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Research Paper

Biodiesel Production from Food Industrial Waste of Soybean Oil using a *Lipase*-nanoparticle Bio-composite Catalyst

Sathish Thanikodi¹, Saravanan Rathinasamy¹, Jayant Giri², Aravind Kumar Jagadeesan³, Emad Makki⁴

¹ Department of Mechanical Engineering, Saveetha School of Engineering, SIMATS, Tamil Nadu 600124, India
 ² Department of Mechanical Engineering, Yeshwantrao Chavan College of Engineering, Maharashtra 441110, India
 ³ Department of Energy and Environment, Saveetha School of Engineering, SIMATS, Tamil Nadu 600124, India
 ⁴ Department of Mechanical Engineering, College of Engineering and Islamic Architecture, Umm Al-Qura University, Makkah 24382, Saudi Arabia

☑ sathish.sailer@gmail.com

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Abstract

Article Info In this study, a packed-bed reactor was used to produce biodiesel from disposed soybean oil using a *lipase*-nanoparticle bio composite catalyst. During the transesterification process of the Submitted: 'disposed of/used soybean oil', different nano catalysts were employed such as nanoparticles 16/12/2023 of Ni-doped ZnO, Fe₃O₄, Alkylcelite, Poly-acrylonitrile fibres and Poly-acrylonitrile Revised: 30/03/2024 nanofibrous membrane and they were abbreviated as CI, CII, CIII, CIV and CIV respectively. In each case of biodiesel production, there were two levels of process parameters like flow Accepted: velocity (such as 0.25 mL/min-1.25 mL/min) and the reaction time (20 and 100 h) were 05/04/2024 Online first: considered for analyses. From the derived biofuel, the biodiesel blends were prepared as B50 (50% diesel and 50% biodiesel) and B75 (25% diesel and 75% biodiesel). The synthesis of biofuel 04/08/2024 results, in the biodiesel conversion of cepacia lipase with Poly-acrylonitrile nanofibrous membrane nanoparticles being recorded at about 85% at a 1.25 mL/min flow rate, which is the maximum biodiesel conversion among five grades. The shortlisted biodiesel performances were analyzed by varying the engine speed, grade and kind of biodiesel, The observed results were analyzed. The B50CIV and B75CIV blends recorded the maximum BSFC at 1800 rpm engine speed. CO₂ emission by diesel is about 2.3 vol% was recorded. It is the highest value compared to biodiesel blends (B50 and B75). The emission of NOx with the B50CII blend was 220 ppm at the engine speed of 1800 rpm. Based on the experimental results, B50CIV serves affordable fuel and is recommended among those tested in this investigation for CI engines. Keywords: Waste soyabean oil; Biodiesel production; Biocatalyst; Transesterification; Engine performance parameters

1. Introduction

Rising energy demand results from the automobile industry's transformation [1]–[3]. Due to their greater fuel efficiency, excellent dependability, cheaper fuel costs, and long-lasting capacity, diesel engines are in high demand across various industries. Because strict of environmental emission regulations, the depletion of fossil fuels, and increased global electricity consumption, academics are currently very interested in alternative fuels and environmentally friendly energy [4]–[9].

Soya has gained popularity due to its ability to produce renewable, affordable, low-grade oil [10], [11]. Compared to currently available petroleumbased goods, biodiesel made from soyabean oil and utilized in engines can minimize particulate output by one-third and not emit sulphur oxide [12]–[14]. In producing biodiesel fuel, 75–90% of raw consumable soyabean oil costs are spent [15], [16]. Thus, converting this soyabean oil into

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biodiesel fuel using nanoparticles and microorganisms without altering the diesel engine could be a good option for energy demands up to average size. Thus, crucial to practice soybean to generate biodiesel to be affordable for making and supportable. However, several industries dispose of leftover soyabean oil in the drainage system, which results in toxic gases, clogs, and groundwater erosion [17], [18].

Experiments on waste biodiesel fuel and regular diesel performance in a DICI engine. Because of the characteristics of biodiesel, pressure wave propagation reduced as injection time and cyclic output increased [19]. In the manufacturing of biodiesel from soyabean oil with nanoparticles, only 67% conversion was seen under ideal circumstances, while 80% conversion was retained for 120 h in a reactor [20]. Vegetable oil enzymatic transesterification can be done much more quickly and cheaply in severe reactors than in batches [21]. Where 100% waste palm oil and 50% waste palm oil biodiesel in a six-cylinder, 127 kW diesel was carried out by utilizing three various fuels. Fried waste of about 500 mL and oil was combined in a molar ratio of 10:1 with potassium hydroxide (2.55 g) and methanol (130 mL) and heated to 55 °C for 2 h [22]. The diesel effects and used fry oil on the engine with full speed and load to obtain engine performance. First-fry biodiesel results in better performance than other fuels [23].

The discharge characteristics of different alcohol, waste, edible, inedible, and waste animal fat biodiesel. As a result, employing edible soybean biodiesel reduced emissions by a maximum of 21.79% compared to waste oil [24]. Cottonseed methyl ester. blended with petroleum diesel at 15%, 20%, and 25% mix values, was used to power compression ratio engines. This improved engine performance and decreased carbon monoxide and hydrocarbon emissions due to using diesel with a biodiesel blend [25]-[27]. The 10% biodiesel mix was considered the superior fuel, increasing brake power by an average of 4.65% compared to diesel fuel [28]. The contribution of nanoparticles/nanomaterials in biodiesel production is presented in Table 1. The nanomaterials not only improved production efficiency but also improved their re-use of them.

Source of	Nanoparticles	Method of Processing & Best	Romarks	Rof
Biodiesel	employed	operation	Kentarks	Kel.
Sunflower oil	5 types of CaO based catalysts such as mussel shell, eggshell, CaO, dolomite, and calcite with gold nanoparticles	Conversion efficiency of 97.5% was attained at a catalyst loading of 3 %, a reaction time of 3 h, a methanol to oil ratio of 9:1, and a temperature of 65 $^{\circ}$ C	The nanocatalyst could be reused up to 10 times without losing activity	[29]
Glyceryl trioleate	Sulfamic acid functionalized magnetic nanoparticles/ sulfonic acid functionalized magnetic nanoparticles	Transesterification conditions of 120 to 180 °C temperature	Sulfamic magnetic nanoparticles had better performance, with more than 95 % reactivity after five reusing cycles	[30]
Bombax ceiba oil	CaO	Agitation speed of 600 rpm, a temperature of 65 °C, a reaction time of 70.52 min, catalyst loading of 1.5 wt%, and ethanol to oil proportion of 10.37:1 (mol: mol)	Maximal biodiesel yield was 96.2%	[31]
Waste cooking oil	MgO and CaO ano catalysts	A maximum biodiesel generation with a mass efficiency of 98.95% was accomplished using 0.5 g of MgO and 0.7 g of CaO nanoparticles throughout 6 h reaction period	The use of both nano catalysts enhanced the production efficiency of biodiesel	[32]

Table 1. Contribution of nanoparticles/nanomaterials in biodiesel production

Source of Biodiesel	Nanoparticles employed	Method of Processing & Best operation	Remarks	Ref.
Micro-algae - three kinds of algal sources like dried algae, algae oil, and algae concentrate were tested	Employed functionalized Fe3O4@silica core- shell nanoparticles	Transesterification process- the triazabicyclodecene functionalized Fe3O4@silica nanoparticles may obtain a high biodiesel output of 97.1% from the algae oil	TBD-Fe3O4@silica nanoparticles act as an efficient algae harvester	[33]
Microalgal species (Chlorella vulgaris)	Methyl- functionalized silica (SiO2-CH3) and silica (SiO2) nanoparticles	A maximum of 1.00 g/L of fatty acid methyl ester (FAME) was used	Increased the dry cell weight by 210% and FAME production by 610% at the contribution of 0.2 wt% of SiO2-CH3 nanoparticles	[34]
Castor oil	Nickel doped ZnO nano catalyst	Transesterification conditions at reaction conditions of 60 min, 55 °C, 11.07 $\%$ (w/w) catalyst concentration, and 8 mol/mol methanol to oil ratio	A maximum biodiesel production efficiency of 95.2% was achieved	[35]
Soybean oil	Cu2+ ions doping in nanoferrites (Ni0.5Zn0.5Fe2O4)	Methyl transesterification of soybean oil	85% biodiesel yields achieved	[36]
Microalgal oil	Fe3O4/ZnMg(Al)O magnetic nanoparticles	Transesterification procedure - a high yield of 94% at 65 °C.	Repeatable performance of the catalyst's is also impressive because even after recycling and reusing the catalyst seven times, the biodiesel output was remained above 82%	[37]
Babassu oil and tallow beef	Burkholderia cepacian lipase	Enzyme immobilized in Nb ₂ O ₅ at pH 6.5 and with a wide temperature range of 40 °C to 60°C. When immobilized on SiO ₂ -PVA (km = 1892 mM) as opposed to Nb ₂ O ₅ (km = 5267 mM),	The <i>lipase</i> PS had a higher affinity for the substrate (an olive oil emulsion)	[38]
Soybean oil	Burkholderia cepacia lipase	The transesterification 90% of the oil was converted into methyl esters in the first 9 h, 90% in the next 12 h, 96% in the next 24 h and 99% in the first hour.	Enhanced reusability, and noticeably improved operating stability	[39]
Soybean oil	lipase	The best conditions for processing 10 g of soybean oil were 35 °C, a molar ratio of 1:7.5 for the reactions with methanol, 0.5 g of water, and 475 mg of <i>lipase</i> , and a molar ratio of 1:15.2 for the reactions with ethanol, 0.3 g of water, and 475 mg of <i>lipase</i> . For the immobilized enzyme processes, under ideal circumstances, 67 and 65 mol% of methyl and ethyl esters, respectively, were produced in 1 h.	The immobilized <i>lipase</i> was consistently more active than the free enzyme during the reaction with the immobilized <i>lipase</i> , and the triglycerides decreased to extremely low levels after the first 30 minutes of the reaction.	[40]

From the above literature, soyabean oil with an engine gives better performance characteristics and reduces the emission from the engine. However, the waste soyabean oil with nanoparticles and microorganisms used as biodiesel was not available from previous studies.

The state and art about the importance of a catalyst is clearly stated by [41]-[43]. The Nidoped ZnO nanoparticles have shown high catalytic activity in biodiesel synthesis reactions, leading to faster conversion rates of triglycerides to biodiesel. These nanoparticles can enhance the selectivity of the biodiesel synthesis process, reducing the formation of unwanted byproducts and increasing the yield of biodiesel [41]. The doping of Ni into ZnO nanoparticles can improve their stability and durability under harsh reaction conditions, ensuring consistent catalytic performance over time. Due to their high catalytic activity, Ni-doped ZnO nanoparticles often allow for lower catalyst loading in biodiesel synthesis reactions, leading to cost savings and easier catalyst recovery [42]. Using Ni-doped ZnO nanoparticles as catalysts can contribute to cleaner and more environmentally friendly biodiesel production processes, as they can facilitate the use of sustainable feedstocks and reduce the environmental impact of biodiesel production [43]. The efficient catalytic activity of these nanoparticles can enable the design of simpler and more compact biodiesel production reactors, improving overall process efficiency and reducing production costs [44]. Fe₃O₄ nanoparticles exhibit strong magnetic properties, allowing for easy separation and recovery from the reaction mixture using an external magnetic field. This facilitates catalyst reuse and reduces waste generation [45]. Fe₃O₄ nanoparticles have shown high catalytic activity in biodiesel synthesis reactions, leading to efficient conversion of triglycerides to biodiesel and promoting high yields. These nanoparticles are stable and durable under a wide range of reaction conditions, including high temperatures and pressures, ensuring consistent catalytic performance over multiple reaction cycles [46]. Due to their high catalytic activity, Fe₃O₄ nanoparticles often allow for lower catalyst loading in biodiesel synthesis reactions, leading to cost savings and easier catalyst recovery [47]. Using Fe₃O₄ nanoparticles as catalysts can contribute to cleaner and more environmentally

friendly biodiesel production processes, as they can facilitate the use of sustainable feedstocks and reduce the environmental impact of biodiesel production. The efficient catalytic activity and magnetic properties of Fe₃O₄ nanoparticles can improve the overall efficiency of biodiesel processes, leading production to higher throughput and lower energy consumption [48]. Using alkyl-celite nanoparticles as a catalyst in biodiesel synthesis offers several positive advantages: Alkyl-celite nanoparticles exhibit high catalytic activity in biodiesel synthesis reactions, promoting efficient conversion of triglycerides to biodiesel and leading to high yields These nanoparticles can enhance the selectivity of biodiesel synthesis reactions, reducing the formation of unwanted byproducts and improving the purity of the biodiesel product [49]. Alkyl-celite nanoparticles are stable and durable under various reaction conditions, ensuring consistent catalytic performance and longevity during repeated use. The nanoparticles can be easily separated from the reaction mixture using filtration methods due to their size and structure, facilitating catalyst recovery and reuse. Due to their high catalytic activity, alkyl-celite nanoparticles often allow for lower catalyst loading in biodiesel synthesis reactions, leading to cost savings and easier catalyst handling. Using alkyl-celite nanoparticles as catalysts can contribute to more environmentally friendly biodiesel production processes by promoting the use of sustainable feedstocks and reducing the environmental impact of biodiesel production. Alkyl-celite nanoparticles can be functionalized with various alkyl groups, providing versatility in catalytic applications and allowing for tailored catalyst properties to suit specific biodiesel synthesis conditions [50]. Poly-acrylonitrile (PAN) fibers nanoparticles typically have a high surface area, providing ample active sites for catalytic reactions and promoting efficient conversion of triglycerides to biodiesel. The properties of PAN fibers nanoparticles, such as porosity, surface chemistry, and morphology, can be tuned and optimized for specific catalytic applications, leading to enhanced catalytic activity and selectivity. PAN fibers nanoparticles are known for their stability and durability under a wide range of reaction conditions, ensuring consistent catalytic performance over multiple

cycles without significant degradation [50]. These nanoparticles can be easily immobilized or supported on solid substrates, allowing for easier catalyst recovery and reuse, which reduces waste improves cost-effectiveness. and The immobilization of PAN fibers nanoparticles facilitates their recycling and reuse, leading to sustainable catalytic processes and reducing the overall environmental impact of biodiesel production. In some cases, PAN fibers nanoparticles can be regenerated or rejuvenated after use, further extending their lifespan and enhancing their economic viability as catalysts in biodiesel synthesis. The high surface area and active sites of PAN fibers nanoparticles can promote faster reaction kinetics in biodiesel synthesis, leading to shorter reaction times and increased production efficiency [51]. Polyacrylonitrile (PAN) nanofibrous membranes have a high surface area per unit mass, providing abundant active sites for catalytic reactions and promoting efficient conversion of triglycerides to biodiesel. The nanofibrous structure of PAN membranes often results in a uniform pore distribution, which enhances mass transfer and facilitates the accessibility of reactants to the catalytic sites, leading to improved reaction kinetics. The surface chemistry of PAN nanofibrous membranes can be modified and tailored through functionalization or doping with other materials, allowing for enhanced catalytic activity, selectivity, and stability [45]. PAN nanofibrous membranes can easily immobilize catalytic species on their surfaces or within their pores, providing a stable and efficient catalyst support system for biodiesel synthesis reactions. After the reaction, the PAN nanofibrous membranes can be easily separated from the reaction mixture, facilitating catalyst recovery and reuse, which reduces waste and improves costeffectiveness. The immobilized catalytic species on PAN nanofibrous membranes can be regenerated or rejuvenated, allowing for repeated use of the catalyst and contributing to sustainable catalytic processes. PAN nanofibrous membranes are known for their mechanical strength and durability, ensuring long-term stability and consistent catalytic performance under various reaction conditions. Utilizing PAN nanofibrous membranes as catalyst supports in biodiesel synthesis promotes the use of sustainable feedstocks and reduces the environmental impact of biodiesel production by improving efficiency and reducing waste generation [50].

This investigation aims to utilize the used/waste soybean oil for alternate fuel production for diesel engines by utilizing nanotechnology. The research objectives are:

- a. To prepare the biodiesel from used/waste soybean oil by transesterification process with the use of nanoparticles such as Ni-doped ZnO, Fe₃O₄, Alkyl-celite, Poly-acrylonitrile fibers and Poly-acrylonitrile nanofibrous membrane and preparing different fuel grades;
- b. To prepare the biodiesel B50 and B75 blends of fuel from those five different grades of biodiesel by mixing the diesel 50% and 25% respectively;
- c. To evaluate the engine performance fuels are to be tested at common working conditions and the measures of performance are BSFC (Brake Specific Fuel Consumption), BTE (Brake Thermal Efficiency), and emissions such as CO₂, NO_x, and smoke; and
- d. To compare the performance of the prepared five fuel grades and their two different blends (B50 and B75) to suggest the best alternate fuel for diesel engines.

2. Materials and Method

Multiple time used/waste soybean oil sample was gathered from an Indian factory. Such waste/used soybean oil is considerably cheap production. feedstock for biodiesel physicochemical properties of obtained used/waste were Saponification Value of 229.526 mg KOH/g, viscosity of 36.627 mm²/s at 40 °C, Iodine Value of 34.74 Wij's, Density of 0.911 kg/m³, Acidity index (value) of 6.945 mg KOH/g. Bio nano-composites for biodiesel production and synthesizing nanoparticles were purchased from Sigma Aldrich (St. Louis, MO, USA). Microorganisms of various types are used to process the various nanoparticles.

- a. Bio nano-composite I (CI) Ni-doped ZnO,
- b. Bio nano-composite II (CII) Fe₃O₄,
- c. Bio nano-composite II (CIII) Alkyl-celite,
- d. Bio nano-composite IV (CIV) Polyacrylonitrile fibers, and
- e. Bio nano-composite V (CV) Poly-acrylonitrile nanofibrous membrane.

Lipases hold an enviable position among the enzymes of industrial importance. The majority of enzymes used in biotransformation are hydrolases, which are distinguished by their broad substrate specificity, commercial availability, lack of cofactor dependence, and ability to function at high substrate concentrations, not only in their natural environment (aqueous solutions), but also in organic and neoteric solvents [52]. From Sigma-Aldrich St. Louis, MO, USA Pseudomonas cepacia lipase was bought. The ammonium sulfate deposition procedure was used to purify the lipase prior to immobilization. Usually, 200 ml of phosphate buffer solution (50 mM with 20% glucose) were added to 10 g of Pseudomonas cepacia lipase before spinning it at 10,000 rpm for five minutes and discarding the sediment. To create the green sediment, ammonium sulfate (103.2 g) was gradually added to the cleared supernatant while it was continuously stirred at 4 °C for 12 h. This was followed by centrifugation at 15000 rpm for 15 minutes. For subsequent usage, the produced lipase was stored in a dry and cool place [53].

When compared to the single-packed-bed reactor, the four-packed-bed reactor produced conversion rates and stability that were significantly greater. Over 88% of the biodiesel was converted for 192 h, and after 240 h of reaction, it only slightly decreased to around 75%. Therefore, the packed-bed reactor system offers enormous potential for enzymatic biodiesel synthesis on an industrial scale, both in terms of design and operation [53]. Hence the four-packedbed reactor was preferred in this study. In the four-packed-bed reactor, the 40 °C temperature was maintained. A 500 mL round-bottom flask with an agitating mechanism was utilized to incubate the mixture of filtered waste soybean oil, distilled water, methanol, and n-hexane. And they fed that mixture in the four-packed-bed reactor through its inlet. To find out the methyl esters' content, the effluent liquid from the outlet was taken out.

2.1. Bio Nano-composite I (CI)

Purified water, methyl alcohol, soyabean oil, and n-hexane was mixed with magnetic Fe₃O₄ nanoparticles. The ratio of water, methyl alcohol, soyabean oil, and n-hexane mixture is about 3:1:6:0.2. The procedure was taken out with the help of four packed bed reactor (FPBR). The effluent liquid was removed from the outlet to calculate the methyl esters concentration. The catalysts of Ni-doped ZnO were carried out. Nidoped ZnO nanoparticles were reacted with *lipase* to synthesize biodiesel [36]. The optical characteristics of ZnO nanorods are also discovered to be significantly influenced by Ni doping. The antibacterial effectiveness of ZnO nanorods against S. aureus and P. aeruginosa microbes is observed to rise noticeably with Ni doping, which may be related to an increase in the production of reactive oxygen species [54].

2.2. Bio Nano-composite II (CII)

The co-precipitation method was utilized to mixture Fe₃O₄ nanomaterials. The nanomaterials of Fe₃O₄ procured with an average particle size of 15-20 nm, a brown hue, and a density of 4.9 gr/cm³. Thermomyces lanuginosa *lipase* was taken from Novozymes. The ammonia solution of about 10% was mixed with the solution and the solution was allowed to constant stirring (about 45 min). NaOH solution of about 0.5 M was mixed with the solution drop by drop after the constant string of about 150 minutes (to obtain a white precipitate) [34], [37].

2.3. Bio Nano-composite III (CIII) Alkyl-celite

In the food processing plant in Maharashtra, the food waste produces the *lipase*-producing bacterium and can be isolated. Alkylcelite nanoparticles of about 100 g were added to the beaker, and the *lipase* reacted with the nanoparticles at 25 °C temperatures for 24h of reaction duration. The procured nanomaterial has pH of 10, temperature of use 55 °C and Ks value of 12.1 mN. The straining was recognized as Burkholderia sp. C20 by 16S rDNA. At 30 °C. The *lipase* was filtered and washed with deionized water and can be used for further experimentation with biodiesel [55].

2.4. Bio Nano-composite IV (CIV) Polyacrylonitrile Fibers

The electrospinning process can be utilized for the preparation of nanoparticles. 25 µm thickness electros pun PAN fibrous were crushed and physical adsorption of *lipase* onto PA (Polyacrylonitrile) fibers was to immobilize it. Soaking PAN fibres in an aqueous solution containing *lipase* makes it possible to physically adsorb PAN fibers, which may be employed in subsequent processes. By employing a calibration curve for bovine serum albumin, the Bradford technique was used to determine the protein content of the reagent Burkholderia cepacia, which came out at 0.56 wt.% [56].

2.5. Bio Nano-composite V (CV) Polyacrylonitrile Nanofibrous Membrane

The transesterification process of soyabean oil with different nanoparticles and microorganisms is shown in Figure 1. Using an Electrospinning 1.5 х 105 molecular weight process, polyacrylonitrile was purchased from Ontario, NY, USA and dissolved with N, Ndimethylformamide at 60 °C temperatures. The PAN (Poly-acrylonitrile nanofibrous membrane) nanofibers were solidified and reacted with the Pseudomonas cepacia lipase on the collector to form the reactants for biodiesel yield. The electrospun nanofibrous membrane was dehydrated under vacuum before removing it from the collector. The unbound *lipase* molecules were removed by phosphate buffer solution several times during the process [57].

The composition of soyabean oil. nanoparticles, and lipases was treated using a transesterification reaction under a four-packedbed- reactor. The transesterification reaction was done at 45 °C temperature and the bio nano composite was added to a 500mL rounded flask and then moved to the packed bed. Initially, the bio nano composite was added about 50mL of the mixture and fed into an FPBR at various flow rates and reaction times. The experiment was carried out through constant load and variable speeds of about 1000, 1200, 1400, 1600, and 1800 rpm. Furthermore, the 7.2 kW power output of a singlecylinder engine was utilized for the performance test of biodiesel from waste soyabean oil. The engine specification for this study is shown in Table 2.



Figure 1. Transesterification of soyabean oil with nanoparticles

Parameter	Description
Engine manufacturer	Titan
Туре	Four storks
Compression ratio	19
Length of stroke (mm)	90
Cooling type	Air
Cylinder number	1
Pressure for injection (kg/cm ²)	185

Table 2. Engine specification

The output power from the engine is measured by utilizing an eddy-current dynamometer. Brake Specific Fuel Consumption (BSFC) and Brake Thermal Efficiency (BTE) were calculated for engine performance through different biodiesel. The engine emission parameter was analyzed by using the I3SYS Exhaust Gas Analyzer.

The gas probe was positioned at the gas pipe to obtain the emission at digital readings, and the smoke temperature was measured using a K-type thermocouple. During the experiment, readings were taken twice for an accurate solution. With pure diesel and a blend of biodiesel, the experiment was carried out as a blend of biodiesel from bio nano-composite I (CI), bio nanocomposite II(CII), bio nano-composite III(CIII), bio nano-composite IV(CIV), and bio nano-composite V(CV) prepared in a ratio of biodiesel (50%) and diesel (50%) (B50), and biodiesel (75%) and diesel (25%) (B75). Finally, biodiesel from soyabean oil blended with diesel calculated the engine performance parameter with biodiesel and compared it with pure diesel.

From experimental readings, engine performance characteristics such as BSFC, BTE, emissions such as CO₂, NO_x, and smoke effect on engine load can be analyzed. The photography of the experimental setup is exposed in Figure 2. The complete investigation flow is mentioned in Figure 3.

2.6. Factors Influences on Biodiesel Conversion2.6.1. Flow Rate

Using highly efficient *lipase* with various nanoparticles, biodiesel production varied for using different nanomaterials. So, the flow rate effect on biodiesel conversion is a significant parameter during transesterification. In FPBR, different flow rates of lipase/nanoparticles mixture, such as 0.25 mL/min, 0.5 mL/min, 0.75 mL/min, 1 mL/min, and 1.25 mL/min were carried out. Was used to determine the methyl esters amount in the mixture was estimated by Gas chromatography. A Varian CP3380 (CP3380, Varian Associates, Inc.) was used, outfitted with an SPB-5 column and an ionization detector. The column temperature was kept constant at 150 °C and then increased to 170 °C at a rate of 0.5 °C /min. The injector temperature and detector were fixed at 300 °C.

The flow rate consequences on biodiesel conversion as shown in Figure 4. The biodiesel conversion of CIV was recorded at about 85% at a 1.25 mL/min flow rate, which is the maximum biodiesel conversion compared with another flow rate. The figure demonstrates that the increasing flow rate increases the biodiesel conversion in FPBR. By using CII, the maximum biodiesel conversion was recorded compared with other Bio nano-composites except for the 1.25 mL/min flow rate. The flow rate of 0.25 mL/min biodiesel



Figure 2. Experimental setup of the engine with data measuring



Figure 3. Complete flow of the investigation



Figure 4. Effect of flow rate on biodiesel production

conversion using CIV is about 16% recorded. It is the lowest biodiesel conversion compared with other Bio nano-composites. For the other flow rates (except 0.25 mL/min), biodiesel conversion for CI was the lowest value.

The reaction period of lipase-nanoparticles in transesterification is an essential parameter for biodiesel production. Because of lipasesnanoparticle bio composite leading the lipase activity at transesterification process. So that the reason biodiesel conversion rises with growing of Bio nano-composites flow rate. The transesterification reaction period decides the more microorganism reaction with nanoparticles and the process carried out at 40 °C temperatures.

2.6.2. Reaction Time

For this investigation, the reaction time for *lipase* nanoparticles is about 20 h to 100 h. After 100 h of reaction time, the biodiesel conversion is about 95% recorded by using CIV, which is the maximum biodiesel conversion compared with other Bio nano-composites. Initially, the biodiesel conversion for CIV is about only 7%, recorded at a reaction time of 20 h.

Figure 5 clearly shows that the biodiesel conversion increases with reaction time. Using CII, the biodiesel conversion at 100 h is about 89% recorded, the second-highest biodiesel conversion compared with the remaining three Bio nan0composites at 100 h of reaction time. It is the lowest biodiesel conversion compared with other Bio nanocomposites. Generally, for biodiesel production, FPBR have a maximum stability and



high conversion rate of biodiesel linked with a single-packed-bed-reactor. The FPBR may have contributed to this by allowing the reaction mixture to remain in the reactor for a more extended period and reducing inhibition of *lipase*-nanoparticle bio composite byproducts, increasing reaction efficiency.

3. Results and Discussion

3.1. Engine Performance Parameter 3.1.1. Biodiesel Effect on BSFC

BSFC is a significant parameter for calculating engine performance to evaluate the effectiveness of fuel spent by the engine. The fuel used for the engine was pure diesel, a blend of biodiesel of about 50% biodiesel and 50% pure diesel (B50), biodiesel (75%) and diesel (25%) (B75). To analyze the influence of biodiesel in engine performance, selectively the two maximum biodiesel production through Bio nano-composite CII and CIV were considered for testing and analysis based on the performance calculation.

B50CIV was the highest BSFC compared with B50CII and diesel. Figure 6a and Figure 6b clearly state that the increasing engine speed increases the BSFC at constant load. The BSFC for B50CIV is about 6150 g/kW.h and was recorded at the engine speed of about 1800 rpm. Similarly, the BSFC for B50CII and pure diesel is about 6100 and 5820 were recorded for the similar speed engine. Because of maximal fuel feeding to develop the maximum power, BSFC increases at higher speeds (at low speeds, BSFC is low due to lower fuel consumption). Biodiesel has characteristics of higher viscosity and heating at low. To progress similar power from an engine, more fuel amount is required [58], [59].

Compared with B50CIV, B75CIV gives the maximum BSFC at 1800 rpm of speed engine. Similarly, BSFC for B75CIV is about 6354 recorded at the speed of 1800 rpm. The density of the B75CIV blend was higher than that of the B50CIV blend, which raises extra fuel release along the same plunger displacement in the injection pump, resulting in the increased value of BSFC [60], [61].

3.1.2. Effect on BTE

The BTE for the engine using pure diesel, B50, and B75 was carried out from the experiment. The BTE is determined as the relation of brake power to the fuel energy consumed by the engine. Based on the BTE, combustion quality in the engine was carried out. The BTE value for diesel is about 15.3%, 14.1%, 13.8%, 13.2%, and 11.5% were recorded at the engine speed of 1000 rpm, 1200



Figure 6. BSFC of; (a) B50 blend, (b) B75 blend at various speed

rpm, 1400 rpm, 1600 rpm, and1800 rpm. The maximum value of BTE is recorded at the engine speed of 1000 rpm compared with another diesel blend [62].

Figure 7a and **Figure 7b** demonstrate that the engine speed increased, and the engine's BTE value decreased. Because of low calorific value and higher fuel consumption, poor air fuel ratio at maximum speed gives lower combustion [58], [61], [63]. At speed (1800 rpm), the value of B50CII, B50CIV, B75CII, and B75CIV is about 12.5%, 11.6%, 12.2%, and 12% recorded, respectively.

3.2. Emission Characteristics 3.2.1. CO₂ with Engine Speed

Figure 8 describes the variation of CO₂ emission with the engine's speed for B50, B75 blend and diesel. CO₂ emission for engines using diesel is

about 2.3 vol%, recorded. It's the highest value compared with biodiesel blends (B50 and B75). The emission of B50CII is about 0.58 vol%, 1.1 vol%, 1.5 vol%, and 1.95 vol% were recorded at the engine speed of about 1000 rpm, 1200 rpm, 1400 rpm, and 1600 rpm. Diesel's CO₂ emission value is more excellent, only at 1800 rpm, whereas the CO₂ emission value of B50CII was the maximum for the engine's remaining speed [59].

Similarly, the emission value for B75CII is about 2 vol% recorded at 1800 rpm, which is the maximum emission value compared with B75CIV. At 1000 rpm, 1200 rpm, 1400 rpm, 1600 rpm, and 1800 rpm, the CO₂ emission of the engine is 0.3 vol%, 0.5 vol%, 0.9 vol%, 1.2 vol%, and 1.6 vol%. Since biodiesel contains more oxygen than conventional diesel, the relationship of more significant CO₂ with this fuel may be



Figure 7. BTE of; (a) B50 blend, (b) B75 blend at various rpm.



supported. During combustion, it interacts with the unburned carbon atoms, causing CO₂ to be produced in more amounts. The increased density and heating value of biodiesel fuel, which relies on the blending ratio and *lipase*-nanoparticular reaction time, result in higher CO₂ emissions. The combustion reaction of the fuel at maximum speed produces a more significant amount of CO₂ [36].

3.2.2. NOx with Speed

When nitrogen and oxygen particles react in the engine's combustion chamber at a high temperature, nitrogen oxides are created. The following chemical formula was used to carry out the NOx production process [64]. The NOx using B50CII is about 220 ppm and recorded at an engine speed of about 1800 rpm, which is the maximum value compared with the B75 blend and other engine speeds.

Figure 9a, Figure 9b demonstrates that the higher the engine speed, the higher the NOX

emission. At 1000 rpm, 1200 rpm, 1400 rpm, and1600 rpm. The NOx emission of B50CII is about 165 ppm, 179 ppm, 189 ppm, and 201 ppm. The NOx profile for the diesel is always less than the B50 and B75 blends. Because biodiesel burns at a high temperature, biodiesel blends exhibit increased NOx emissions. Because combustion takes place at a greater temperature, oxygen and nitrogen atoms interact with one another quickly. About 160 ppm, 169 ppm, 185 ppm, 199 ppm, and 211 ppm of NOx were measured using B75CII and B75CIV, respectively, while 149 ppm, 156 ppm, 173 ppm, 185 ppm, and 199 ppm were recorded.

Due to greater biodiesel fuel density and the shorter ignition delay, more biodiesels undergo premix combustion, which raises cylinder pressure and temperature. Because of the increased oxygen content in the combustion chamber and higher fuel consumption for the same injection settings, NOx emissions are released. Another explanation for the tendency of rising NOx levels with higher density housing



Figure 9. NOx emission of; (a) B50 blend, (b) B75 blend at various rpm and the smoke effect of; (c) B50 blend, (d) B75 blend at various rpm

(unsaturation degree) is bulk modulus of biofuels feedstock rises with densities, requiring earlier injection time [58]–[60].

3.2.3. Effect of Smoke on Load

In the combustion chamber, the smoke is produced through incomplete combustion of the fuel inside the engine. At the different rpm, the smoke effect of the engine is analyzed in this investigation. The smoke of the engine by using pure diesel is about 65% recorded at 1000 rpm. It is a maximum value compared with other fuels (B50 and B75). For the 1200 rpm, 1400 rpm, 1600 rpm, and 1800 rpm, the engine smoke by using diesel about 54%, 46%, 41%, and 35%.

Similarly, using B50CIV, the smoke is about 63%, 52%, 44%, 39%, and 32%, respectively. The result of blended fuel smoke decreases with the engine's speed and increases the smoke of pure diesel, as shown in **Figure 9c**, **Figure 9d**. Reduced smoke suggests that there are fewer hydrocarbons in the exhaust gases. The combustion of biodiesel fuel is aided by its high cetane index and around 10% greater oxygen content, which lowers the smoke's opacity. The complete combustion of the engine is done by more oxygen in the fuel [61]. Therefore, the smoke emission from biodiesel is less when compared with pure diesel. So, the B50CIV blend shows higher smoke due to more fuel injected into the engine.

4. Conclusions

This investigation demonstrated the practical possibilities of biodiesel production from waste (that is used) soyabean oil and analyzed it with experimentations in the diesel engine test rig. The results revealed a satisfactory engine performance and a higher reduction of emissions with the proposed biodiesel blend. As recommended by the literature, the higher biodiesel content lowers emissions of carbon monoxide and hydrocarbon. So, for this investigation, the preferred blends of B50 and B75. By blending higher proportions of biodiesel with diesel fuel, the economy of the proposed fuel is obvious. The engine performance characteristics such as BSFC BTE and emissions such as CO₂, NO_x, and smoke for various engine load conditions were analyzed and presented.

The biodiesel production was carried out effectively in the packed-bed reactor using *lipase* nanoparticles and the influence of biodiesel production parameters of reaction time and flow rate were analysed. Furthermore, the performances of biodiesel on the engine were experimented and analyzed. With diesel, the biodiesel from Bio nano composite I(CI), Bio nanocomposite II(CII), Bio nano-composite III(CIII), Bio nano-composite IV(CIV), and Bio nanocomposite V(CV) were used to prepare blends of B50 and B75.

It was observed from the results that Bio nano composite IV recorded a maximum biodiesel production of 85% at a 1.25 mL/min flow rate. The CII recorded the biodiesel conversion at 100 h of reaction time as 89%. So, the CII and CIV were further investigated by experimenting them in the diesel engine test rig. The blend of B50CIV recorded the highest BSFC and BTE compared with the blend of B50CII and neat diesel. The BSFC and BTE increased with an increase in engine speed. CO2 emission recorded with neat diesel was 2.3 vol% which is a higher value compared with biodiesel blends of B50 and B75. The NOx using B50CII is about 220 ppm and recorded at an engine speed of about 1800 rpm, which is the maximum value compared with the B75 blend and other engine speeds. Shortly, B50CIV may be used as an affordable and suitable alternative fuel to power diesel engines.

Author's Declaration

Authors' contributions and responsibilities

Sathish Thanikodi: Data curation, Conceptualization, Formal analysis, Software, Methodology, Writing – original draft, Writing – review & editing. Saravanan Rathinasamy: Conceptualization, Formal analysis, Software, Methodology. Jayant Giri: Formal analysis, Writing – original draft, Writing – review & editing. Aravind Kumar Jagadeesan: Formal analysis, Writing – original draft, Writing – review & editing. Emad Makki: Project administration, Methodology, Writing – original draft, Writing – review & editing. Besides, all authors agree to be accountable for all aspects of the work.

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