

Investigation of biodiesel from Canola oil deodorizer distillate using dual biocatalyst

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Article Received: 01 January 2020

Article Accepted: 27 February 2020

Article Published: 08 March 2020

ABSTRACT

Depleting nature of nonrenewable energy sources and continuous environmental tribulations make the mankind to think differently regarding alternative renewable energy sources. In this regard, present research investigation contributes biodiesel from canola oil deodorizer distillate (Codd) using Lipase AY Amano 30 (*Candida rugosa*) and Novozyme 40013 (*Candida antarctica*) in the presence of methanol. Initially the neutral glycerides present in Codd were hydrolysed using lipase Amano AY 30 in the presence of water. The hydrolysed Codd was then esterified with methanol using non-specific immobilized enzyme NS 40013 for the production of biodiesel. The characteristics of final product were compared with diesel fuel and it showed good results. This bioprocess technology using biohydrolysis and bioesterification is a novel technology for biodiesel production from cheap raw materials like Codd.

Keywords: Canola oil deodorizer distillate, *Candida rugosa*, *Candida antarctica*, Biohydrolysis, Bioesterification.

1. INTRODUCTION

Alternative energy sources are one of the major issues in today's world. Depleting nature of conventional energy sources along with the continuous degradation of surrounding environment create a lot of attention for the requirement of renewable environment friendly fuel. Researchers and academicians are trying to produce alternative renewable energy sources from different cheap raw materials. In this regard, vegetable oil refinery by products like fatty acid distillates or deodorizer distillates are utilized as cheap raw materials for the production of alternative energy sources like biodiesel in the presence of chemical catalyst or biocatalyst. 98% biodiesel from palm fatty acid distillate was prepared by Saimon et. al [1] through microwave-assisted sulfonated glucose acid catalyst. Lokman et. al [2] also optimized production of biodiesel from palm fatty acid distillate using the same catalyst. Reactive distillation method was also utilized by Budiman et. al [3] for the production of biodiesel from palm fatty acid distillate.

Present author tried to optimize the production of biodiesel from other distillate like soybean oil deodorizer distillate and studied the kinetics of the process technology [4]. Present author also successfully produced biodiesel from non edible oils like Karanja oil [5] and Jatropha Curcas oil [6-7] in the presence of non specific enzyme. Praveen et. al [8] also studied the process optimization and reaction kinetics of palm fatty acid biodiesel and successfully conducted the production process. Ilgen et. al [9] investigated the production of biodiesel from canola oil using Amberlyst -26 catalyst by varying different reaction parameters. Many researchers tried to produce biodiesel from other cheap sources like waste oil [10-11], animal fats [12-15] etc. But very few researchers utilized the bioprocess technology for the production of biodiesel from Codd.

In the present research investigation, Codd was utilized for the production of biodiesel through biohydrolysis and bioesterification technology. This process technology is clean, safe, environment-friendly and easy to separate. Moreover, biocatalyst may be recycled which helps to reduce the process cost. So, initially Lipase AY Amano 30 (*Candida rugosa*) was used for optimization of biohydrolysis of neutral glycerides present in Codd and finally Novozyme 40013 (*Candida antarctica*) was used for bioesterification of fatty acids and methanol to get biodiesel.

2. EXPERIMENTAL

2.1 MATERIALS

CODD was obtained from Emami Agrotech Ltd, Haldia, West Bengal. The enzymes used in the present study were Lipase AY Amano 30 which was a crude lipase from *Candida rugosa* with activity <30000 units/g and Novozyme 40013, an immobilized non specific lipase from *Candida Antarctica* with ester synthesis activity of 10000 propyl laurate unit/g. The chemicals monoglycerides and diglycerides were purchased from Scientific and Laboratory Instrument Co., Kolkata. Except otherwise specified all other chemicals were A.R. Grade.

2.2 BIOHYDROLYSIS OF CODD

Initially, CODD used for the production of biodiesel was centrifuged for the removal of solid impurities and gums. These causes the solid impurities to settle down and are removed from CODD. After that, for hydrolysis of neutral glycerides present in CODD, it was taken in a 500 ml stoppard Erlenmeyer flask and water (60% by weight of neutral glycerides) containing Amano 30 lipase powder (5% w/w) was added to it. The reaction mixture was magnetically stirred with a 1 inch Teflon coated stir bar at 40⁰C in a temperature controlled bath for 6 hrs. After 6 hrs, the hydrolysis reaction was complete and 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 CODD was collected for further reaction.

The hydrolysed CODD was then taken in an Erlenmeyer flask and heated up to 80⁰C to drive off moisture by continuous stirring for about 1 hr. After that methanol and enzyme Novozyme 40013 were added to it. The mixture was then continuously stirred for 4 hrs using solvent hexane fitted with a water condenser. 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 and yield of product was monitored by column chromatographic method. TLC was done by using a silica-gel G plate (0.2 mm thick) with hexane-diethyl ether-acetic acid (90:10:1) as a developing solvent. Column chromatographic method was employed using silicic acid as an adsorbent and 160 ml of hexane-diethyl ether (99:1) as eluting solvent. Values are reported as mean \pm s.d., where n=3 (n=no of observations).

3. RESULTS AND DISCUSSIONS

3.1 ANALYSIS OF CODD

The composition of fatty acids, neutral glycerides and unsaponifiable matters present in CODD was shown in Table 1. It was observed from Table 1 that CODD contains higher amount of FFAs which mainly includes oleic acid and linoleic acid. Among other acids, linolenic acid shares maximum amount though it contains unusual acids like arachidic acid and eicosenoic acid. Neutral glycerides namely triacylglycerols (TAG), diacylglycerols (DAG) and monoacylglycerols (MAG) are also an important part of CODD which contributes 19.05 \pm 0.116% in the composition. CODD contains little amount of unsaponifiable matters which mainly includes sterols, hydrocarbons and tocopherols. Before enzymatic hydrolysis, CODD was thoroughly bleached to remove peroxides.

Table 1: Analytical characteristics of CODD

Component	Amount (%w/w)	Component	Amount (%w/w)
FFA (Total)	77.25±1.313	Neutral glycerides	19.05±0.116
Palmitic acid	7.15±0.256	TAG	37.26±0.136
Oleic acid	51.67±0.157	DAG	43.06±0.179
Linoleic acid	26.46±0.078	MAG	19.45±0.112
Linolenic acid	6.14±0.013	Unsap. Matters	3.1±0.003
Arachidic acid	2.02±0.005	Sterols	48.21±0.106
Eicosenoic acid	3.14±0.014	Tocopherols	13.26±0.135
Stearic acid	1.44±0.012	Hydrocarbons and others	37.67±0.121
Myristic acid	1.38±0.008		

3.2 EFFECT OF CONCENTRATION OF WATER FOR BIOHYDROLYSIS REACTION

Biohydrolysis of neutral glycerides present in CODD is actively dependent on the water content in the reaction mixture. So for the identification of optimum water content required for biohydrolysis, the reaction was performed at 40°C for 6 hrs in the presence of 5% Amano 30 enzyme by varying the amount of water from 30% to 70% by weight of neutral glycerides as shown in Fig. 1. It has been observed from the Fig. that increasing concentration of water from 30% to 60% enhances the rate of hydrolysis but after 60% concentration of water, % FFA did not enhance. This may be due to the fact that the active sites of enzyme is bound with substrate or water until the sites are available after that enhancement of water content did not increase the rate of biohydrolysis. So 60% is the optimum water content for this biohydrolysis reaction.

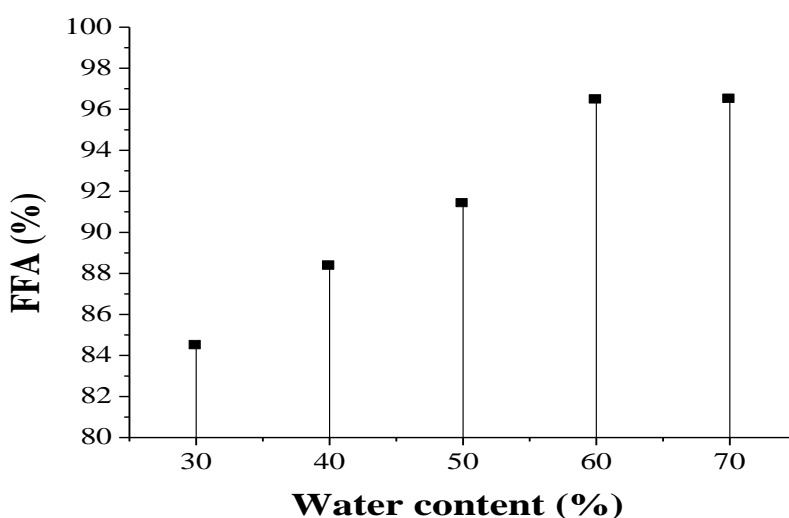


FIG 1: EFFECT OF WATER CONTENT OF BIOHYDROLYSIS REACTION OF CODD

(TEMPERATURE – 40°C, NS 40013 – 5%, TIME – 6 HRS)

3.3 EFFECT OF TEMPERATURE FOR BIOHYDROLYSIS REACTION

Temperature has a strong influence on the activity of enzyme as each enzyme can withstand up to a certain temperature beyond which it has been deactivated. For identifying the optimum temperature for the biohydrolysis reaction, the reaction mixture of CODD and water were treated in the presence 5% Amano 30 enzyme for 6 hrs by varying the temperature from 20⁰C to 50⁰C as shown in Fig. 2. It has been observed from the Fig. that enhancing temperature from 20⁰C to 40⁰C increases the rate of biohydrolysis but after that increasing temperature did not enhance the % FFA. So 40⁰C is the optimum temperature for this biohydrolysis reaction.

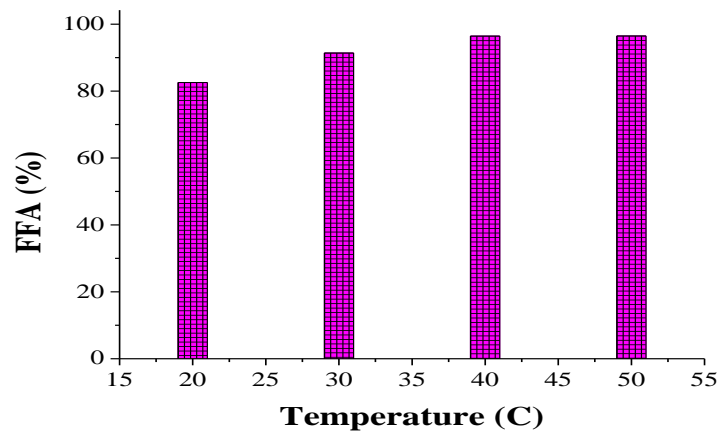


FIG 2: EFFECT OF TEMPERATURE OF BIOHYDROLYSIS REACTION OF CODD

(WATER CONTENT – 60%, NS 40013 – 5%, TIME – 6 HRS)

3.4 EFFECT OF REACTION TIME FOR BIOHYDROLYSIS REACTION

For the identification of optimum duration of reaction, the biohydrolysis reaction has been carried out between CODD and water (60%) in the presence 5% Amano 30 enzyme at 40⁰C from 1 to 6 hrs as shown in Fig. 3.

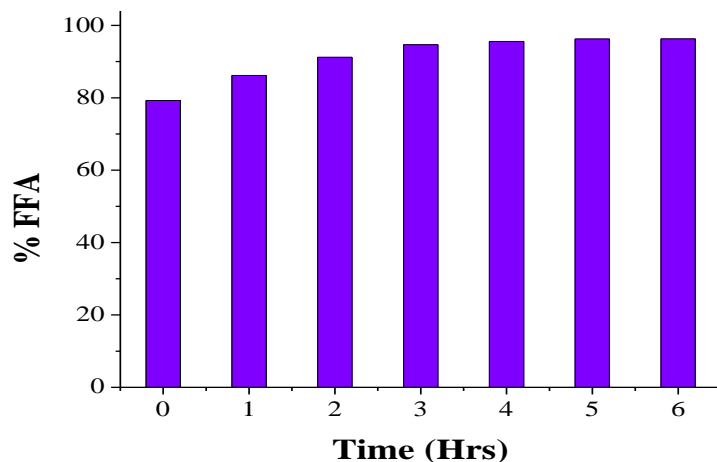


FIG 3: EFFECT OF REACTION TIME OF BIOHYDROLYSIS REACTION OF CODD

(WATER CONTENT – 60%, NS 40013 – 5%, TEMPERATURE – 40⁰C)

It has been observed from Fig. 3 that initially the degree of biohydrolysis of neutral glycerides was very rapid. Within 6 hrs, it was almost complete. Further enhancement of reaction time did not contribute any encouraging results. Initial high rate of hydrolysis reaction is due to the rapid complex formation between the glyceride molecules and active sites of enzyme molecules. So within 3-4 hrs, maximum conversion has been achieved and 6 hrs is the optimum time for maximum conversion.

3.5 EFFECT OF ENZYME CONCENTRATION FOR BIOHYDROLYSIS REACTION

Enzyme plays a significant role for successful conversion of biohydrolysis reaction. For the effect of concentration of enzyme as catalyst, the biohydrolysis reaction between CODD and water (60%) was performed for 6 hrs by varying the concentration of enzyme from 1% to 5% at 40⁰C as shown in Fig. 4. It has been observed from the Fig. that lower concentration of enzyme could not hydrolyse neutral glycerides completely. This may be due to insufficient amount of active sites of enzyme compared to higher amount of substrate. So the reaction would be incomplete. Increasing the concentration of enzyme up to 5% actually supplies the required amount of active sites of enzyme which completes the binding of all substrates and ultimately contributes full conversion of reaction. Beyond 5% concentration of enzyme, conversion did not enhance. This may be due to agglomeration of enzyme which actually make unavailable of all the active sites. So 5% is the optimum concentration of enzyme for the present biohydrolysis reaction.

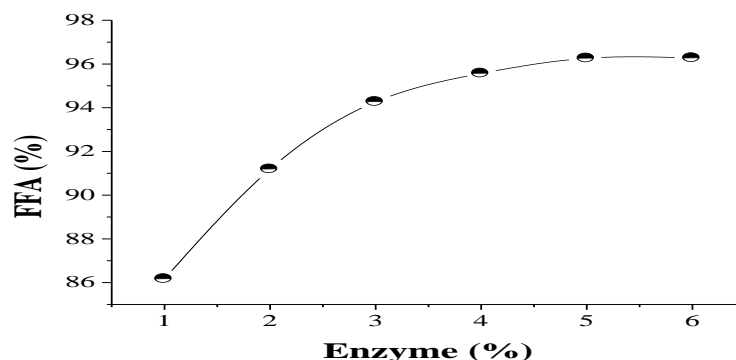


FIG 4: EFFECT OF ENZYME CONCENTRATION FOR BIOHYDROLYSIS REACTION OF CODD

(WATER CONTENT – 60%, TIME – 6 HRS, TEMPERATURE – 40⁰C)

3.6 BIOESTERIFICATION OF HYDROLYSED CODD

Bioesterification between hydrolysed CODD and methanol has been performed for the preparation of biodiesel as depicted in Fig. 5. It shows a typical analysis of time study vs percent conversion of biodiesel from hydrolysed CODD and methanol w.r.t molar ratio of substrates, concentration of enzyme, mixing intensity of reaction mixture and temperature of bioesterification reaction. It has been observed from Fig. that 4 hrs is the optimum time duration through which maximum conversion has been achieved. Here the reaction parameters are 4:1 molar ratio of methanol to hydrolysed CODD, temperature 60⁰C, mixing intensity 600 rpm for 4 hrs and 5% enzyme Novozyme 40013. After that increasing time duration did not enhance the conversion of biodiesel w.r.t. reaction parameters. Maximum conversion has which has been achieved by maintaining these reaction parameters are nearly 94%.

Optimum molar ratio of methanol and hydrolysed CODD was identified as 4:1 beyond which increasing time or molar ratio did not enhance the percent conversion.

This may be due to the fact that enhancing molar ratio decreases the time between two collisions for the substrates which ultimately affect the conversion. Temperature is a significant parameter of any reaction especially for enzymatic reaction. Each enzyme has a specific temperature beyond which it would be deactivated. 60°C temperature is the optimum temperature in the present research investigation for enzyme Novozyme 40013 at which it contributes maximum conversion and beyond which it does not enhance the production due to its deactivation.

Mixing intensity or stirring rate is another important criteria for enzymatic reaction. Increasing mixing intensity actually enhance the no of collision between substrates and enzymes through which enzyme-substrate complex has been formed. This complex formation is essential for enzymatic reaction which ultimately gives the product. Optimum mixing intensity which has been identified was 600 rpm for the present bioesterification reaction. Increasing beyond this rpm probably hampers the complex formation which declines the product formation. Optimum concentration of enzyme was identified as 5% beyond which percent conversion of biodiesel did not increase. This may be due to the agglomeration of enzyme which blocks the active sites and ultimately hampers percent conversion.

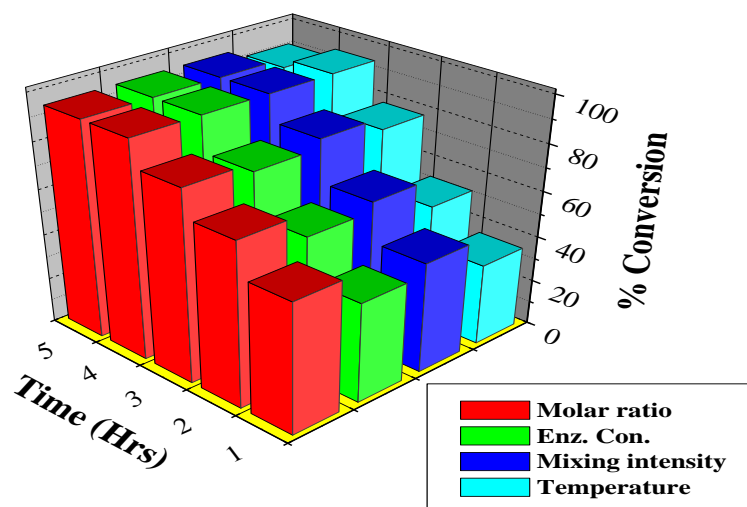


FIG 5: TYPICAL ANALYSIS OF TIME STUDY VS CONVERSION OF BIOESTERIFICATION REACTION BETWEEN HYDROLYSED CODD AND METHANOL

3.7 CHARACTERISTICS OF CODD BIODIESEL

Good quality biodiesel is useful for the performance and emission characteristics of the diesel engine. In our study, biodiesel is comparable with diesel fuel as shown in Table 2. It shows that flash point and fire point are quite high for biodiesel than conventional diesel fuel indicating that biodiesel can be used safely from fire hazards. Calorific value is somewhat less in case of biodiesel that diesel fuel but with regard to other characteristics, it is good and can be used instead of diesel fuel or with mixture of diesel fuel.

Table 2: Characteristics of CODD biodiesel

Characteristics	Biodiesel	Diesel fuel	Test method
Density (gm/cc)	0.852±0.001	0.840	ASTMD-4052-96
Flash point (°C)	217±0.748	56	ASTMD-93
Fire point (°C)	222±0.635	62	-----
Kinematic viscosity @40°C (Cst)	4.74±0.011	3.02	ASTMD-445
Specific gravity	0.879±0.004	0.85	-----
Calorific value (Kcal/Kg)	3674±1.985	4285	ASTM-6751
Acid value (mg/KOH)	0.56±0.014	0.36	ASTMD-64-01
Moisture (%)	0.02	0.02	-----
Cetane number	40±0.211	49	ASTMD-6751

4. CONCLUSION

Bioprocess technology has been adopted in the present research methodology for the preparation of alternative fuel, biodiesel from cheap raw material like canola oil deodorizer distillate. Initially biohydrolysis reaction parameters have been optimized and later utilized for canola oil deodorizer distillate and water in the presence of enzyme Amano 30. Nearly 98% conversion has been achieved and all neutral glycerides were hydrolysed. Finally biodiesel has been produced successfully (96% conversion) through bioesterification reaction between hydrolysed canola oil deodorizer distillate and methanol in the presence on enzyme Novozyme 40013. This bioprocess technology for biodiesel production from canola oil deodorizer distillate through biohydrolysis and bioesterification reaction in the presence of biocatalysts is a novel technology which can encourage the future researchers to mitigate the scarcity of fossil fuel in near future.

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