ISSN: 0974-2115
www.jchps.com Journal of Chemical and Pharmaceutical Sciences

A comparative study of Lipase/base catalyzed biodiesel production from Banalities aegyptiaca

N. Sunil Naik*, B. Balakrishna
Department of Mechanical Engineering, University College of Engineering, J. N. T. University, Kakinada.
***Corresponding author: E-Mail: sunilnaik.n@gmail.com**

ABSTRACT

In this study, biodiesel synthesis from balanites aegyapatia oil by alkaline and lipase catalyzed transesterification is investigated. The oil content extracted from the balanites aegyapatia kernel by solvent extraction is found to be 44-51%. The free fatty acid profile of balanites aegyapatia oil comprises mainly linoleic acid (43.69%), oleic acid (32.89%) and palmitic acid (17.1%). The optimum reaction conditions for alkaline catalyzed transesterification of balanites aegyapatia oil were found to be 1.26% potassium hydroxide catalyst concentration, 8:1 molar ratio (methanol: oil), reaction temperature of 65^0C and reaction time 2.5 h, whereas for transesterification reactions catalyzed by xylanase lipase, the optimized conditions are, 1% xylanase concentration, 8:1 molar ratio (methanol: oil), reaction temperature of 40^0C for 4 h reaction time. The optimum reaction conditions (i.e., reaction time, reaction temperature, methanol/oil molar ratio and the water content) on fatty acid methyl ester yield were considered.

KEY WORDS: Banalities aegyptiaca oil, Tranesterification, free fatty acid profile, alkaline catalyzed, Lipase catalyzed.

1. INTRODUCTION

Biodiesel is normally produced from vegetable oils, animal fats and waste cooking oils through transesterification process. At present, biodiesel is mainly produced using alkaline catalysts such as NaOH and KOH and whereas the acid and heterogeneous catalysts are used depending upon the unsaturated fatty acids profile of selected feedstock Hassan (2015). The problems associated with catalyst processing are reaction with water, saponification, unpredictable secondary reactions which are difficult to separate; unpurified glycerol is obtained as by-product which is expensive process for further purification process. Thus, by using enzymatic catalysis these processing problems can be minimized.

Enzymatic transesterification processes is carried out in the presence of lipase Feng Su (2006), which motivated researchers to focus on this area in order to minimize the processing problems and to enhance yield rate relative to that of chemical transesterification.

Previously many types of research regarding lipase reactions with extracted oil are published, but when compared to our work, there is apparent peculiarity. Haseen (2015), compared the alkaline transesterification reactions with lipase Novozym 435 (1 to 6%, based on oil weight) in a closed flask at 150 rpm with varying parameters such as 4:1 to 9:1 molar ratio, temperature range (25^0C to 50^0C) for 1 to 6h and concluded that enzymatic reactions show higher conversions yielding up to 97.8%. Feng (2006), carried out reactions in a crapped flask with 0.22 g (10 wt % based on oil weight) *candida rugosa* lipase at 40^0C with 200 rpm in a shaking bed for 24 h and concluded that the yield % of 95.4 Feng Su (2006), Meng (2015) reported that enzymatic synthesis of waste cooking oil shown significant improvement on low-temperature performance and concluded that optimum conditions for 90.4% yield are lipase weight 14% (w/w), reaction temperature 40^0C with shaking speed of 200 rpm. Luis (2015) studied the production of biodiesel from Nannochloropis gaditana oil using fungal lipases and concluded that immobilized lipase from hexagonal structured materials achieved more fatty acid methyl ester (FAME) content. The operating variables are optimized to the reaction temperature of 40^0C for 24h with 500:1 oil: lipase ratio and 8:1 ethanol: oil mass ratio respectively. Tran, studied the catalytic transesterification of coconut oil with two lipases (*Candida rugosa* and *Porcine pancreas*) for better yield biodiesel production and results shown that catalytic ability of candida rugosa leads to better yield. The optimum conditions for the lipase reaction are carried out in a magnetic stirrer at 40^0C for a period of 4h with 250 rpm at the molar ratio of 6:1 Tran (2015). Mohamad (2016), Synthesized enzymatic biodiesel reaction for rapeseed oil with three varied methanol proportions added at regular intervals of reaction time and reported that purity of prepared biodiesel is about 96-97%. The optimum conditions for better yield by adding 5% Novozyme on (m/v) basis at 37^0C with shaking speed of 200 rpm. Muhammad (2016), utilized the lipase catalyzed transesterification of Eruca sativa oil with Novozyme- 435 and *A. niger* lipase respectively and concluded that methodology employed for Novozyme- 435 depicted to be 98.3%. The reaction conditions for the employed methodology found to be 0.75% Novozyme-435 concentration, at a molar ratio of 6:1, for 60h reaction time at 32.5^0C reaction temperature. Prateek (2015), synthesized the lipase transesterification with surfactant lipase suspended inside the reaction vessel and incubated for 24h at 40^0C followed by rotating speed of 250 rpm with added buffer and stated that the yield percentage was 15%. Changhwan (2016), Stated that catalytic reaction of coconut oil using Novozyme 435 yields up to 99.8% mainly consists of triglycerides which are not suitable for engine testing.

Changhwan (2016), reported that the optimum conditions for high purity transesterification process carried out at 50^0C for 50 h, at 5:1 molar ratio adding 1g lipase for 100 ml of oil agitated at 350 rpm.

The main aim of this paper is to study the transesterification reaction using balanites aegyapatia kernel oil and methanol in the presence of alkaline catalyst NaOH and lipase catalytic process using Xylanase.

2. METHODOLOGY

Materials: Balanites aegyapatia seeds were manually collected from arid regions of Rajasthan, India. Xylanase, X2753, 10g, Sigma is purchased from a local Authorized dealer, Kakinada, India. The physicochemical properties and fatty acid profile of Balanites aegyapatia oil are listed in Table: 1 and Fig. 1 respectively.

Table.1. Physical properties of Balanites aegyapatia kernel oil

Property	Value	Property	Value
Density (Kg/m^3)	874.8	Water content (mg/kg)	214
Viscosity (mm^2/s)	4.46	Ash content % (Max)	0.001
Acid value (Mg KOH/gm)	1.26	Carbon residue (%)	0.19
Flash point (^{0}C)	>160	Cetane number	42

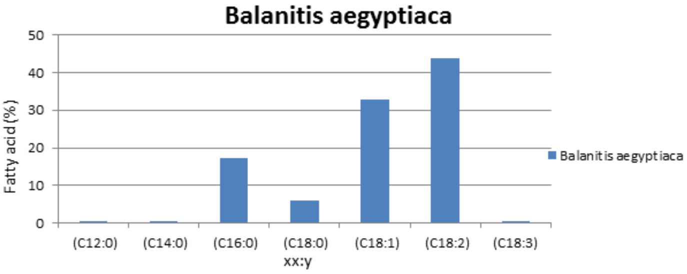

Figure.1. Fatty acid composition of Balanites aegyapatia kernel oil

Extraction procedure: After collecting the seeds, they are crushed manually and the pulp, shell, and kernel (nut) are separated. Since the moisture content of separated seeds is high, they are dried outside for 72 h or can use tray dryer at a constant temperature of 60˚C. Then the seed samples are grinded into fine particulate and the grinded particulates are wrapped in a filter paper carefully and placed into the timble for further oil extraction process in a Soxhlet apparatus.

Transesterification Process: The enzymatic catalytic reactions are carried out in a conical flask with steady shaking speed and the optimum operating conditions such as the molar ratio of 8:1 (methanol: oil), reaction temperature at 40˚C for 4 h reaction time and xylanase 1 % based on oil weight. Initially, the methanol/oil ratio is blended in proportions of 8:1 in a conical flask at a constant stirring speed of 350 rpm for 30 min with 1 g of xylanase enzyme. After that, 1g of phosphate and sodium boride buffers are added in order to maintain the pH of the mixture at above operating conditions. Then the reaction mixture is allowed to run at a constant stirring speed of 350 rpm for 4 h at 40˚C. A thin layer of separation is observed at the end of the process for further calculation of the yield obtained Tran (2015). The experimental setups of enzymatic reaction carried out are shown below in Fig.2 and Fig.3 respectively. The optimum operating reaction conditions for alkaline transesterification are carried out at a molar ratio of 8:1 (methanol: oil), the reaction temperature of 65˚C, the reaction time of 2.5 h, and 1.26 wt.% KOH catalyst loadings Sunil (2016).

Table.2. Comparison of alkaline and enzymatic transesterification process

Process	**Feedstock**	**Molar ratio**	**Solvent**	**Catalyst Concentration**	**Reaction conditions**	**Yield (%)**
Alkaline	Balanites aegyapatia	8:1	Methanol	KOH (1.26 wt. %)	Reaction temperature of 65˚C, the reaction time of 2.5 h.	89
Enzymatic (Immobilized)	Balanites aegyapatia	8:1	Methanol	Xylanase (1g wt. %)	Constant stirring speed of 350 rpm for 4 h at 40˚C	98.3

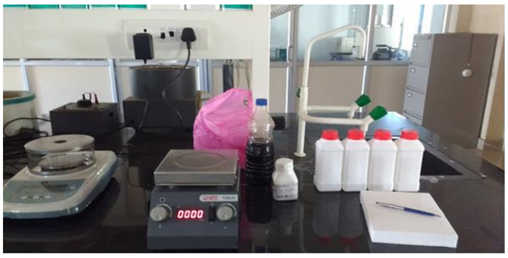

Figure.2. Enzymatic transesterification process setup

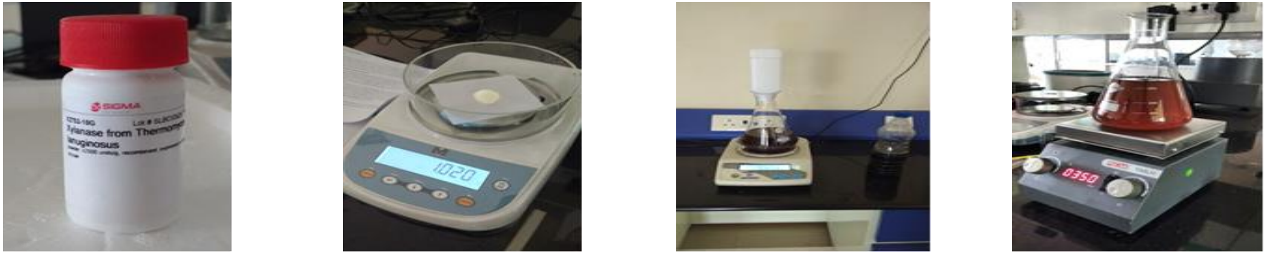

Figure.3. Stages of Enzymatic transesterification process: (A) Xylanase lipase; (B) Weighing of lipase; (C) Blending of BAO and methanol in conical flask as per molar ratio; (D) Conical flask mounted on magnetic stirrer at constant stirring speed of 350 rpm

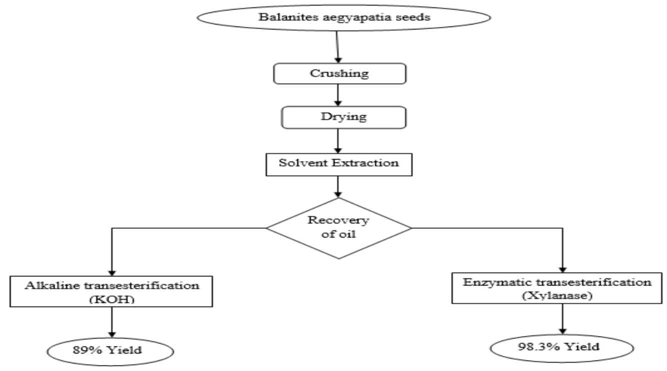

Fig.4. Flow chart of complete biodiesel production process

3. RESULTS AND DISCUSSION

The Balanites aegyapatia seeds contain 44-51% (w/w) of oil. The extracted oil has the ability to run effectively in CI diesel engine Carmen (2012). A single step alkaline transesterification process is carried out in present work. Deshmukh (2009), explained the steps used for esterification process in an appropriate way. The fatty acid profiles of BAO, used for transesterification reactions, are given in Fig.1. The statistics confirmed that the total saturated fatty acids C (14:0), C (16:0) and C (18:0) is nearly 23.16%. Whereas, the total unsaturated fatty acids: C (16:1), C (18:1), C (18:2) and C (18:3) are about 76.75%, while the total polyunsaturated fatty acids: C (18:2) and C (18:3) results in 43.76% respectively.

Effect of reaction time: The effect of reaction time on alkaline transesterification of KOH and the enzymatic transesterification of BAO with xylanase immobilized lipase is studied and tabulated in Table.2. In this study, using immobilized lipase xylanase, a maximum yield rate of 98.3% resulted for 2.5 h whereas, for the alkaline catalyst process, the maximum yield rate is slightly decreased while increasing the reaction time up to 4 h. This may be due to the presence of water which triggers in the formation of soapy contaminants Hassen (2015). So, the reaction time mainly depends on the ester yield rate, and extending the reaction time further affects the yield rate.

Effect of reaction temperature: The reaction temperature is a significant factor in both alkaline and enzymatic catalytic process. Higher temperatures will direct to higher lipase deactivation. Besides, at a lower temperature, the reaction mixture could not dissolve well as BAO is in a semisolid stage. Meng (2015), reported that the optimal conditions for the enzymatic reaction of fusel alcohol and waste cooking oil (WCO) esters were at 40˚C. The optimal temperatures with the two types of catalysts were 65˚C and 40˚C when using KOH and Xylanase, respectively as shown in Table.2. A maximum yield of 98.3% resulted at optimum temperature of 40˚C using xylanase lipase. The decline in FAME yields was observed using alkaline catalyst when the reaction temperature exceeds 65˚C. This may be due to high reaction temperature which mainly targets the strength of the catalyst and also due to high chemical reaction activity of unsaturated fatty acids.

Effect of methanol/oil ratio: The molar ratio of BAO is considered to appraise the effect of methanol on the transesterification process carried out using KOH and Immobilized Xylanase. However, the molar ratio of two different types of catalysts was carried out at 8:1 (methanol: oil) ratio and found that the yield rate obtained by using an alkaline catalyst (KOH) is about 89% whereas for the lipase catalytic reaction it is about 98.3%. This decrease in yield rate by base catalyst might be due to methanol prevention between alkyl ester and glycerol separation which leads to evident loss of yield percentage due to soap formation Idris (2016). But, in the case of lipase catalyst process methanol ensures that enzymatic reactions are carried out with ease and protects the enzymes from denaturalization.

Effect of water content: In lipase catalytic process, water plays a vital role and has major effects on lipase activity and stability. From the above Fig.1, the free fatty acid profile of BAO consists of nearly 76.75% and so plenty of water would be formed after the BAO reacted with alcohols. The excess water could slow down the transesterification reaction because of the reaction equilibrium. The effect of water content on alkaline transesterification reaction is highly sensitive and results in secondary unpredictable reactions leading to soap formation whereas, for immobilized lipases, water is the fundamental participant to trigger the enzymes and does not form soapy contaminants thus yielding better yield rate. A precise quantity of water is clearly necessary for keeping the lipase active in the reaction system Meng (2015). So, it can be concluded that the yields of esters very totally under different water contents.

4. CONCLUSION

In this study, methyl esters are transesterified from balanites aegyapatia oil (BAO) using two types of alkaline and lipase-catalyzed reactions. Consequently, enzymatic transesterification process resulted in higher methyl ester rate compared to that of alkaline transesterification reactions. Under the optimum conditions, the methyl ester yield rate by lipase-catalyzed transesterification yields up to 98.3%. However, there are some major drawbacks using alkaline catalyzed reactions such as soapy formation by reacting with water, secondary unpredictable reactions which are difficult for separation process, low quality of byproducts needs to be purified further which is a cost effective process which results in decrease in yield rate whereas, the lipase catalyzed transesterification process of BAO results in significant advantages, that is, tolerant to high water concentrations and does not form any soapy contaminants, efficiently converts the feedstocks with higher yields, enzyme recovery and reusability, and results in higher quality of by-products. Due to balanites aegyapatia high oil yield content from the kernel and conventional feedstock, the physical properties of BAO might be useful for biodiesel production.

5. ACKNOWLEDGEMENTS

The authors are thankful to University college of Engineering, Kakinada, JNTUK Kakinada for economic assistance through Process TEQIP-II.

REFERENCES

Carmen Sotelo Montes, Dimas Agostinho da Silva, Rosilei Garcia A, Graciela Ines Bolzon de Muniz, John C Weber, Calorific value of Prosopis africana and Balanites aegyptiaca wood, Relationships with tree growth, wood density and rainfall gradients in the West African Sahel, Biomass and Bio energy, 35, 2012, 346 – 353.

Changhwan Woo, Sanghoon Kook, Evatt R Hawkes, Peter L, Rogers, and Christopher Marquis, Dependency of engine combustion on blending ratio variations of lipase-catalysed coconut oil biodiesel and petroleum diesel, Fuel, 169, 2016, 146–157.

Deshmukh S.J, Bhuyar L.B, Transesterified Hingan (Balanites) oil as a fuel for compression ignition engines, Biomass and Bio energy, 33, 2009, 108 – 112.

Feng Su, Cheng Peng, Guan-Lin Li, Li Xu, Yun-Jun Yan, Biodiesel production from woody oil catalyzed by *Candida rugosa* lipase in ionic liquid, Renewable Energy, 90, 2006, 329-335.

Hassen Mohamed Sbihi, Imededdine Arbi Nehdi, Lahssen El Blidi, Umer Rashid, Saud Ibrahim Al-Resayes, Lipase/enzyme catalyzed biodiesel production from *Prunus mahaleb*, A comparative study with base catalyzed biodiesel production, Industrial Crops and Products, 76, 2015, 1049–1054.

Idris Atadashi Musa, The effects of alcohol to oil molar ratios and the type of alcohol on biodiesel production using transesterification process, Egyptian Journal of Petroleum, 25, 2016, 21-31.

Luis Fernando Bautista, Gemma Vicente, Álvaro Mendoza, Sara Gonzalez, Victoria Morales, Enzymatic production of biodiesel from *Nannochloropsis gaditana* microalgae using immobilized lipases in mesoporous materials, Energy & Fuels, 29, 2015, 4981–4989.

Meng Wang, Kaili Nie, Feng Yun, Hao Cao, Li Deng, Fang Wang, Tianwei Tan, Biodiesel with low temperature properties, Enzymatic synthesis of fusel alcohol fatty acid ester in a solvent free system, Renewable Energy, 83, 2015, 1020-1025.

Mohamad Y, Firdaus Zheng Guo Sergey Fedosov N, Development of Kinetic Model for Biodiesel Production Using Liquid Lipase as a Biocatalyst, Esterification Step, Biochemical Engineering Journal, 105, 2016, 52-61.

Muhammad Waseem Mumtaz, Hamid Mukhtar, Usman Ali Dilawer, Syed Makhdoom Hussain, Majid Hussain and Munawar Iqbal, Biodiesel production from Eruca sativa oil catalyzed by Novozyme-435 and lipase, Biocatalysis and Agricultural Biotechnology, 5, 2016, 162-176.

Prateek Rastogi, Ranjitha Jambulingam, Vijayalakshmi S, Michael Donatus S, Extraction and characterization of oil from the seeds of Jatropha Curcas using supercritical CO_2 and Soxhlet extraction process, Applied Mechanics and Materials, 787, 2015, 809-814.

Sunil Naik N, Balakrishna B, Experimental evaluation of a diesel engine fueled with *Balanites aegyptiaca* (L.) Del biodiesel blends, Biofuels, 2016.

Tran Thi Be Lan, Phan Ngoc Hoa, Lipase Catalysis for Transesterification Produces Biodiesel Using Coconut Oil as Main Raw Material Source, Biological and Chemical Research, 2015, 258-267.