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Enzymatic Preparation of Biodiesel from Sal (Shorea Robusta) Oil and its Characterisation

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Abstract: Fuel from renewable sources is urgently needed due to the scarcity of non-renewable sources of energy as well as for saving our mother earth. Sal oil has been utilized for this purpose in the present research investigation. Sal oil and methanol are transesterified for the production of biodiesel in the presence of 7.5% biocatalyst, Novozyme 40013 (Candida antarctica) at 1:6 molar ratio of oil to methanol at a reaction temperature of 63°C for 9 hrs of continuous stirring with 550 rpm of mixing intensity. 94.17% conversion was achieved through this transesterification reaction. The physicochemical properties of the sal-biodiesel were characterized and it is almost at par with diesel fuel which ensures its use as fuel without modification of the engine. The study shows that sal oil can be an important cheap source for the production of renewable fuel in the future world.
Keywords: Sal oil, Biodiesel, Candida antarctica, Transesterification,

I. INTRODUCTION

Cheap renewable energy sources are supposed to be the most thought-provoking issue in the present world. Among the renewable energy sources, biodiesel is a significant alternative energy source and achieved a lot of attention by the scientists, researchers and academicians [1,2,3,4,5]. Vegetable oils like mustard, ricebran, sunflower, soybean, safflower, Jatropha Curcas [6-9], Karanja (*Pongamia pinnata*) [10,11], Mahua (*Madhuca Indica*) [12,13], Undi, Castor etc are being used as raw materials for the production of biodiesel. The processes which are being adopted are based on chemical or bio catalytic method and higher conversion of biodiesel is obtained in different methodology. Sal (*Shorea Robusta*) oil is another potential oil that may be introduced for this purpose which has the capability to contribute a big portion in the renewable energy sector.

Sal seed oil is an edible oil extracted from the seeds of Shorea robusta. Shorea robusta is known as the Sal tree in India. Sal is the most famous of all the species of Shorea (Dipterocarpacceae) found in India and occurs in two main regions separated by the Gangetic Plain, namely the northern and central Indian regions. Sal oil is not only useful as source of renewable fuel but the leaves and bark are widely used to treat many diseases like leprosy, wounds, ulcers, cough, gonorrhoea, headache, diarrhoea and vaginal discharges [14]. The extracted plant resin has astringent, carminative and stomachic properties useful for different health purposes [15-18]. So utilisation of sal tree in different areas are tremendous. Biodiesel from sal oil is also a wonderful application which is importantly useful for the renewable energy sources. In India, there is big opportunity for sal oil production because it has 10 million hectors Sal (Shorea Robusta) forest. From this forest, 180 million kg non-edible seeds are grown annually [19]. This indigenous seeds may be a large possibility to fulfil the biodiesel mission of India. Fig. 1 shows the sal tree and sal trees.


Fig. 1 Sal tree and sal seeds

Biodiesel from sal oil and its optimum reaction parameters have been tried by many researchers. Pali and Kumar [20] prepared biodiesel from sal oil by using chemical catalyst like potassium hydroxide and also the yield was good. Pali et al [21] also used response surface methodology for process optimization of biodiesel from sal seed oil. Chhibber et al [22] made a comparative study on Sal (Shorea Robusta) biodiesel and diesel oil. But little study have been made for the production of biodiesel from sal using enzyme catalyst. Present authors have tried to prepare biodiesel from sal oil using enzyme catalyst along with the optimization of the reaction parameters using non-specific enzyme Novozyme 40013 as catalyst. 94.17% conversion has been achieved and the properties of sal methyl ester were compared with diesel standard which showed satisfactory results.

II. MATERIALS AND METHODS

Sal oil was collected from Angura Oils Pvt Ltd, Kolkata, West Bengal. Novozyme 40013, an immobilized nonspecific lipase from *Candida antarctica* was used as catalyst in the reaction with ester synthesis activity of 10000 propyl laurate unit/g. Methanol was purchased from Scientific and Laboratory Instrument Co., Kolkata. Except otherwise specified all other chemicals were A.R. Grade.

A. Transesterification Of Sal Oil

500 mL of sal oil was filtered and taken in an Erlenmeyer flask and heated up to 80 °C to drive off moisture by continuous stirring for about 1 h. After that, methanol was added to it for transesterification reaction through stepwise manner in an appropriate proportion using solvent hexane at a specified temperature for 9 hours. Enzyme Novozyme 40013 was added in definite proportion (w/w) to the reaction mixture. The progress of reaction or production of biodiesel was monitored by thin layer chromatographic (TLC) method and the typical yield of each reaction product was determined separately by column chromatography using silicic acid as an adsorbent and 160 mL of hexane diethyl ether (99:1) as eluting solvent. At the end of the reaction, the product was filtrated through separating funnel to remove the enzyme and allowed to separate. The lower layer was then evaporated under vacuum in order to remove excess methanol and the final product was collected. The enzyme was washed with hexane, dried and reused for the next experiment. Biodiesel characterization was done according to the American Standard Testing Method (ASTM). Values are reported as mean ± s.d., where n = 3 (n = no of observations).

B. Gas Chromatographic Analysis

Fatty acid composition of sal was determined by a gas liquid chromatographic (GLC) method after converting into methyl ester. The HP 5890A GLC was connected with a HP 3390A data integrator. The GLC was fitted with a glass column (1.83 m X 3.175 mm id) packed with 10% DEGS supported on Chromosorb – WHP (100/200 mesh) of HP make. The oven temperature was programmed from 100 to 190 °C at 5° per min. The injector and detector block temperatures were maintained at 230 and 240°C, respectively. IOLAR-2 nitrogen was used as the carrier gas (flow rate 30 mL/min). The fatty acid esters peak was identified and calibrated with standard methyl esters. Data were represented an averages of three determinations.

III. RESULTS AND DISCUSSIONS

A. Identification of reaction parameters w.r.t. Molar ratio of methanol and sal oil

Different molar ratios of alcohol to sal oil have been analysed from 2:1 to 8:1 (MeOH: sal oil) for the production of sal-biodiesel in the presence of 7.5% biocatalyst with a mixing intensity of 550 rpm for 9 hours at a temperature of 63^{0}C. It has been observed that 6:1 ratio contributes maximum production of biodiesel as shown in Fig. 2. Further enhancing the ratio of alcohol to mahua oil does not increase the conversion which is evidenced from Fig. 2. It may be due to the fact that increasing the amount of alcohol creates more separation between the reactants or less contact time with the biocatalyst.

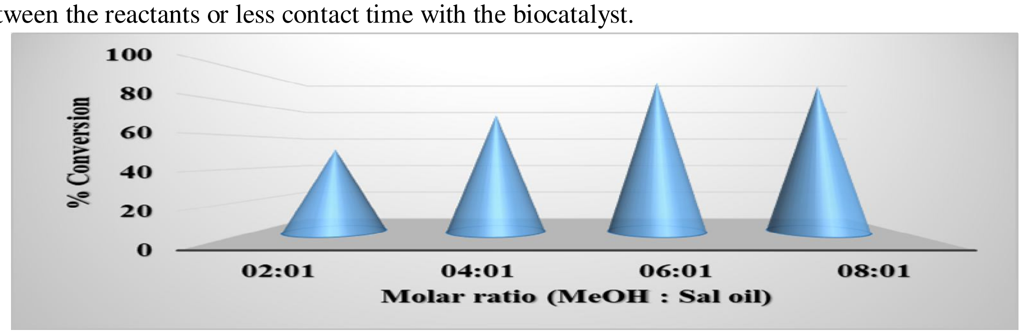

Fig. 2 Analysis of molar ratio of alcohol to sal oil for biodiesel production

B. Identification of reaction parameters w.r.t. temperature

Activation energy of a reaction measures the conversion rate or percentage conversion of the product and hence it is directly related to the temperature of the transesterification reaction between oil and alcohol. Reaction temperature has been analysed from 30 to 70^{0}C in the presence of 7.5% biocatalyst with a mixing intensity of 550 rpm for 9 hours maintaining 6:1 molar ratio of methanol and sal oil. It has been observed from Fig. 3 that 63^{0}C is the optimum temperature which contributed maximum conversion. Enhancing the temperature beyond 63^{0}C does not increase the product percentage. This may be due to the fact that each enzyme shows its maximum efficiency at a certain temperature and after that deactivation occurs which goes to low conversion rate.

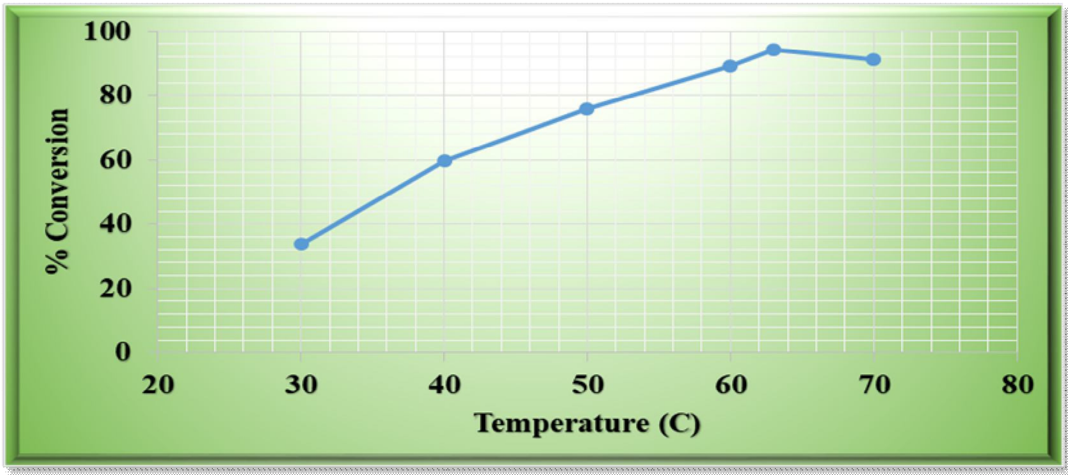

Fig. 3 Analysis of temperature for biodiesel production

C. Identification of reaction parameters w.r.t. concentration of enzyme

Concentration of catalyst (here enzyme) plays a big role for the conversion of reaction. In the present research investigation, rate of transesterification reaction has been analysed w.r.t. enzyme concentration from 4.5 to 8.5% (w/w) at 63^{0}C in the presence of 7.5% biocatalyst with a mixing intensity of 550 rpm for 9 hours maintaining 6:1 molar ratio of methanol and sal oil. It has been observed from Fig. 4 that maximum conversion (94.17%) was achieved at 7.5% concentration of enzyme. Beyond this concentration, conversion decreases and less biodiesel was obtained. This may be due to the fact that at higher concentration of enzyme, agglomeration may occur which prevents the opening of active sites of it. As active sites are the centre of reaction of an enzyme, its blockage reduces the conversion rate.

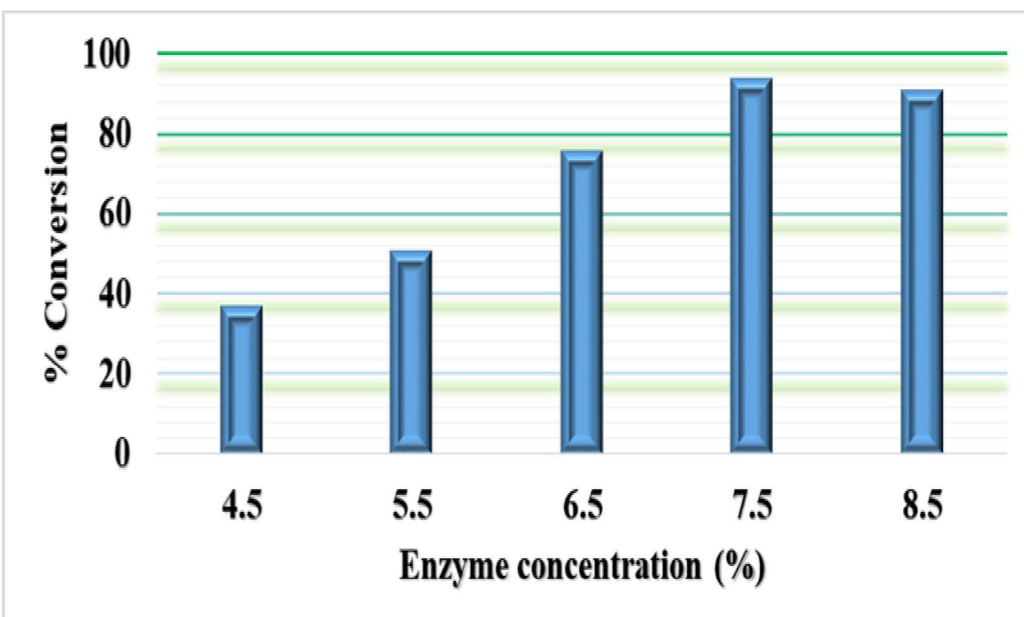

Fig. 4 Analysis of concentration of enzyme for biodiesel production

D. Identification of reaction parameters w.r.t. mixing intensity

Mixing intensity of a reaction helps to enhance the contact between the reactants so that the reaction can proceed smoothly. In the presence of enzyme as a catalyst, mixing intensity increases the contact between active sites and reactants which is essential for the transesterification reaction. Analysis of mixing intensity has been analysed by applying 250 to 650 rpm for the reaction mixture and it was observed from Fig. 5 that 550 rpm is the ideal intensity for maximum production of biodiesel as shown in Fig. Beyond that, conversion rate decreases. This may be due to the fact that enhancing mixing intensity decreases the contact or collision of the active sites of enzyme with the reactants.

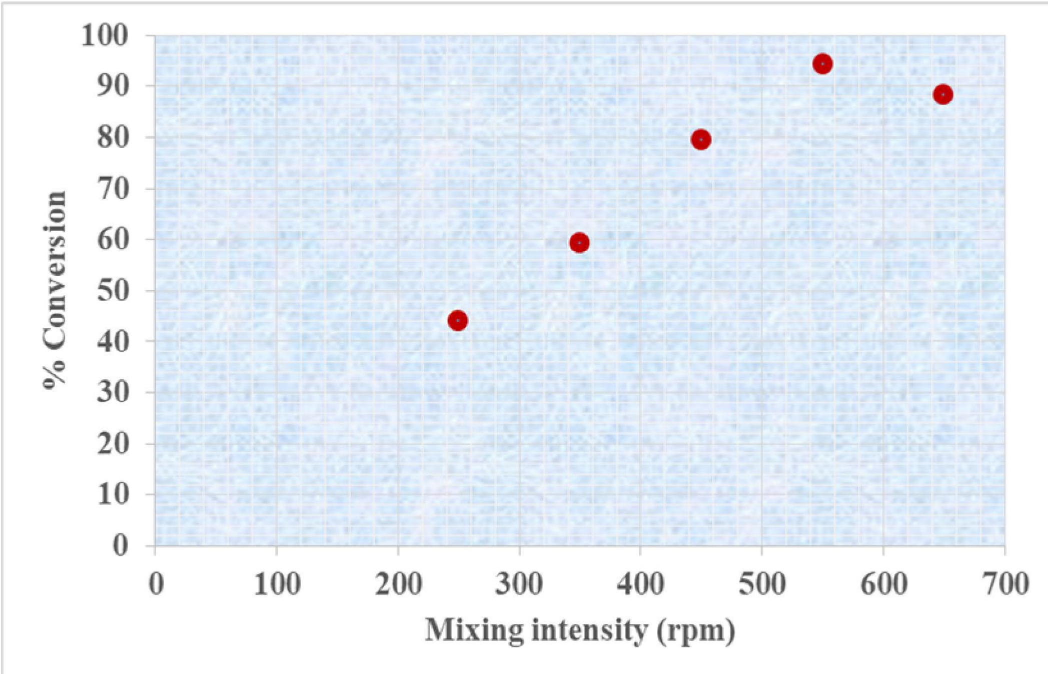

Fig. 5 Analysis of mixing intensity for biodiesel production

During the progress of the transesterification reaction, biodiesel or methyl ester has been generated from sal oil through some intermediates like diacylglycerols (DAGs) and monoacylglycerols (MAGs). Triacylglycerols (TAGs) and free fatty acids (FFAs) of the reaction decrease with time. Biodiesel production also increases with the decrement of intermediates as shown in Table 1 It has been observed from Table 1 that initially the rate of reaction is higher and as the reaction proceeds, the conversion of biodiesel reaches maximum with a time duration of 9 hrs.

TABLE I
COMPOSITION OF REACTION MIXTURE AT DIFFERENT TIME INTERVALS

Material	0 hr	3 hrs	5 hrs	7 hrs	9 hrs
TAG	79.08±0.117	53.17±0.078	31.19±0.162	12.49±0.045	1.75±0.006
DAG	5.27±0.021	4.01±0.018	2.18±0.006	1.26±0.015	1.02±0.009
MAG	2.11±0.009	1.56±0.029	1.07±0.005	0.78±0.002	0.36±0.006
FFA	12.56±0.018	5.66±0.013	3.11±0.01	1.78±0.011	1.01±0.005
Methyl ester or biodiesel	0.00	53.31±0.091	71.29±0.117	89.16±0.051	94.17±0.056

E. Characteristics of sal-biodiesel

Physicochemical properties of sal-biodiesel was compared with diesel fuel as shown in Table 2. It has been observed from Table 2 that the characteristics of biodiesel are comparable with biodiesel standards in most of the properties. Higher flash point of sal biodiesel is significant and desirable also due to safe handling than diesel fuel. The calorific value of diesel fuel is higher than biodiesel but with regard to other characteristics, sal biodiesel can be used safely without modification of engines.

TABLE 2
COMPARATIVE CHARACTERISTICS OF SAL BIODIESEL

Properties	Diesel fuel	Sal biodiesel	ASTM D6751
Density at 15^{o}C(Kg/m3)	841	876±0.301	860-900
Calorific value (MJ/Kg)	45	39±0.103	------
Kinematic viscosity at 40^{o}C (mm^2/s)	3.17	3.7±0.013	1.9-6.0
Flash point (^{o}C)	63	203±0.163	Min 130
Cetane no	51	57±0.191	--------

IV. CONCLUSIONS

Sal oil has been utilised as a potential source for the production of renewable energy sources like biodiesel. It can be exploited as a cheap raw material for this purpose which is easily available. Process parameters have been identified in the present study utilising the enzyme Novozyme 40013 as a catalyst. Enzyme catalyst can be recycled many times which helps to reduce the process cost. So the present methodology utilising the sal oil as raw material may be a good source as renewable energy in future.

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