Environmental Science and Pollution Research (2019) 26:11371–11386
https://doi.org/10.1007/s11356-019-04556-w

RESEARCH ARTICLE

Generation of biodiesel from industrial wastewater using oleaginous yeast: performance and emission characteristics of microbial biodiesel and its blends on a compression injection diesel engine

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Received: 9 July 2018 / Accepted: 13 February 2019 / Published online: 23 February 2019

Abstract

Microbial-derived biodiesel was tested on a lab scale CI diesel engine for carrying out exhaust emission and performance characteristics. The performance, emission, and combustion characteristics of a single cylinder four stroke fixed compression ratio engine when fueled with microbial bio-diesel and its 10–30% blends with diesel (on a volume basis) were investigated and compared with conventional diesel. The bio-diesel was obtained from microbes which were grown by combining distillery spent wash with ligno-cellulosic hydrolysate at nutrient deprived conditions. The microbes consumed the wastes and converted the high strength waste water into lipids, which were trans-esterified to form bio-diesel. Testing of microbial bio-diesel blends with ordinary diesel at different loading pressures and the emission characteristics were compared. Results indicate that with increasing of the blends, reduction of HC and CO emissions were observed, whilst brake thermal efficiency maxed out at 20% blending. Further increase of blends showed a tendency of increasing of both emissions in the exhaust stream. The Brake Specific Fuel consumption was observed to decline with blending until 20% and then increased. The nitrogen oxide emissions, however, were found to increase with increasing blend ratios and reached a maximum at 20% blend. The escalation of HC, CO, CO_2, and NOx emissions was also observed at higher blending ratios and higher engine loads. The performance studies were able to show that out of the three blends of biodiesel, 20% biodiesel blend was able to deliver the best of reduced hydrocarbon and carbon monoxide emissions, whilst also delivering the highest Brake thermal efficiency and the lowest Brake Specific Fuel consumption.

Keywords Microbial biodiesel · Biodiesel blends · Emission reduction · Thermal efficiency · Specific fuel consumption

Abbreviation

CDI	Compression direct ignition
HC	Hydrocarbons
BTE	Brake thermal efficiency
BSFC	Brake specific fuel consumption
BMEP	Brake mean effective pressure
FAME	Fatty acid methyl esters
ASTM	American Society for Testing and Materials
DSW	Distillery spent wash
LCBH	Lignocellulosic biomass hydrolysate
CDI	Compression direct injection
FTIR	Fourier transform infra-red spectroscopy
TAG	Tri-acyl glycerol
MTCC	Microbial type culture and collection
CDF	Conventional diesel fuel
CO	Carbon monoxide
CO_2	Carbon dioxide
NO_x	Nitrogen oxides

Introduction

Current scenario and need for bio-diesel

Energy is the basis upon which modern society was and is being built. Everything from transportation to electricity generation requires energy. Modern energy needs are satisfied by

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usage of fossil fuels which regrettably fill the atmosphere with greenhouse gases. These cause global warming, climate change, desertification, and a host of other environmental damages (Umair 2015). Transportation alone contributes to 60% of the fossil fuel consumption as of 2014 (Sakthivel and Kasimani 2018). Current and future emission regulations are stringent and expected to become more stringent (Euro and BS). As a consequence, the transport sector is undergoing rapid transformation in order to comply with these regulations.

In addition, fossil fuel demand is continuously increasing globally, the result of which is the rapid depletion of fossil fuel deposits (Nabi and Hustamed 2010; Bhaskar et al. 2016). Recent decades have seen the interest in renewable fuels increasing dramatically due to demand of energy and the depletion of fossil fuel (ASTM 2008). Biodiesel has been receiving more attention in the last few decades due to its ability to replace fossil fuels, which are extrapolated to be exhausted by 2030. This offers the opportunities for developing domestic resources by effective use of domestic products. Replacing of oil consumption with renewable biomass energy might be one of the approaches to reduce dependence on petroleum-based fuels (U.S. Congress 2007). Biodiesel is a carbon neutral fuel and more eco-friendly than fossil fuels, since it releases carbon that was absorbed from the atmosphere and hence does not contribute to the rise of greenhouse gases (Robbins et al. 2011; U.S. EPA 2002). Nowadays, the search for alternative Feedstocks of biofuels presents a major roadblock in environmental and political challenges worldwide (Raj et al. 2010). Although there are a number of literatures for research on engine performances and its emissions by using biodiesel, especially after 2000, only fewer research has been done on microbial biodiesel. Hence, the study becomes important from an energy point of view, when industrial waste is used for generating usable fuel and thereby reduction of pollution load is achieved.

The very first diesel engine invented by Rudolph diesel was designed to be run on esterified vegetable oils only. Biodiesel can be used in diesel engines, since properties are similar to the conventional diesel fuel in terms of performance and engine modification is not required (Yanowitz and McCormick 2009). Despite having the above advantages, biodiesel has many disadvantages when it comes to fuel properties like lower calorific value, lower power output, and emission of higher contents of nitrogen oxides (NOx).

The interest in biodiesel for usage in diesel engines is its reduced life-cycle emissions with respect to Green House Gases (Danilo et al. 2006) McCormick 2007 was able to point out that Biodiesel blends could reduce greenhouse gas life-cycle emissions . According to Moser et al. 2009, biodiesel and blends of diesel (B10, B20, etc.) has a strong and beneficial effect on exhausts of hydrocarbons (HC), carbon monoxide (CO), and particulate matter (PM). Dorado et al. (2003) tested a direct injection diesel engine with olive oil methyl esters and reported a constant combustion efficiency compared to conventional diesel, with an insignificant reduction in brake specific fuel consumption (BSFC), reduction of 58.9% in CO, 8.9% in CO_2, 37.5% in NO, and 32% in NOx. Adaileh and Alqdah (2012) tested biodiesel made from waste cooking oil with a four-stroke DI diesel engine and found an increase of BSFC when compared to diesel. The study also obtained an increase in brake thermal efficiency and CO_2 emission, reduced CO, smoke (70%), and HC emission (63%), while also reducing NOx (Volpato et al. 2012). Brahma et al. (2017) studied Pongamia biodiesel blends in a compression ignition engine and found out an increase of brake thermal efficiency (BTE) with increasing of blends and increasing of loads.

The more accepted reasons in reduction of emissions particularly CO, CO_2, hydrocarbons, sulfur dioxide (SO_2), particulates, and smoke can be attributed to the presence of sufficient oxygen in biodiesel (Kegl 2008); the notion is due to the fact that the higher content of oxygen in biodiesel leads to complete combustion of fuels thereby reducing the amount of emitted gases (Roy et al. 2013). Complete combustion can convert CO into carbon dioxide (CO_2). From Nabi (2009a) study, it is clear that the decrease in emissions of carbon monoxide (27%), hydrocarbon (27%), nitrogen oxides (5%), and smoke (52%) is at full load when operated with biodiesel (Nabi et al. 2009b).

The NO_X from biodiesel are variable with increase in some and decrease in some studies (Fernando et al. 2006). Biodiesel emissions vary with sources and regions (Hoekman et al. 2011; Sun et al. 2010). But generally, biodiesel combustion tends to be consistent with a rise of NOx emissions (Kent and Curtis 2012). The result showed the greater reduction of NOx for fish oil biodiesel, and the brake thermal efficiency was found to be higher. Gupta et al. (2013) focused on the use of two non-edible oils mahua oil and processed waste fish oil.

Boehman et al. have also reported relationships between FAME density, viscosity, and NOx emissions (Boehman et al. 2004; Özener et al. 2012; Monyem et al. 2001). With FAME having higher density than conventional diesel, equivalent volume injection results in greater mass injection of biodiesel, although there is lesser energy injections. This argument that biodiesel blends deposit less soot has been verified by many authors who are working in this research. According to Sinha and Agarwal (2010), the effect of B20 on wear of engine components inside the cylinder and observed the following changes. It was observed that there were lesser carbon deposits on the injector tip, cylinder head, and piston crown of biodiesel-operated engine due to the lowered soot deposition during biodiesel combustion (Pandey et al. 2012; Ren and Li 2011). B20 blend of biodiesels is beneficial since it balances the property differences with conventional diesel, e.g., performance, emission benefits, and cost. Further, no major modification is required for B20 blends in automotive engines. It is also reported in Agarwal (2005) and Agarwal et al. (2003) that biodiesel improves carbon deposits in combustion chamber.

Microbial lipids for use as starter material for biodiesel production

Biodiesel preparation is done by reacting a lipid raw material with a short chain alcohol under homogeneous (acid or base), ultra-sonication (Patel et al. 2018) or heterogeneous (alkali salts) catalysis. The lipids are usually long chain fatty acids (> C16), and the short chain alcohols are either methanol or ethanol. The process is called transesterification. Transesterification process is carried out using plant oils, animal fats, waste oils, and microbial lipids. Most work on biofuels has been conducted on plant oils and animal fats (Ketterer et al. 2014), but the usage of food crops in this manner leads to food versus fuel complications (Sivakumar and Thompson 2012).

Recent attention to Feedstocks has been one of exploiting oleaginous microbes as future feedstocks for biodiesel production (Meeuwse et al. 2013). The oleaginous microbes accumulate lipids to more than 20% of their cell dry weight in the late log phase (Muniraj et al. 2013), under high carbon to nitrogen ratio (Meng et al. 2009). Studies on Oleaginous microbes have been performed on organisms such as yeasts (Ratledge 2004; Rossi et al. 2011), fungi, and bacteriae (Alvarez et al. 2000) for improving lipid accumulation (Bellou et al. 2016). Hence, oleaginous microbes have been identified as an alternative competent sources to produce biofuels (Teresa et al. 2010; Singh and Singh 2010). Microorganisms possess several advantages over plants for lipid accumulations, such as short life cycles, demand less space, and can be grown at any location irrespective of climate of any season (Ramalingam et al. 2010).

Microbial lipids grown using an organic materials as growth media can shorten the gap between demand and supply of the market (Lee et al. 2008; Gui et al. 2008; Du et al. 2008; Ratledge and Wynn 2002). Fortunately, oleaginous microbes can also utilize carbon-rich wastes for lipid production (Rittmann 2008; Wu et al. 2012; Rossi et al. 2011; Poontawee et al. 2017; Gong et al. 2015; Kitcha and Cheirsilp 2011; Vieira et al. 2016). Hence, utilizing microbial biomass by growing them in industrial wastewaters containing high organic content media reduces the waste load (Jiayin et al. 2016; Salamaa et al. 2017). This approach presents a dual advantage of cutting down on the organic load of waste stream and also generating energy as lipids (Jin 2015).

The microbe used in the current study has been variously reported as *Candida pulcherrima*, *Rhodotorula pulcherrima*, *Torulopsis pulcherrima*, *Saccharomyces pulcherrimus*, *Cryptococcus castellanii*, and *Torulopsis pulcherrima*. Recent naming puts the scientific name as *Metschnikowia pulcherrima* which is a non-ascomycetous yeast belonging to the family *Metschnikowiaceae* which is found in fruits, flowers, and nectars (Sitepu et al. 2013). The target organism was a previously non-oleaginous organism that was used in preventing the post-harvest decay of various fruits (Türkel et al. 2014) and for controlling wine yeasts (Oro et al. 2014). But, under altered growth conditions such as lowered temperature, high carbon-to-nitrogen (C/N) ratio and limitation of few salts triggered the microbe to convert consumed sugars into storing lipids as tri-acyl glycerol (TAGs) or triglycerides (Santomauro et al. 2014). The organism also has the ability to tolerate high sugar levels in its environment. The fact that it can inhibit a variety of microbes (except aspergillus) from contaminating its culture was taken into consideration for choosing *M. pulcherrima* for the study as observed by Sipiczki (2014). Since oleaginous yeasts are sensitive to contamination and the organism was able to grow under non-sterile conditions (Santomauro et al. 2014), additionally, the oils produced by yeasts is similar in composition to vegetable oils with unsaturated fatty acids making up more than 85% of the total lipids. The lipids stored by the organisms have a high triglycerides content, comprising Oleic (18:1), Palmitoleic (C16:1), linoleic (18:2), Linolenic (18:3), and arachidic (20:0) (Sitepu et al. 2013). *M. pulcherrima* is also expected to produce oils that are future substitutes for palm oil, thus preventing the destruction of rain forests for growing palm trees (Whiffin et al. 2016). Microbial oils/biodiesels containing mostly oleic acids are considered great for usage as biodiesel (Wu et al. 2011).

In this study, oleaginous microbes were grown in distillery spent wash and growth conditions were altered for producing lipids. Then from the lipids, biodiesel was produced. Biodiesel obtained from microbes was blended with conventional diesel and used for testing of engine performance using a compression direct injection (CDI) engine. The power output, exhaust gas emissions, and other effects were studied by combustion of various blends of biodiesel and were compared with those of conventional diesel (Shahir et al. 2015). The operating conditions were monitored using various sensors and kept close to the real combustion and airflow conditions inside a working conventional diesel engine.

Materials and methods

Microbial mass culturing

The test organism *Metschnikowia pulcherrima* bearing the serial number of MTCC 632 was obtained from Microbial Type Culture and Collection (MTCC) located in Chandigarh, India. The culture arrived as vacuum dried powders stored in vacuumed glass ampoules. From the dormant powders, the microbes were revived in distilled water after 24 h and subsequently used for preparing stock and subcultures of *M. pulcherrima* in standard culture media. The cultures were kept at 25 °C, 150 rpm, and at pH 4 inside an incubator cum shaker. From these cultures, the organisms were used for inoculation into distillery spent wash (DSW).

All inoculation studies were performed under sterile conditions of the Laminar Flow Chamber, and hence there was no detection of contamination.

The industrial wastewater distillery spent wash (DSW) was obtained from a local distillery plant. The initial carbon to nitrogen ratio was low in the range of 9 to 9.4 and not suitable for lipid accumulation. The carbon to nitrogen ratio of the distillery spent wash was increased by pre-treatments from 9.4 to 35 (study methods not shown here). The additional glucose was added to the DSW in the form of lignocellulosic hydrolysate obtained by acid hydrolysis of wood waste biomass.

A 5 l bench top fermenter supplied from BIO-AGE equipments was used for culturing of microbial biomass in mass amounts for lipid extraction. The microbes were grown in pre-treated distillery spent wash which possessed high recalcitrant abilities in the form of extreme biological oxygen demand, total solids, total sugars, and fermentation products (Farid et al. 2012; Satyawali and Balakrishnan 2008).

Lignocellulosic biomass hydrolysate studies

Lignocellulosic biomass hydrolysate (LCBH) was prepared using waste wood chips under acid catalysis as the methods adopted by Šantek et al. (2018). The wood particles were sieved under various sizes (600, 300, 150, 75 μm), and the smallest sized particles (75 μm) were used for depolymerisation. The depolymerisation of lignocellulosic biomass was performed by combining dilute acid hydrolysis and hydrothermal pre-treatments to dissolve the cellulose from the wood fibers (Ruan et al. 2014; Huang et al. 2009). The acid concentration was kept as 3% *v*/*v*, and the weight of 4 g (*w*/*v*) was used for optimum C/N ratio (Subhash and Mohan 2015). The lignocellulosic biomass hydrolysate was prepared after the above solution was treated hydrothermally to a temperature of 121 °C and a pressure of 15 pounds per square inch for 20 min. Then, after the hydrothermal treatment, the solution was left to stand for 2 h to allow the hydrolysis to proceed to completion. After 24 h, the hydrolysate was filtered with the help of Whattman filter paper and then neutralized using base solutions (Šantek et al. 2018).

The hydrolysate was mixed with DSW in various ratios (10, 20, 30, 40, and 50%) to find out the optimum range for microbial growth (data not shown). The 50% dilution was found out to be the best ratio for microbial lipid accumulation. Hence, the same ratio was followed for bulk culturing of oleaginous yeast biomass in the fermenter.

The 5-l bench top fermenter was constructed of glass, and the metallic lid was provided with provisions for air supply, sampling, pH, and foam control. Several probes were used for monitoring pH, temperature, media introduction, air supply, and a cooling jacket for lowering the temperature to below 20 °C. The motor at the top took care of keeping the media under agitation conditions.

Single stage lipid productions have been reported by Jin et al. (2015), but the yields were lower than expected. Hence, as studied by Slininger et al. (2016), a two-stage batch process involving the introduction of acid hydrolysate was found to be advantageous. The entire unit was filled with the constituted media and autoclaved prior to inoculation. The yeasts were inoculated on day 1 and allowed to grow under constant growth conditions. Figure 1 shows the experimental fermenter set up used in the study. The after growth for 3 days lignocellulosic hydrolysate (1:1 ratio) was added to increase the C/N ratio to 55. The growth conditions are given in Table 1.

The combination of high C/N ratio and a reduction of temperature (< 15 °C) triggered the non-oleaginous yeasts into a stressful state where *M. pulcherrima* cells begun to accumulate TAGs by rapid consumption of glucose in the lignocellulosic hydrolysate (Santomauro et al. 2014; Chuck et al. 2014.). After the growth period, separation of yeast biomass was accomplished through centrifugation. The wet biomass was dried in a halogen moisture analyzer at 105 °C for 2 h. The biomass was ruptured and lipids were extracted from them by usage of mechanical pre-treatment and solvent extraction (Wang et al. 2012).

Transesterification process (acid-catalyzed)

There are several methods for direct transesterification of oleaginous biomass as done by Demirbas (2008) and acid catalysis using sulfuric acid and methyl alcohol were performed for

Fig. 1 Experimental set up of bench-top fermenter used for culturing microbes

Table 1 Growth conditions before and after stationary phase

Parameters	During growth phase	During stationary phase
Temperature	25 °C	< 15 °C
pH	4	5
C/N ratio	35	55
Rotation	150 rpm	60 rpm

the transesterification process. The microbial biomass was added to conical flasks containing transesterification mixture in 1:20 ratio. The mixture was stirred at 600 rpm and held at 90 °C for 90 min (Thliveros et al. 2014). After 90 min, water and hexane were added to form separated layers. The upper layer which contained the fatty acid methyl esters (FAME) was purified by adding equal volumes of petroleum ether and 0.9 M sodium sulfate solutions. The final purification steps involved the centrifugation to separate the FAME and non-FAME layers. The FAME layers were left overnight for the evaporation of petroleum ether, and purified FAME was got.

Biodiesel properties

The fuel properties are very important for bio-based fuels since the characteristics tend to vary much in comparison to conventional diesel fuel. The microbial bio-diesel was studied for the various physicochemical and energy properties for usage in CDI engines. Parameters such as viscosity, heating values, energy content, elemental composition, cetane number, density, and flash point were measured for the study. The fuel property information were determined by methods adhering to the guidelines set by ASTM D7544.

Fourier transform infra-red spectroscopy

Fourier transform infrared spectroscopy (FTIR) was helpful to non-destructively identify the various molecular functional groups of microalgal and yeast lipids (Dean et al. 2010; Vongsvivut et al. 2013; Ami et al. 2014). The instrument used for the above purpose was PerkinElmer spectrum two SP10S/W which operated in the middle infra-red range of 4000 to 400 cm^{-1}. The accessory of Horizontal Attenuated Total Reflection making use of Zinc Selenite crystal was used for characterizing microbial FAME. The spectrum was obtained using resolutions of 2 with scanning accumulations of 16. The various compounds were identified through the in-built software called FLUKA which returned 20 matching spectra of which those matching 85% or more were considered for results.

Biodiesel blends

The obtained biodiesel was blended with conventional diesel to produce blends. A high-speed homogenizer was used for producing the blends of biodiesel with conventional diesel. The conventional high-speed diesel fuel was procured from Bharath Petroleum petrol pump, Coimbatore. The blends ranged from 0%, 10%, 20%, and 30% *v*/*v* of biodiesel and named respectively as B0, B10, B20, and B30.

Engine set-up

The emission and performance testing of the biodiesel blends were done on single cylinder four stroke diesel engine, and its results were compared with that of neat diesel operation. The engine was made up of single cylinder with a displacement of 661 cm^3. The setup had provisions for monitoring the air supply, amount of fuel entering the combustion chamber and the like. The inputs from the test are captured by using data acquisition system provided by National Instruments, and the data are processed by EngineSoft software. The information about the engine set-up is given in Table 2. The exhaust gas from the engine operation was analyzed with AVL MDS-250 gas analyzer. Before starting the engine, the coolant and lubricant circuits were checked for proper working. The engine was started and warmed up by running at rated speed for 20 min without any load in neat diesel fuel. After that, biodiesel blends were fed to the fuel line through separate tank. The performance and emission readings are noted for different loading conditions after stabilizing the engine speeds at respective loads. All the readings are taken in triplicate to ensure the reliability in results and discussions.

Brake thermal efficiency

It is defined as the average pressure exerted by the engine on the piston through all the four strokes of the engine cycle. If N is the number of revolutions per second, and n_c the number of

Table 2 Engine specifications

Make	Kirloskar
No. of cylinders	1
Stroke	4
Type of injection	Direct injection
Cylinder bore diameter	87.5 mm
Cylinder stroke diameter	110 mm
Compression ratio	17.5
Injection timing	23° bTDC
Engine speed	1500 rpm
Rated output power	3.5 kW
Cooling system	Water cooling

revolutions per cycle, the number of cycles per second is just their ratio (W) which can be expressed by

$$w = \frac{Pnc}{N}$$

Brake specific fuel consumption

The BSFC is a measure of the efficiency of the engine in using the fuel supplied to produce work. It is desirable to obtain a lower value of BSFC meaning that the engine uses lesser fuel to produce the same amount of power. It is calculated by

$$\mathrm{BSFC}\ (\mathrm{g/kWh}) = W_{\mathrm{f}}/P_{\mathrm{b}}$$

where W_{f} = fuel consumed (g/h).
P_{b} = brake power (kW) which can be calculated by:

$$P_{\mathrm{b}} = P_{\mathrm{g}}/\eta_{\mathrm{g}}$$

where P_{g} = load (kW) at generator end

$$\eta_{\mathrm{g}} = \text{efficiency of the generator}$$

Uncertainty analysis and instrument details

Uncertainty of a measurement is a measure of the errors that may exist in the results obtained from the analyzing equipment. Uncertainty analysis helps to judge the fitness values obtained from the equipment. The uncertainty analysis enables that the accuracy of the measured values of the instrument is reliable (Sakthivel and Kasimani 2018). If the errors exceed a certain percentage, then the reliability of the obtained results are questionable. The uncertainty analysis was carried out by taking the mean values with error values falling in p = 0.05 or 95% confidence level (Imdadul et al. 2016). All the experiments were conducted in triplicates, and the average values were taken to ensure the reliability of results. Table 3 lists the uncertainties of the instruments used in the present study.

Results and discussion

Characteristics of microbial lipid and fame yield

From 6 l of combined wastewaters, 204 g of dry biomass was obtained, forming a 34 g/l of yeast yield. Lipid extraction by Hara and Radin method gave 76 g of lipid from 204 g of dry biomass, accounting for 37.2% of biomass dry weight as lipids. The transesterification method was successful in turning up to 85% of lipid into FAME. Hence, the yield was 65 ml of FAME. This amounts to 10.8 ml of FAME per liter of culture media. Direct methanolysis was able to convert 20 g of dry biomass into 8 ml of FAME. The initial calorific value, flash point, and cetane number were found out for pure microbial biodiesel. The characteristics of microbial biodiesel are all given in Table 4.

Fuel properties are indispensable for analyzing different alternative fuels when operating the engine and evaluating fuel combustion (Yasin et al. 2013). From Table 4, it is clear that the cetane number of biodiesel is higher than diesel. The density is higher, so is the viscosity. Hence, the flow of biodiesel through the fuel lines, injectors, and cylinder will be affected more when compared to diesel. Higher viscosity also affects atomization, droplet size, and lubricative properties of biodiesel in the engine parts.

In general, biodiesel fuel density is higher than diesel, thereby the specific fuel consumption increases for the same engine power (Ali et al. 2013). Its calorific value is at the low end of the diesel spectrum, implying that the engine output power reduces with the same specific fuel consumption (Uddin Shahab et al. 2012). From Table 4, it is evident that biodiesel fuel's flash point is higher than diesel fuel which ensures safe handling and storage of fuel (Yasin et al. 2017). The oxygen content is higher than conventional diesel, meaning the combustion will be started readily without any ignition delay and the combustion would proceed to completion.

FTIR spectral analysis

From Fig. 2, it can be seen that there are alkyl stretches in the region of 2800–2500 cm^{-1} implying the presence of C–C and C=C bonds. This is indicative of alkene compounds with double bonds in their molecules. A deep peak at 1735–1750 cm^{-1} indicates the presence of fatty acid ester (C=O) in the molecule, indicating the presence of the target molecule. At the peak of 720–725 cm^{-1} represents that the molecule has more than four methylene (CH_2) chains in its backbone, implying the presence of a long chain (> 6 carbon) hydrocarbon groups in the molecule. All these arrive at a conclusion that the molecule has a long chain unsaturated fatty acid methyl ester as compounds in it. The internal library search by FLUKA reveals that the presence of many methyl esters is given in Table 5.

Gas chromatography mass spectrometry technique was used to find all the molecules present in the microbial lipids which corroborated the presence of the above compounds. The spectrometry data revealed the presence of unsaturated fatty acids which matched with the FTIR result (data not shown).

Performance testing on CDI engine

Biodiesel was found to have varying efficiencies with respect to blends, and it was seen that the efficiency was rising with increasing blends. However, the increase in efficiency reached

Table 3 Purpose, accuracy, and uncertainties of the various instruments used in the study

Instruments used	Parameters measured	Accuracy	Uncertainty
PerkinElmer FTIR Spectrum Two SP10S/W	Analysis of the functional group of compounds present in FAME sample (wavenumbers in cm^{-1})	< 1% of transmittance value	± 0.008
pH meter	Determination of pH of the LCBH media	± 0.02 pH	± 1.20
Kinematic viscometer	Determination of the kinematic viscosity of FAME (centistoke)	< 3% of measured value, calibrated	± 1.27
Density meter	Determination of density of the FAME sample (g/cm^3)	± 0.012 g/cm^3	± 0.29
Thermocouple	Determination of reactor temperature (°C)	± 1 °C	± 0.11
Bomb calorimeter	Determination of calorific value of the FAME (MJ/kg)	± 0.05%	± 1.24
Pensky-Martens apparatus	Determination of flash point of the FAME (°C)	± 2.1 °C (less than 200 °C)	± 1.68
Pour point apparatus	Determination of pour point of the sample (°C)	± 1.1 °C	± 2.85
Engine testing	Brake thermal efficiency	± 0.50%	± 0.05
	Brake specific energy consumption	± 0.051 MJ/kWh	± 1
Emission testing	CO	± 0.01 vol%	± 2
	CO_2	± 0.031 vol%	± 0.5
	HC	± 1 ppm	± 2
	NOx	± 1 ppm	± 2
	Smoke	± 0.1%	± 1.25

a maximum at B30 and with increase of biodiesel blends was seen to be decreasing. Various parameters and their effects when handling biodiesel blends are discussed in terms of BTE, BSFC, and emissions.

Brake thermal efficiency

Table 6 shows the brake thermal efficiency of B0, B10, B20, and B30 blends.

As seen from the table, it is observed that the brake thermal efficiency increases from diesel through to B20; then at B30, it decreases at all pressures. The increasing trend is due to the property of biodiesel having better combustion efficiency than diesel.

The behavior of BTE as seen from the Table 6 and Fig. 3 shows that the blend ratio of 20%. Microbial FAME is seen to increasing with increased brake mean effective pressure (BMEP) from 1.16 all the way to 4.11 bars.

The brake thermal efficiency is the lowest for conventional diesel and increases up to B20 and then decreases. The increase in BTE is high for lower loads; then there is a tendency to increase at the highest load. The decrease of BTE is due to the fact that the methyl esters have lower calorific value than the pure diesel. The overall increase ranged from 1.377 to 12.87%. At lower loads, the rise of BTE was small and had a tendency to increase with increasing of loads and with increasing of biodiesel blends ratio up to B20. Increasing of ratio to B30 saw a decrease in BTE but still remained higher than those of conventional diesel. Lowest BTE among all the blends was arrived at B30 at the BMEP of 1.11 for all fuels, and the BTE follows a pattern of decreasing and then increase due to the fact that higher blends have more numbers of methyl esters. The low efficiency may be resulted as low volatility, slightly greater viscosity, and higher density of the FAME and also due to presence of higher unsaturated

Table 4 Properties of microbial biodiesel and commercial diesel

Properties	Unit	Method	Commercial diesel	Microbial bio-diesel
Density (at 15 °C)	kg/m^3	ISO 3675	820–840	962.5
Viscosity (at 40 °C)	mm^2/s	ISO 3104	2.6–6	13.1
Calorific value	MJ/kg	ASTM D4809	40–45	40.3
Flash point	C	ISO 2719	75	112
Pour point	C	EN 116	20	6
Cetane number		ISO 5165	49	52
C mass fraction	% kg/kg	ASTM D5291	85	78
H mass fraction	% kg/kg	ASTM D5291	15	14.5
O mass fraction	% kg/kg	–	0	7.5

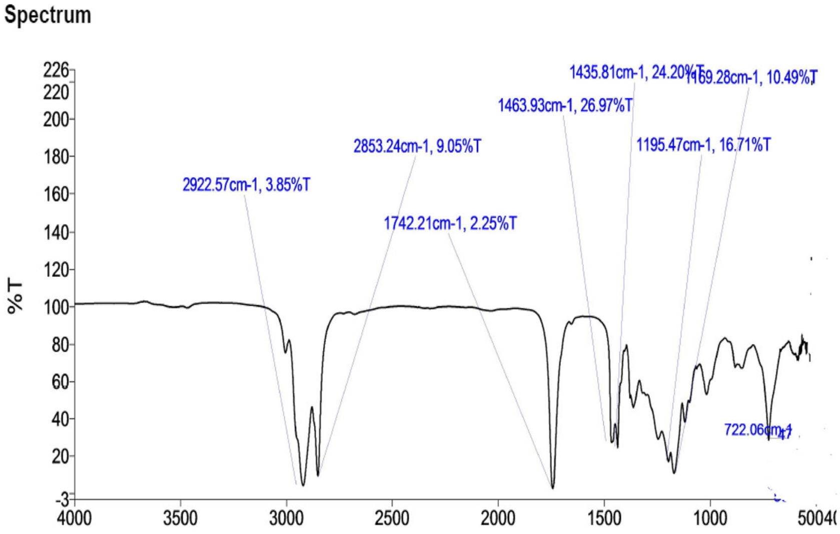


Fig. 2 FTIR spectral profile of biodiesel

Table 5 FTIR library search results

Library hit	Molecular formula	Description
Methyl linoleate	$C_{17}H_{31}COOCH_3$	Methyl ester of linoleic acid
Methyl palmitoleate	$C_{15}H_{29}COOCH_3$	Methyl ester of palmitoleic acid
Methyl oleate	$C_{17}H_{33}COOCH_3$	Methyl ester of oleic acid
Methyl ricinoleate	$C_{17}H_{33}COOCH_3$	Methyl ester of ricinoleic acid

compounds which affects mixture formation of the fuel and leading to slower combustion.

The increase was highest for B20 blends at 6.51% as compared to diesel as was observed by Buyukkaya (2010). Hence, the BTE of the blends was higher than CDF for all blends, and B20 gave the maximum value out of all others.

Brake specific fuel consumption

Table 7 is the calculation results based on the data collected from diesel engine testing which is ranged from B0 to B30. However, the specific fuel consumption increased differently, while the biodiesel blend ratio was increased.

Table 6 Brake thermal efficiency of the FAME at different blends and loads

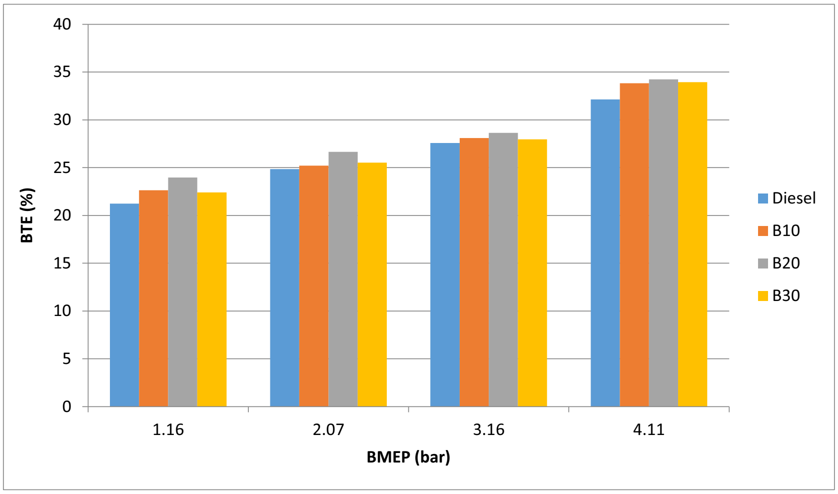
BMEP (bar)	Diesel	B10	B20	B30
1.16	21.24	22.65	*23.97*	22.41
2.07	24.85	25.21	*26.65*	25.52
3.16	27.58	28.1	*28.65*	27.96
4.11	32.14	33.84	*34.25*	33.96

All emission values are given in parts per minute

BSFC had a tendency to get reduced as the BMEP increased from 1.16 to 4.11 bars. The BSFC also followed a pattern of going downhill as the blend increased from B0 to B20 and then increased. From Fig. 4, it can be observed that the specific fuel consumption is directly correlated to the biodiesel blending percentage. In other words, the specific fuel consumption increased when the biodiesel blending percentage increased as observed byHao et al. (2017). From the increment percentage, it can be spotted that the B30 has the highest SFC value and it consumes more fuel to produce 1 kW of power when compared to conventional diesel (B0). With methyl esters having higher Cetane Index (CI) than diesel, delay period gets reduced and it leads to effective combustion which reduces HC emissions to a greater extent (Vallinayagam et al. 2013), but since the amount of fuel delivered during the ignition delay period increases because of high injection rate, the BSFC increases with injection pressure.

With B20 as the load increased, BSFC increases from 2 to 8.4%, then plunges, and goes up again. Similar trend is seen with B30 although it is higher than B20. The higher BSFC of those higher percentage blends ratio is due to the fact that the biodiesel has lower calorific value than the conventional diesel as observed by Arbab et al. (2013). Despite the better combustion of biodiesel compared to the conventional diesel,

Fig. 3 BTE % profiles for four fuels at each of four loads of the engine



the oxygen takes up space in the blend and hence increases the BSFC.

Hence, the lower ignition delay coupled with lower calorific value of biodiesel has been found to consume more fuel for similar power output; thus, an increase of BSFC is observed for all the blends. This is similar to various studies reporting that biodiesel and its blends cause an increase of fuel consumption when used in diesel engines.

Exhaust emission characteristics

The exhaust streams are the end results of combustion occurring in the ignition chamber. The results for concentration of various gases in the exhaust stream and their reduction or increment with various blends of biodiesel are discussed below.

CO emissions

The CO portions of the exhaust streams are seen as in Table 8 and Fig. 5.

From the Table 8, it is seen that the CO emissions resulting from the exhaust chamber of diesel are higher than the biodiesel blends at lower BMEP. From the values shown in Fig. 5, the emissions decrease as the blend ratio increases at all loads, but only up to B20 (2.6% to 21.4%). After that, the increase in blending causes an increase of emissions (7.03 to 13.66%) although the values are lesser than CDF.

Table 7 Brake-specific fuel consumption of all blends with varying loads

BMEP (bar)	Diesel	B10	B20	B30
1.16	30.26	30.02	*29.65*	29.99
2.07	25.36	24.98	*23.24*	23.91
3.16	21.36	21.03	*20.52*	21
4.11	13.35	12.98	*12.58*	12.87

All emission values are given in parts per minute

As the load increases, the emissions start to go downhill steadily with all the fuels, though the pattern does not follow a linear pattern. This is presumed to be due to higher combustion pressures inside the chamber. But proportionally, the reduction gets higher as load increases for B20 (11.7–21.4%). this was also observed by Agarwal and Atul (2010).

The blends all give off lesser CO than CDF at same loads, as they contain fewer carbon atoms than the CDF. But since the BSFC reduces, the emissions tend to rise again as seen for Blend B30. B30 has highest CO emissions among the blends but is still lower than CDF and for reasons cited above B20 has the lowest concentrations of CO (11 to 21.4%) when compared to CDF.

Hence, the higher injection pressures that have positive effect in reducing CO emissions were obtained but only until the B20 blend. Muralidharan et al. (2018) found out that the increasing of CO emissions correlates with increase of blends beyond B20 and is implicated to the poor atomization of fuel due to higher viscosity of the blend.

CO_2 emissions

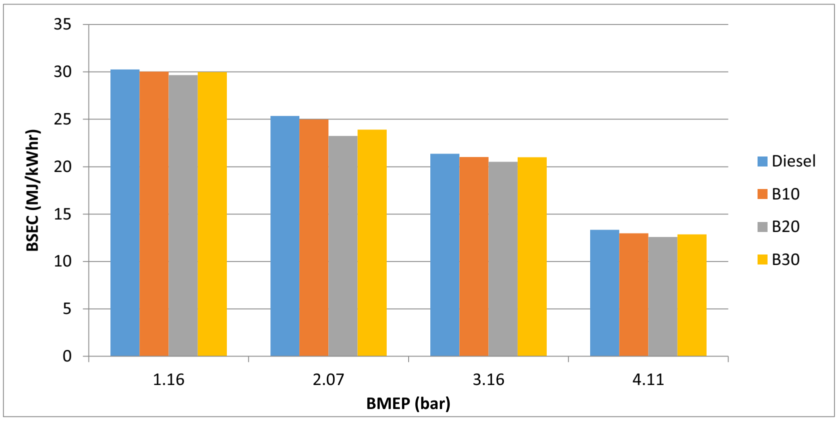
The CO and CO_2 emissions are in a trade-off relationship since for the same molecules, the combustion varies with oxygen content (Knothe 2005) Heywood 1988, had featured an equation to factor in the oxyen intake for diesel as well as biodiesel blends as shown.

$$C_{16}H_{31}COOCH_3 + 25\ O_2 \rightarrow 17\ CO_2 + 1\ CO + 17\ H_2O$$

$$C_{18}H_{38} + 25\ O_2 \rightarrow 15\ CO_2 + 3\ CO + 17\ H_2O$$

From the above equation, it is clear that the CDF have higher carbon to hydrogen ratio than the esters as observed by Liaquat et al. (2012). There is also a trade-off between CO_2

Fig. 4 Brake specific energy consumption of all blends with varying loads



and CO in combustions between FAME and CDF. Hence, burning of blend fuels releases more CO_2 for the same air intake. As the oxygen content increases, the complete combustion also increases consequently leading to higher CO_2 in the exhaust gases.

The CO_2 emissions from the combustion of the four blends are shown in Table 9 and Fig. 6; CO_2 emissions are expected to be truncated when using blends of biodiesel, and it is also reflected in the values shown.

From the Table 9, the CO_2 emissions are decidedly higher than CDF at same BMEP and is principally known to be due to the fact of better combustion of all the ester molecules ultimately leading to higher CO_2 molecules in the blends than from CDF emissions.

From Fig. 6, as the loads begin to go higher, so do the emissions. The greater emissions with greater blends is observed to be somewhat modest at the lowest pressures and at 3.16. The cumulative increase is highest at BMEP of 2.07 and with the highest load, the cumulative emission is lowest even though it carries the highest emission of all the fuel blends. It can be concluded that the emissions are lower at lower injection pressures.

The amount of CO_2 in the analyzed exhaust gases are observed to follow an ascending pattern for blends up to B20 and then they descend down starting at B30. The decrease of CO_2 with increasing of blend ratio is due to the fact the volume occupied by higher blends increased and hence the emissions decrease but the power output also decreases (Shahabuddin et al. 2012). Hence, more fuel is burned for producing same power and the lower elemental carbon to hydrogen ratio of the blends.

Table 8 CO emission profiles of the diesel and biodiesel blends

BMEP(bar)	Diesel	B10	B20	B30
1.16	1.39	1.28	*1.18*	1.2
2.07	1.28	1.21	*1.13*	1.19
3.16	1.12	1.09	*0.96*	1.01
4.11	0.84	0.78	*0.66*	0.77

All emission values are given in parts per minute

NO_X emissions

Many authors pointed out that there is an increase of NOx emissions when biodiesel is used in CI engines (Miller and Bowman 1989; Fennimore 1971; Sairam et al. 2013). Md. Saiful et al. (2014) pointed out that the NOx emissions are a function of total oxygen in the chamber, temperature, compressibility, and pressure. Also, the higher cetane number of biodiesel and the reduced ignition delay can lead to higher NOx emissions, since the residence time of the burning mixture inside the cylinder increases.

And, the current study that was carried out with microbial biodiesel delivered a similar result.

The NOx emissions are given in Table 10 and graphically represented in Fig. 7.

From the Table 10, the NOx emissions are found to surge and the higher emissions at BMEP 1.16 where there is an increase of 15.5%, 31.47%, and 22.4%, respectively. From Fig. 8 for a BMEP of 2.07, the blends are found to release higher amounts of NO_X, viz., 8.27, 10.48, and 9.37%, respectively. At higher BMEP of 3.16, the blends are found to give-off emissions in the ranges of 3.5%, 14.22%, and 17.2%, respectively. Here, the trend is an increasing of emissions with the blend ratio rather than decrease at the B30 blend.

At even higher BMEP, the emissions are proportionally lower, viz., 2.4%, 8.2%, and 8.98%, respectively. The trend seen is smooth incline as compared to lower BMEP. Mueller et al. (2009) had the theory behind the increase of NOx being due to the higher combustibility and oxygen content of biodiesel that oxidizes the nitrogen in the incoming air. Rise of injection pressure tends to surge NOx emissions due to the greater heat release rate in the premixed stage of combustion.

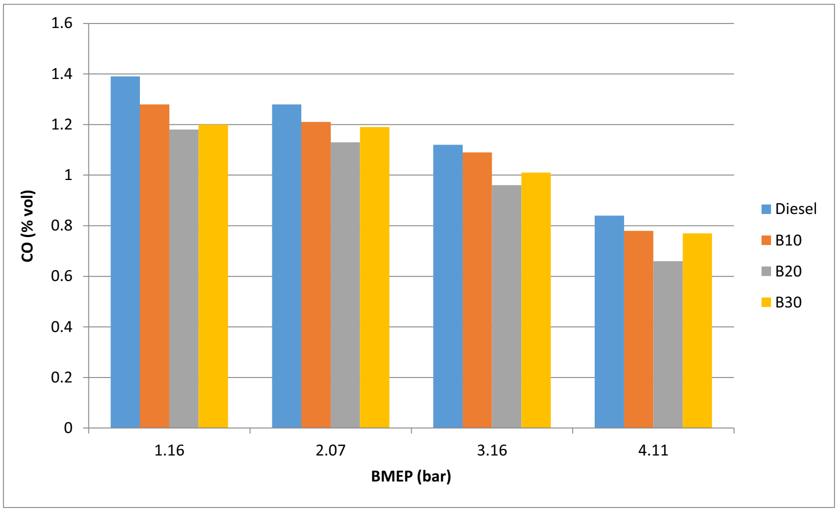


Fig. 5 CO emission profiles comparison with diesel and biodiesel blends

Hence, considering the emissions with respect to NOx, the propensity is to increase with increase of blending and with increase of BMEP, as observed by Roy et al. (2013), although the emissions are proportionally lower at higher loadings. Gaurav and Sharma (2013) was able to come to similar conclusions.

HC emissions

Unburnt hydrocarbons in the exhaust gas is a measure of incomplete combustion in the combustion chamber. There is plenty of evidence to conclude that the usage of biodiesel blends provides the advantage of reduced HC emissions as observed by Singh and Shrivastava (2012), for the entire loading range.

And, it is seen here with microbial biodiesel blends as well. The emission values of hydrocarbons (HC) from the engine testing are given in Table 11.

As seen from the Fig. 8, the HC at the exhaust stream tend to reduce with addition of blending and with increasing BMEP.

At lower loadings from B10 to B20, the cutting down rises from 15.97 to 37.2%, the reduction is less in B30 18.34%. Hence, the maximum reduction is seen at B20. And, as studied by earlier, the increase of blending decreases the HC, but after B20, the reduction is lesser as was observed by Sonar et al. (2015) and Damanik et al. (2018) who also obtained a reduction of HC when using biodiesel blend of B20.

Similar trends are seen with higher loadings of 2.07 (8.9, 20.68, 11.03%) with maximum reduction of 20.68% being obtained at B20 blend. And, with 3.16, the maximum reduction occurs at B20 with 23.21% than CDF. At the highest load, we obtain reductions of 8%, 21.33%, and 6.66% reductions. As with the previous loads and blends, it is at B20 and there is maximum reduction of HC exhaust (21.33 to 37.22%).

The results are similar to those obtained for CO emissions where emissions are lowest for B20 with addition of loads but also that the emissions get winded down at higher blends. Adaileh and Alqdah were able to conclude that higher CO_2 in the exhaust was due to the higher oxygen content of the methyl esters causes complete burning of the hydrocarbon molecules in the blend. This trend was also confirmed by McCormick et al. 2006.

Table 9 CO_2 emission values for all blends with different loading pressures

BMEP (bar)	Diesel	B10	B20	B30
1.16	5.9	6.2	*6.5*	6.25
2.07	6.8	7.2	*7.8*	7.4
3.16	8.2	8.5	*8.9*	8.4
4.11	9.3	9.4	*9.8*	9.5

All emission values are given in parts per minute

Smoke

Smoke is a characteristically diesel engine property as there are particulate matters and sulfur oxides that cause smoke. The smoke results are given in Table 12 and graphically in Fig. 9.

As seen from the Table 12, it is clear that the emissions reduce as the loadings and blends increase.

From the table, it is clear that the reduction of smoke at low loads is given as 7.6 to 18% for the lower two loads and the trend at higher loadings is 0.4 to 10.8%.

From Fig. 9, the smoke opacity reduces from CDF to B30 in a decreasing pattern.

The tendency along the column of B20 is that the opacity reduction percentage is inversely proportional to load additions. Hence, at the lower load of 2.07, there is consistent reduction percentage of smoke.

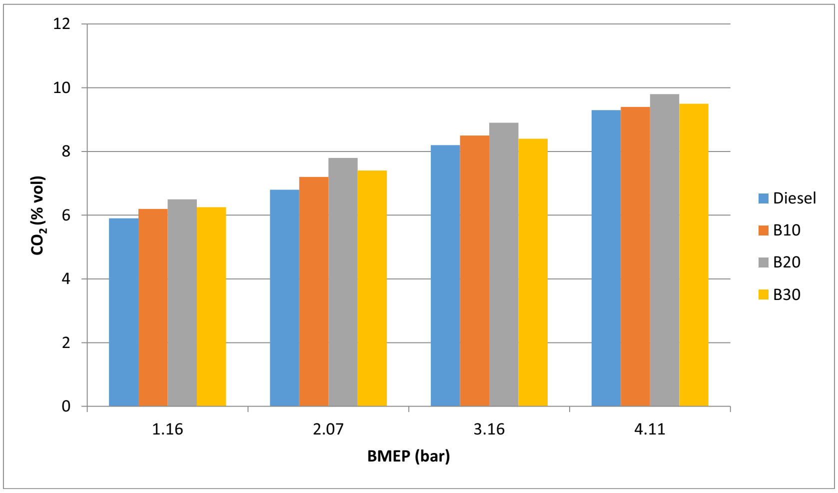


Fig. 6 CO_2 emission profiles comparison with diesel and biodiesel blends

Table 10 NOx emission profiles for all blends with different loading pressures

BMEP (bar)	Diesel	B10	B20	B30
1.16	521	602	*685*	638
2.07	725	785	*801*	793
3.16	935	968	*1068*	1096
4.11	1169	1198	*1265*	1274

All emission values are given in parts per minute

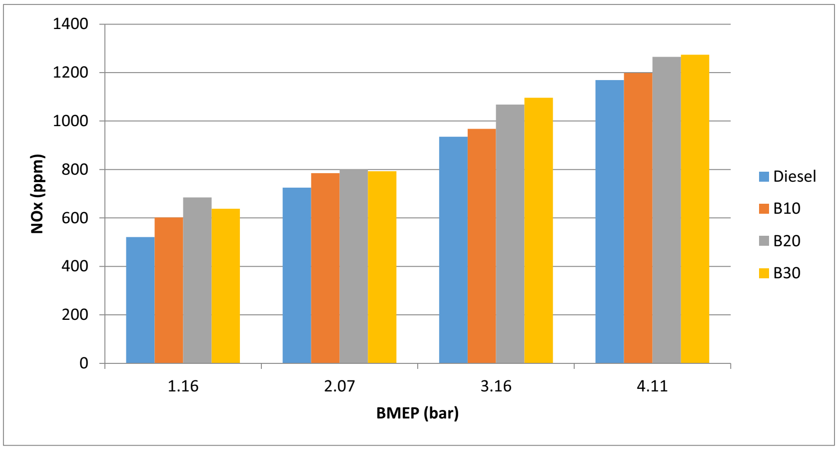


Fig. 7 NOx emission profiles comparison with diesel and biodiesel blends

Table 11 UHC emission profiles for all blends with different loading pressures

BMEP (bar)	Diesel	B10	B20	B30
1.16	169	142	*126*	138
2.07	145	132	*115*	129
3.16	112	98	*86*	101
4.11	75	69	*59*	70

All emission values are given in parts per minute

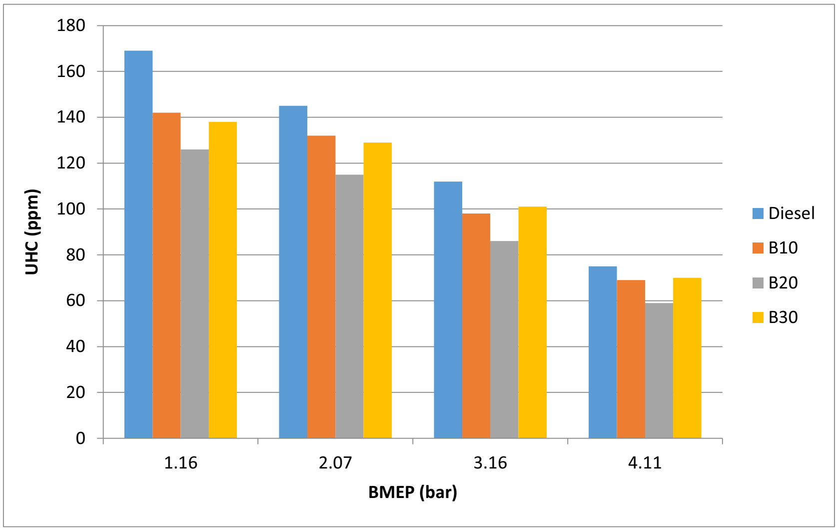


Fig. 8 HC emission profiles comparison with diesel and biodiesel blends

Table 12 Smoke emission profiles for all blends with different loading pressures

BMEP (bar)	Diesel	B10	B20	B30
1.16	39	36	*33*	32
2.07	37	33.2	*31.68*	31.99
3.16	31	29.32	*27.65*	28.35
4.11	27	26.87	*25.32*	24.68

All emission values are given in parts per minute

As with the rest of the exhaust emissions results above, it is also observed that the maximum reduction of smoke is seen with B20 blends as was arrived at by Buyukkaya (2010). This is consistent with study by Mahmudula et al. (2017), who reported a smoke opacity reduction up to 20% when using biodiesel lends in lower load conditions. Higher blends and higher pressures had caused an increase of smoke due to the factors like poor atomization (higher viscosity) of the fuel leading to a rich mixture which explains why there is comparatively less reduction of smoke in the exhausts.

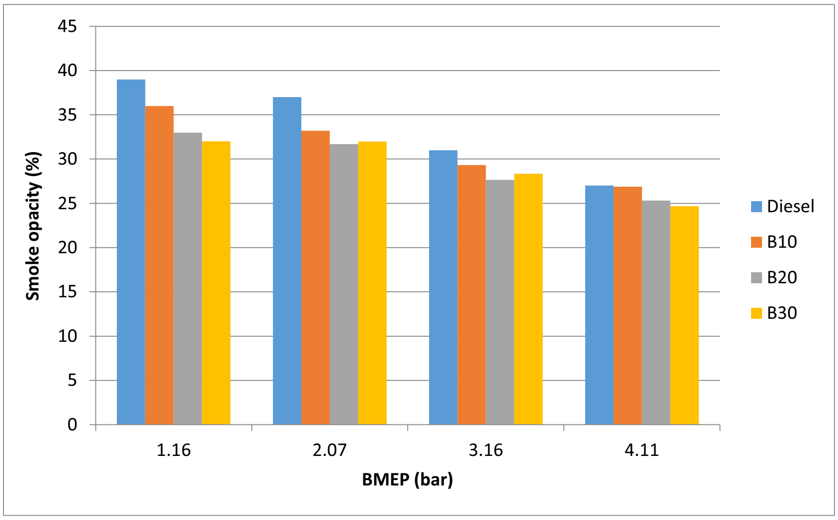


Fig. 9 Smoke opacity profiles comparison with diesel and biodiesel blends

Conclusions

The study on alternative biofuels has led to the transesterification of microbial oils which were grown on industrial wastewaters. Pollution load of wastewaters was minimized by 60% by the oleaginous microbial growth. The FAME derived from the yeast had properties that agreed well within the international standards set for bio-diesel.

Different blends of microbial biodiesel with diesel fuel were used as fuel in a compression ignition engine, and the performance and emission characteristics were analyzed. Lower percent of blends (B5, B20) give a good improvement in the engine power although there was a slight increase of fuel consumption. Lower percent blending also had reduced BSFC. Furthermore, the study found out that blend usage had improved exhaust emissions of carbon monoxide, hydrocarbons, and smoke by 21.4%, 31.7%, and 18%, respectively, when using the blend of B20 when compared to all other blends. But, the study found no considerable reductions in nitrogen oxide emissions with all blends as was always the case with biodiesel combustion in diesel engines.

Hence, the microbial biodiesel has the potential to be used as twin advantages of waste minimization and energy generation. From the study, it was concluded that B20 blend yielded the best performance on basis of brake specific fuel consumption. And, the blend of B20 may be the best blends for usage as combustion fuel in diesel engines.

Acknowledgements The authors would like to thank Centre of excellence for environmental studies in Government College of Technology for funding this research. We also thank the Department of Mechanical Engineering, GCT, for running tests using CDI engine.

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