Supplementary Information for

Obtention of biodiesel through an enzymatic two-step process. Study of its performance and characteristic emissions.

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Supporting Information

METHODS.

Typical Procedure for the Preparation of Perchloric Acid Immobilized on Silica Gel ($HClO_4$-SiO_2).[1]
To a suspension of silica gel (23.75g, mesh no. 230-400) in Et_2O (50 mL) was added $HClO_4$ (1.25g, 12.5mmol, 1.78mL of a 70% aq solution of $HClO_4$) and the mixture was stirred magnetically for 30min at rt. The Et_2O was removed under reduced pressure (rotary evaporator) and the residue heated at 100°C for 72h under vacuum to afford $HClO_4$-SiO_2 (0.5mmol g^{-1}) as a free-flowing powder.

Purification of WCO.
WCO (177.3g) was treated with 7.3g of bentonite under agitation at 75-80°C for 60 min. The mixture was then filtered over Celite, which was washed with n-hexane. After separating the phases, the oil was dried on a stove at 80°C for 48 h. The purified oil (159.6g) was stored at room temperature in a dark glass bottle.

Thin-Layer Chromatography (TLC) Development.[2]
Sample volumes of 2 µL were applied to the plate, and the TLC was developed with a mixture of heptane: diethyl ether: acetic acid (80:20:1, v/v/v). Compounds were detected either under UV light (at 254 nm) or by spraying a mixture of acetic acid:water: sulfuric acid, 80:16:4.

Determination of enzymatic hydrolysis efficiency.[3]

TABLES.

Table S1. Key technical data of test engine

Item	Specification
Motor type	Hatz 1B20-6
Bore (mm)	69
Stroke (mm)	62
Rod length (mm)	104
Crank length (mm)	31
Maximum torque (N m)	7
Maximum speed (rpm)	3500
Output	approx. 2.0 kW
Compression	21:1
Oil volume	0.9 litre

Table S2. Technical specifications of the gas analyzer used in the present study

Measurement	Range	Accuracy
O_2	0-25 %	±0.1% ±5%
CO	0-10 %	±0.06% ±5%
CO_2	0-20 %	±0.5% ±5%
NO_x	0-5000 ppm	±25ppm ±4%
HC	0-10000 ppm	< 2000 ppm; ±12 ppm ±5% > 2001 ppm; ±12 ppm ±10%

Table S3. Key properties of the test fuels

PROPERTY	D(100)	D80B20	D50B50	D25B75	B(100)
MOLECULAR FORMULA	C12-C25	-	-	-	-
STOICHIOMETRIC AIR/FUEL RATIO	14.7	-	-	-	12.5
LOWER HEATING VALUE (MJ KG-1)	45.0	44.02	42.65	41.47	40.3
DENSITY AT 40°C (G ML-1)	0.803	0.818	0.840	0.859	0.878

VISCOSITY AT 40°C (MM2 S-1)	2.40	2.79	3.06	3.51	4.29
CETANE NUMBER	52	52.58	53.45	54.17	54.9
OXYGEN CONTENT (%)	0	-	-	-	11

FIGURES.

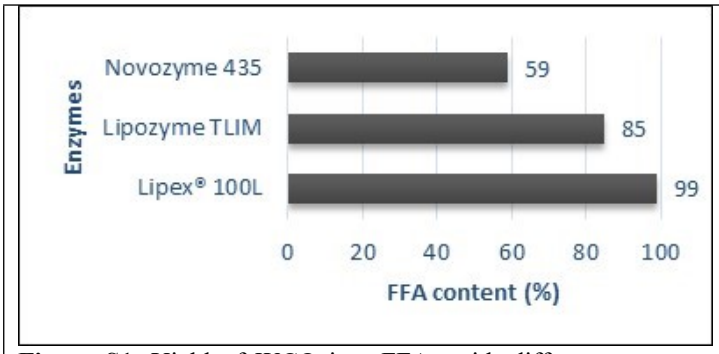

Figure S1. Yield of WCO into FFAs with different enzymes. Reaction conditions: 2 g of WCO; 2 mL of 50 mM phosphate buffer pH 7; 2% (w/w) lipase; 36 h, 35°C and 250 rpm

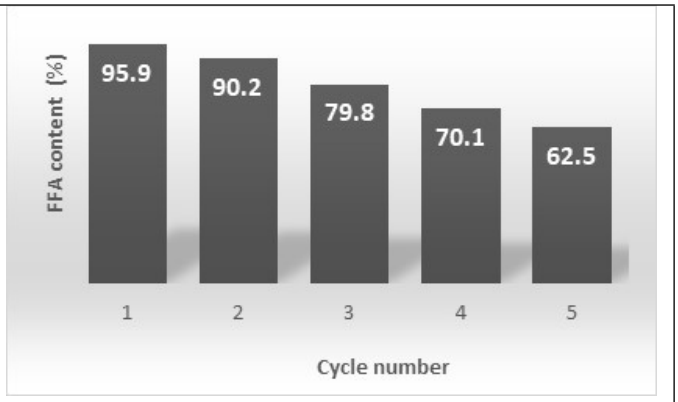

Figure S2. Effect of cycle number on biodiesel production for immobilized lipase. Reaction conditions: 2 g of WCO; 2 mL of 50 mM phosphate buffer pH 7; 10% immobilized lipase; 36 h, 35°C and 250 rpm.

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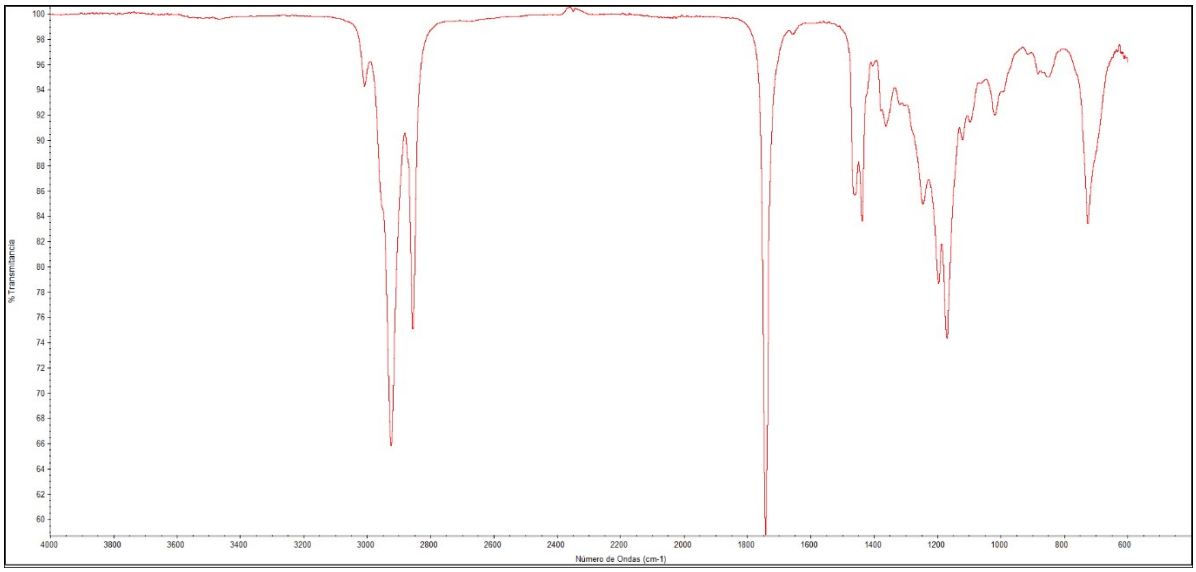


Figure S3. Biodiesel IR spectrum.

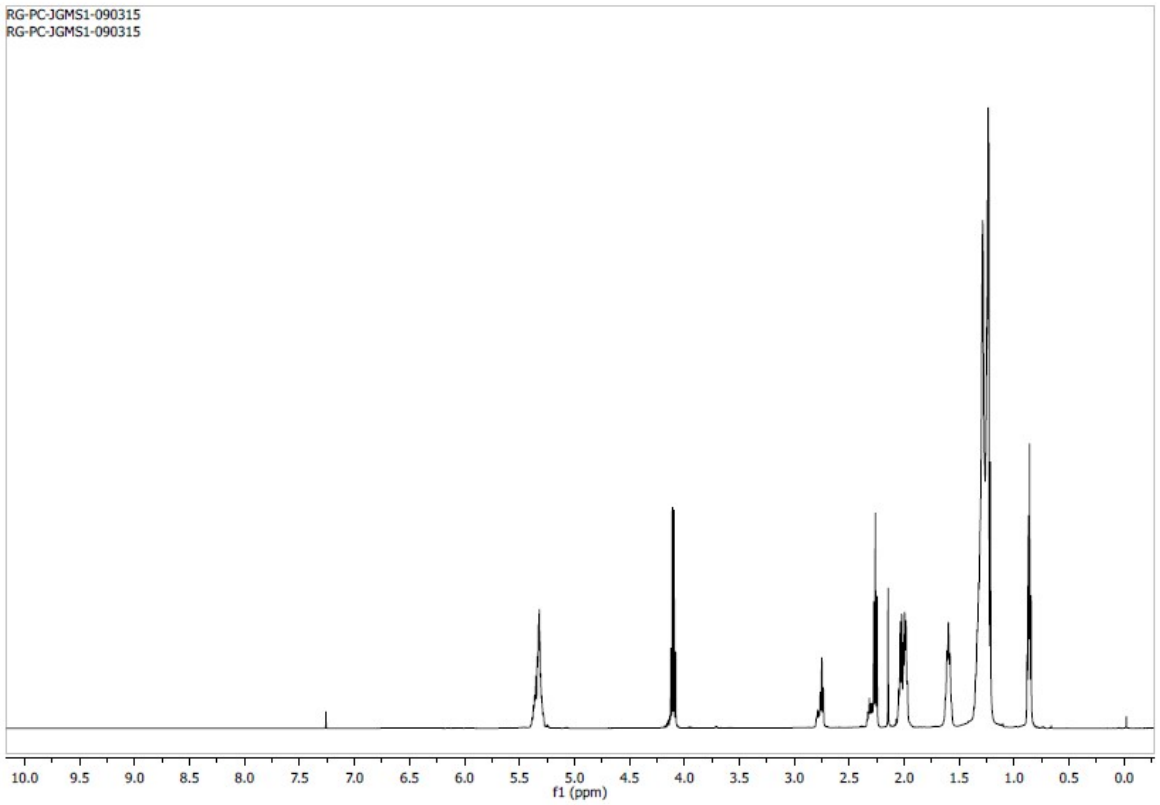


Figure S4. Biodiesel ^{1}H NMR spectrum (400 MHz, $CDCl_3$, room temperature).

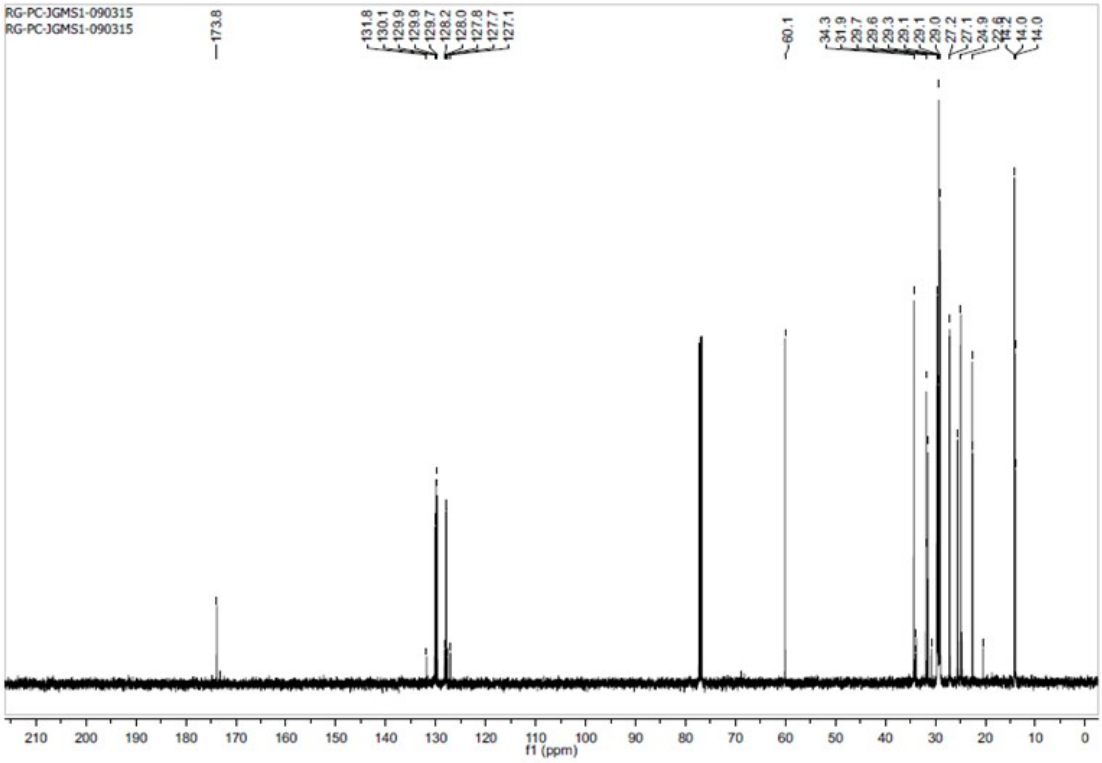


Figure S5. Biodiesel ^{13}CNMR spectrum (400 MHz, $CDCl_3$, room temperature).

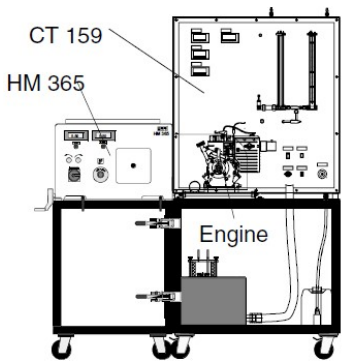


Figure S6. Schematic diagram of diesel engine experimental setup.

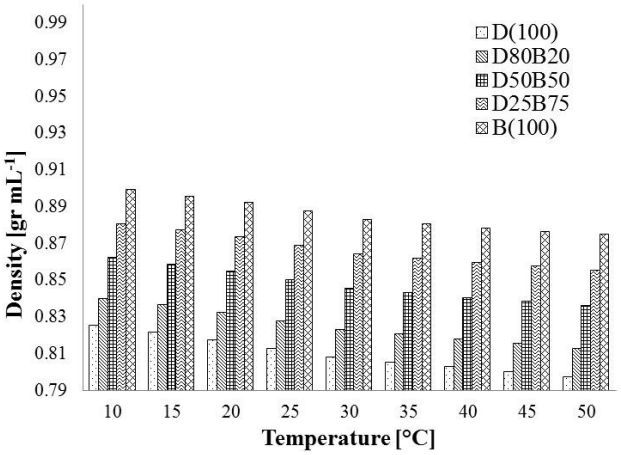


Figure S7. Effect of temperature on the density of diesel, biodiesel, and its blends.

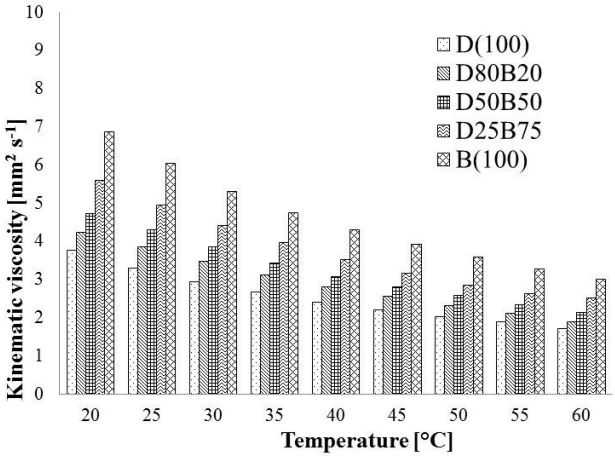


Figure S8. Variation of Kinematic Viscosity of diesel, biodiesel, and its blends with Temperature

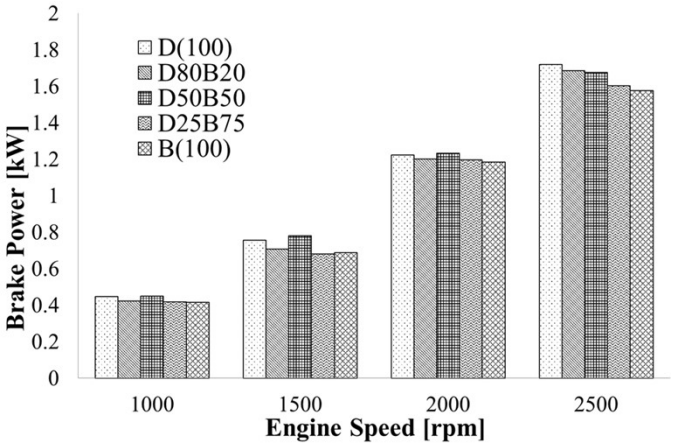


Figure S9. Variation of brake power vs. engine speed for different biodiesel blends.

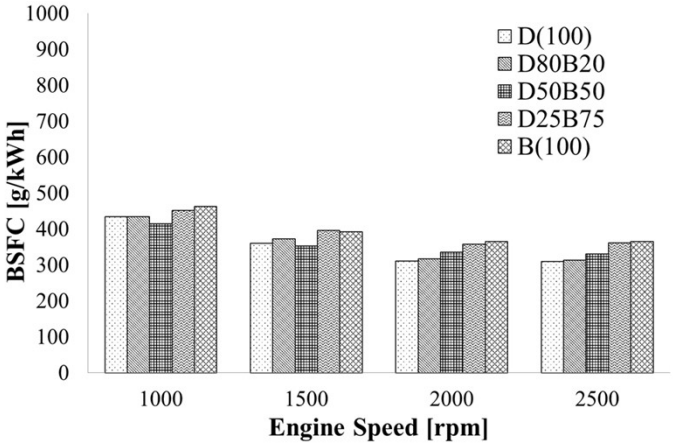


Figure S10. Variation in brake specific fuel consumption at full load as a function of engine speed.

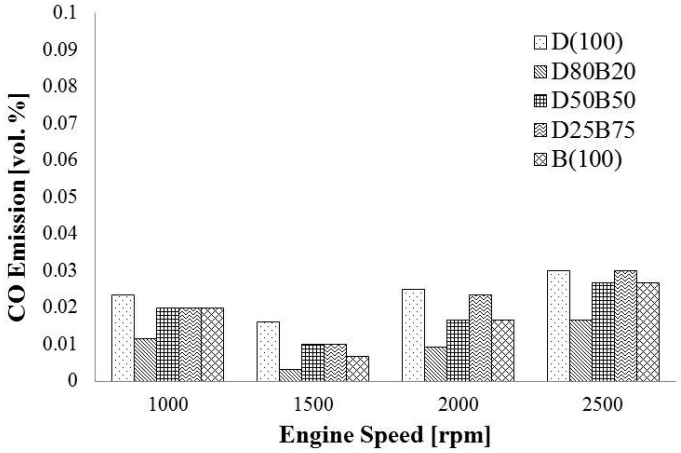


Figure S11. Variation in CO emission depending on engine speed and fuel.

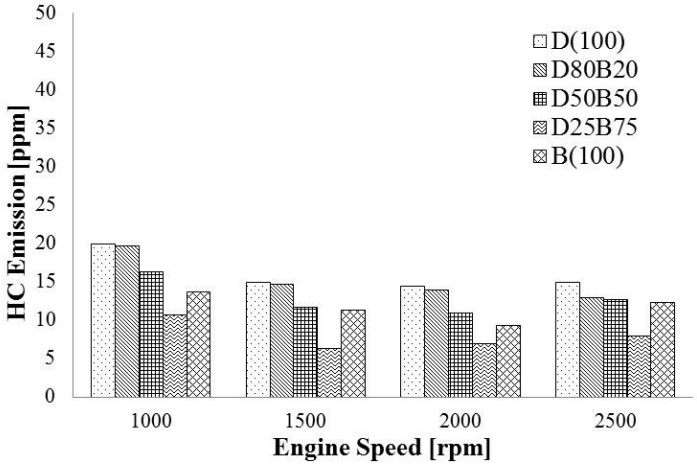


Figure S12. Variation in HC emission with engine speed.

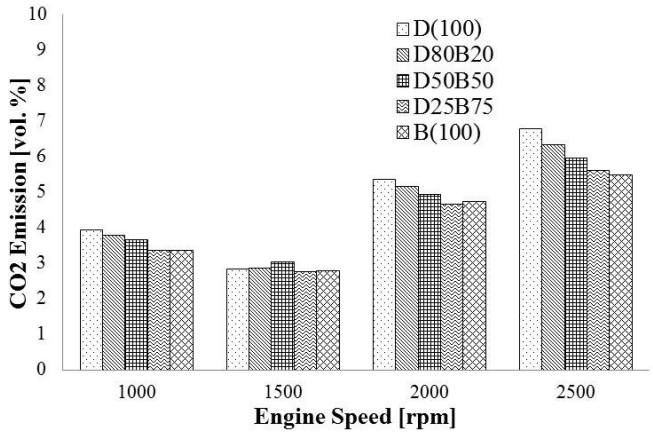


Figure S13. Variation in CO_2 emission with engine speed.

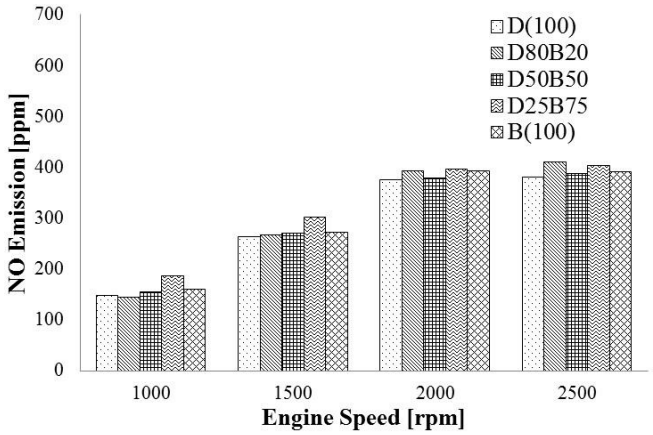
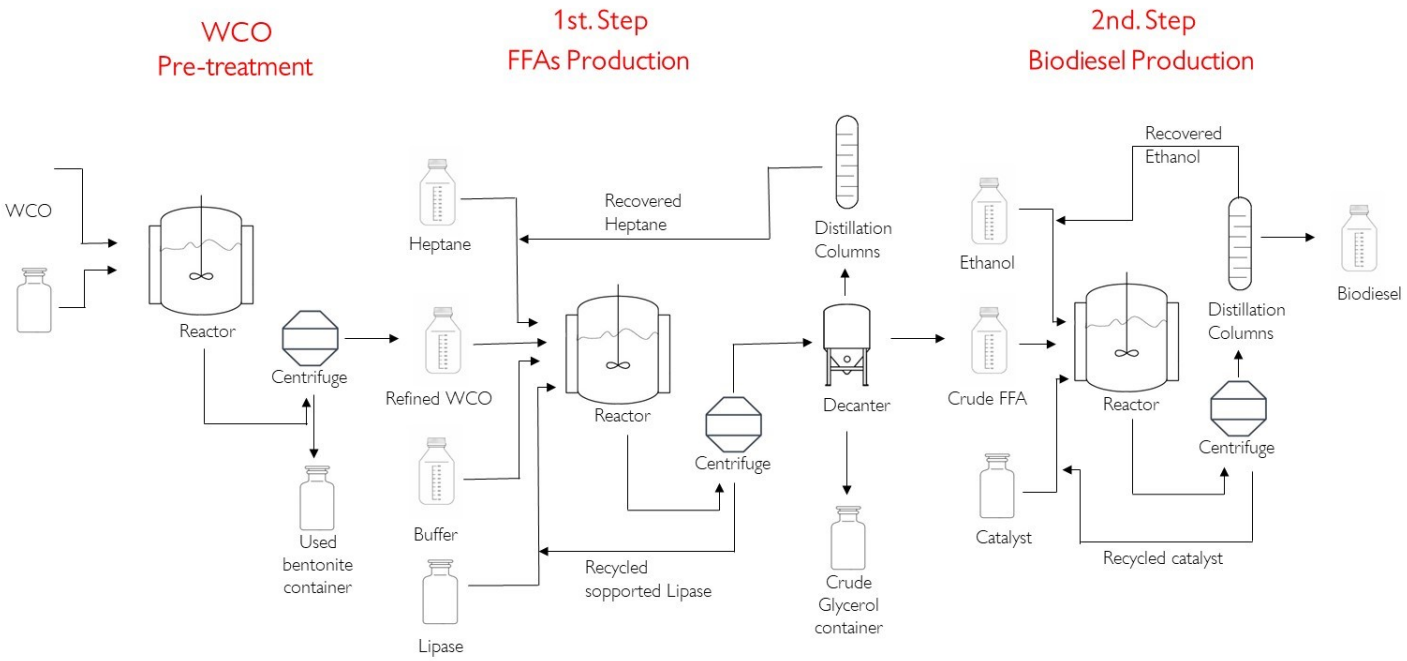


Figure S14. Variation in NO_x emission with engine speed.



Scheme S1. Biodiesel production process with enzyme catalyst.

References

1. A.K. Chakraborti, R. Gulhane. Perchloric acid adsorbed on silica gel as a new, highly efficient, and versatile catalyst for acetylation of phenols, thiols, alcohols, and amines. *Chem. Commun*., 2003, **22**, 1896-1897.
2. M. Dołowy, A. Pyka, Chromatographic methods in the separation of long-chain mono- and polyunsaturated fatty acids. *Journal of Chemistry*, 2015, **2015**, Article ID 120830, 20 pages. https://doi.org/10.1155/2015/120830.