200 rpm, as were tests of a reference fossil diesel. The relatively low constant engine speed of 1200 rpm was chosen so as to allow for longer durations of engine operation on the restricted quantities of wet slurry diesel emulsions available. The volume of fuel injected per cycle was kept constant in the case of each test fuel, and the engine load, defined by the indicated mean effective pressure (IMEP), was allowed to vary accordingly. Algal slurry/diesel emulsions prepared from lyophilised algal biomass (Section 2.1.3) were tested alongside a reference fossil diesel at a constant start of injection (SOI) of 12.1 CAD BTDC (before-top-dead-centre) ±1.3 CAD. The tests of algal slurry diesel emulsions prepared from wet algal slurry (Section 2.1.3) were conducted alongside a reference fossil diesel at a constant SOI of 31.6 CAD BTDC ±0.4 CAD. The SOI for the tests of the algal slurry diesel emulsions prepared from wet algal slurry was advanced relative to those emulsions prepared from lyophilised algal biomass, as during the engine tests of the latter (Section 3.2.1) the SOI utilized of 12.1 CAD BTDC ±1.3 CAD was found to be insufficient for fuel and air mixing to a degree representative of typical compression ignition combustion prior to the fuel auto-ignition.

2.3. Scanning electron microscope (SEM) analysis

Samples of the algal slurry/diesel emulsion following high pressure injection and particulates from the engine exhaust gas were examined using SEM (Hitachi S-3400N field emission scanning electron microscope utilizing a range of magnification between 170× and 20,000×, and with an acceleration voltage of 10 kV to 20 kV). To collect a sample of the algal slurry diesel emulsion post-injection (but prior to combustion), the fuel injector was removed from the engine and positioned so that the emulsion was injected into a closed vessel at an injection pressure equivalent (~320 bar) to that used during the engine tests. A small volume (3–4 mL) of the emulsion was then poured through a 0.22 micron glass fiber filter and allowed to dry under ambient conditions for 24 h. Exhaust particulate samples were collected by partially diverting the exhaust gases through a 0.22 micron glass fiber filter 700 mm downstream of the engine exhaust valves. In both instances, a subsection of the filter papers was prepared for SEM analysis by gold deposition on the filter surface.

Table 3
Properties of algal cultures at various cultivation conditions grown for measurement of growth parameters only. Cultures were maintained till early stationary phase and each condition was set up at least in triplicate. Values are the mean of measured values ± standard deviation.

Culture condition	OD_{750nm}	Dry weight (g/L)	Maximum specific growth rate (h^{-1})
TAP (light)	4.98 ± 0.75	0.78 ± 0.20	0.087 ± 0.005
10% N TAP (light)	2.64 ± 0.68	0.35 ± 0.15	0.080 ± 0.007
TAP + glucose (light)	15.32 ± 0.71	3.62 ± 0.26	0.104 ± 0.007
TAP + glucose (dark)	16.10 ± 0.89	3.75 ± 0.38	0.099 ± 0.007

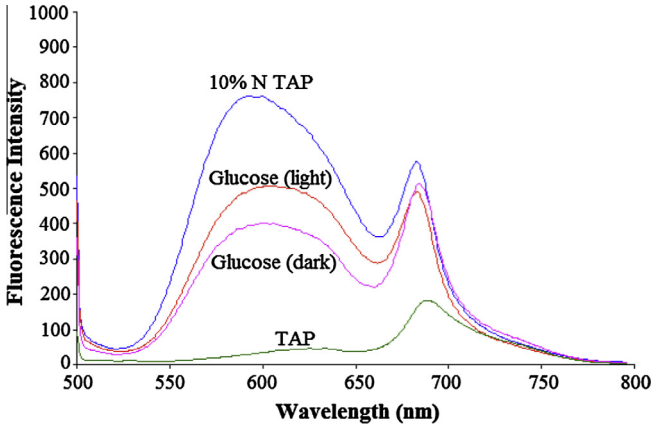


Fig. 2. Nile red fluorescence curves of cultures from four different growth conditions assayed at stationary phase. Nile red was added to a final concentration of 2 μg/mL and fluorescence measured using a Perkin-Elmer LS-55 Luminescence Spectrometer with the excitation wavelength set at 510 nm and the emission scanned between 520 and 800 nm. Peaks at around 580 nm show the content of neutral lipids within the cells qualitatively. The curves shown are representative of assays of three separate cultures for each condition.

3. Results and discussion

3.1. Preparation of algal biomass slurries

Algal biomass was produced under four culture conditions, as described in Section 2.1.1, and harvested via two different methods (Section 2.1.2), to investigate the influence of the biomass physical properties on the characteristics of the slurries to be utilized for the preparation of biomass and diesel emulsions.

3.1.1. Growth rates and lipid content of algal cultures under varying conditions

Table 3 shows the final biomass concentration and maximum specific growth rate for each culture condition. The cultures in acetate-containing medium (TAP medium) with added glucose, grown either under light or dark conditions, produced almost four fold more biomass than those in the standard TAP medium, while the final biomass concentration in the nitrogen depleted medium was approximately half of that in standard TAP medium (Table 3). Similarly, the growth rates of the TAP + glucose cultures (in light or dark) were appreciably higher than those of the TAP medium and 10% N TAP medium, with the rate in 10% N TAP slightly lower than for TAP medium. These results indicate that for applications requiring large amounts of algal biomass the addition of glucose to the culture medium may be an effective strategy.

The cellular neutral lipid content of algal biomass obtained from the four culture conditions (Section 2.1.1) was evaluated through measurement of fluorescence intensity using the lipophilic dye, Nile red. Algal cells obtained from each culture were re-suspended to an equivalent biomass concentration before staining with Nile red. Fig. 2 shows the fluorescence intensity (representative of the lipid content profile) of the four different cultures.

As can be seen in Fig. 2, the cultures grown with added glucose in both light and dark conditions have elevated levels of cellular lipids compared to those grown in standard medium, while algal cells cultured in N-depleted medium have an even higher lipid content, as indicated by a higher fluorescence. However, the very low biomass production rate of cultures in N-depleted medium (Table 3), resulted in a lower overall lipid yield than the cultures with glucose, and was also observed to take significantly longer to reach the stationary phase than all other cultures. Differences in the composition of algal biomass were also visible in the appearance of the culture broths, with cultures in the standard medium appearing a dark green color, while those in the N-depleted medium had a yellow hue. These phenotypic changes are a well-established stress response of green algae to nitrogen limitation, where growth is retarded, storage lipids accumulate and the chlorophyll-containing photosynthetic apparatus is broken down [47]. Interestingly, the addition of glucose appears to induce lipid accumulation under non-stress conditions; cultures grown in the TAP + glucose mediums accumulated more lipids than the those grown in the TAP medium and so also looked pale, but with a much higher cell density apparent than cultures in N-depleted medium.

3.1.2. Algal biomass recovery

The measurement of the algal slurry volumes following dewatering (via centrifugation, or flocculation followed by centrifugation) allowed for calculation of the increase in biomass concentration achieved following the centrifugation process and the final water content of the algal slurries. Table 4 shows the measured volumes and densities following dewatering of the algal slurries utilized in preparation of the algal slurry diesel emulsions (Section 3.1.3), and also the calculated biomass and water contents of the slurries.

Table 4
Calculated and measured (mean of ten values) concentration parameters of the algal slurries obtained in the centrifugation process or flocculation with chitosan followed by the centrifugation process and subsequently utilized for preparation of algal slurry diesel emulsions. All algae were cultured in shake flasks under continuous light (~65 μmol photons/[m^2 s]) at 25 °C with continuous shaking (120 rpm).

Culture and harvesting conditions	Algal slurry volume (mL/L culture)	Algal slurry density (g/mL)	Dry biomass in algae slurry (g/mL slurry)	Water content in algal slurry (%)
TAP	2.50 ± 0.28	0.99 ± 0.08	0.17 ± 0.09	82.8
10% N TAP	1.25 ± 0.09	1.09 ± 0.04	0.34 ± 0.05	68.8
TAP + glucose	3.13 ± 0.21	0.97 ± 0.05	0.37 ± 0.05	61.9
TAP + flocculation	2.88 ± 0.24	1.14 ± 0.04	0.45 ± 0.07	60.5

The repeated washing steps resulted in a significant loss of algal biomass, reducing the biomass yield available for diesel blending relative to that measured in the algal cultivations (Table 3). In Table 4, it can be seen that the efficiency of the dewatering process varied among the cultures. Algal slurry obtained from the cultures in standard TAP medium contained the highest level of water; while per unit volume, algal slurry from N-depleted medium with lipid rich cells contained a similar amount of biomass as cultures in medium with glucose. The addition of chitosan as a flocculant showed very little effect on the subsequent centrifugation process relative to direct centrifugation of non-flocculated cells from TAP medium (Table 4). The dewatering level was improved very slightly by the flocculation process, as is apparent from the smaller volume but higher density of the algal slurry and the higher concentration of algal biomass in the slurry. The concentrated wet algal slurry was washed with distilled water, and was either dried by lyophilisation before blending with diesel, or used directly for blending without drying.

The cell sizes of the different cultures were monitored by a Mastersizer 3000 (Malvern Instruments Ltd.). Fig. 3 shows the particle size distribution curves of varying particle sizes and also a reference cellulose powder. It can be seen in Fig. 3 that relative to the particle size distribution curve of the TAP culture, the distribution curves of cultures in 10% N TAP and TAP with glucose moved to a larger particle size. Algal cells cultured in standard TAP medium displayed a mean particle diameter of approximately 3.3 μm, while cells cultured under conditions that induce lipid accumulation displayed a larger mean particle diameter of approximately 4.4 μm in diameter (a calculated increase in cell volume of approximately 2.4 fold). No difference in cell size was observed between cells grown in the presence of glucose and those growing in N-limited medium, despite the slightly lower lipid content of the former (Fig. 2).

3.1.3. Surfactant pack forming stable algae/diesel emulsions

The functionality of a surfactant is highly dependent on the surfactant chemical structure, which has direct implications for the stability of an emulsion system utilizing surfactants. The selection of surfactants screened for formation of stable algal biomass slurry/diesel emulsions was based on previous studies of surfactants for commercial water/diesel emulsions (a water-in-oil emulsion system) [36]. Of the two major emulsion systems, i.e. oil/water emulsions and water/oil emulsions, the suspension of algal cells in fossil diesel is more comparable to a water/oil emulsion as the surfaces of algal cells are highly hydrophilic. As a result, this work aimed to obtain an algae/diesel emulsion similar to a water/oil system, and therefore it was preferable to generate a pack of surfactants with higher oil solubility [48].

In preliminary experiments, a group of commonly used surfactants with varying properties, including sorbitan monooleate (Span80), Polyoxyethylene (20) sorbitan monooleate (Tween80), octyl phenol ethoxylate (Triton X-100), cetyltrimethylammonium bromide (CTAB), were tested for effectiveness in obtaining stable algal biomass slurry/diesel emulsions, with several alcohols (including methanol, ethanol, butan-1-ol) examined for suitability as co-surfactants. Due to the hydrophilic and negative charged nature of the algal surface, stable algae/diesel emulsions were finally prepared from the algal biomass slurries specified in Table 4 using a combination of non-ionic Span80 and cationic CTAB as surfactants, and butanol as co-surfactant. Table 5 shows the composition of the final surfactant pack which was selected based on optimum emulsion stability with a minimum surfactant requirement.

For every 10 mL of emulsion prepared for the engine tests (described in detail in Section 3.2), 1.5 mL of the surfactant pack specified in Table 5 was utilized regardless of the algal biomass concentration. Fig. 4 shows the appearance of an algae/diesel emulsion produced with the final surfactant pack relative to a water/diesel emulsion and also pure fossil diesel.

The algae/diesel emulsion consists of hydrocarbon petroleum diesel and algae slurry in an emulsion in which the diesel is the continuous form. Fig. 5 shows the appearance of an algae/diesel emulsion under a microscope at a magnification of x100, and readily apparent is an even distribution of algal cells throughout the emulsion.

3.2. Combustion and emissions characterization of algae/diesel emulsions

3.2.1. Engine tests of algae/diesel emulsions prepared from dry algal biomass

In order to investigate the impact of the presence of algae cells within a fuel emulsion on combustion characteristics in a modern compression ignition engine (Section 2.2), dry biomass of *C. sorokiniana* cultured in TAP medium was obtained through centrifugation followed by lyophilisation (as specified in Section 3.1.2) and utilized to form emulsions of a surfactant pack (as specified in Table 5) to reference fossil diesel volumetric ratio of 1.5:8.5,

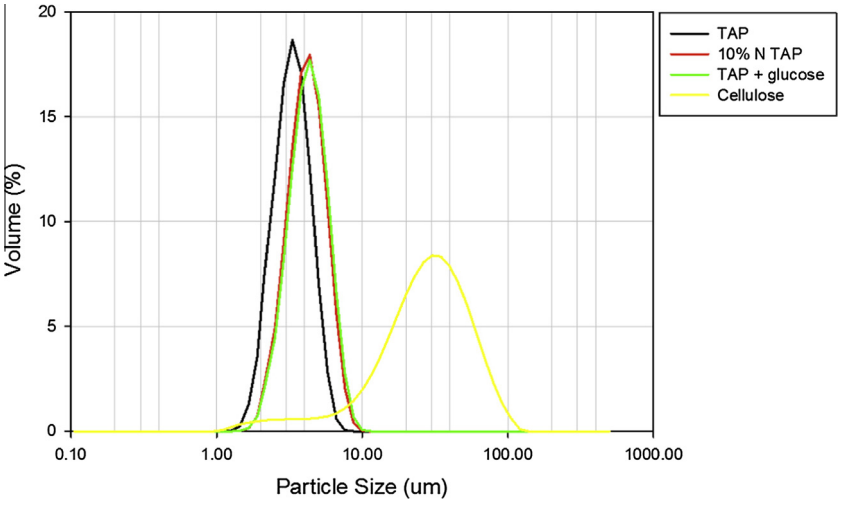


Fig. 3. Particle size distributions of algal cells cultured in different media and wet cellulose powder solution. Cultures were grown in shake flasks under continuous light (~65 μmol photons/[m^2 s]) at 25 °C with continuous shaking (120 rpm).

Table 5
Final compositions of the surfactant pack used in the preparation of algae/diesel emulsions for engine combustion.

	Volume (mL)	Weight (g)
Span80	0.45	0.44
CTAB	–	0.30
Butan-1-ol	0.60	0.49
Water	0.45	0.45
Total	1.50	1.68

containing either 5% w/v or 7.5% w/v dry algal biomass. A control emulsion with an equivalent volume of diesel and surfactants, but no algal biomass, was also prepared.

Fig. 6 shows the in-cylinder pressure and apparent net heat release rates of the dry algal biomass emulsions of varying algae content, and also the reference fossil diesel. The term 'Diesel start' is used in Figs. 6, 9 and 17 to refer to the testing of the reference fossil diesel immediately prior to testing of the algal slurry diesel emulsions, while 'Diesel end' refers to a test of the reference fossil diesel immediately following the test of the algal slurry emulsions. While all of the emulsions tested passed through the fuel injector, immediately apparent in Fig. 6a, is a significantly lower peak in-cylinder pressure for the emulsion containing 7.5% w/v dry algal biomass relative to the same volume of control emulsion of zero algal biomass content, the emulsion containing 5% w/v dry algal biomass and the reference diesel. It can be seen in Fig. 6b that for all of the emulsions, and the reference diesel, the majority of heat release takes place during diffusion mode combustion. This is likely attributable to the late SOI employed in the engine tests of the dry algal biomass emulsions (12.1 CAD BTDC), which may have resulted in insufficient time for fuel and air premixing prior to the start of combustion [29]. Also apparent in Fig. 6b are lower rates of heat release rate for the end test of the reference fossil diesel relative to those observed during the test of the reference fossil diesel prior to testing of the dry algal biomass emulsions, possibly suggestive of a detrimental effect of the emulsions on the operation of the fuel injector.

Fig. 7 shows the maximum fuel pressure and IMEP of the dry algal biomass emulsions and reference diesel with constant fuel volume injection. In Fig. 7, and where also present in the following figures, the limits of the error bars shown are plus and minus one standard deviation from the mean value (the value displayed on the plots), taken from repeat experimental runs of the same test fuel. In Fig. 7a, it can be seen that as the dry algal biomass content of the emulsions increased from 0% w/v, to 5% w/v and 7.5% w/v there is a linear increase in the maximum fuel pressure from approximately 330 bar to 380 bar. This increase in the maximum fuel line pressure relative to the reference fossil diesel and the control emulsion (Fig. 7a) suggests an increase in the density of the dry algal biomass emulsions as the percentage w/v of dry algal biomass is increased. Notwithstanding the high degree of variability in IMEP (~±0.5 bar) observed in constant volume injection of the reference diesel (Fig. 7b), significantly less work output was produced by the engine from the emulsion containing 7.5% w/v dry algal biomass relative to the control emulsion and that containing 5% w/v dry algae. This is in agreement with the observed lower in-cylinder pressures and heat release rates (Fig. 6a and b) of the 7.5% w/v dry algal biomass emulsions, and suggests that at levels greater than 5% w/v, the presence of dry algal biomass does not contribute to energy release during combustion, and or reduces the efficiency of energy release from the liquid fuel components.

Fig. 8 shows the particle number emissions and total particulate mass emissions of the dry algal biomass/diesel emulsions and reference diesel. Visible in Fig. 8a is an increase in the peak number of particulates emitted with an increasing level of algal biomass present in the emulsion. Apparent also is a reduction in the peak particle number diameter from approximately 100 nm to 40 nm with an increase of the level of dry algal biomass from 5% (w/v) to 7.5% (w/v) (Fig. 8a). Furthermore, the increase in the level of dry algal biomass present resulted in a decrease in the total mass of particulates emitted (Fig. 8b).

3.2.2. Engine tests of algae/diesel emulsions prepared from wet slurry

Following engine tests of algae and diesel emulsions prepared from dry biomass (Section 3.2.1), experiments were conducted with emulsions prepared from wet algae slurries cultured and harvested under various conditions so as to ascertain the influence of the biomass physical properties on the combustion characteristics of the resultant emulsions with diesel. To determine a suitable water content that would not impede combustion for the preparation of emulsions from wet algae slurry, a series of preliminary engine experiments were conducted with emulsions containing the reference fossil diesel, the surfactant pack and varying levels of water (0, 10%, 20% and 30% v/v). The engine experiments were conducted with an earlier start of injection at 31.6 CAD BTDC (Section 2.2.2) and constant volume of fuel injection, and found that while combustion continued at water contents up to 30% (v/v), at levels greater than 20% (v/v) the duration of ignition delay was significantly increased by approximately 8 CAD. Therefore, a water content of 15% (v/v) was chosen for the subsequent preparation

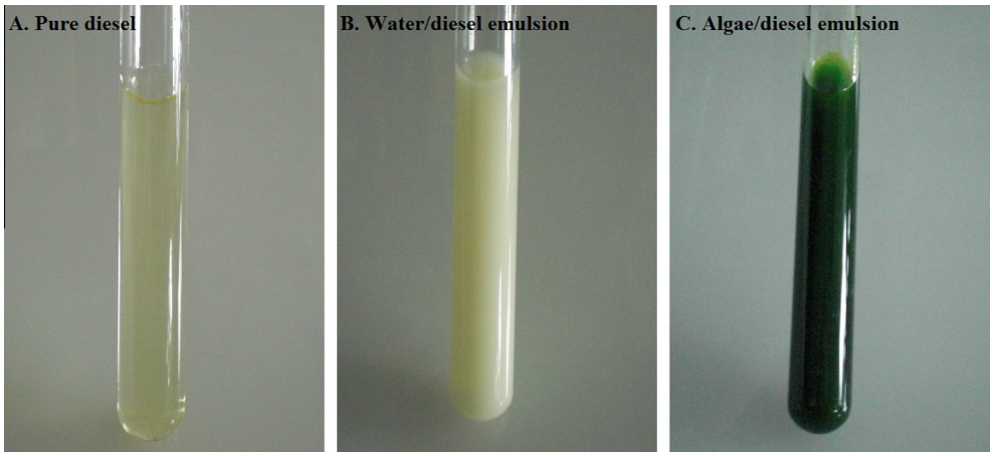


Fig. 4. Algae/diesel emulsion samples prepared for engine combustion. A. Pure diesel; B. Water/diesel emulsion with water content of 10% (v/v); C. Algae/diesel emulsion using wet algae slurry with algae slurry content of 10% (v/v) and dry algae biomass of about 2% by weight.

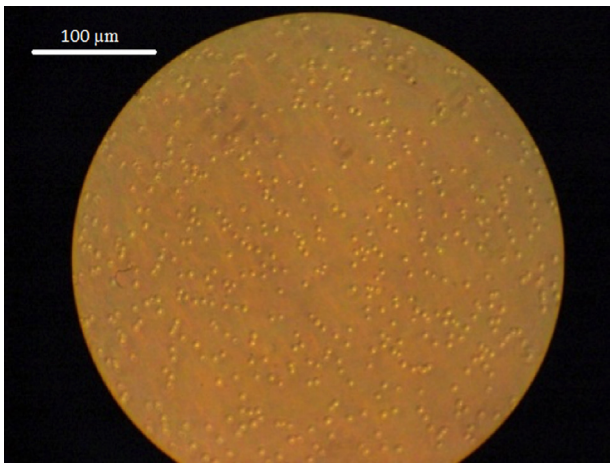


Fig. 5. Microscope observation of algal cells cultured from TAP medium suspended in the diesel with an algal biomass concentration of ~0.5% w/w in the suspension.

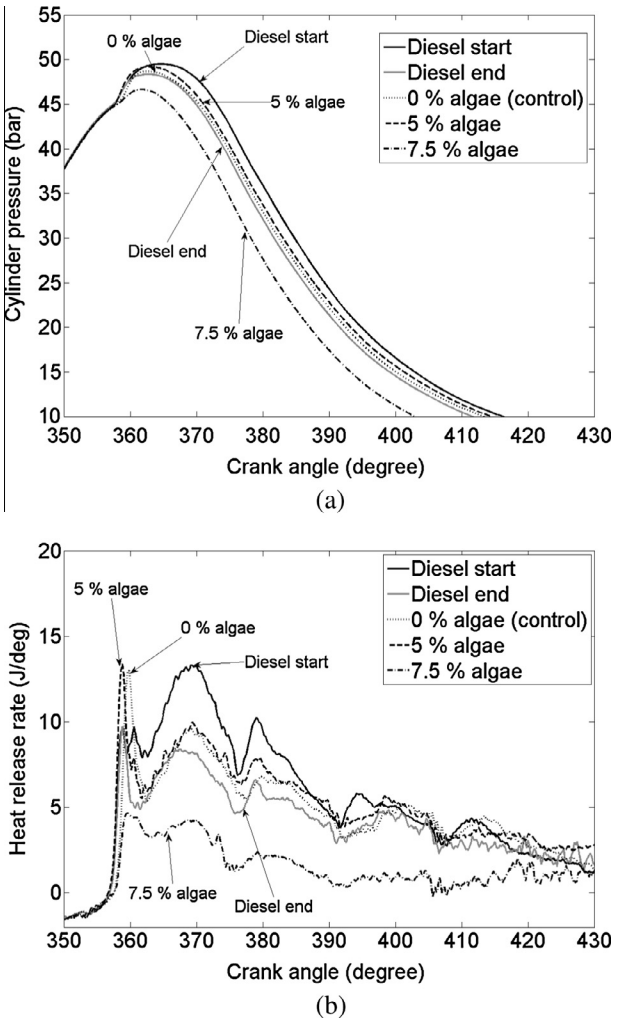


Fig. 6. (a) In-cylinder pressure and (b) apparent net heat release rates of the dry algal biomass diesel emulsions and reference diesel.

of the emulsions from wet algae slurry; 1.5 mL of algal slurry was used for every 10 mL algae/diesel emulsion, with a resultant algal biomass concentration of between 2.7 and 6.6% (w/w) dependent on the density of the algal slurry.

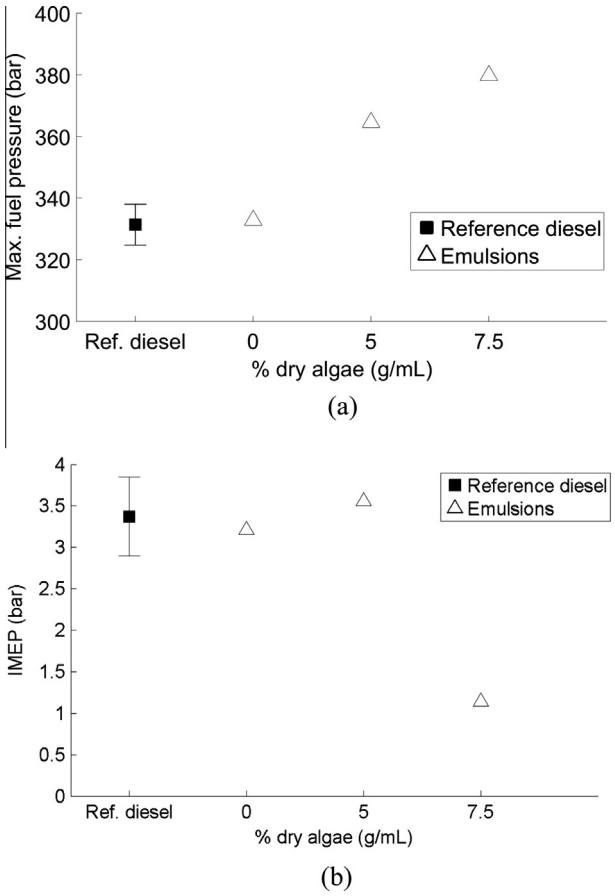


Fig. 7. (a) Maximum fuel pressure and (b) indicated mean effective pressure (IMEP) of the dry algal biomass diesel emulsions and reference diesel.

Table 6 shows the properties of the four emulsions from wet algal slurry prepared, and also those of a control emulsion prepared from only water, diesel and the surfactant pack, and an emulsion containing dry cellulose powder of mean particle diameter of 20 µm in place of algal biomass. While in the preparation of each emulsion, the mass of algae slurry was maintained at approximately 1.5 g per 10 mL, the varying water contents of the slurries resulted in a range of final algal concentrations. Algal slurry obtained from cultures grown in TAP medium retained the highest water content, resulting in an emulsion with the lowest concentration of algal biomass (~2.7% w/w), approximately half the biomass concentration of the other emulsions (Table 6). The emulsion of cellulose was prepared from a water based slurry containing 40% cellulose by weight.

Fig. 9 shows the in-cylinder pressure and apparent net heat releases for a constant volume of fuel injection of the wet algal slurry, control and cellulose emulsions, and reference diesel. It can be seen that at the earlier SOI employed (31.6 CAD BTDC) relative to the tests of dry algal biomass emulsions (Section 3.2.1), all of the fuels tested exhibit energy release during both premixed and diffusion combustion (Fig. 9a and b). Also apparent are higher peak heat release rates in the case of the algal slurry emulsions, relative to both the reference fossil diesel and control and cellulose emulsions (Fig. 9b).

Fig. 10 shows the maximum fuel line pressure during fuel injection for the wet slurry diesel emulsions and reference fossil diesel. It can be seen that all of the emulsions were subject to fuel line pressures approximately 20–30 bar higher than that experienced by the reference diesel (Fig. 9). This can likely be attributed to the higher densities of the wet slurry diesel emulsions (Table 6)

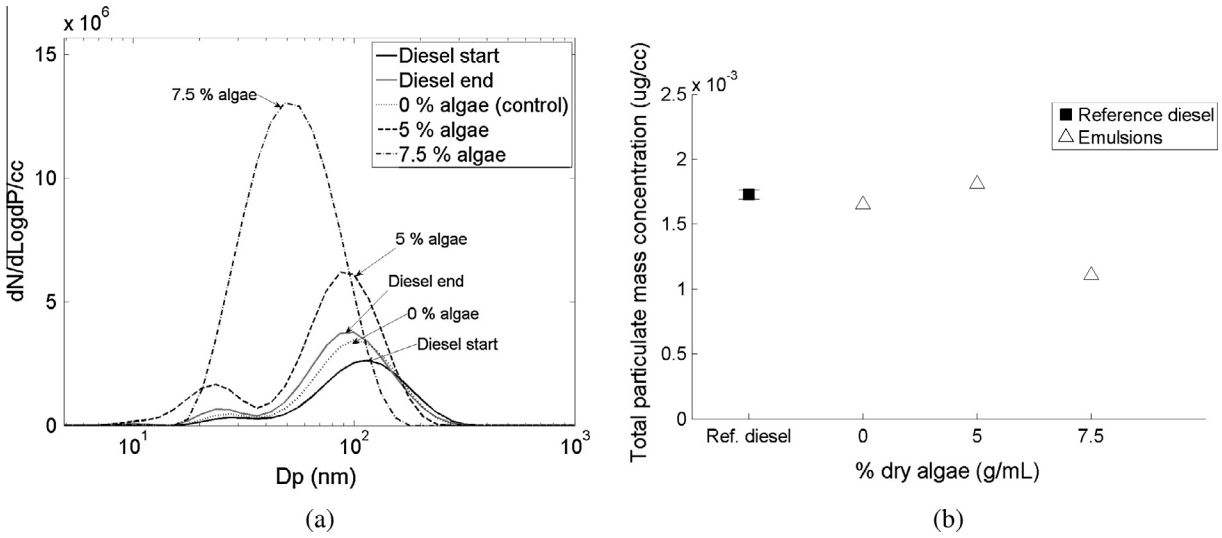


Fig. 8. (a) Particulate number emissions and (b) total particulate mass emissions of the dry algal biomass diesel emulsions and reference diesel.

Table 6
Characterization of the algae/diesel emulsions prepared from wet algal slurries cultured in different mediums.

Sample (Every 10 mL)	Total weight (g/10 mL)	Density (g/mL)	Algal slurry weight (g)	Dry algal biomass (g/10 mL)	Algal concentration (% m/m)	Lower heating value (MJ/kg)	Lower heating value (MJ/L)
Control emulsion	8.73	0.873	–	–	–	32.54	29.29
TAP	8.91	0.891	1.41	0.24	2.7	33.76	30.08
10% N TAP	9.05	0.905	1.55	0.48	5.3	35.43	32.06
Glucose	9.07	0.907	1.57	0.60	6.6	37.10	33.65
Flocculation	8.98	0.898	1.48	0.59	6.6	34.14	30.66
Cellulose	8.75	0.875	1.25	0.50	5.7	31.58	27.63

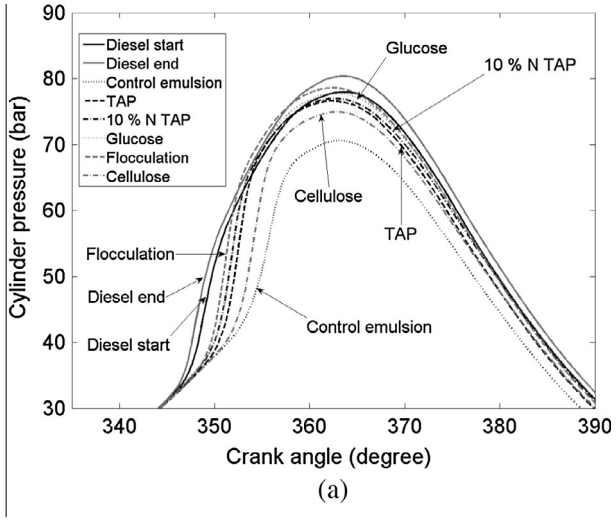
relative to the reference diesel fuel tested (which possessed a measured density of 0.835 g/mL).

Fig. 11 shows the engine IMEP produced during the tests of the wet slurry diesel emulsions and the reference fossil diesel. It can be seen that for a constant volume of fuel injection, all of the emulsions produced a lower engine work output than the reference fossil diesel. Of the emulsions tested, the control emulsion containing no algal biomass or cellulose produced the lowest IMEP, while the highest work output was obtained by the emulsions prepared from glucose grown algal slurry (Fig. 11), which also contained the highest concentration of algal biomass and possessed the highest measured lower heating value of all the emulsions (Table 6). It should be noted that the much reduced range of variation in IMEP observed during the tests of the wet slurry diesel emulsions (Fig. 11) relative to that observed during the tests of the dry algal biomass emulsions (Fig. 7b), can likely be attributed to the earlier SOI utilized in the tests of the former (Section 2.2.2).

Fig. 12 shows the engine IMEP produced during tests of the algal slurry diesel emulsions and reference diesel relative to the lower heating value of each of the fuels. In the case of the algal slurry emulsions, it can be seen that for a constant volume of fuel injection, engine output (IMEP) increases with an increasing lower heating value, with the highest IMEP obtained from the glucose grown algal slurry and the lowest from the TAP algal slurry (Fig. 12). The lower IMEP of the glucose algal slurry relative to that of the reference fossil diesel, despite a higher lower heating value on a volumetric basis, would suggest the presence of the algal slurry and/or surfactant pack to have adversely affected the efficiency of energy release during combustion (Fig. 12). Meanwhile, the slightly higher IMEP of the cellulose emulsion relative to the TAP grown algal slurry emulsion, despite a lower energy content (Fig. 12) is potentially attributable to the later start of combustion in the case of the cellulose emulsion (Fig. 9b), resulting in heat release closer to TDC and at a smaller in-cylinder volume (which can be expected to reduce rates of heat transfer to the cylinder walls). It is also interesting to note the significantly larger particle diameter of the cellulose powder relative to the algal cells utilized in preparation of the algal slurry diesel emulsions (approximately 15 μm and 4 μm respectively, Fig. 3), as it might tentatively have been assumed that a larger particle might combust more slowly and less efficiently than a smaller particle of higher surface area to volume ratio.

Fig. 13 shows the duration of ignition delay of the wet slurry diesel emulsions and reference diesel, where ignition delay is the period between SOI and SOC (defined as the first appearance of positive heat release following SOI). In Fig. 13, it can be seen that all of the wet slurry diesel emulsions displayed a longer duration of ignition delay than the reference fossil diesel. This is likely attributable to the presence of water and biomass in the wet slurry emulsions, which are effectively displacing diesel fuel and are unlikely to contribute positively to the rates of the low temperature reactions that lead to the accumulation of ignition promoting radicals and increasing temperatures towards autoignition [31,49]. The evaporation of water can also be expected to reduce in-cylinder temperatures to a greater extent than the evaporation of diesel, given the higher enthalpy of vaporization of water, 43.99 kJ/mol [50], relative to typical diesel components, such as *n*-heptane with an enthalpy of vaporization of 35.95 kJ/mol [51].

Fig. 14a and b shows the CO exhaust emissions of the wet slurry diesel emulsions and the reference fossil diesel, and the same



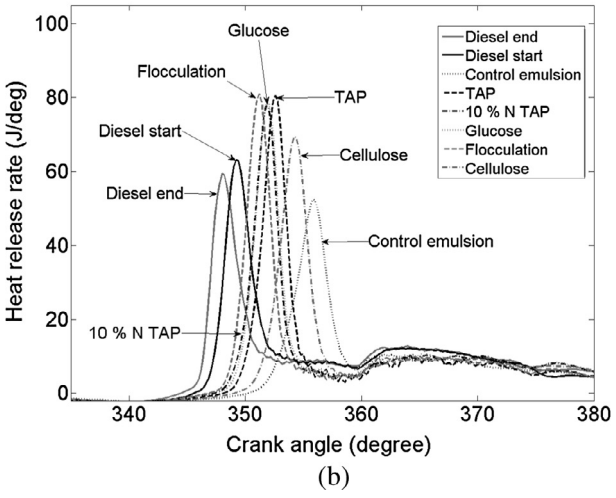


Fig. 9. (a) In-cylinder pressure and (b) apparent net heat release rates of the wet slurry diesel emulsions and reference diesel.

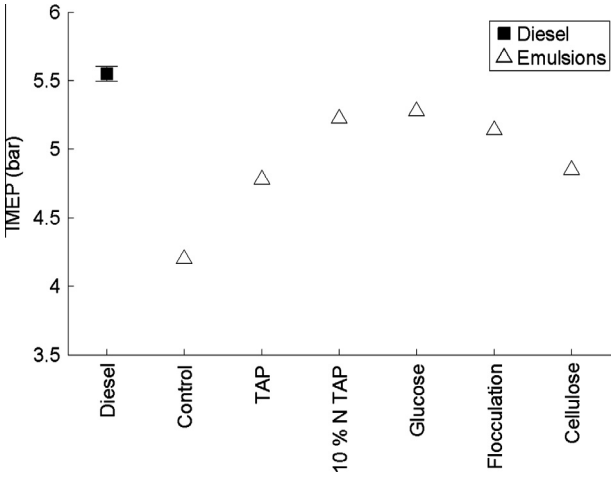


Fig. 11. Engine IMEP of the wet slurry diesel emulsions and reference diesel.

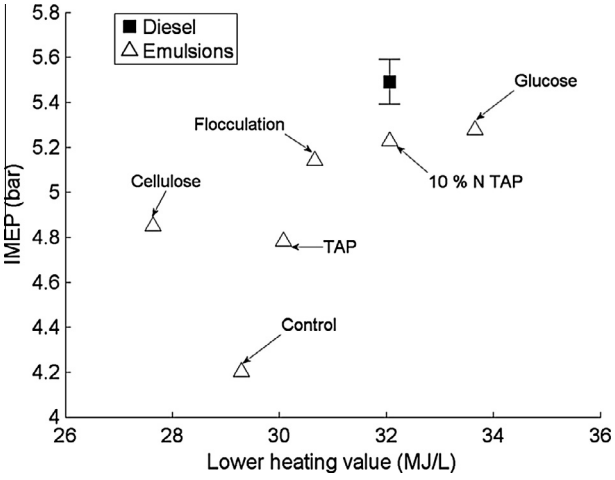


Fig. 12. Engine IMEP of the wet slurry diesel emulsions and reference diesel relative to the test fuel lower heating value.

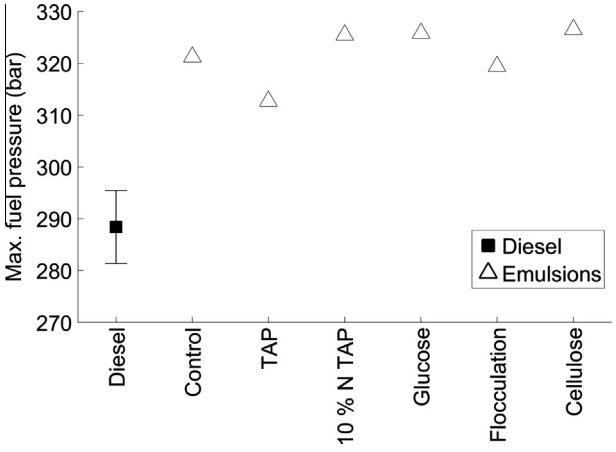


Fig. 10. Maximum fuel pressure of the wet slurry diesel emulsions and reference diesel.

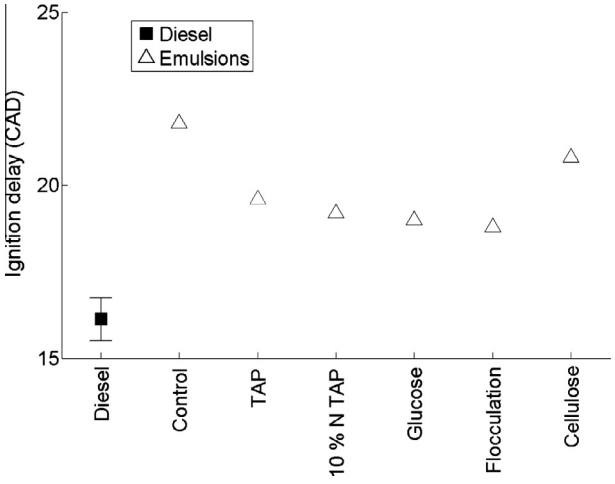


Fig. 13. Ignition delay duration of the wet slurry diesel emulsions and reference diesel.

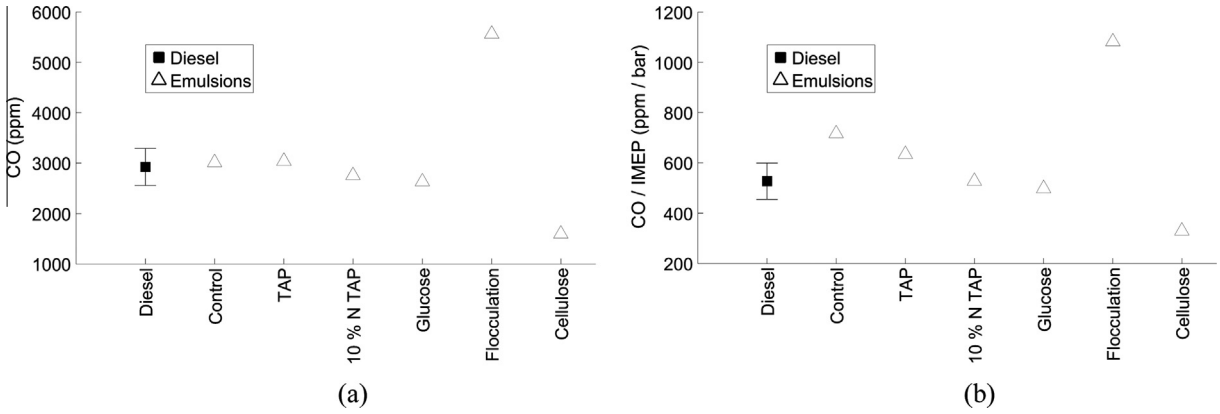


Fig. 14. (a) Measured CO exhaust emissions, and (b) CO exhaust emissions normalized with respect to the engine IMEP, of the wet slurry diesel emulsions and reference diesel.

emissions normalized with respect to the engine IMEP produced by each fuel (Fig. 11). It can be seen that while the CO emissions of the TAP, 10% N TAP and glucose cultured algal biomass emulsions are equivalent to those from the reference diesel, those from the flocculation emulsion are significantly higher (Fig. 14a and b). It is plausible that the flocculation of the algal slurry may have led to larger agglomerates of algal cells present in the subsequently prepared emulsion relative to those prepared from algal slurry concentrated by centrifugation alone, and that some of the flocculating agent (chitosan) may have persisted as solid particles. Therefore, given that CO is known to form in fuel rich areas of the combustion chamber [44] (where insufficient oxygen is present for complete oxidation of fuel carbon), it is suggested that the possible presence of algal cell aggregates, or chitosan particles in the emulsion prepared through flocculation, may inhibit fuel and air mixing relative to the other emulsions considered. It can be seen that the control emulsion (and to a lesser extent the emulsion produced from TAP cultured algal biomass) emitted higher levels of CO than the other emulsions when normalized with respect to engine IMEP (Fig. 14b), suggesting the lower in-cylinder temperatures attributable to the lower engine IMEP produced by these emulsions (Fig. 11) to have decreased rates of fuel carbon oxidation.

Fig. 15a and b shows the THC exhaust emissions of the wet slurry diesel emulsions and the reference fossil diesel, and the same emissions normalized with respect to the engine IMEP produced by each fuel (Fig. 11). Apparent in Fig. 15a and b, is that all of the wet slurry emulsions emitted higher levels of THC relative to the reference diesel. The control emulsion, containing no algal biomass or cellulose, emitted the highest levels of THC (Fig. 15), and also produced the lowest engine IMEP for a constant volume of fuel injection (Fig. 11). Exhaust emissions of THC are the result of incomplete fuel combustion, levels of which increase with reduced in-cylinder temperatures, which in turn decrease with lower engine IMEP and heat release (Fig. 9b).

Fig. 16 shows the NOx emissions of the wet slurry diesel emulsions and reference diesel, and the same emissions normalized with respect to the engine IMEP produced by each fuel (Fig. 11). With and without the exhaust emission levels normalized with engine IMEP (Fig. 16a and b), it can be seen that all of the emulsions produced lower levels of NOx than the reference fossil diesel tested, with the control and cellulose emulsions emitting lower levels of NOx than any of the algae slurry emulsions. The lower NOx emissions of the wet slurry diesel emulsions relative to the reference fossil diesel is in agreement with several previous studies of water diesel emulsions [32,33,35]. The production of NOx during compression ignition combustion is known to be dominated by the thermal oxidation of nitrogen [52,27], and has been observed previously to correlate strongly with peak heat release rates [29]; an increase in which increases in-cylinder temperatures and thus NOx formation rates. However, in Fig. 9b it can be seen that all of

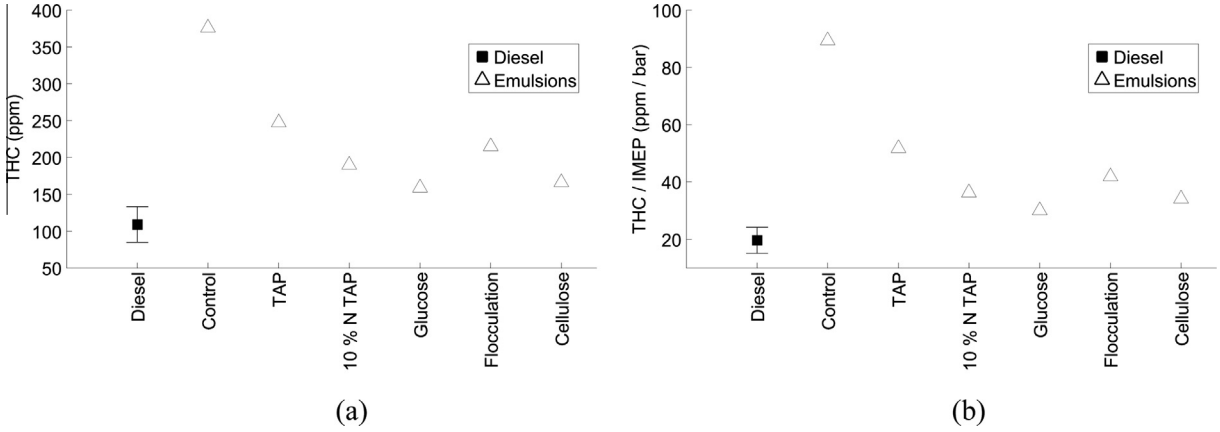


Fig. 15. (a) Measured THC exhaust emissions, and (b) THC exhaust emissions normalized with respect to the engine IMEP, of the wet slurry diesel emulsions and reference diesel.

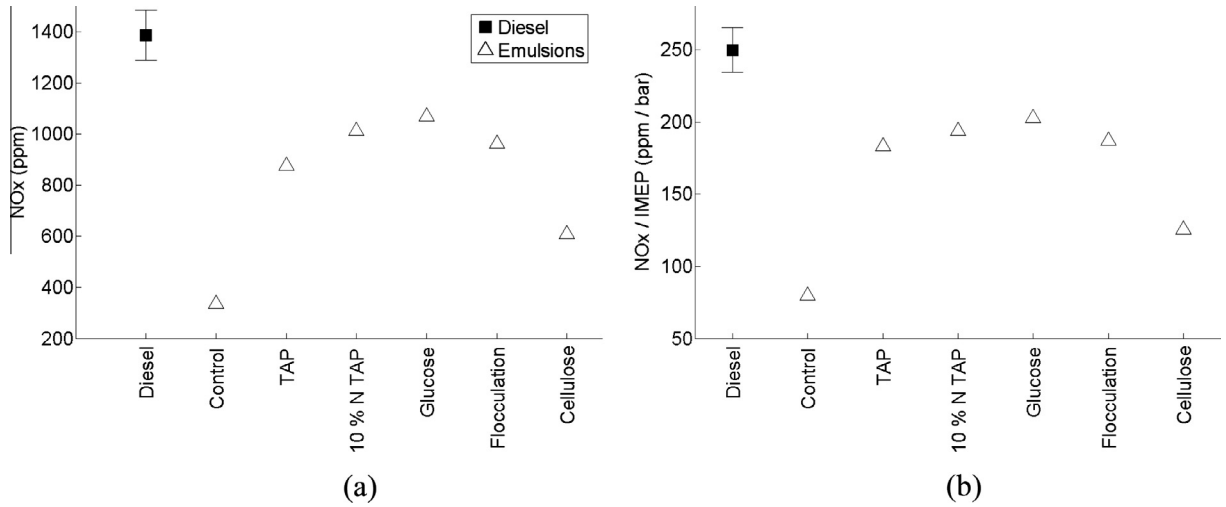


Fig. 16. (a) Measured NOx exhaust emissions, and (b) NOx exhaust emissions normalized with respect to the engine IMEP, of the wet slurry diesel emulsions and reference diesel.

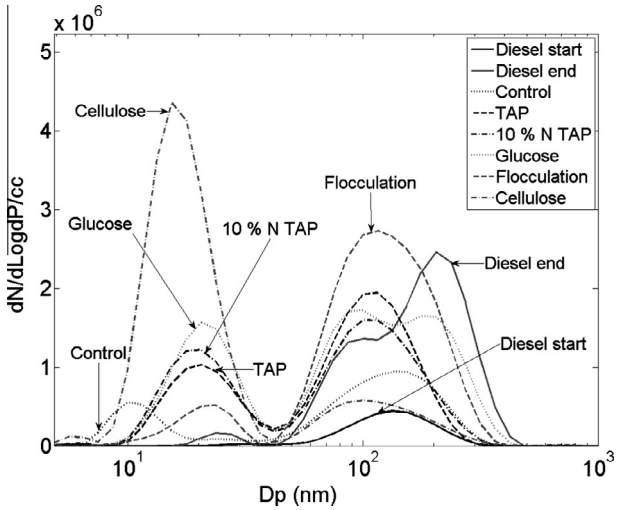


Fig. 17. Particle number emissions of the wet slurry diesel emulsions and reference diesel.

the algal slurry emulsions, and also the cellulose emulsion, displayed a higher peak heat release rate than the reference fossil diesel, which produced higher levels of NOx than all of the emulsions (Fig. 16). It is therefore suggested that the evaporation of the emulsion water content resulted in a decrease in local in-cylinder temperatures, thereby reducing NOx production (Fig. 16), despite higher apparent net peak heat release rates (Fig. 9b).

Fig. 17 shows the particle number emissions of the wet slurry diesel emulsions and reference diesel. Immediately apparent in Fig. 17 is that all of the emulsions (and reference fossil diesel) produced peaks of particulates of around 10–20 nm in diameter and of around 100 nm diameter. It can be seen that the cellulose slurry emulsions produced significantly higher numbers of particulates of diameter 10–20 nm than the other wet slurry emulsions and reference diesel (Fig. 17). Notwithstanding the range of variability as indicated by the diesel start and diesel end tests (Fig. 17), it can also be seen that the flocculation algae slurry emulsion produced the highest number of particles of diameter 100 nm.

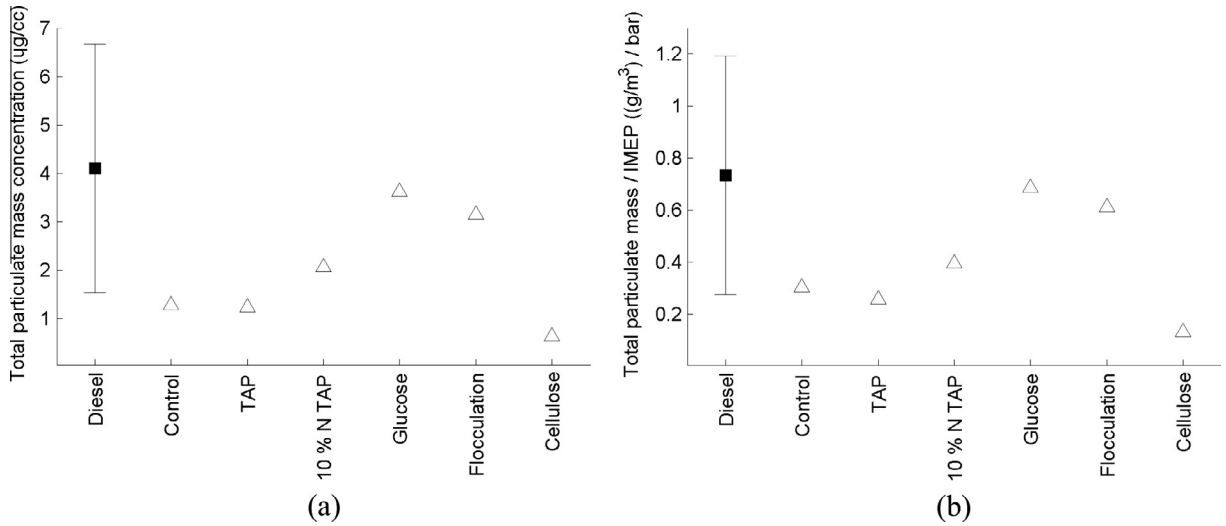


Fig. 18. (a) Measured total particulate mass exhaust emissions, and (b) total particulate mass exhaust emissions normalized with respect to the engine IMEP, of the wet slurry diesel emulsions and reference diesel.

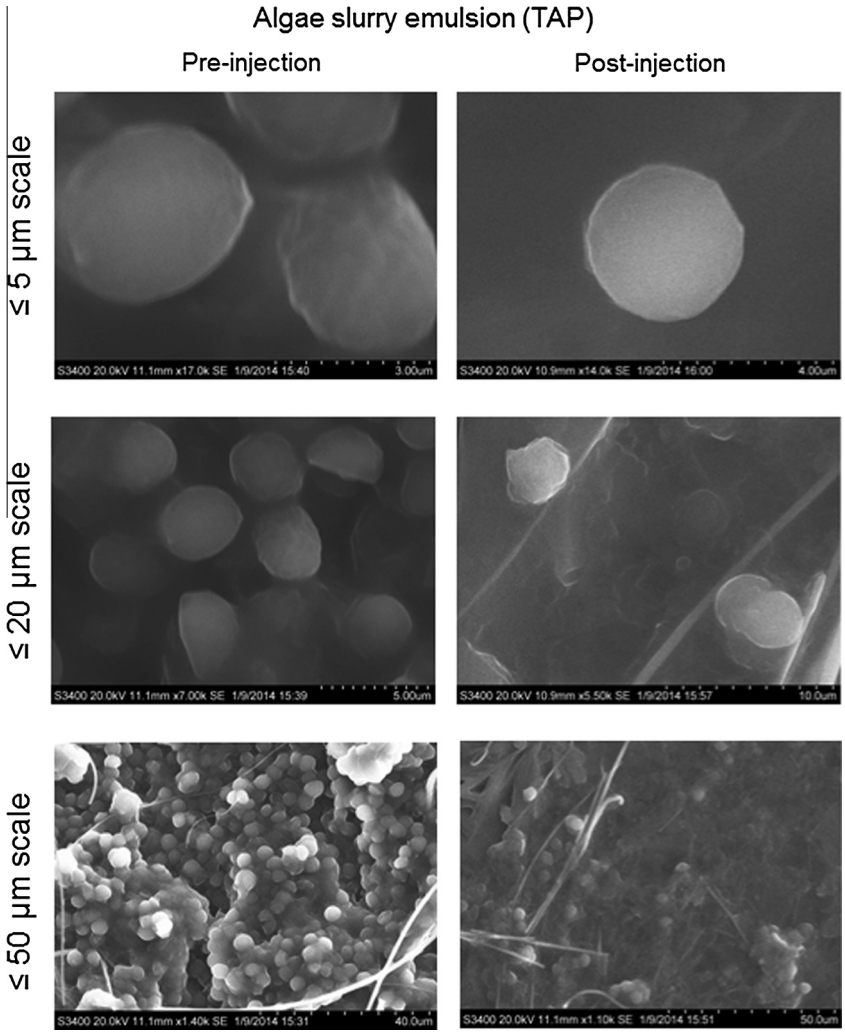


Fig. 19. SEM imaging of algal slurry emulsion (TAP) before and after high pressure fuel injection.

Fig. 18 shows the total particulate mass emitted as measured in the engine exhaust, and also normalized with respect to engine IMEP, for the wet slurry diesel emulsions and reference diesel fuel. While the significant range of uncertainty represented by the variation in total particulate mass emitted by the reference fossil diesel fuel prevents the drawing of any definitive conclusions, it can be seen that, excluding the reference fossil diesel, the highest levels of PM were emitted by the glucose algal slurry emulsion and the flocculation algal slurry emulsion (Fig. 18a and b).

3.3. SEM analysis of emulsions post-injection and exhaust particulate matter

Electron microscope techniques, such as SEM and transmission electron microscopy (TEM), have been widely used to characterize both particulate matter produced during diesel combustion [53,54], and for microalgal ultrastructure and morphological analysis [55,56]. In the current study, SEM analysis was undertaken so as to determine whether (a) rupturing of individual algal cells occurred during high pressure fuel injection, and (b) if any intact algal cells were present in the engine exhaust following combustion.

Fig. 19 shows SEM images of an algal slurry emulsion which had not been subject to any high pressure conditions following preparation, and those of the same emulsion following high pressure injection by the fuel injector utilized in the engine tests (Section 3.2). Apparent both before and after high pressure injection of the emulsion, are individual algae cells that appear whole, with no cell fragments visible (Fig. 19). This observation would suggest a significant fraction of the algae cells to have passed through the fuel injector at high pressures without rupturing or disintegration. Given the persistence of whole algae cells during fuel injection, and dependent on SOI and fuel injection pressures, it can be speculated that fuel spray impingement on the piston bowl and combustion chamber walls could potentially lead to erosion of these combustion chamber surfaces by the high velocity impact of algae cells over extended periods of engine operation.

Fig. 20 shows SEM images of particulate matter collected from the engine exhaust during testing of the control, TAP slurry, flocculated and nitrogen limited emulsions. No individual algae cells are visible in the particulate matter of any of the emulsions, indicating whole cells to have not persisted to the engine exhaust (Fig. 20). However, while from visual inspection, the morphology of the particulate matter present (especially visible at the 10 µm images present in Fig. 20) is typical of that produced during fossil diesel compression ignition engine combustion [44,53,54], no compositional analysis of the collected particulate matter was undertaken. Therefore, the possibility that algal cell fragments may be present and have impacted on both the composition and morphology of the particulate matter cannot be entirely discounted.

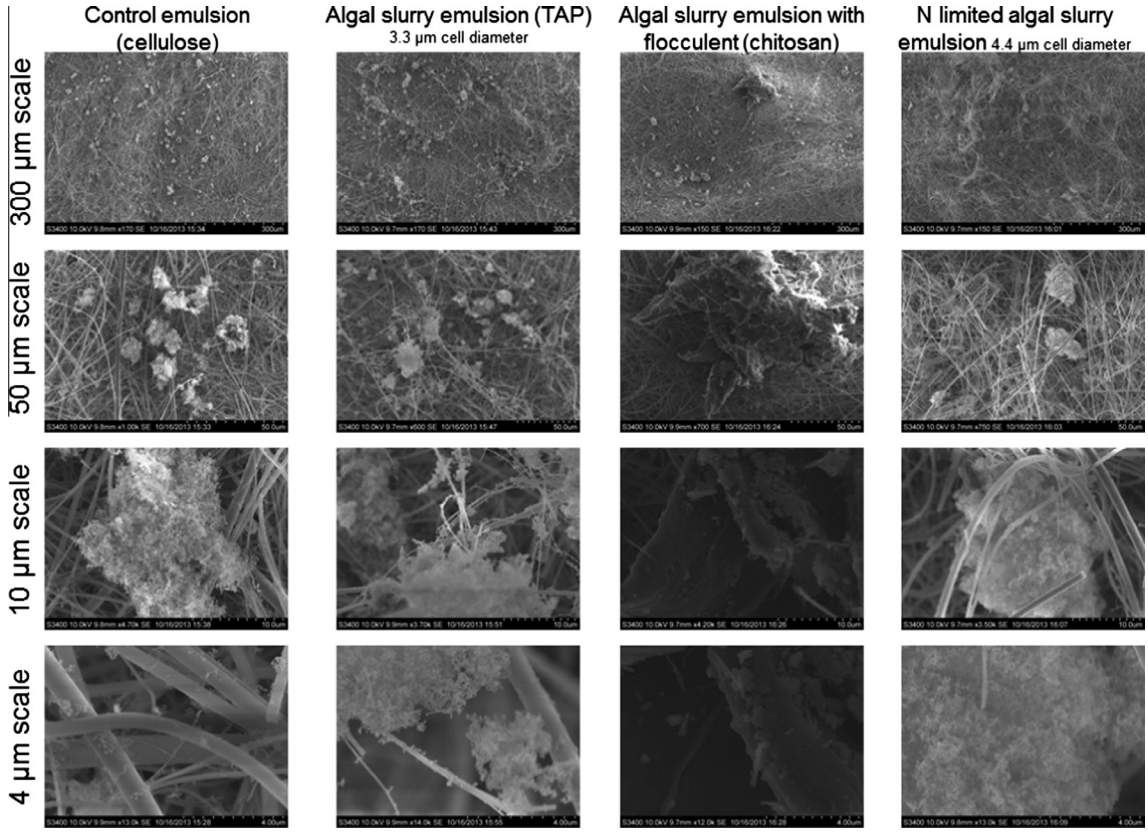


Fig. 20. SEM imaging of emulsion combustion engine exhaust gas particulate matter.

4. Conclusions

The preparation and combustion of wet algal slurry/fossil diesel emulsions was investigated as a means of utilizing the energy content of microalgae while minimizing the cost and energy input of using algae as a biofuel feedstock. As the algal biomass concentration of these emulsions was significantly higher than those reported in previous investigations, this required the development of a novel surfactant package to ensure that stable emulsions could be maintained. From the assessment of algal biomass under different culture conditions and the combustion and emissions characterization of subsequent wet algal slurry/fossil diesel emulsions in a modern compression ignition engine (equipped with a mechanically closed fuel injector as preliminary tests with solenoid valve fuel injectors resulted in immediate failure of the injector), the following conclusions can be drawn:

1. Stable wet algal biomass slurry and diesel emulsions of up to 6.6% wt/wt algae biomass content can be prepared using a novel surfactant pack of non-ionic Span80, cationic CTAB and butanol.
2. The use of a glucose enriched growth medium to induce lipid accumulation in the algal cells was found to result in an algal slurry diesel emulsion of higher algal biomass content and lower heating value than the traditional approach of nitrogen depletion, although the latter resulted in algal cells of higher lipid content.
3. The engine work produced during combustion of the wet algal slurry diesel emulsions was observed to increase with measured lower heating values, suggesting the calorific content of the algal biomass to be making a positive contribution to energy release during compression ignition combustion.
4. All of the wet algal slurry/diesel emulsions emitted lower levels of NOx and particulate mass relative to a reference fossil diesel, with only the emulsion prepared from flocculated algae emitting higher exhaust levels of CO.
5. Individual algal cells persist intact following high pressure direct diesel injection. However, no whole algal cells are visible in samples of exhaust particulate matter, suggesting breakdown of the cells occurs during combustion.

The experimental investigations undertaken suggest that stable emulsions of wet algae slurry with fossil diesel can be formed, and further release useful energy during the course of combustion in a modern compression ignition engine without significant impact on the exhaust emission of pollutants. These results therefore highlight the possibility of such emulsions as a means of utilizing the renewable energy content of microalgae to partially displace that from liquid fossil fuels in internal combustion engines. However, as it is likely to be undesirable to transport significant volumes of a wet algal biomass slurry over any considerable distance (given the energy requirement of doing so), it is suggested that the utilization of algal biomass slurry emulsions might be most appropriate for micro-power generation [57] in the vicinity of sites at which the algal biomass is produced. This could for example include industrial facilities which undertake remediation of liquid effluents, such as those for domestic waste water treatment [58] or acid mine drainage [18], and which are able to utilize micro-algae for the removal of excess nutrients or metal containments.

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