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Research Paper

LIPASE CATALYZED BIODIESEL PRODUCTION FROM *DELONIX REGIA* OIL

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The enzymatic transesterification of *Delonix regia* oil was investigated in the present study. Oil was extracted from *D. regia* seeds using *n*-hexane as solvent and various physico-chemical characteristics of the oil was studied. Lipase from *Candida rugosa* was immobilized on celite and transesterification reaction was performed using free and immobilized lipase. Three-step addition of methanol was employed to avoid the enzyme inactivation and higher yield of methyl esters (>90 %) was obtained after 24 h of reaction. The conversion of the oil into fatty acid methyl esters (biodiesel) was confirmed by Gas Chromatography (GC) analysis. Various parameters such as oil to methanol molar ratio, reaction temperature and amount of catalyst were optimized to obtain higher yield of biodiesel.The best conditions for maximum biodiesel yield was obtained with oil/methanol molar ratio of 1:4, temperature of 50°C, and enzyme concentration of 20 % (w/w).

Keywords: *Delonix regia*, Immobilized lipase, Transesterification, Biodiesel

INTRODUCTION

Depletion of limited fossil fuel resources, rising crude oil prices and increasing concerns about the environmental effects of burning fossil fuels has evoked interest in researchers to look for a better alternative fuel. Biodiesel is a promising alternative to conventional diesel as it is renewable, bio-degradable, non-toxic and has reduced emission properties (Demirbas, 2009; Ma and Hanna, 1999; Knothe *et al*., 2006). Plant derived oils are attracting increased attention in the production of biodiesel as they are renewable, widely available from a variety of sources, are carbon dioxide neutral and have energy content close to that of diesel fuel. Biodiesel is mainly produced through transesterification reaction where triglycerides present in the vegetable oil react with an alcohol in the presence of a catalyst to form fatty acid alkyl esters (biodiesel) and the by-product, glycerol. Conventional method of biodiesel production involves chemical method where an acid (e.g. H_2SO_4) or alkali (e.g. NaOH) is used as the catalyst. Acid catalyzed reaction leads to the corrosion of the equipment whereas alkaline catalyst has the disadvantages of soap formation, difficulty in product recovery and

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alkaline wastewater formation. Lipase catalyzed biodiesel production shows great potential as an environmental friendly method of biodiesel synthesis as it can address the above mentioned problems associated with chemical catalysis.

Delonix regia, commonly known as Gulmohar, is a medium sized ornamental tree found in tropical countries (Jungalwala and Cama, 1962). The trees produce bunches of scarlet red flowers during the month of April to July and fruits ripen in August to October (Figure 1). The seeds contain 7-9% oil content (Adewuyi, 2010; Hosmani and Hosmani, 1995) and the seed meal is rich in protein content (Amata and Nwagu, 2012, Prakash and Misra, 1988). Okey and Okey (2013) have reported the optimization of biodiesel production from *D. regia* using sodium methoxide as chemical catalyst. The present study is aimed at lipase mediated transesterification of *Delonix regia* seed oil for biodiesel production.

Figure 1: *Delonixregia* and Its Seeds

Delonix regia

D. regia seeds

MATERIALS AND METHODS

Materials

Dried pods of *Delonix regia* were collected from the trees grown in Bharathiar University campus and the seeds were manually separated from the pods. The samples were dried in an oven at 105°C for 24 h and then it was milled with the help of coffee grinder to obtain fine powder. Lipase from *Candida rugosa* was obtained from Sigma-Aldrich, St. Louis, MO, USA. All other chemicals were purchased from Hi-Media, Mumbai and were of analytical grade.

Proximate Analysis of *D. regia* Seeds

The proximate composition of the seed in terms of moisture, lipids, ash, crude protein, crude fiber and carbohydrate content was determined according to the methods of the Association of Official Analytical Chemists (AOAC, 2002). Moisture content was determined by heating 2.0 g of the seed sample to a constant weight in a crucible placed in an oven maintained at 105 °C. Ash was determined by the incineration of 1.0 g samples placed in a muffle furnace maintaned at 550 °C for 5 h. The organic nitrogen content was determined by Kjeldahl method and the crude protein content was calculated by multiplication of the organic nitrogen by a factor of 6.25; lipid content was obtained by extracting 30.0 g of the sample in a soxhlet apparatus using *n*-hexane

as the solvent. Total carbohydrate was obtained by the difference.

Extraction of Oil and Physico-Chemical Characterization

The extraction of *D. regia* oil was carried out using solvent extraction. The oil was extracted from the ground seed powder using *n*-hexane as the solvent for 6 h. The extracted oil was filtered and excess solvent was removed using a rotary evaporator. Finally, the oil was heated at 100°C for 1 h in order to remove any water molecules, cooled and stored for subsequent physico-chemical analysis. The color and state of the oil at room temperature was visually observed. The kinematic viscosity was measured using Brookfield DV-II Pro viscometer. AcidValue (AV), Free Fatty Acids (FFA), Iodine Value (IV), Saponification Number (SN) and Peroxide Value (PV) were measured by AOAC (2002) methods.

Lipase Immobilization

C. rugosa lipase (100 mg) was dissolved in 1 mL of 20 mM sodium phosphate buffer solution (pH 7.3) and mixed with 250 mg of Celite-545. The immobilized lipase was collected by filtration and washed twice with *n*-hexane to remove any free enzyme. The immobilized lipases was then dried and stored at 4°C for further use.

Immobilized Lipase Mediated Transesterification of *D. regia* Oil

Immobilized enzyme mediated transesterification was carried out in a 100 mL capped flask on a shaking incubator. *D. regia* oil was mixed with methanol followed by the addition of the immobilized lipase enzyme. A three-step addition of methanol was employed to avoid the enzyme inactivation. The reaction was carried out at 200 rpm for 24 h. The effect of varied oil to methanol mole ratio (1:2 to 1:6), reaction temperature (30-60 °C) and enzyme concentration (5-30% w/w) was studied. After completion of the reaction, the mixture was transferred to a separating funnel and was kept for 16 h. After 16 h, the mixture separated as an upper layer containing Fatty Acid Methyl Esters (FAME or biodiesel) and a lower layer containing glycerol and unreacted methanol. The lower layer was discarded and the ester phase was collected. The fatty acid methyl esters formed were analyzed by Gas Chromatography (GC).

RESULTS AND DISCUSSION

Proximate Composition of the *Delonix regia* Seeds

The proximate composition of the *D. regia* seeds is presented in Table 1. The present study shows that the seeds of *D. regia* contains significant amount of lipids (9.82%), crude proteins (16.57%), crude fibers (8.32%) and carbohydrates (54.71%).

Table 1: Proximate Composition of *D. regia* seeds

Constituents	Value (%)
Moisture	7.91
Ash	2.14
Fiber	8.32
Carbohydrate	54.71
Lipid	9.82
Protein	16.57

Oil Extraction and its Physico-chemical Characterization

In the present study, soxhlet extraction using *n*-hexane as the solvent was employed and the yield of the oil was found to be 9.82%. The oil content is slightly higher than the values reported previously (Adewuyi *et al.,* 2010; Arora *et al.,*

2010; Hosamani and Hosamani, 1995; Gunstone *et al.,* 1972). The physico-chemical properties of the extracted oil is presented in Table 2.

Table 2: Physico-chemical Properties of *D. regia* Seed Oil

Property	Value
Density (g/cm^3)	0.933
Kinematic viscosity @40p C cst	36.65
Acid value (mg KOH/g)	4.88
Free Fatty Acids (%)	2.45
Iodine value (g iodine/100 g)	117.46
Peroxide value (mg O_2/g oil)	3.82
Saponification value (mg KOH/g)	198.23

Acid Value (AV) is the measure of total acidity of the oil and the present study reveals that *D. regia* seed oil contains a high acid value (4.88 mg KOH/g) which will result in formation of soap during chemical transesterification. Thus, enzymatic transesterification is preferred over chemical catalysis for biodiesel production. The Peroxide Value (PV) of the extracted oil was found to be significantly high (3.8 mg O_2/g oil) indicating its susceptibility to oxidative rancidity. Iodine Value (IV) is a measure of total unsaturation of a fatty material and the higher value of iodine number (117.46 g iodine/100 g) indicates the presence of more unsaturated fatty acids. Saponification Value (SV) is an indication of the molecular weights of triglycerides in oil and it value was found to be 198.2 mg KOH/g indicating the presence of normal triglycerides. Previous reports on the fatty acid composition of *D. regia* seed oil reveals the presence of higher amount of unsaturated fatty acids such as linoleic acid and oleic acid (Hosamani and Hosamani, 1995; Adewuyi *et al*., 2010) and hence, the values of the physico-chemical properties (AV, IV, PV and SV) in the present study is in accordance with these reports.

Transesterification of *D. regia* oil Using Immobilized *C. antarctica* Lipase

In the present study, methanolysis of *D. regia*oil was performed in a water-free environment as previous studies by Nelson *et al.* (1996) and Shimada *et al.* (1999) reported that pre-activation with water was not necessary for immobilized *C. antarctica* lipase. Kose *et al.* (2002) reported higher yield of methyl esters (91.5%) without using co-solvents during immobilized *C. antarctica* lipase-catalyzed alcoholysis of cotton seed oil. Hence, the present study was investigated in a solvent free medium. It is well known that at least three molar equivalents of methanol are required for the complete conversion of the vegetable oil to its corresponding methyl ester. However, addition of more than 1 molar equivalent of methanol will inactivate the activity of lipase (Shimada, 1999). Hence a three-step addition of methanol was employed to avoid the enzyme inactivation. During the first step addition of methanol (1:1 oil/methanol molar ratio), the ester conversion reached 30.58 % at 7 h. The second molar equivalent of methanol was then added and the ester conversion reached 61.36 % after 7 h (total, 14 h). A third molar equivalent of methanol was added again and the reaction continued. After 24 h of incubation, 92.37 % of the oil was converted to its corresponding methyl esters.

Fatty Acid Methyl Ester (FAME) Analysis

Biodiesel produced by lipase catalyzed transesterification is shown in Figure 2. The chromatogram of GC analysis of the FAME is shown in Figure 3. The major fatty acid methyl esters were methyl palmitate (20.68 %), methyl sterate (12.04%), methyl oleate (44.30%) methyl linoleate (8.53%), and methyl arachidate (14.45%). The presence of higher amount of

methyl esters of oleic acid in the biodiesel indicates that *D. regia* is an ideal feedstock for biodiesel production.

Figure 2: Biodiesel Synthesized from *D.regia* oil

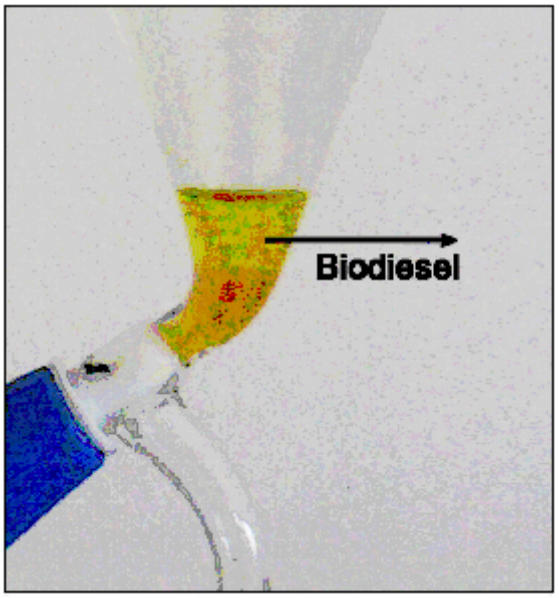
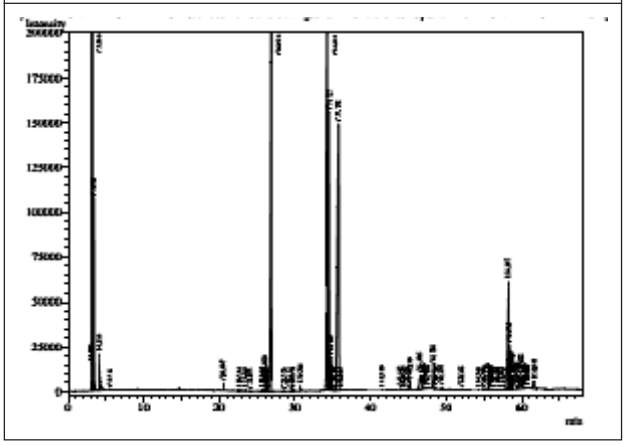


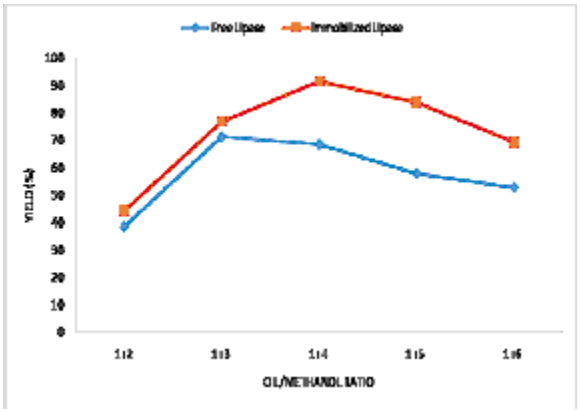
Figure 3: GC Analysis of Biodiesel



Effect of Oil to Methanol Molar Ratio

The optimum level of methanol concentration for the maximum yield of biodiesel was investigated by varying the oil to methanol molar ratio from 1:2 to 1:6. Three-step addition of methanol was performed to avoid enzyme inactivation. Figure 4 shows the effect of oil to methanol molar ratio on biodiesel synthesis. The highest methyl ester yield was obtained at the oil to methanol molar ratio of 1:4. At higher methanol concentrations (1:5 and 1:6), there was a gradual decrease in the methyl ester yield which may be due to the inactivation of the enzyme by methanol. Our results coincides with the reports of Kose *et al.* (2002), who has obtained maximum yields of methyl esters with 1:4 methanol to oil molar concentration during the immobilized *Candida antarctica* lipase-catalyzedalcoholysis of cotton seed oil in a solvent free medium.

Figure 4: Effect of Oil/methanol Ratio on Biodiesel Synthesis



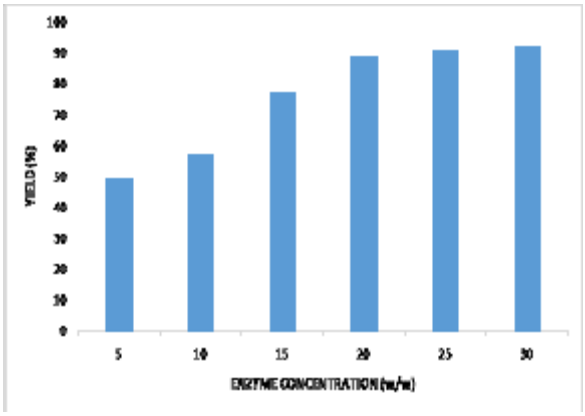
Effect of Enzyme Concentration

The effect of enzyme concentration on the yield of methyl ester is presented in Figure 5. The yield of methyl esters was found to be increasing by increasing the enzyme concentration up to 30 % (w/w). There was no significant difference in the yield of methyl esters above 20%(w/w) enzyme concentration.

Effect of Reaction Temperature

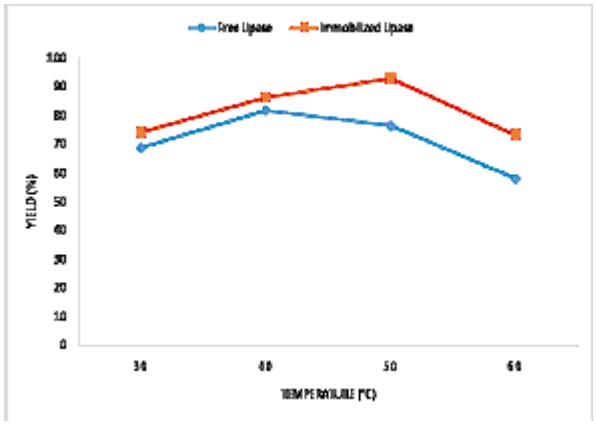
To study the influence of temperature on the yield of biodiesel, lipase catalyzed transesterification

Figure 5: Effect of Enzyme Concentration on Biodiesel Synthesis



was carried out at different temperatures of 30, 40, 50 and 60 °C. The reaction was performed at a methanol/oil molar ratio of 4:1 and 20 % (w/w) enzyme concentration. The result is presented in Figure 6. The highest yield was obtained at the reaction temperature of 50°C. There was a decrease in methyl ester yield at 60°C which may be due to the deactivation of the enzyme caused by high temperatures.

Figure 6: Effect of Reaction Temperature on Biodiesel Synthesis



CONCLUSION

The present study investigated the feasibility of *D. regia* oil in immobilized lipase catalyzed biodiesel production. The results revealed that *D. regia* seeds contained significant amount of oil (9.82%) that can be converted to corresponding methyl esters by using lipase as biological catalyst. It was observed that a higher amount of biodiesel conversion (92.37 %) was obtained using immobilized enzyme. Also the immobilized enzyme was found to be stable in a wide range of temperature (30-50°C) and higher oil to methanol molar ratio (1:4). In conclusion, *D.regia* oil can be used as a feedstock for green synthesis of biodiesel.

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