Recycling Technology - A Cost Effective Approach for the Synthesis of Alternative Fuel

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ABSTRACT

Recycling is an effective technology for minimization of process cost. Recycling of biocatalyst along with recycling of used oil is a new technique for the preparation of alternative fuel. Preparation of alternative fuel through cost minimization is supposed to be the most challenging job in the present academicians and researchers. Biodiesel is one of the most important alternative fuels in the near future and it attracts considerable attention as environment friendly, renewable and non-toxic fuel. In the present research investigation, waste cooking oil (WCO) is utilized as cheap raw materials for this purpose and enzyme recycling technology has been adopted to prepare biodiesel. Recycling of enzyme is a novel technology which can reduce the process cost. In our study, nonspecific enzyme Novozyme 435 (Candida antarctica) is utilized and recycled ten times for the transesterification reaction of WCO and methanol maintaining definite reaction parameters like alcohol to oil molar ratio, reaction temperature, mixing intensity and biocatalyst concentration. The physical properties of WCO methyl ester and diesel fuel have been compared and it shows significant results. So recycling of enzyme for the production of alternative fuel from recycled oil can be utilized to mitigate scarcity of non-renewable fuel in the future world.

Keywords: Waste cooking oil, Biodiesel, Candida antarctica, Biocatalyst, Recycling.

1. Introduction

Alternative fuel is one of the important issues in the present researchers and academicians throughout the world. To prepare it from cheap sources through cost effective methodology is another prime thinking nowadays which spreads in scientists and technologists of different areas of like chemistry, biology, mathematics, engineering fields and many more. Technological development is only useful to the whole of mankind whenever the product or production cost is minimum. Preparation of alternative fuel or biodiesel, its use and emission analysis is studied by many researchers using various techniques [1-9]. One of the cost effective methodologies can be obtained by selecting cheap raw materials. In this regard, recycled oil or WCO plays an important role for the production of alternative fuel. Many researchers use WCO for biodiesel production which ensures cost effective fuel. Present author also identified WCO for production of alternative fuel using bioprocess technology [10].

Karmee et. al [11] also identified WCO as cost effective feedstock for alternative fuel in Hong Cong. WCO has also been utilized for biofuel production by many researchers and scientists using different chemical and biochemical catalyst [12-18]. Another method of minimizing cost for biodiesel production is recycling of catalyst. Chemical catalyst is difficult to reuse due to complex separation and isolation procedure. Biocatalyst can easily be recycled for this purpose. Reuse of biocatalyst for the synthesis of biodiesel has been applied by the present author by taking rice bran oil fatty acid distillate as raw material [19]. But rice bran oil fatty acid distillate has been used as soap making process and some other purposes.

Babaki et. al [20] used 20 cycles of immobilized enzyme from Thermomyces lanuginosus and studied the effect of water, t-butanol and blue silica gel content for biodiesel production. Aguieiras et. al [21] investigated the reuse of immobilized lipases in biodiesel synthesis and identified the influence of different solvents in lipase activity. Azócar et. al [22] reutilized lipase in an anhydrous medium with enzyme reutilization to produce biodiesel with low
acid value. Chen et al. [23] studied the optimization of continuous biodiesel production in packed bed reactor by reusing enzyme. Manurung et al. [24] recycled Lipozyme RM IM to produce biodiesel from Crude Palm Oil (CPO) as feedstock. But very few studies have been made regarding the production of biodiesel using recycled raw materials with recycling of enzyme. In the present research investigation, WCO have been identified as recycled raw material for biodiesel production with the reuse of enzyme Novozyme 435 (Candida antarctica) after optimization of reaction conditions. Results showed that ten times of recycling of enzyme has been successfully done by applying 6:1 molar ratio of MeOH and WCO at 60°C temperature for 8 hrs with 8% biocatalyst.

2. Experimental

2.1 Materials

WCO was collected from different cafeterias, fast-food centres and restaurants of Madhyamgram, Kolkata, West Bengal. The enzymes used in the present study was Novozyme 435, an immobilized non specific lipase based on immobilization via interfacial activation of lipase B from Candida antarctica on a resin, Lewatit VP OC 1600 with ester synthesis activity of 10000 propyl laurate unit/g and it was a kind gift of Novozyme South Asia Pvt. Ltd. Bangalore, India. 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 Optimization of Reaction

For optimization of transesterification reaction, initially 250 ml of WCO 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, reaction was carried out by adding methanol in an appropriate proportion using solvent hexane fitted with a water condenser and stirred by a magnetic stirrer for 8 hours maintaining other reaction conditions in the presence of 8% (w/w) immobilized enzyme Novozyme 435 (w/w). Stepwise addition of methanol was allowed to minimize the deactivation of enzyme.

2.3 Product Analysis

For the analysis of the products, definite amount of samples were withdrawn into a capped vial at suitable intervals, immediately immersed in boiling water for at least 5 minutes to denature the enzyme and stop the reaction. After that the samples were centrifuged for 15 min to remove immobilized lipase. The supernatant part was taken in hexane and no leaching of enzyme was observed in this part. It was then evaporated to dryness and the products were isolated and their amounts were determined by thin layer chromatographic (TLC) method. TLC was done by spotting the lipid mixture on a silica-gel G plate (0.2 mm thick) using hexane-diethyl ether-acetic acid (90:10:1) as a developing solvent. The lipid spots were identified by iodine absorption with triacylglycerol (TG), diacylglycerol (DG), monoacylglycerol (MG) and BD as standard. The composition of WCO esters was determined by column chromatography using silicic acid as an adsorbent and 160 mL of hexane-diethyl ether: 99:1 as eluting solvent. The enzyme was washed with hexane, dried and reused for the next experiment. During recycling of enzyme, same procedure has been followed for identification and determination of WCO esters (biodiesel). Biodiesel characterization after each batch of experiment was done according to the American Standard Testing Method.
Values are reported as mean ± s.d., where n=3 (n=no of observations). Enzyme recycling was done ten times in laboratory scale for preparation of biodiesel. After each esterification process, the enzyme was collected through filtration. It was then washed with solvent hexane and vacuum dried. Used enzyme was then reused further for biodiesel synthesis maintaining identical parameters and process conditions.

3. Results and Discussions

3.1 Analysis of WCO

The physicochemical properties of WCOs for ten batches are shown in Table 1. Ten batches of WCO were identified as Batch no. 1, 2, 3, 4, 5 and so on. The characteristics of each batch was determined based on free fatty acids (FFAs), iodine values, neutral glycerides, kinematic viscosity, moisture and peroxide value.

Table 1: Physicochemical properties of WCO

| Batch No. | Neutral glycerides (wt%) | Iodine value (I2/100g) | FFA (%) | Moisture (%) | Kinematic Viscosity at 40°C (mm²/s) | Peroxide value (meq/kg) |
|-----------|--------------------------|------------------------|---------|--------------|------------------------------------|------------------------|
| 1         | 88.78±0.108              | 87.57±0.139            | 6.21±0.027 | 0.2456       | 28.67± 0.135                      | 20.49± 0.166           |
| 2         | 89.23±0.111              | 101.56±0.167           | 5.57±0.017 | 0.2011       | 32.46± 0.179                      | 8.23± 0.175            |
| 3         | 89.72±0.154              | 89.91±0.177            | 5.06±0.011 | 0.2032       | 30.71± 0.131                      | 12.59± 0.123           |
| 4         | 90.57±0.187              | 88.98±0.167            | 4.30±0.017 | 0.1837       | 31.67± 0.123                      | 12.34± 0.183           |
| 5         | 91.11±0.109              | 89.99±0.172            | 4.37±0.012 | 0.1934       | 27.38± 0.157                      | 12.61± 0.113           |
| 6         | 90.23±0.186              | 102.45±0.163           | 4.58±0.027 | 0.2148       | 28.40± 0.159                      | 8.23± 0.127            |
| 7         | 89.45±0.138              | 99.67±0.167            | 5.29±0.012 | 0.2025       | 23.90± 0.198                      | 8.59± 0.129            |
| 8         | 91.88±0.184              | 103.78±0.191           | 3.86±0.016 | 0.1639       | 29.98± 0.185                      | 8.23± 0.135            |
| 9         | 92.05±0.195              | 96.26±0.168            | 3.47±0.011 | 0.1612       | 32.78± 0.164                      | 11.34± 0.108           |
| 10        | 91.34±0.188              | 90.69±0.111            | 4.71±0.032 | 0.1954       | 29.89± 0.191                      | 12.39± 0.123           |

It has been found from Table 1 that the neutral glycerides of ten samples are in the range of 89.23±0.111 to 92.05±0.195%. This value indicates hydrolysis of oils during prolonged cooking. Iodine value of WCO samples are in the range of 87.57±0.139 to 103.78±0.191 I2/100g. The upper limit of iodine value is defined as 120 by EN14214 due to polymerization tendency of high iodine value fuels. In addition, the strong relationship between the oxidative instability with high iodine number is observed for the biodiesels. In our study, iodine values of all WCO samples are within the maximum limit. The properties like kinematic viscosity (23.90 ± 0.198 to 32.78 ± 0.164 mm²/s at 400C) and FFA content (3.47±0.011 to 6.21±0.027%) are found to vary significantly. For WCO
samples, it is found that kinematic viscosity and FFA content increase with the darkness of oil colour. This may be due to the fact that during cooking process at high temperature, some additional volatile and non-volatile compounds are generated in the oil causing darker the oil colour and the higher the oil viscosity and FFA content. Peroxide value of WCO samples are found to be in the range of 8.23± 0.127– 20.49± 0.166 meq/kg. Higher value of peroxide value affects the activity of catalyst specifically biocatalyst. So before processing, peroxide value should be low for WCO samples.

3.2 Optimization of Reaction Parameters

Initially, biodiesel preparation from WCO has been optimized and the optimized parameters have been applied for ten batches during recycling. Optimization of the transesterification reaction in laboratory scale between WCO and methanol has been carried out by using enzyme Novozyme 435. The optimized parameters identified are 6:1 molar ratio of MeOH and WCO, 60°C temperature, 8% enzyme for 8 hrs of reaction duration.

FIG. 1: ANALYSIS OF MOLAR RATIO OF MEOH TO WCO FOR BIODIESEL PRODUCTION
[TEMPERATURE: 60°C, TIME: 8 HRS AND ENZYME NS 435: 8% (W/W)]

FIG. 2: ANALYSIS OF TEMPERATURE FOR BIODIESEL PRODUCTION
[MOLAR RATIO: 6:1 (MEOH:WCO), TIME: 8 HRS AND ENZYME NS 435: 8% (W/W)]
Fig. 1 and Fig. 2 show the optimization of molar ratio of methanol: WCO and temperature of reaction respectively for biodiesel production. It has been observed from Fig. 1 that 6:1 molar ratio is the optimum ratio of methanol and WCO for maximum conversion of biodiesel. Enhancement of amount of alcohol did not increase the rate of production due to the decrement of possible no of collisions between the molecules. 60°C temperature is the ideal temperature after which rate of conversion decreases probably due to the inactivation of enzyme at higher temperature.

**FIG. 3: ANALYSIS OF ENZYME CONCENTRATION FOR BIODIESEL PRODUCTION**

[MOLAR RATIO: 6:1 (MEOH:WCO), TIME: 8 HRS AND TEMPERATURE: 60°C]

**FIG. 4: ANALYSIS OF REACTION TIME FOR BIODIESEL PRODUCTION**

[MOLAR RATIO: 6:1 (MEOH:WCO), ENZYME NS 435: 8% (W/W) AND TEMPERATURE: 60°C, CONVERSION MEASURED AT 2, 4, 6, 8 AND 10 HRS]

Optimization of concentration of enzyme and duration of transesterification reaction were identified as shown in Fig. 3 and Fig. 4 respectively. Increasing concentration of enzyme enhances the conversion of reaction and optimization was observed at 8% concentration of enzyme as shown in Fig 3. Further enhancement of enzyme
concentration does not increase the conversion of biodiesel and it may be due to the agglomeration of enzyme at its higher concentration which ultimately decreases the availability of active sites of enzymes. Production conversion vs time has been analysed in Fig. 4 through a pie chart diagram. It has been observed from the Fig. that optimization of reaction time goes for 8 hrs where 92.37% conversion was achieved. Further continuation of reaction has a very minor impact for production efficiency.

### 3.3 Recycling of Enzyme

After optimization of reaction parameters, recycling of enzyme has been done for ten times with ten new batches of WCO and methanol. Time study analysis w.r.t percent conversion of biodiesel is shown in Table 2.

#### Table 2: Time study analysis w.r.t. percent conversion of biodiesel

| Batch no | 2 hrs | 4 hrs | 6 hrs | 8 hrs | 10 hrs |
|----------|-------|-------|-------|-------|--------|
| 1        | 42.64±0.102 | 59.38±0.127 | 80.49±0.162 | 92.35 ±0.209 | 92.45±0.235 |
| 2        | 42.60±0.094 | 59.34±0.143 | 80.33±0.173 | 92.24 ±0.193 | 92.27±0.217 |
| 3        | 42.44±0.115 | 59.22±0.149 | 80.21±0.155 | 92.17 ±0.222 | 92.18±0.202 |
| 4        | 42.37±0.136 | 59.02±0.173 | 80.17±0.168 | 92.01 ±0.194 | 92.03±0.194 |
| 5        | 42.05±0.129 | 58.91±0.142 | 80.13±0.151 | 91.89 ±0.188 | 91.91±0.176 |
| 6        | 41.93±0.119 | 58.83±0.162 | 80.03±0.172 | 91.81 ±0.173 | 91.83±0.206 |
| 7        | 41.88±0.107 | 58.76±0.147 | 79.91±0.169 | 91.73 ±0.210 | 91.77±0.213 |
| 8        | 41.72±0.111 | 58.66±0.145 | 79.82±0.166 | 91.66 ±0.179 | 91.62±0.201 |
| 9        | 41.54±0.137 | 58.47±0.152 | 79.77±0.158 | 91.58 ±0.180 | 91.49±0.212 |
| 10       | 41.33±0.128 | 58.38±0.163 | 79.69±0.173 | 91.39 ±0.204 | 91.32±0.216 |

Here, the percent conversion of biodiesel was observed from the amount of methyl ester through chromatographic method at definite time interval i.e. 2 hrs, 4 hrs, 6 hrs, 8 hrs and 10 hrs. The optimized parameters were maintained in all ten batches for esterification reaction. It has been observed from Table 2 that reusing of enzyme as catalyst for the esterification reaction is very much effective. The same enzyme may also be used even after ten batches which may help to decrease the process cost.

Table 2 shows that percent conversion of WCO to biodiesel in Batch 1 was 92.35±0.209% after 8 hrs of reaction while after using 10 times, the same reused enzyme can convert 91.39±0.204%. So conversion efficiency of enzyme is still active after ten batches and it can be recycled many more times which helps a lot to minimize the process cost. Initial percentage of FFA is different for different batches as the WCO was collected from different sources. The characterization of biodiesel was done for all ten batches and these are compared with diesel fuel and biodiesel standard. Table 3 shows the different comparative characteristics of ten batches of biodiesel. It has been
observed from the table that preparation of biodiesel can be successfully done using recycled oil and recycled enzyme and properties of biodiesel for each batch has close proximity with biodiesel standard and diesel fuel. Cetane number and flash point are comparatively better in case of biodiesel than diesel fuel as observed from the Table. So use of biodiesel as fuel is safer than diesel fuel. Other properties like specific gravity, kinematic viscosity, density and cloud point are quite similar. Calorific value of biodiesel is somewhat less than diesel fuel though it is in the range of biodiesel standard. So biodiesel prepared from WCO and methanol using recycled enzyme has a good conformity with diesel fuel and is in good agreement with biodiesel standard. This work also proves that the efficacy of enzyme remains almost equal even after using many times which helps to reduce the process cost.

**Table 3: Comparative characteristics of biodiesel with diesel fuel**

| Material | Specific gravity (15°C) | Kinematic Viscosity (mm²/s) | Density at 150°C (kg/m³) | Calorific value (MJ/kg) | Cloud point (°C) | Flash point (°C) | Cetane number |
|----------|-------------------------|-----------------------------|--------------------------|-------------------------|-----------------|-----------------|---------------|
| BD Std.  | 0.86 - 0.90             | 1.96 - 6.0                  | 865 - 900                | 33-40                   | 5               | >120            | >40           |
| Diesel  | 0.82 - 0.95             | 1.3 – 4.1                   | 820 - 860                | 45                      | -----           | 60 - 80         | 50            |
| Batch 1 | 0.84±0.001              | 4.96±0.007                  | 880.6±0.312              | 37.46±0.117             | 5.3±0.003       | 129±0.138       | 51±0.134      |
| Batch 2 | 0.83±0.002              | 4.91±0.002                  | 881.6±0.273              | 37.33±0.121             | 5.4±0.001       | 130±0.133       | 50±0.132      |
| Batch 3 | 0.84±0.001              | 4.98±0.010                  | 883.6±0.310              | 37.01±0.132             | 5.3±0.002       | 126±0.124       | 51±0.129      |
| Batch 4 | 0.82±0.006              | 4.96±0.002                  | 885.6±0.276              | 37.51±0.114             | 5.3±0.004       | 126±0.151       | 56±0.145      |
| Batch 5 | 0.87±0.002              | 4.92±0.011                  | 884.6±0.317              | 37.80±0.109             | 5.4±0.003       | 5.4±0.003       | 53±0.185      |
| Batch 6 | 0.81±0.001              | 4.93±0.007                  | 882.6±0.257              | 36.89±0.099             | 5.2±0.002       | 128±0.137       | 55±0.149      |
| Batch 7 | 0.82±0.002              | 4.94±0.008                  | 885.6±0.311              | 36.97±0.128             | 5.3±0.004       | 128±0.129       | 52±0.174      |
| Batch 8 | 0.84±0.004              | 4.90±0.009                  | 883.6±0.311              | 37.07±0.125             | 5.1±0.001       | 129±0.135       | 50±0.128      |
| Batch 9 | 0.87±0.001              | 4.97±0.009                  | 881.6±0.276              | 37.27±0.103             | 5.5±0.003       | 127±0.144       | 51±0.152      |
| Batch 10| 0.88±0.004              | 4.93±0.004                  | 883.6±0.307              | 36.88±0.118             | 5.6±0.002       | 122±0.128       | 53±0.148      |

**4. CONCLUSION**

Recycling of enzyme using recycling oil for the preparation of alternative fuel is a novel idea which reduces the process cost as well as contributes a clean process technology. Waste cooking oil is used as cheap raw material in this methodology which also helps to produce low-priced biodiesel. Activity of enzyme is slightly reduced for
recycling of biocatalyst though it does not hamper the productivity of the process. Initial process parameters have been optimized which is applied in recycling technology. In our study, ten times recycling have been successfully completed with desired conversion of biodiesel. This recycling technology for enzymatic production of biodiesel from recycled raw materials is useful to mitigate the scarcity of fossil fuels in near future. Our process technology contributes a new ray of knowledge to the future researchers to think differently for the production of environment friendly alternative fuel.

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