Environmental Science Processes & Impacts

The role of microalgal biodiesel composition on diesel engine exhaust particle emissions and their oxidative potential

Journal:	*Environmental Science: Processes & Impacts*
Manuscript ID:	EM-ART-03-2015-000125.R1
Article Type:	Paper
Date Submitted by the Author:	11-Jul-2015

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Combustion engines are the major sources of ultrafine particles in urban areas. Biodiesel in diesel engines can reduce this harmful pollutant to some extent. Among many biodiesel feedstocks, microalgae are considered to be the most promising feedstock to meet future biodiesel demand. This study investigates the influences of microalgal biodiesel chemical composition on engine exhaust particle emissions. The outcome of this research provides new insight into the optimum chemical composition of microalgal biodiesel that would minimise diesel particle emissions. It could be useful in formulating microalgal biodiesel composition, or even setting a standard which will ensure better engine performance with lowest possible emissions.

Particle Emissions from Microalgae Biodiesel Combustion and Their Relative Oxidative Potential

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Abstract:

Microalgae are considered to be one of the most viable biodiesel feedstocks for the future due to their potential for providing economical, sustainable and cleaner alternatives to petroleum diesel. This study investigated the particle emissions from a commercially cultured microalgae and higher plant biodiesels at different blending ratios. With a high amount of long carbon chain lengths fatty acid methyl esters (C20 to C22), the microalgal biodiesel used had a vastly different average carbon chain length and level of unsaturation to conventional biodiesel, which significantly influenced particle emissions. Smaller blend percentages showed a larger reduction in particle emission than blend percentages of over 20%. This was due to the formation of a significant nucleation mode for the higher blends., In addition measurements of reactive oxygen species (ROS), showed that the oxidative potential of particles emitted from the microalgal biodiesel combustion were lower than that of regular diesel. Biodiesel oxygen content was less effective in suppressing particle emissions for biodiesels containing a high amount of polyunsaturated C20-C22 fatty acid methyl esters and

generated significantly increased nucleation mode particle emissions. The observed increase in nucleation mode particle emission is postulated to be caused by very low volatility, high boiling point and high density, viscosity and surface tension of the microalgal biodiesel tested here. Therefore, in order to achieve similar PM (particulate matter) emission benefits for microalgal biodiesel likewise to conventional biodiesel, fatty acid methyl esters (FAMEs) with high amounts of polyunsaturated long-chain fatty acids (≥ C20) may not be desirable in microalgal biodiesel composition.

1.1 Introduction

Biodiesel is considered to be a potential alternative fuel for use in compression ignition (CI) diesel engines. It is compatible with existing engine technology, without any significant modifications and it also provides emission benefits, including a reduction in carbon footprint emissions. Biodiesel produced from either renewable vegetable oils or animal fats is considered as neutral in terms of carbon emissions [(1)]. In addition, numerous studies report low CO, HC and particulate matter (PM) emissions from biodiesel [(2-4)], and while some show an increase in NOx emissions [(5, 6)], others report no significant change [(6, 7)]. Despite these advantages, the consumption of biodiesel is not widespread. The main barrier to wide-spread use is a higher price compared to petroleum diesel. In addition, the use of vegetable oil biodiesels raises food *versus* fuel conflicts, since most commercial biodiesel feedstocks are also used as either human or animal food. Therefore, in order to ensure a sustainable future for biodiesel, it is necessary to find feedstocks that will be able to address these problems. Microalgae are considered to be one of the most promising feedstock alternatives, which have potential to provide a viable solution for overcoming present barriers.

Microalgae are considered to be a third generation biofuel feedstock, due to higher yields and relatively low land requirement for production. Practically, microalgae can be grown in any

place where there is sufficient sunshine and water of low quality (industrial tailing dams, secondary treated sewage, saline/brackish), including infertile land not suitable for the cultivation of other biodiesel feedstocks and food producing crops [8]. Among photosynthesising organisms, microalgae are the fastest growing and they can complete an entire production cycle within a few days [9]. According to some estimates, annual oil production from microalgae ranges from 20,000 to 80,000 L per acre, depending on species and production method, which is 7–31 times higher than that of the highest oil-producing terrestrial crop (palm) [9]. The required land footprint is also 10–340 times smaller than that of their terrestrial counterparts. Therefore, some estimates suggest that oil production from microalgae can be up to 200 times higher than the most efficiently produced vegetable oils [10].

Although microalgae production, oil extraction and oil characterisation has been extensively studied, very few have focused on engine performance and emissions from microalgae biodiesel. Recently, Makarevičiene et al. [11] and others [12-14] investigated engine performance and emission characteristics using low blends of microalgae biodiesel (up to B30), but none of the studies conducted detailed particle emission measurements. In addition, there are many varieties of microalgal species available and the fatty acid profile of biodiesel produced from those species can be significantly different *per se* and is strongly influenced by growth conditions. Some studies suggest that variations in the fatty acid profiles of biodiesel can affect the performance and emission profiles [15, 16]. The fatty acid composition of microalgae can be controlled either by selecting species with ideal fatty acid profiles, genetic modification of a species, typically aimed at improved growth and/ or fatty acid (lipid) production, or by manipulating growth conditions. However, before embarking on the use of genetically modified microalgae or adding costs for controlling growth conditions, it is necessary to determine which fatty acid compositions will provide optimal output with the

lowest possible emissions. In order to address this knowledge gap, we conducted detailed particle emission measurements for a number of different blends of a microalgal biodiesel (engine performance analysis is reported in Islam et al [17]).

Diesel particle emissions have been in the spotlight in recent years since the International Agency for Research on Cancer (IARC) included particulate matter emitted from diesel engine exhaust as carcinogens. Our previous study [18] investigated particle emissions from biodiesel with a FAME carbon number ≤ 18 and a high degree of poly-unsaturation. This study established particle emissions dependence on carbon chain length and degree of unsaturation of the biodiesel fatty acids, as well as oxygen content. Biodiesel with FAME carbon numbers of more than 18 and a high degree of poly-unsaturation have not been studied to date. Therefore, PM emissions from a microalgal biodiesel with high amounts of C20 and C22 polyunsaturated fatty acids (PUFAs) were investigated and compared to B20 blends of vegetable biodiesels (cotton seed oil (CSO) and waste cooking oil (WCO)). This study is a continuation of our previous study [18] with the aim of investigating the influence of a biofuel with high amounts of very long chain poly-unsaturated FAMEs on exhaust particle emissions.

1.2 Materials and Methods

Experimental measurements were performed on a turbo-charged common rail engine typically used in passenger cars. Detailed specifications of the engines are given in Table 1. A two-stage dilution system, as shown in Figure 1, was used for emission measurements, where two ejector diluters (Dekati DI-1000) were connected in series. Exhaust was sampled after the exhaust manifold *via* a 0.5 meter long stainless steel tube. A fraction of the exhaust was then transferred to gas analysers *via* a copper tube fitted with a HEPA filter and water trap. The rest of the sampled gas was sent to the diluter for dilution, followed by particle

measurement. A CAI 600 series CO_2 analyser and a CAI 600 series CLD NO_x analyser were used for raw CO_2 and NO_x measurements. A SABLE CA-10 recorded CO_2 concentrations from diluted exhaust. Particle number size distribution was measured with a DMS-500 (Cambustion Ltd) without the heated sample line connected. Particle mass was calculated from DMS 500 data by using a re-inversion tool in the DMS data analysis suite (version UIv 7.11), as suggested by Jonathan et al. [19]. In this case, a density factor of 2.2×10^{-15} and a power coefficient of 2.65 and 5.2×10^{-16} and 3 were applied to accumulation mode particles and to nucleation mode particles, respectively. In addition, a TSI DustTrak 8530 measured PM. Oxidative potential (OP) of PM (nmol of ROS per mg of PM) was based on the mass concentration of reactive oxygen species (ROS). Profluorescent nitroxides (PFN) are very powerful optical sensors which can be used as detectors of free radicals and redox active substances. The probe itself is poorly fluorescent; however, upon radical trapping, or redox activity, a strong fluorescence is observed [20]. Therefore, a BPEA (bis(phenylethynyl) anthracene-nitroxide) molecular probe was used for the measurement of OP (potency of PM to induce oxidative stress). Samples for ROS measurements (n=2) were collected by bubbling the aerosol through an impinger containing 20 mL of 4 μM BPEA solution (containing dimethyl-sulfoxide (AR-grade, supplier and details) as a solvent), followed by fluorescence measurements with a spectrophotometer (Ocean Optics). The amount of BPEA reacting with the combustion aerosol was calculated from a standard curve obtained by plotting known concentrations of the methanesulfonamide adduct of BPEA (fluorescent) against the fluorescence intensity at 485 nm [21, 22].

Microalgal biodiesel, derived from the dinoflagellate *Crypthecodinium cohnii* (Martek, Singapore) was tested for three blending ratios of 10%, 20% and 50% biodiesel to petroleum diesel (v/v) (supplied by Caltex Australia), designated as A10D90, A20D80 and A50D50, respectively. A single batch of diesel was used to prepare all blends. In addition to neat

diesel, a 20% blend of waste cooking oil (WCO) and cotton seed oil (CSO) biodiesel, designated as WCO20D80 and CSO20D80, were used as reference fuels with shorter carbon chain lengths and different level of saturation. All blends were prepared in volumetric flasks and then poured into the custom built engine fuel tank. The engine was operated at a maximum torque speed of 2000 rpm and under four different loads (i.e. 25%, 50%, 75% and 100%). Due to a very limited amount of algal biodiesel only measurement with reference diesel were repeated twice, at the beginning and end of the campaign. The observed variability was below 10% for all particle parameters (PM, PN, CMD, etc.) and this ensured that with the sampling system used we could obtain reproducible results. All the other measurements with microalgal biodiesel were conducted only once.

The fatty acid profile of the used microalgae, CSO and WCO biodiesel are published in [17] and are provided for convenience in the supporting information (SI) Table SI-1. The microalgal biodiesel was dominated by long chain poly-unsaturated fatty acids resulting in longer average carbon chain length (20.38) and higher average degrees of unsaturation (3.46) compared to the other two biodiesels tested (CSO and WCO). Also, this microalgal biodiesel did not contain any mono-unsaturated fatty acids (MUFA), but had a poly-unsaturated fatty acids (PUFA) content of ~69%. Average carbon chain length and average unsaturation for the CSO was 18.94 and 1.47 respectively, followed by 18.78 and 1.03 for WCO. WCO biodiesel was composed of a higher fraction (67%) of MUFA, whereas CSO biodiesel was composed of a higher fraction (51%) of PUFA.

Important physical properties of the pure microalgae and WCO biodiesel can be found in Islam et al [17], and is provided for convenience in Table SI-2 alongside with CSO biodiesel for comparison. Elemental compositions and relevant properties of the blends used in engine testing are shown in Table 2. As suggested by Benjumea et al [23], all these blend properties are calculated from the measured pure fuel properties by using the Grunberg–Nissan mixing

rule [24]. Viscosity, density and NBP increased with the increase of biodiesel contents in the blends, where HHV and CN decreased. Among the biodiesel blends, microalgal biodiesel blends had higher viscosity, density and NBP than WCO and CSO for the same blending ratio. Despite these differences, all of the relevant properties were found to be within the range of biodiesel standard ASTM 6751-12 or EN 14214, although the CN of the pure microalgal biodiesel was slightly lower than prescribed in the ASTM standard.

1.3 Results and Discussion

1.3.1 Specific particulate matter (PM) emissions

Brake-specific particulate matter (PM) emissions from the reference diesel and different blends of biodiesels are shown in Figure 2. PM emissions were calculated from DMS data using a re-inversion tool in the DMS data analysis software. The microalgal biodiesel blends-PM emissions were load-dependent, where PM emissions were lower than petroleum diesel for all blends and loads except for A20D80 at 75% load where there was no significant difference. In addition, reductions among blends were not consistent. Smaller blends, A5D95 and A10D90, consistently showed reduction in PM for all of the measured loads. Higher blends of A20D80 and A50D50 showed significant reduction only for 100% load and some smaller reduction for the other loads. However, when considering total particulate matter (TPM) emissions, which refers to the sum of the accumulation and nucleation mode PM, higher blends show less of a reduction (see Figure SI-1). For the majority of the loads there is a small or no change in the TPM for A20D80 and A50D50. The reason for this is the presence of the nucleation mode for higher blends (see Figure 4.).

In comparison to the microalgal biodiesel blends, both accumulation mode PM and TPM emissions from WCO20D80 and CSO20D80 biodiesels were found to be lower than for the A20D80 blend, with TPM emissions from CSO20D80 being ≥ 50% lower than WCO20D80,

except at 25% engine load, where the difference was not that pronounced (Fig. 1 and Fig. SI-1). DustTrak TPM measurements, as shown in the supporting information (Figure SI-2), followed exactly the same trend as for the accumulation mode PM calculated from DMS measurements. Therefore, it is reasonable to assume that the DustTrak is not capable of detecting PM from the nucleation mode, as reported in other studies [25].

A few recent studies [12, 13] tested a B20 blend of biodiesel from the green freshwater microalga *Chlorella vulgaris* and reported lower smoke opacity and soot emissions than diesel. However, information on the FAME composition of the feedstock used was not reported. It can be seen from other studies that FAME compositions of *Chlorella vulgaris* contain fatty acids with a carbon chain length of ≤ 18 [26, 27]. Taking into account the results presented in this study, which are contrary to the work of Patel et al. [12], and excluding the variations in the engine operating parameters, the reason for the difference could be in the FAME profile of the biodiesel used. Due to the lack of detailed FA profile information provided, this very important phenomenon should be investigated in more detail. This should be done by testing microalgae biodiesels of different origins using the same or a similar engine and keeping all other parameters unchanged, so the impact of biodiesel chemical composition on the overall emission pattern can be determined.

Biodiesel literature [23, 28-30] suggests linear correlation between blend properties and pure biodiesel properties and their blending ratio. Study on microalgae biodiesel further support this [31]. Likewise pure microalgal biodiesel, the diesel-microalgal biodiesel blends tested had a higher density, viscosity, boiling point, surface tension and lower cetane number than the reference diesel, and the other two biodiesels for the same blending ratio. In addition, the average carbon chain length and average unsaturation of the microalgal biodiesel was also higher than for the other two biodiesels, while the oxygen content was almost the same.

Therefore, as per our previous study [18], higher PM emissions were expected from the microalgal biodiesel blends than for the CSO and WCO blends. Schönborn et al. [16] also tested pure C22:0 FAME in their custom-made engine system, and found PM emissions to be almost the same as for diesel. In terms of carbon number, the microalgal biodiesel tested in this study was similar to the one used in Schönborn et al. [16], except that it contained high amounts of by C22:5 and C22:6. The effect of biodiesel poly-unsaturation on PM emissions is not clear yet. Although one study reported an increase in PM emissions with the increase of biodiesel degree of unsaturation [32], others showed a small decrease or no significant change [18, 33, 34]. On the other hand, a decrease in PM emissions for lower blends of microalgae biodiesel (B5 and B10) might be due to their oxygen content, while the change of the other properties (i.e. viscosity and boiling point) typically responsible for increased PM emissions may have been insufficient to produce an effect at such low blend ratios.

1.3.2 Particle number (PN) emission

Before discussing specific PN emissions, it is worth mentioning that DMS 500 provides a separate log normal PSD spectrum for both nucleation and accumulation mode particles. Nucleation or accumulation mode number concentration is actually an integrated number of that particular PSD spectrum. The total PSD spectrum is the best fit for the nucleation and accumulation mode spectrum, and the integrated total number under this best fit spectrum is considered as the total particle number concentration. In the presence of a nucleation mode peak, total particle number (TPN) is dominated by nucleation mode particles. Therefore, a trend in TPN emissions among different biodiesel blends is not expected here (Figure SI-3). An almost 10-fold increase in TPN emissions was observed for the A50D50 blend, which was predominantly driven by the presence of nucleation mode particles. However, as shown in Figure 3, a trend similar to that presented for particle mass, was observed for accumulation mode PN emissions. Accumulation mode PN from the microalgal blends decreased for 5%

and 10% blends, and then increased for 20% and 50% blends, except at 100% engine load, where it consistently decreased with the increase in biodiesel content. Unlike the microalgal biodiesel blends, TPN from WCO and CSO blends was found to be slightly lower than from the reference diesel and this followed the trend for TPM emissions. This finding indicates that WCO and CSO blends did not contribute as much to nucleation mode particles compared to the microalgal blends, although the measurement conditions were the same for the overall duration of tests. This can be explained by the difference in chemical composition and physical properties of the tested biodiesels, as outlined above. , and The higher boiling point, due to high amounts of C22:5 and C22:6, of the microalgal biodiesel blends tested could result in unburned fuel escaping from the combustion process and staying in the exhaust as volatiles and semi-volatiles, along with other partially oxidised substances [35]. These volatiles and semi-volatiles could also have a higher boiling point and lower saturation vapour pressure, which means that they are more prone to condense, and form nucleation mode particles, than low boiling point substances under the same conditions [36]. In addition, the presence of fewer accumulation mode particles/soot for the microalgal biodiesel blends could also enhance this process [37, 38].

1.3.3 Particle number size distribution

Particle size distributions (PSD) for the different blends of biodiesel are shown in Figure 4. Due to the presence of a large nucleation mode for some blends, the whole PSD spectrum has been shown as an inset, with the main graph clearly presenting the variation among the different blends. The microalgal biodiesel blends consistently exhibited 20 nm nucleation mode peaks at 100% load. The peak of the nucleation peak was positively correlated with the increase in microalgae biodiesel content, being highest in the A50D50 blend (almost 10-fold). WCO and CSO blends did not produce such nucleation mode peaks, although their accumulation mode size was well below that of the reference diesel. On the other hand, at

50% engine load, a nucleation mode peak was only observed for A50D50. Other blends of the microalgal biodiesel produced the same accumulation mode peak as the reference diesel, however the A20D80 peak was higher than the diesel peak. Schönborn et al. [16] also found similar nucleation peaks in their measurements using pure C22:0. As summarised above, the higher density, viscosity and surface tension, as well as low volatility due to the microalgal fatty acid profile could have led to the formation of excessive partially oxidised semi-volatile substances. Upon cooling, these semi-volatiles then either nucleate to form new particles or condense on the surface of existing soot particles. For example, the amount of soot produced from A50D50 did not have a large enough surface area where the semi-volatiles could condense, therefore those semi-volatiles were more likely to undergo nucleation. However, in the case of A20D80, the higher levels of C22:6, which has a low volatility and mixing tendency, may also be responsible for excessive soot formation, which is likely to have occurred under part load conditions. Therefore, while A20D80 can be expected to produce some semi-volatiles under part load conditions, it was not likely to be enough to trigger nucleation, since there was enough soot surface area on which it could condense.

1.3.4 Relationship between fuel oxygen content and particle emissions

Fuel-bound oxygen plays an important role in combustion, soot oxidation and subsequent PM reduction. It either prevents in cylinder soot formation or oxidises already formed soot particles. In our previous study [18], reductions in PM and PN were observed to be inversely correlated with biodiesel oxygen content, regardless of variations in other properties. However, a slightly different trend was observed in terms of the microalgal biodiesel blends tested here. As shown in Figure 5 accumulation mode PM and PN emissions decreased with increasing oxygen content at 100% engine load, however this was not the case for TPM. To the contrary, although a number of studies showed consistent reductions in PM with increased biodiesel oxygen content [18, 39], both TPM and TPN increased for the 20% and 50%

microalgal biodiesel blends in this study, which have a relatively higher oxygen content. Some studies [40, 41] reported increased nanoparticle emissions, which might be due to the increase in the nucleation mode particles. Barrios et al. [42] used oxygenated additives (i.e. Ethyl Tertiary Butyl Ether (ETBE) and Diglyme (Bis (2-methoxy ethyl ether)) which consistently resulted in reduction in accumulation mode particles with increasing blending ratios, whereas nucleation mode particles followed the opposite trend. Several other reports also demonstrate reductions in PM in the presence of oxygenates [18, 43, 44], with some studies suggesting that soot produced from oxygenated fuels possesses more oxygen functional groups [2]. This would make biodiesel soot more reactive, which could result in reductions in PM [45]. Others suggest that oxygen atoms in the ester molecule decompose into two separate reactive oxygen carriers, which then contribute to reductions in soot-precursors [46]. Therefore, while the positive effect of fuel-bound oxygen on particle emissions is well established, none of the previous studies have tested biodiesels with the same FAME content as those tested here. Only one study, by Schönborn et al. [16], reported diesel-like TPM emissions from biodiesel having 22 carbon atoms in their FAME. Their study also demonstrated that biodiesel oxygen content did not effectively reduce TPM emissions when the carbon number was $\geq$22. Therefore, the generally accepted opinion that biodiesel oxygen content is the main driving force behind reduced TPM emissions might not always hold true, especially for biodiesels having a carbon number $\geq$22 in their FAME.

1.3.5 Influence of microalgae biodiesel on nucleation mode particle formation

Particle nucleation in engine exhaust emission measurements is a very complex process. It largely depends on dilution conditions (i.e. pressure, temperature, humidity etc.) and the saturation vapour pressure of volatile substances present in the exhaust. A small change in dilution conditions can either promote or reduce nucleation and it is this uncertainty that makes nucleation mode particle measurement difficult to reproduce [47-50]. Large variations

are mainly due to the exponential relationship between saturation vapour pressure and temperature [51]. Therefore, small changes in the cooling gradients will cause large changes in the saturation vapour pressure. Considering the above-mentioned circumstances, the European Union (EU) Particle Measurement Program (PMP) excluded nucleation mode particles from their particle number-based emission standards (i.e. EURO5/6). Keeping in mind the complexities of measurements involving nucleation mode particles, we kept the dilution system settings constant for the entire measurement period in this study. Despite this, nucleation in microalgae biodiesel measurements was repeatedly observed, especially with the higher blends. As shown in Figure 6(a), specific nucleation mode PN increased linearly with increasing microalgal biodiesel content. On the other hand, WCO20D80 and CSO20D80 were not observed to produce nucleation. This could indicate that the unusual chemical composition of the microalgal biodiesel blends tested here played a role in triggering the nucleation. This is likely explained by the above mentioned significant differences of the microalgal biodiesel and the WCO and CSO biodiesel blends. Therefore, it is likely that the density, viscosity and boiling point will increase with increasing blend ratios [52, 53]. Figure 6(b) shows the thermos-gravimetric analysis (TGA) of the diesel and biodiesel blends used in this study which clearly demonstrates that the microalgal biodiesel blends are less volatile than CSO and WCO biodiesel blends, showing a significant mass fraction even at temperatures above 350°C.

A fuel with relatively high density, viscosity, boiling point and low volatility could cause poor atomisation and improper in-cylinder mixing with air [53, 54], which results in the presence of unburned hydrocarbon and partially oxidised semi-volatiles in the exhaust. In addition, the boiling point of these volatiles/semi-volatiles from the unburned fuel is also expected to be higher, as indicated by lower saturation vapour pressure. This low saturation vapour pressure could also be responsible for a higher tendency for the gas to particle

partitioning. Therefore, it is reasonable to assume that the microalgal biodiesel blend properties in this regard are influenced by the blend ratios which should correlate positively with gas to particle partitioning and increased nucleation mode particle numbers. This is supported by some of the studies demonstrating a positive correlation between nucleation mode particle increase and the carbon number of the biodiesel molecules [16, 55]. Fischer et al. [15] observed the presence of nucleation mode particles when using canola biodiesel containing a higher amount of glycerol, a finding that was further supported by one of our earlier studies [40]. Relevant properties of glycerol i.e. density, viscosity and boiling point are also higher than diesel and commercial biodiesel [56], therefore it could be speculated that biodiesels with substantial amounts of either ≥ 22 carbon number FAME molecules or impurities (i.e. glycerol) could induce nucleation mode particles in the engine exhaust. Lubricating oil could also be a strong contributor to nucleation mode particles[57], however probably not in this case as we did not observe nucleation for 20% blends WCO and CSO biodiesel.

1.3.6 Oxidative potential of particles emitted from microalgae biodiesel blends

The measurement of oxidative potential (OP), based on the ROS concentration of PM, can be used as a good indicator for reactivity and toxicity [58]. An in-house-developed profluorescent molecular probe BPEAnit was applied in a unique, rapid and non-cell-based way to assess particulate OP [20, 59]. Based on data provided in the literature [22] there are some uncertainties as to which chemical species are responsible for the measured redox potential and overall toxicity. Generally, there is a consensus that the organic fraction is a carrier of ROS [60]. Alternatively, ROS can be formed as a consequence of organic species reactivity within the cell environment [61]. The latter can be also considered as a secondary organic species.

The oxidative potential of the tested microalgal biodiesel blends was smaller compared to diesel (Figure 7). ROS concentrations were measured at two different loads: at idle load and

50% load. It was expected that idle emissions would result in the emission of higher concentrations of ROS, as previously observed [62]. This result can be explained by the possible contribution of the combusted lubricating oil to overall OP. Furthermore, biodiesel content of the blends lowered OP significantly in respect to the value measured for diesel. Oxidative potential for B10 and B50 was very low, very close to a detection limit for the performed ROS measurements. B20 had the highest OP of the blends and it can be attributed to the accuracy of the measurement of the mass. This result suggests that OP and associated toxicity of the particles can be lowered by blending with the microalgal biodiesel. Further experiments should be conducted to get a more detailed perspective on this.

1.4 Conclusion

This study investigated the particle emission behaviour of microalgal biodiesel blends as a fuel with a high carbon chain length (20.38) and unsaturation (3.46) compared to conventional biodiesel feedstocks, such as WCO and CSO blends. Results showed that the fuels with smaller percentages of the C22 FAMEs showed a consistent reduction in both PM and TPM (A5B95 and A10B90) while higher blends did not show such a clear trend with similar TPM emissions as diesel. Particle emissions from the 20% microalgae biodiesel blends were significantly higher than 20% WCO and CSO biodiesel blends. This study also demonstrated that the increased biodiesel oxygen content was less effective in suppressing TPM emissions, if the biodiesel blend contained high percentages of FAMEs with a carbon number $\geq$22 and a high degree of poly-unsaturation. Such biochemical composition of biodiesel blends could also trigger a significant increase in nucleation mode particle emissions, but lower OP and particle associated toxicity compared to diesel, irrespective of blend ratio. In contrast, biodiesel blends with a FAME carbon chain length of $\leq$18 were less prone to produce nucleation mode particles, unless blends contain a significant amount of impurities (i.e. glycerol). It is possible that the very low volatility and high boiling point of

the microalgal biodiesel blends tested here in conjunction with other properties (i.e. high density, viscosity and surface tension) were the driving forces for the formation of nucleation mode peak. Therefore, FAMEs with ≥22 carbon atoms in biodiesel might not be as desirable as FAMEs with ≤22 carbon atoms. A significant caveat to our measurements is the lack of sufficient amount of algal biodiesel that prevented us of collecting a larger number of repeated measurements and improving the statistical significance of the conclusions.

The desired FAMEs composition in biodiesel could be ensured through appropriate species selection, the genetic modification of a target species or by the manipulation of microalgae growth conditions.

1.5 Acknowledgement

The authors sincerely acknowledge the technicians in the Engine Lab at the University of Queensland (UQ), as well as PhD students in Queensland University of Technology (QUT) Mr. Md. Jahirul Islam and Ali Mohammad Pourkhesalian for their valuable support during the experimental work. The authors also acknowledge the North Queensland Algal Identification/culturing Facility (NQAIF) at JCU, for providing the microalgal biomass and access to their analytical facilities. The authors also acknowledge EcoTech Biodiesel Company for providing waste cooking oil methyl ester for this experiment and Caltex refinery for their valuable fuel property tests.

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List of Figures:

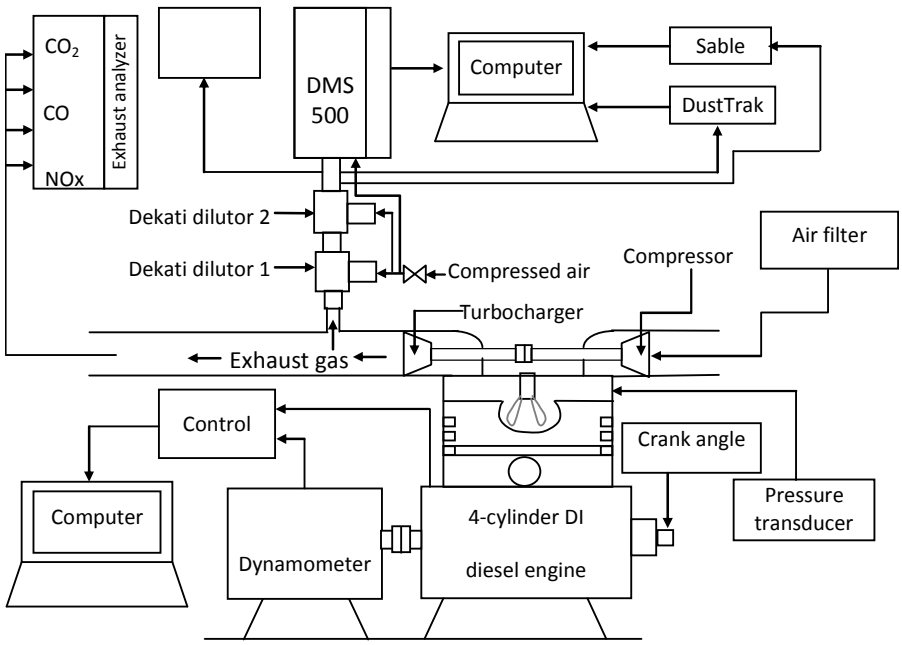

Figure 1; Schematic of experimental set up

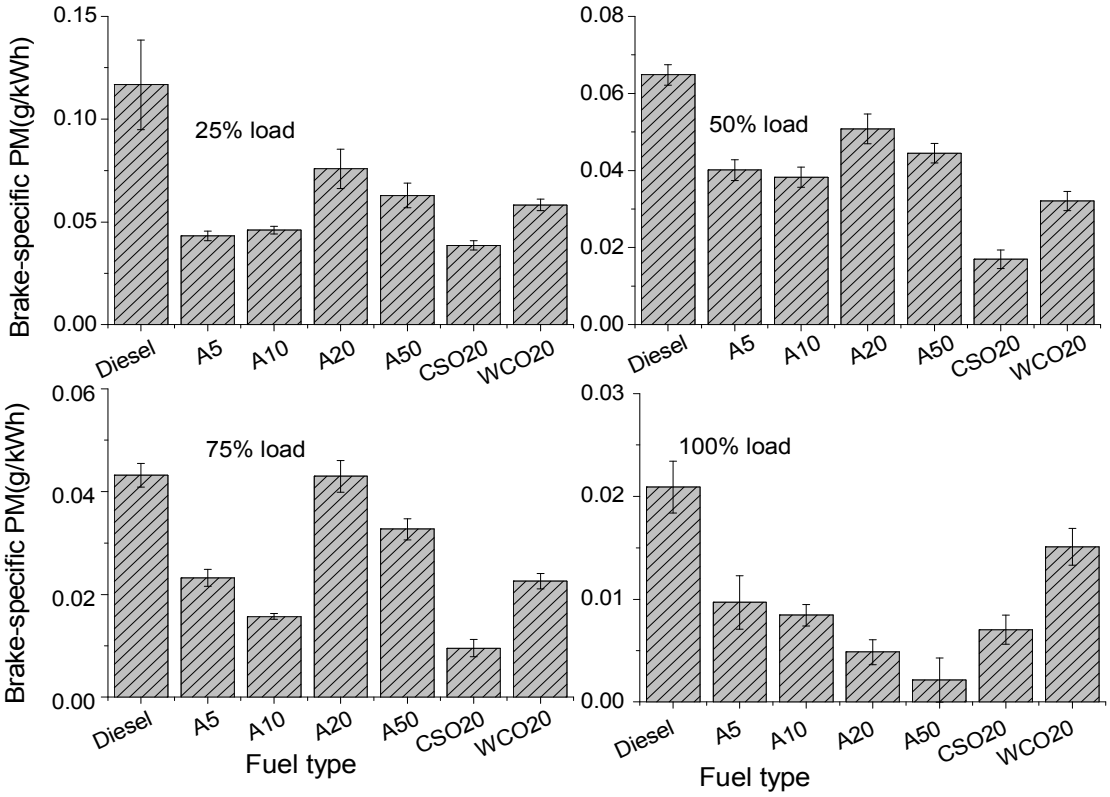

Figure 2: Brake-specific accumulation mode PM emissions of diesel and biodiesel blends

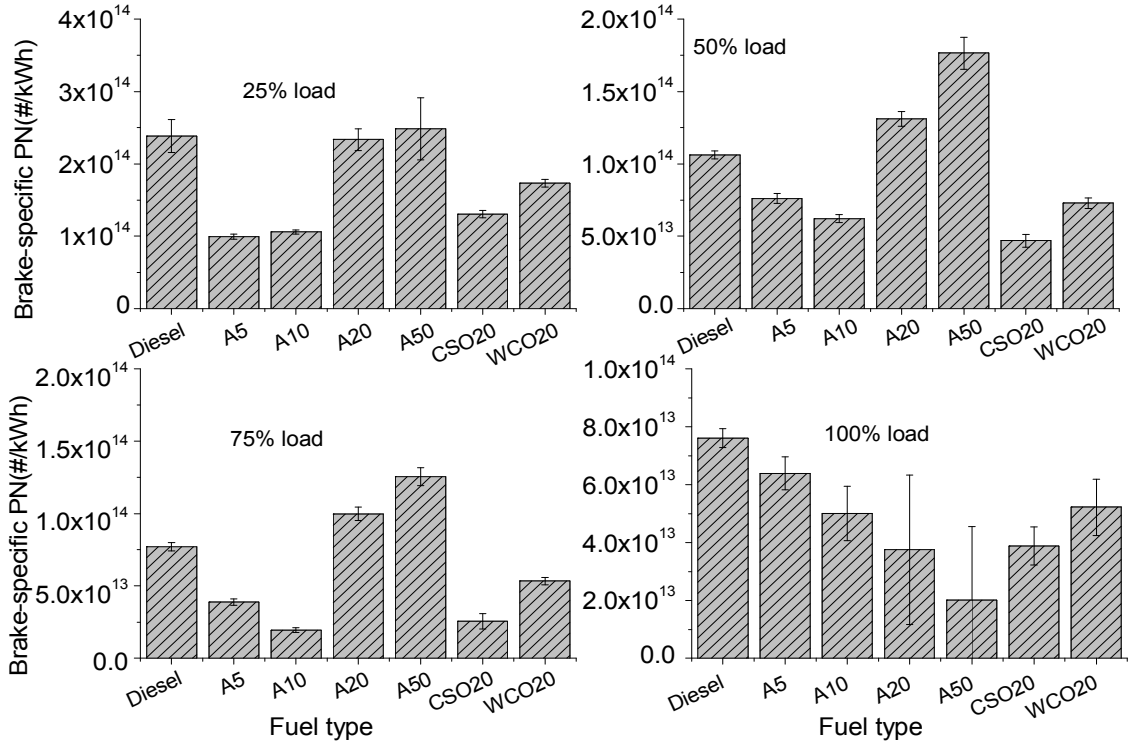

Figure 3: Brake-specific particle number emission (accumulation mode) for diesel and biodiesel blends

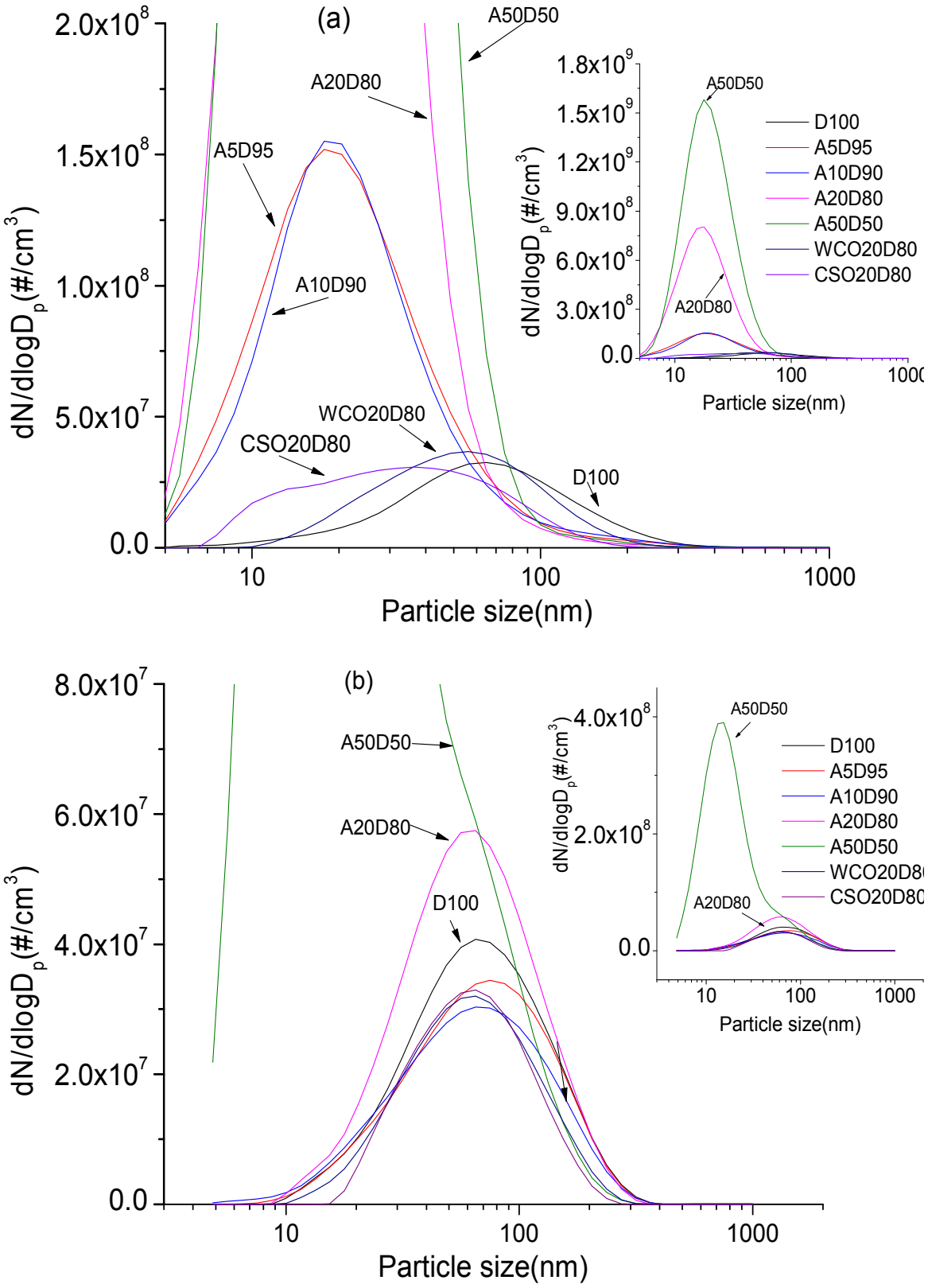

Figure 4: Particle size distribution of diesel and biodiesel blends at 100% (a) and 50% (b) loads

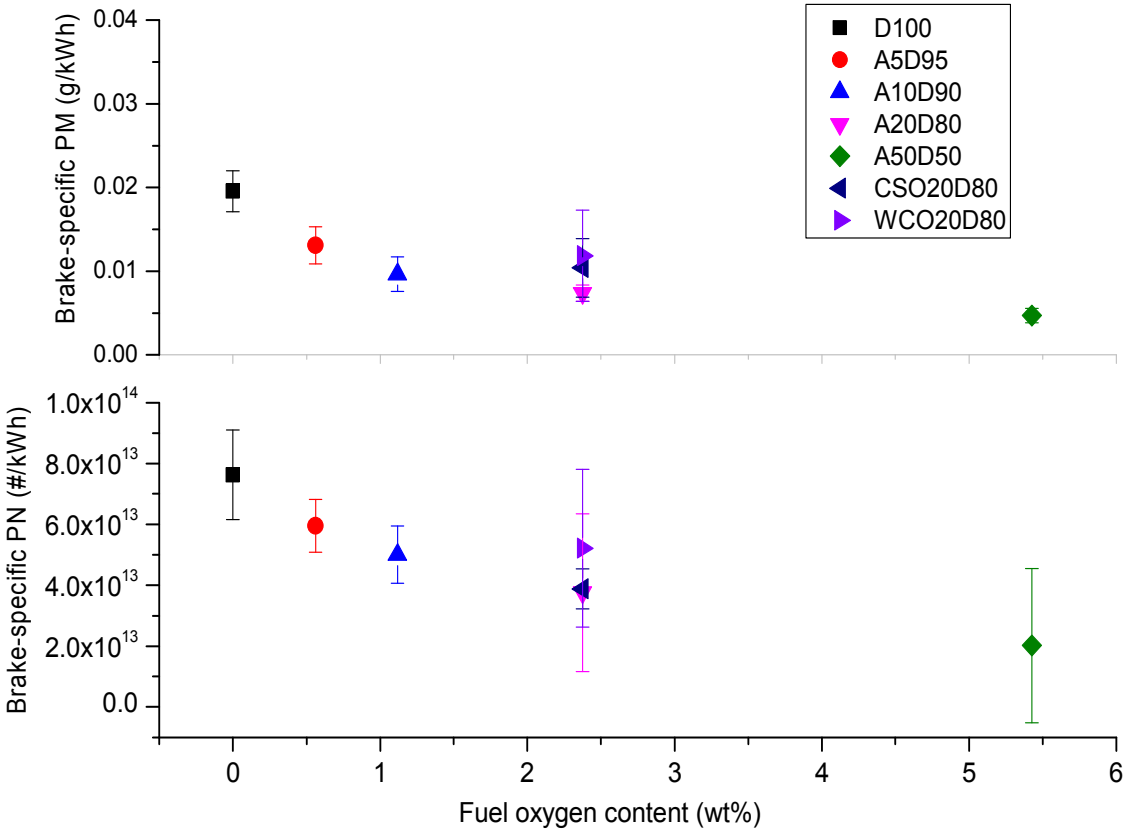

Figure 5: Relationship between accumulation mode PM and PN emissions with fuel oxygen content at 100% load for diesel and biodiesel blends

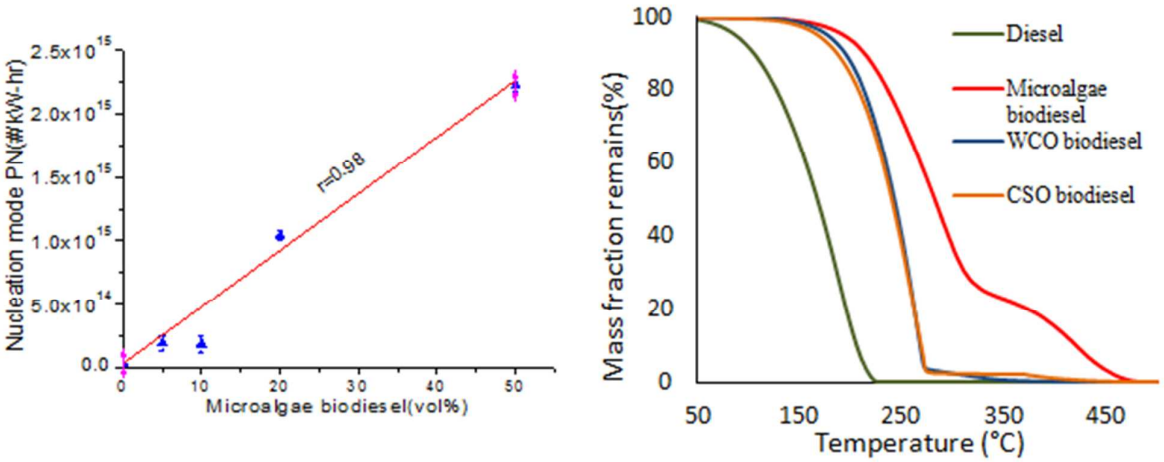

Figure 6: Effect of biodiesel blends on nucleation mode particle (a) and TGA analysis of the diesel and biodiesel blends used (**b)**

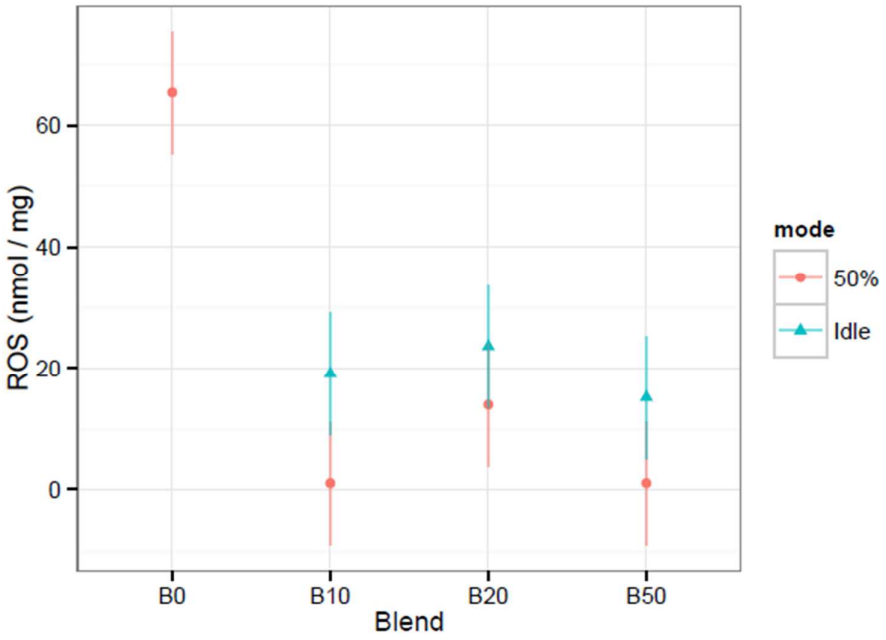

Figure 7: Oxidative potential of particles produced from diesel and microalgal biodiesel blends combustion

Table 1: Test engine specifications

Model	Peugeot 308 2.0 HDi
Cylinders	4
Compression ratio	18
Capacity	2.0 (L)
Bore × Stroke	85 × 88 (mm)
Maximum power	100 kW @ 4000 rpm
Maximum torque	320 Nm@ 2000 rpm
Aspiration	(Turbocharged) Intercooled
Fuel injection system	Common rail (Multiple fuel injection)
	Injection pressure: 1600 bar
Dynamometer	Froude Holfmann AG150 eddy current dyno
Emission Certification	Euro-IV

Table 2: Elemental compositions and important physical properties of the biodiesel blends used for engine testing

	A05D95	A10D90	A20D80	A50D50	CSO20	WCO20	Diesel
Elemental composition							
Carbon (wt%)	85.59339	85.18983	84.39171	82.067	84.17697	84.14855	86.67
Hydrogen (wt%)	13.04125	12.93331	12.71985	12.09809	12.90075	12.95812	13.15
Oxygen (wt%)	0.560893	1.117584	2.21855	5.425364	2.375388	2.365388	0
Relevant physical properties							
Viscosity(mm^2/s)	2.761	2.882	3.124	3.85	2.946	3.076	2.64
Density (Kg/l)	0.8436	0.8472	0.8544	0.876	0.848	0.846	0.84
HHV (MJ/kg)	45.62365	45.3203	44.7136	42.8935	44.4264	44.6924	45.927
NBP (^oC)	145.5	151	162	195	146	148	140
CN	50.3	50.1	49.7	48.5	57.8	52.12	50.5

HHV: Higher heating value, NBP: Normal boiling point, CN: cetane number