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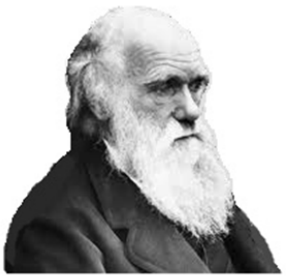

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Biodiesel from coconut acid oil using *Candida rugosa* and *Candida antarctica* lipases

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ABSTRACT

Coconut acid oil (CAO) is a byproduct from the processing of coconut oil. CAO has a tremendous potential in the preparation of alternative fuel as biodiesel. In the present research investigation, CAO has been utilized for the preparation of biodiesel using Lipase AY Amano 30 (*Candida rugosa*) and Novozyme 40013 (*Candida antarctica*) in the presence of methanol using hexane as solvent. Initially, Lipase AY Amano 30 has been used for the biohydrolysis of neutral glycerides present in CAO, under the influence of water at 40^0C. Henceforth, the hydrolysed CAO was bioesterified with methanol in the presence of non-specific immobilized enzyme NS 40013 for the production of alternative fuel. The characteristics of CAO biodiesel (CAOB) was compared with diesel fuel and good outcome has been revealed. So cheap raw materials like CAO has been selectively utilized for the production of alternative fuel through a novel technology which may be useful for the partial replacement of present non-renewable fuels.

***Keywords*:** Coconut acid oil, *Candida rugosa, Candida Antarctica*, Biohydrolysis, Bioesterification

1. INTRODUCTION

The continuous depleting phenomenon of non-renewable fuel reserves along with environmental degradation make renewable fuel energy resources more demanding and realistic in the present world. The most feasible way to solve this issue is to utilize alternative eco-friendly fuel. Biodiesel creates an attractive option in this situation due to the easy availability of raw materials, eco-friendly nature, no requirement of engine modification and also its cost-effectiveness [1-4]. Biodiesel has also the advantage of being essentially free of

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sulfur, making it a cleaner burning fuel than petroleum diesel. In many cases, biodiesel has similar physical and chemical properties with diesel fuel but it can sometimes be superior than that of the latter due to its higher flash point, ultralow sulfur concentration, better lubricating efficiency and better cetane number. Different researchers used different raw materials for the production of alternative fuel in the form of biodiesel by applying different process technologies. They also used chemical catalyst or biocatalyst and compared various properties of the biodiesel with diesel fuel.

Kareem *et al*. [5] used palm oil and palm kernel oil for the enzymatic production of biodiesel. Ge *et al*. [6] reviewed that canola oil is a source of biodiesel production. Biodiesel production through different types of reactors was studied by Zahan [7] using different technology. Xuan *et al.* [8] used ultrasound-assisted catalytic transesterification process for biodiesel production. Al-muhtaseb *et al.* [9] identified a specific catalyst from biomass-derived waste date pits which was used for the production of alternative fuel. Another catalyst was also identified by Sulaiman *et al*. [10] for biodiesel production from fish bone.

Kurniati *et al*. [11] applied Electromagnetic Induction for the production of Calophyllum Inophyllum biodiesel using a novel process technology. The present authors also used different resources like soybean oil deodorizer distillate [12], Karanja oil [13], Jatropha Curcas oil [14-15], waste cooking oil [16], canola oil deodorizer distillate [17] and palm fatty acid distillate [18] for the production of biodiesel using lipase as catalyst. Coconut oil was also used by many researchers for the production of biodiesel [19-22]. The oil is also widely used in making soaps and cosmetics. CAO, a by product of coconut oil refinery industry, is mainly utilized in soap manufacturing process. But CAO can be alternatively utilized for the production of alternative fuel. Very few research works [23-26] have been identified by utilizing CAO for the production of biodiesel.

Based on this idea, CAO has been utilized as a main raw material for the production of biodiesel through the use of enzyme technology. This process technology includes less process hazards, easy handling, environment-friendliness and minimization of byproducts. Moreover, biocatalyst, used in this process, may be recycled many times which helps to reduce the process cost. So, in the present research investigation, the first step includes application of Lipase AY Amano 30 (*Candida rugosa*) for the biohydrolysis of neutral glycerides present in CAO and second step includes conversion of biohydrolysed CAO to biodiesel by applying Novozyme 40013 (*Candida antarctica*) lipase through bioesterification reaction between hydrolysed CAO and methanol followed by a comparative study of fuel characteristics with diesel fuel.

2. EXPERIMENTAL

2. 1. Materials

CAO was obtained from Edible Products (I) Pvt. Ltd. The enzyme Lipase AY Amano 30 was a crude lipase from *Candida rugosa* with activity <30000 units/g and a kind gift from AMANO ENZYME Inc, Nagoya, Japan. Novozyme 40013, an immobilized non specific lipase from *Candida antarctica* with ester synthesis activity of 10000 propyl laurate unit/g, was a gift of Novozyme South Asia Pvt. Ltd., Bangalore, India. The chemicals such as monoglycerides and diglycerides were purchased from Scientific and Laboratory Instrument Co., Kolkata. Except otherwise specified all other chemicals were A.R. Grade.

2. 2. Methods

For the biohydrolysis of CAO, initially CAO was centrifuged for the removal of solid impurities. After that, it was taken in a 500 ml stoppered Erlenmeyer flask and water (70% by weight of neutral glycerides) containing Amano 30 lipase powder (5% w/w) was added to it. The reaction mixture containing CAO, enzyme and water was magnetically stirred with a 1 inch Teflon coated stir bar at 40 °C in a temperature controlled bath for 8 hrs. After completion of hydrolysis reaction, the oil layer containing free fatty acids (FFAs) and the water layer containing enzyme and glycerol were separated through centrifugation. The oil layer i.e. hydrolysed CAO was collected for further reaction.

The hydrolysed CAO was then taken in an Erlenmeyer flask for the bioesterification reaction. Methanol and enzyme Novozyme 40013 (5% w/w) were added to it. The mixture was then continuously stirred for 6 hrs using solvent hexane fitted with a water condenser at a temperature of 60 °C. Methanol was added in the mixture in stepwise manner to minimize the deactivation of enzyme. During the esterification reaction, continuous analysis of the sample was done by taking it in a capped vial and separating it from enzyme through centrifugation. The progress of the reaction was done by thin layer chromatographic (TLC) method by using a silica-gel G plate (0.2 mm thick) with hexane-diethyl ether-acetic acid (90:10:1) as a developing solvent. Yield of product was monitored by column chromatographic method using silicic acid as an adsorbent and 160 ml of hexane-diethyl ether (99:1) as eluting solvent. Values are reported as mean ± s.d., where n=3 (n=no of observations).

3. RESULTS AND DISCUSSIONS

3. 1. Analysis of CAO

Table 1 shows the composition of fatty acids, neutral glycerides and unsaponifiable matters present in CAO. It was observed from **Table 1** that CAO contains higher amount of FFAs which mainly includes medium chain fatty acids like lauric acid and myristic acid. Among other acids, palmitic acid, oleic acid, capric acid and caprylic acid predominate. Among the neutral glycerides (30.14%), amount of triacylglycerols (TAG) are much higher (20.45%) than monoacylglycerols (MAG) and diacylglycerols (DAG).

Table 1. Analytical characteristics and fatty acid composition of CAO

Component	Amount (%w/w)	Component	Amount (% w/w)
FFAs (Total)	**69.22**	**Unsap. matters**	**0.6**
Lauric acid (C 12:0)	50.26	**Neutral Glycerides**	**30.14**
Myristic acid (C 14:0)	19.72	Monoglycerides	5.21
Palmitic acid (C 16:0)	7.41	Diglycerides	3.73
Stearic acid (C 18:0)	1.31	Triglycerides	20.45

Oleic acid (C 18:1)	5.54		
Linoleic acid (C 18:2)	2.16		
Caprylic acid (C 8:0)	4.33		
Capric acid (C 10:0)	6.59		

3. 2. Analysis of water concentration in biohydrolysis reaction

Water plays an important role in the biohydrolysis reaction. The neutral glycedrides present in COA is hydrolysed in the presence of enzyme Amano 30 under the influence of water. Optimum water content required for biohydrolysis reaction was identified by performing the reaction at 40 °C for 8 hrs in the presence of 6% Amano 30 enzyme by varying the amount of water from 40% to 80% by weight of neutral glycerides as shown in **Fig. 1.** It has been observed from **Fig. 1** that increasing concentration of water from 40% to 80% enhances the rate of hydrolysis reaction but after 70% concentration of water, rate of production did not change. So here, 70% is the optimum concentration of water for maximum hydrolysis of neutral glycerides present in CAO. This may be due to the fact that enzyme activity depends on the vacant active sites where substrate/s can be attached and reaction proceeds. In the presence of 70% concentration of water, maximum substrate or water could be bound with the active sites of enzyme and optimum hydrolysed product could be obtained.

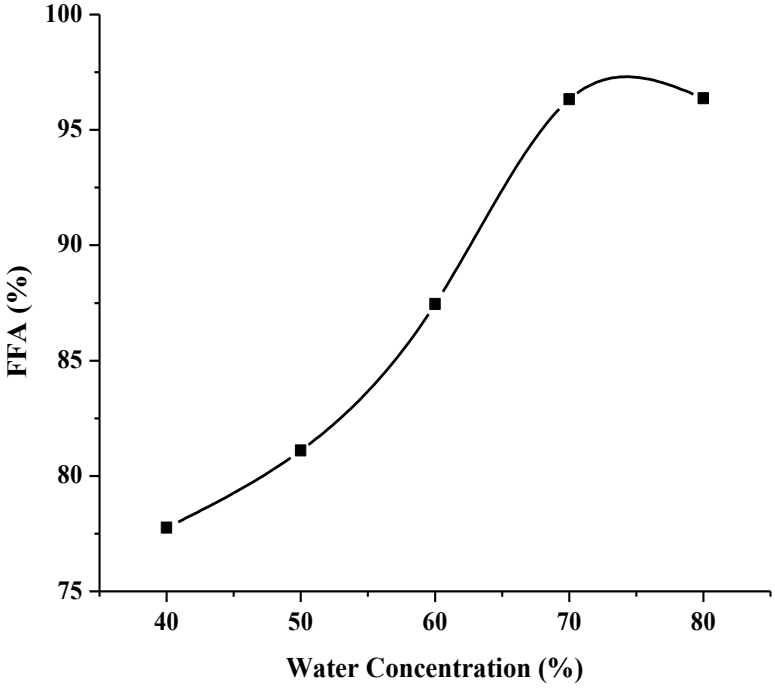

Figure 1. Analysis of water concentration of biohydrolysis reaction of CAO

3. 2. Analysis of enzyme concentration in biohydrolysis reaction

Optimum concentration of enzyme helps in the smooth conduction of reaction. In our study, concentration of enzyme has been increased from 3% to 7% for identifying the optimum level at 40 °C for a period of 8 hrs in the presence of 70% water. It has been observed from the analysis that lower concentration of enzyme could not hydrolyse neutral glycerides completely as shown in **Fig. 2.** Maximum hydrolysis of neutral glycerides was obtained at 6% concentration of enzyme beyond which no development of hydrolysis was observed as shown in **Fig. 2.** Increasing the enzyme concentration beyond 6% enhanced the agglomeration of enzyme which inhibited the rate of forward reaction, so hydrolysis did not increase.

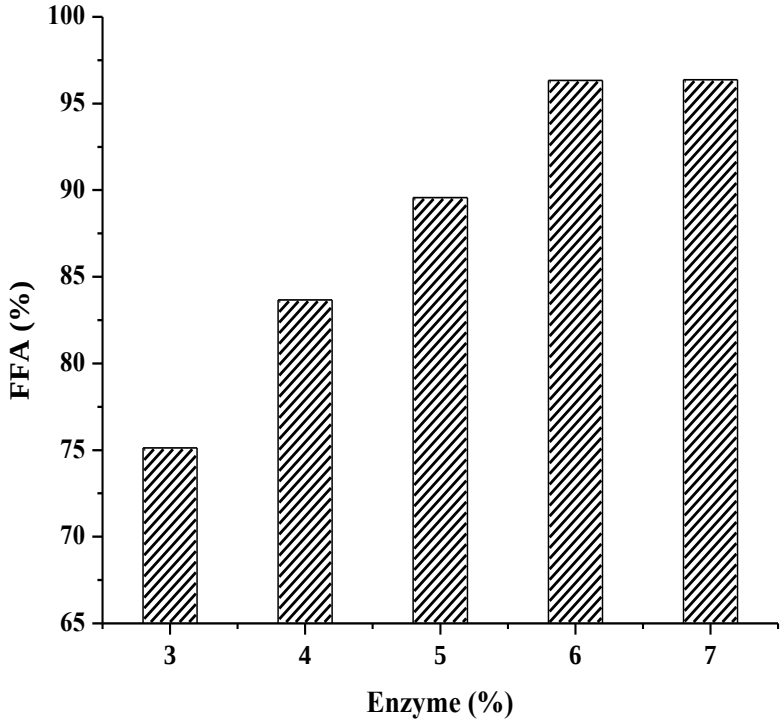

Figure 2. Analysis of enzyme concentration of biohydrolysis reaction of CAO

3. 3. Analysis of glycerides level in biohydrolysis reaction

Biohydrolysis of glyceride has been studied in the presence of 6% enzyme with 70% water in the present research investigation. Analysis showed that glyceride level in the CAO decreased rapidly from the start of the reaction at 40 °C temperature. Glyceride level decreased from 30.14% to 1.31% within 8 hrs of reaction as shown in **Fig. 3.** After that, continuation of reaction did not enhance the hydrolysis rate at the identified reaction parameters.

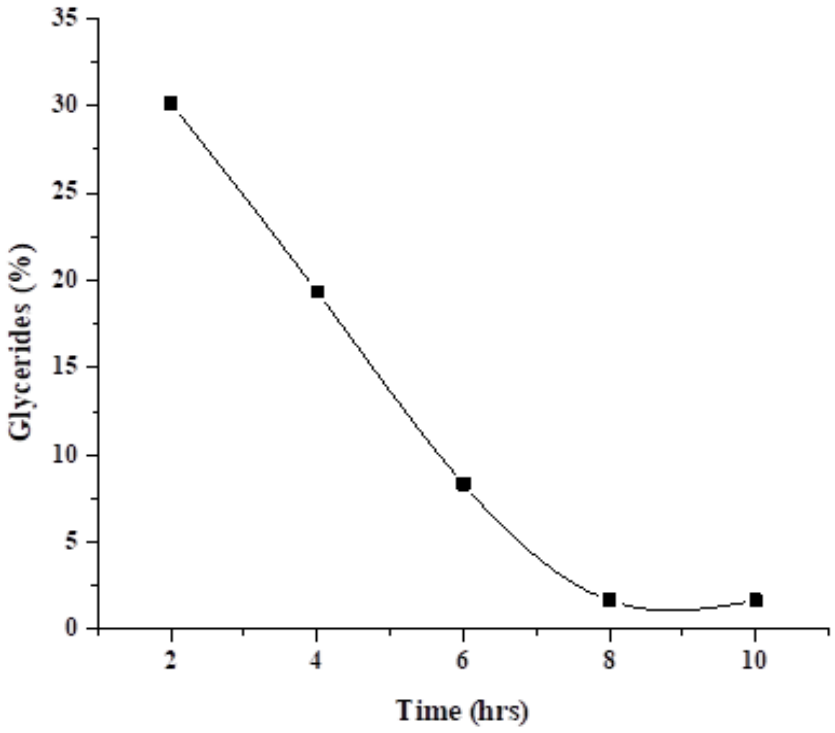

Figure 3. Analysis of glyceride concentration of biohydrolysis reaction of CAO

3. 4. Bioesterification of hydrolysed CAO for biodiesel production

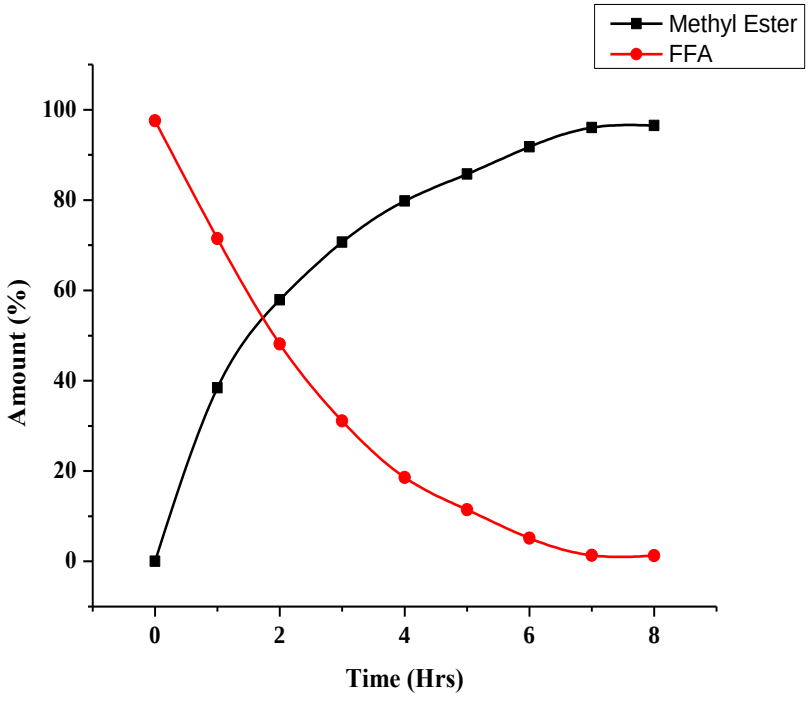

Figure 4. Analysis of bioesterification reaction of hydrolysed CAO.

For the preparation of biodiesel, hydrolysed CAO and methanol were treated at 6:1 molar ratio of methanol and hydrolysed CAO at 60 °C temperature in the presence of 5% enzyme Novozyme 40013 for 8 hrs. This was the identified optimum reaction parameters for bioesterification reaction and 96.24% conversion had been achieved for biodiesel preparation. **Fig. 4** shows a typical time study for the progress of bioesterification reaction with respect to the FFA consumption and methyl ester (biodiesel) preparation for 8 hrs. It has been observed from **Fig.4** that initial reaction rate between FFA and methanol was very fast which was also indicated by the progress of methyl ester production. As the reaction proceeded, the conversion of FFA to methyl ester went slower and after 5 hrs of reaction, the rate of reaction decreased. This was probably due to the low availability of reactants after 5 hrs and more than 80% conversion had been achieved within that time duration.

3. 5. Material balance for biohydrolysis and bioesterification reaction

The material balance of overall reaction has been done by considering 100 gm CAO with respect to FFA, glycerides (TAG, DAG and MAG), methyl ester (biodiesel) and unsaponifiable matters as shown in Table 2. It has been observed from **Table 2** that initially, CAO contained 69.22% FFA along with 30.14% glyceride molecules while those were 97.56% and 1.31% respectively after enzymatic hydrolysis. Through bioesterification of hydrolysed CAO, FFA content came down to 1.48% while biodiesel content in the final droduct was 96.52%. So a good conversion has been achieved through this bioprocess technology for the production of biodiesel from crude CAO.

Table 2. Material balance for biohydrolysis and bioesterification reaction of CAO (100 gm)

Material	CAO (gm)	Hydrolysed CAO (gm)	Bioesterified CAO (gm)
FFA	69.22	97.56	1.48
Glycerides	30.14	1.31	0.97
Unsaponifiable matters	0.6	0.6	0.6
Methyl ester (Biodiesel)	0	0	96.52
Misc.	0.04	0.53	0.43

3. 6. Characteristics of CAO biodiesel

The properties of CAOB like density, calorific value, flash point, cetane number, kinematic viscosity etc have been analysed with diesel fuel as shown in **Table 3**. It has been observed from **Table 3** that there is a good conformity of the properties of CAOB with the diesel fuel. It shows that flash point, cetane number and kinematic viscosity are quite higher than diesel fuel. Higher flash point indicates that biodiesel can be used safely in case of fire hazards than that of conventional diesel fuel. With regard to calorific value, though it is

somewhat lower than diesel fuel but with regard to other properties, CAOB can be used safely instead of diesel fuel or as blended fuel.

Table 3. Characteristics of biodiesel from CAO.

Characteristics	Biodiesel	Diesel fuel	Test method
Density (gm/cc)	0.867±0.005	0.840	ASTMD-4052-96
Calorific value (Kcal/Kg)	3848±1.702	4285	ASTM-6751
Flash point (°C)	157±0.145	56	ASTMD-93
Specific gravity	0.881±0.002	0.85	----------
Cetane number	58±0.211	49	ASTMD-6751
Kinematic viscosity @40 °C (Cst)	3.15±0.021	3.02	ASTMD-445
Acid value (mg/KOH)	0.35±0.007	0.36	ASTMD-64-01

4. CONCLUSIONS

Coconut acid oil has been effectively utilized for the production of biodiesel through bioprocess technology. Initially, biohydrolysis of coconut acid oil converted almost all neutral glycerides to free fatty acids in the presence of enzyme Lipase AY Amano 30 where 97.56% conversion had been achieved. In the 2nd stage of reaction, biodiesel was successfully produced from hydrolysed coconut acid oil through bioesterification reaction in the presence of enzyme Novozyme 40013. Finally, a material balance was done for identifying the minimum process loss. A comparative characteristics with diesel fuel showed that coconut oil biodiesel could be significantly utilised as an alternative fuel. This novel technology is not only helpful for biodiesel production from cheap raw materials but it also encourages the researchers and academicians to think about the mitigation of non-renewable fuels in the near future.

References

[1] D. Huang, H. Zhou, L. Lin, Biodiesel: an Alternative to Conventional Fuel. *Energy Procedia* 16 (2012) 1874-1885

[2] J. Ali, A. Semwal, Biodiesel as an Alternative Fuel for Diesel Engine – An Overview. *International Journal of Applied Engineering Research* 9 (10) (2014) 1159-1168

[3] Y. Putrasari, O. Lim. A Review of Gasoline Compression Ignition: A Promising Technology Potentially Fueled with Mixtures of Gasoline and Biodiesel to Meet Future Engine Efficiency and Emission Targets. *Energies* 12 (2019) 238

[4] S. O. Kareem, E.I. Falokun, S.A. Balogun, O.A. Akinloye, S.O. Omeike, Enzymatic biodiesel production from palm oil and palm kernel oil using free lipase. *Egyptian Journal of Petroleum* 26 (2017) 635–642

[5] J. Ge, S. Yoon, N. Choi, Using Canola Oil Biodiesel as an Alternative Fuel in Diesel Engines: A Review. *Appl. Sci.* 7 (2017) 881

[6] M. K.K.A.Zahan, Technological Progress in Biodiesel Production: An Overview on Different Types of Reactores. *Energy Procedia* 156 (2019) 452–457

[7] S. Xuan, S. Lim, H. Chyuan, Y. Ling, State of the art review on development of ultrasound-assisted catalytic transesterification process for biodiesel production. *Fuel* 235 (2019) 886–907

[8] A. H. Al-muhtaseb, F. Jamil, L. Al-haj, M. Tay, Z. Myint, E. Mahmoud, M.N.M Ahmad, A. O. Hasan, S. Ra, Biodiesel production over a catalyst prepared from biomass-derived waste date pits. *Biotechnol. Rep*. 20 (2018) e00284

[9] S. Sulaiman, N. Fatin, A. Jamaludin, N. A. Kabbashi, Development of CaO/PVA Catalyst from Fish Bone for Biodiesel Production. *Bull. Chem. React. Eng. Catal*. 14 (2019) 153–157

[10] S. Kurniati, S. Soeparman, S. S. Yuwono, L. Hakim. A Novel Process for Production of Calophyllum Inophyllum Biodiesel with Electromagnetic Induction. *Energies* 12 (2019) 3–383

[11] S. Nandi, R. Bhattacharyya, T. K. Ghosh, Process optimization and kinetic of biodiesel production from renewable raw materials. *Saudi Journal of Engineering and Technology* 4 (6) (2019) 248-252

[12] Palestro CJ, Radionuclide imaging of osteomyelitis. *Semin Nucl Med.* 45(1) (2015) 32

[13] S. Nandi, R. Bhattacharyya, Biodiesel from Karanja (Pongamia Pinnata L.) oil using non specific enzyme Candida Antarctica. *Chemical Science Review and Letters* 7 (25) (2018) 118-121

[14] Howard CB, Einhorn M, Dagan R, Nyska M, Ultrasound in diagnosis and management of acute haematogenous osteomyelitis in children. *J Bone Joint Surg Br.* 75(1) (1993) 79

[15] S. Nandi, S. Ganguly, Enzymatic packed bed reactor for biodiesel production from Jatropha Curcas oil. *Journal of Chemical, Biological and Physical Sciences* 6 (4) (2016) 1419-1428

[16] S. Nandi, R. Bhattacharyya, Studies on enzymatic production of biodiesel from Jatropha Curcas oil by varying alcohols. *Journal of Chemical, Biological and Physical Sciences* 6 (1) (2016) 311-320

[17] S. Nandi, R. Bhattacharyya, D. Marik, T. K. Ghosh, Utilisation of waste cooking oil as biodiesel through bioprocess technology. *International Journal for Research in Applied Science & Engineering Technology*8 (4) (2020) 133-137

[18] S. Nandi, R. Bhattacharyya, G. Misra, Investigation of biodiesel from Canola oil deodorizer distillate using dual biocatalyst. *Asian Journal of Applied Science and Technology* 4 (1) (2020) 131-138

[19] S. Nandi, S. Mallick, Parametric optimization of biodiesel synthesis and property modification of biodiesel-diesel blends using Palm fatty acid distillate. *Indian Journal of Natural Sciences* 10 (61) (2020) 26939-26945

[20] Md. A. Hossain, S. M. Chowdhury, Y. Rekhu, K. S. Faraz, M. U. Islam, Biodiesel from Coconut Oil: A Renewable Alternative Fuel for Diesel Engine. *World Academy of Science, Engineering and Technology* 68 (2012) 1289-1293

[21] O. J. Alamu, O. Dehinbo, A. M. Sulaiman, Production and Testing of Coconut Oil Biodiesel Fuel and its Blend. *Leonardo Journal of Sciences* 16 (2010) 95-104

[22] M. S. A. Malik, A. I. M. Shaiful, M. S. M. Ismail, M. N. M. Jaafar, and A. M. Sahar, Combustion and Emission Characteristics of Coconut-Based Biodiesel in a Liquid Fuel Burner. *Energies* 10 (2017) 458

[23] A. M. Liaquat, H. H. Masjuki, M. A. Kalam, I. R. M. Fattah, M. A. Hazrat, M. Varman,M. Mofijur, M. Shahabuddin, Effect of Coconut Biodiesel Blended Fuels on Engine Performance and Emission Characteristics. *Procedia Eng*. 56 (2013) 583–590

[24] Tsegay Hiwot, Determination of oil and biodiesel content, physicochemical properties of the oil extracted from avocado seed (Persea americana) grown in Wonago and Dilla (gedeo zone), southern Ethiopia. *World Scientific News* 58 (2016) 133-147

[25] C. Woo, S. Kook, E. Hawkers, P. L. Rogers, C. Marquis, Engine Combustion and Emission of Coconut Oil Based Biodiesel and Diesel Blends, In Proceedings of the 19th Australasian Fluid Mechanics Conference, Melbourne, Australia, 8–11 September 2014.

[26] P. Nakpong, S. Wootthikanokkhan, High free fatty acid coconut oil as a potential feedstock for biodiesel production in Thailand. *Renewable Energy* 35 (8) (2010) 1682-1687