Chapter-12

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Biolubricant synthesis from waste cooking oil via enzymatic hydrolysis followed by chemical esterification

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Abstract

BACKGROUND: Lubricants manufactured conventionally from non-renewable mineral oil resources are not biodegradable and are liable to cause adverse environmental impacts. Biodegradable vegetable oils present a promising lubricant feedstock alternative. Waste cooking oil (WCO), which otherwise finds no immediate potential utilization can be successfully used to synthesize bio-lubricant. A novel synthetic method was developed by using the two-step process of C. rugosa lipase-mediated hydrolysis of WCO to free fatty acids (FFA) followed by Amberlyst 15H esterification of FFA with octanol. The octyl esters produced was the desired biolubricant.

RESULTS: The effect of different physico-chemical parameters like temperature, catalyst loading, agitation speed, molar ratio of octanol : FFA and the presence of different desiccants on the esterification reaction was examined. The optimum conditions to get maximum yield of biolubricant in minimum time were, octanol : FFA molar ratio = 3 : 1, temperature = 80 °C, catalyst = 2 g and desiccant (preferably silica gel powder) = 50% weight of FFA. Fourier transform infrared spectroscopy confirmed that the product formed was ester.

CONCLUSION: Biolubricant (octyl esters) was prepared efficiently from WCO by the two-step process developed. This novel approach represents a viable means of producing lubricants from wastes which are renewable in nature and can be an alternative to non-renewable mineral oil feedstocks.

Keywords: waste cooking oil; hydrolysis; esterification; biolubricant

INTRODUCTION

The growing impetus for sustainable development has amplified the synthesis of ‘environment-friendly’ bio-based products in recent years. This rising momentum has a holistic approach in bringing together different fields of science to establish new insights in green technology for ending a pollution free environment. Among various renewable feedstocks, vegetable oils provide the basis for production of multifarious environmentally acceptable products. The application of vegetable oils in the manufacture of lubricants is showing marked escalation in research and technology. Conventional lubricants are prepared from mineral oil, which is a non-renewable source. Depleting mineral oil resources with increasing crude oil prices has highlighted the urgent need to manufacture non-mineral oil based lubricants. Vegetable oils represent promising alternatives to other synthetic and mineral-oil based lubricants due to their specific functional attributes such as high viscosity index, high lubricity, high flash point, very low volatility, bio-degradability, etc. It has been claimed that about 90% of the existing lubricants can be replaced by biolubricants. It is so it is time to explore the natural raw materials to formulate biolubricants. European countries like Germany, France and Austria, have already led efforts in this region. Fatty acid alkyl esters having 22 to 26 carbon atoms can serve as biolubricant components. Vegetable oil is converted to fatty acids via hydrolysis. These fatty acids are converted to their corresponding esters with higher alcohols (C8 to C14) in the presence of suitable catalyst, for use as lubricants. Investigations of biolubricant preparation have been conducted using a number of vegetable oils such as sunflower, soybean, castor, rapeseed, palm, jatropha, etc. Both enzymatic as well as chemical catalysts have been found to be effective for biolubricant production. The use of various lower and higher alcohols, namely methanol, ethanol, n-propanol and n-octanol, for the synthesis of methyl, ethyl, propyl and octyl esters by transesterification of vegetable oil has been reported in literature.

Biolubricants are produced mostly from expensive virgin vegetable oils, which accounts for 70–80% of their total production cost. Therefore the need for processing from cheaper feedstocks is of great interest. On the other hand waste minimization and reuse is a vital concern of governments worldwide. In order to reduce defilement of the environment, better ways for waste
utilization are being investigated. To meet these demands waste cooking oil (WCO) can be used as an excellent feedstock for lubricant base stock production.

The novelty of the present study lies in the use of WCO as feedstock for biolubricant production by using the two-step process of *C. rugosa* lipase-mediated hydrolysis followed by Amberlyst 15H esterification with octanol as well as determination of the effect of different physico-chemical parameters on esterification of the fatty acids. Octanol has been selected as the working alcohol since it is the cheapest among the higher chain alcohols (C8 to C14). The advantages of this two-step process developed over single-step alkali/acid or enzymatic transesterification are: feedstock flexibility i.e. acceptance of feedstock with any percentages of FFA and water.\(^\text{15}\)

The hydrolysis of WCO occurs as follows:

\[
(\text{RCO})_2\text{C}_\text{H}_5 + 3\text{H}_2\text{O} \leftrightarrow 3\text{RCOOH} + \text{C}_\text{H}_3\text{O}_2
\]

The esterification reaction takes place as follows:

\[
3\text{RCOOH} + 3\text{ROH} \leftrightarrow 3\text{RCO}_2\text{R} + 3\text{H}_2\text{O}
\]

**EXPERIMENTAL**

**Materials and methods**

The WCO was provided by a local restaurant with an acid value of 2 mg of KOH g\(^{-1}\) of WCO. *Candida rugosa* lipase (powder) and Amberlyst 15H was obtained from Sigma Aldrich, USA and Himedia Laboratories Pvt. Ltd, Mumbai, India, respectively. The saponification value of WCO was determined following ASTM D5558 at 227 – 229 mg of KOH g\(^{-1}\) of WCO. The water content in the WCO was 0.35 wt% (determined following ASTM D95 – 99). 1-octanol (99% pure) was obtained from Merck. All the other chemicals used in the analysis were of analytical grade and purchased from Merck.

A specialized batch reactor (1.5 L) made of stainless steel was employed to perform the esterification reactions in respective operating conditions. The reactor was equipped with the following features: an electrically driven stirrer with a digital display of speed (rpm); a thermostat that continuously maintained the required temperature (digital display), allowing temperature variation within ± 0.5 °C; a sample pouring inlet duct; and an outlet sampling point equipped with a valve. The schematic diagram of the reactor is shown in Fig. 1.

**Hydrolysis of WCO**

WCO was initially filtered (using ordinary filter paper) to remove carbon particles and other suspended impurities and then poured into glass stoppered bottles (500 mL size). The reaction was initiated by adding a specified amount of lipase solution at 30 °C and 250 rpm in a shaker incubator. The bottles were incubated for different times ranging from 1 to 30 h to attain equilibrium. *C. rugosa* lipase was dissolved in deionized water to prepare the lipase solution. The enzyme concentration was kept constant at 1.0 g L\(^{-1}\) (1 g of lipase powder dissolved in 1 L of water). The water to WCO ratio (v/v) was varied in the range 0.5 : 1 to 5 : 1.

**Separation of FFA following hydrolysis**

An excess of n-hexane was added to the reaction mixture after 30 h of reaction. The mixture was centrifuged at 4000 rpm and 30 °C for 15 min. After an initial centrifugation of 10 min the upper layer of free fatty acids in hexane was separated and then again centrifuged into glass stoppered bottles (500 mL size). The reaction was continued for another 5 min for complete separation. The solvent phase (n-hexane) containing the FFA was separated from the glycerol and water layer at the bottom. Anhydrous sodium sulphate was added to the fatty acid – solvent phase to remove any trace of water. The solvent layer containing fatty acids was filtered and hexane was removed by distillation. FFAs from various flasks were then taken and their initial acid value determined (every time). To measure the FFA concentration 20 mL of ethanol–acetone solution (1 : 1; v/v) was added to 5 mL of FFA and titrated against 0.2N KOH using phenolphthalein indicator to get the acid value of FFA. The percentage hydrolysis was calculated using the following formula: \(^\text{16}\)

\[
\text{Percentage hydrolysis} = \frac{\text{Acid value} \times 100}{\text{Saponification value}}
\]

**Esterification of FFA**

Esterification was performed in the reactor taking 500 mL of fatty acid obtained from several hydrolysis batch experiments. Octanol to fatty-acid molar ratio was varied from 1 : 1 to 4 : 1. Addition of Amberlyst 15H catalyst commenced the reaction. The amount of catalyst was varied from 0.2 to 3 g and reaction temperature was varied from 60 °C to 110 °C. The effect of stirrer speed was also studied in the range 450 to 650 rpm. Desiccants like magnesium sulphate, sodium sulphate and silica gel powder were added to the reaction mixture at 50% of the weight of FFA in order to investigate their effects on the reaction rate. Prior to their use, magnesium sulphate and sodium sulphate were dried at 110 °C and Silica gel powder at 180 °C.

**Analysis of feed and products**

The fatty acid composition of the WCO were determined using a gas chromatograph (Agilent 6890, version N.05.03) equipped with FID detector. The analysis was performed using a DB-23 (Agilent) column (60 m × 320 nm I.D). The carrier gas used was nitrogen and the flow rate was 30 mL min\(^{-1}\) with hydrogen as the make-up gas.
the flow rate was maintained at 1.5 mL min⁻¹. Inlet and detector temperatures were kept at 250 °C and the oven temperature was programmed as 150–190–230 °C, with initial increase of 15 °C min⁻¹ to 190 °C and held for 5 min. Then at 4 °C min⁻¹ up to 230 °C and held for 10 min. Oleic acid, palmitic acid, linoleic acid, stearic acid and traces of myristic and erucic acids were found as the constituent fatty acids of the WCO. The average molecular weight of the total FFA from WCO was 271.66 g mole⁻¹.

The esters formed from the reaction with FFA from WCO and octanol is the desired biolubricant (octyl esters). The acid value of the reaction mixture were determined by titration with alkali (0.02 N KOH) at different times from the beginning to the end of the reaction. The acid value was found to be almost zero by the end of the reaction. This ensured complete utilization of the FFA in the esterification reaction. The percentage conversion of FFA to ester was determined by the following formula:\(^1\,^2\)

\[
\text{Conversion} = \frac{AV_0 - AV_t}{AV_0}
\]

where \(AV_0\) is the initial acid value of the sample and \(AV_t\) is the acid value of the sample at time \(t\). Fourier transform infrared spectroscopy (FT-IR) was employed to the final product to confirm that the product formed is ester. FT-IR scans were carried out in a Jasco-670Plus FT-IR fitted with a DLABTGs detector.

Statistical analysis of data

All experiments were completed in triplicate unless stated otherwise and the results are presented as mean ± standard deviation. Graphs were plotted with the mean values including the error bars. Statistical differences of mean values were analyzed (for esterification reactions) using student’s t-test in STATISTICA software. Differences were considered significant when probability value \(p < 0.05\).

RESULTS AND DISCUSSION

Effect of water on hydrolysis

The effect of different water to WCO ratio on the extent of hydrolysis was investigated. Candida rugosa lipase has been reported as one of the most efficient lipase enzymes for hydrolysis of oils and fats.\(^1\,^5\,^17\,^18\) This enzyme was used in the present study to hydrolyze WCO to FFA. Lipase enzyme has a characteristic interfacial activation.\(^1\,^6\) Figure 2 shows that with increasing water to oil ratio from 0.5 : 1 to 5 : 1 the percentage yield of FFA significantly increased, due to increase in water to oil interfacial area, reaching a maximum of 92% at water to oil ratio 4 : 1 with 1 g L⁻¹ enzyme concentration in 30 h. Hydrolysis reaction occurs simultaneously with reverse esterification reaction catalyzed by lipase. A large amount of water is therefore essential to shift the equilibrium towards hydrolysis.\(^1\,^9\,^20\) Further increase in the water to oil ratio from 4 : 1 to 5 : 1 resulted in reduction of FFA yield. This can be attributed to the fact that higher level of water has a negative effect on lipase activity. Shimada et al. reported the dilution effect of lipase enzyme at higher water level which reduces the efficiency of the enzyme to hydrolyze.\(^20\) The main objective of the work being the investigation of esterification reaction, the hydrolysis step was not studied in detail and only a single concentration of enzyme was taken in account that gave sufficient yield of FFA.

Effect of molar ratio of octanol: FFA on esterification

Alcohol : FFA molar ratio is an important parameter to be optimized in an esterification reaction. Esterification of FFA (from WCO) with octanol at different molar ratios was studied. The reaction temperature was set at 80 °C, agitation speed was maintained at 550 rpm and 2 g catalyst was employed. The conversion increased with increase in the octanol : FFA molar ratio (Fig. 3). Similar results have been reported by others.\(^2\,\,^1\,^2\) Higher amounts of alcohol shift the esterification equilibrium to the right thereby increasing the ester yield.\(^1\) Moreover the rate of ester synthesis also increased with time at higher molar ratios. The maximum conversion of 98% was obtained at an octanol : FFA ratio of 3 : 1 in 3 h. Increasing the molar ratio to 4 : 1 did not show any significant decrease in the time (3 h) required to reach equilibrium. Hence 3 : 1 was chosen as the optimum molar ratio.
Effect of varying amounts of catalyst (Amberlyst 15H) were investigated with octanol:FFA ratio of 3 : 1 at 80 °C and 550 rpm. Figure 4 shows the esterification profile with varying amounts of Amberlyst 15 H. The rate of conversion significantly increased with increase in catalyst amount. 0.2 g catalyst gave a maximum conversion of 83.3% after 8 h of esterification, while 2 g of catalyst brought 98% conversion in just 3 h. Therefore it is evident that esterification reaction is very much dependent on the amount of catalyst loading. More catalyst reveals more active sites which participate in the reaction and catalyse the production of lubricant.\(^{15,23}\) With further increase in Amberlyst 15H to 3 g the rate of fatty acid conversion remained the same, i.e. the reaction rate reached a maximum for Amberlyst 15H loading of 2 g.

**Effect of temperature on esterification**

In order to observe the effect of temperature on the production of biolubricant, esterification reaction was carried out at various temperatures ranging from 60 °C to 110 °C. The ester conversion at different temperatures is reported in Fig. 5. The esterification reaction is endothermic hence increase in temperature resulted in an increase in conversion of FFA to esters.\(^{24,27}\) At higher temperatures the rate of reaction was also found to increase giving 98% conversion in minimum time. Maximum conversion was obtained at 110 °C in 1.5 h whereas it took 3 h to achieve almost the same conversion at 80 °C. The color of the final lubricant product mixture was found to be affected by the temperature as well. The color gradually darkened from light yellow to dark brown from 80 °C to 110 °C. Similar findings have been reported by Ozgulsun et al.\(^{21}\) Since, the product darkened at higher temperatures, 80 °C was chosen as the optimum experimental temperature.

**Effect of stirrer speed (rpm) on esterification**

The influence of external resistance to mass transfer was assessed by varying the stirrer speed from 450 to 650 rpm keeping other reaction conditions the same (80 °C, 2 g catalyst, alcohol:FFA ratio 3 : 1). The experimental results are shown in Fig. 6. The increase in agitation speed did not significantly affect either the final conversion percentage of the reaction or the reaction rate. 550 rpm was selected as the optimum stirring speed for further studies.

**Effect of desiccants on esterification**

Water is also produced as a product in the esterification reaction along with the desired lubricant. This product slows down the reaction rate.
Esterification reaction with varying desiccants (50% w of FFA), octanol: FFA 3:1, temp 80°C catalyst 2 g, 550 rpm \( (n = 3, \text{ mean } \pm \text{ S.D.}) \). Percentage conversion are significantly different in all 0.5–3.5 h reaction time intervals for individual desiccant with respect to percentage conversion just before starting the reaction i.e. \( t = 0 \) h, at the level of \( P < 0.01 \) (except \( P > 0.05 \)). Percentage conversions are also compared with respect to the control, i.e. samples without desiccant (other conditions remaining constant), at a particular reaction time, i.e. 0.5–3.5 h at 0.5 h intervals. Values showed significant enhancement of rate of percentage conversion with the addition of desiccant in the reaction mixture, at the level of \( P < 0.01 \) (except \( P > 0.05 \)).

Product analysis

The final reaction product was distilled in a rotary vacuum evaporator to remove the excess water and octanol and then analyzed using FT-IR. Figure 8 illustrates the typical changes that were found in the absorption peak of the product (ester) formed compared with the FFA absorption data. The characteristic \( \text{C}=\text{O} \) stretching for carboxylic acid of FFA gave a peak at 1709 cm\(^{-1}\) that shifted to 1738 cm\(^{-1}\) for the ester. A characteristic \( \text{C} \equiv \text{O} \) bond peak was seen in the ester produced at 1056 cm\(^{-1}\) wavelength\(^9\). FT-IR scans confirmed that the product formed is ester.

Optimization of reaction parameters

The present initial design was done following the classical approach of parametric study (one parameter at a time). The optimum physico-chemical conditions to achieve maximum conversion for such esterification reaction were found to be octanol: FFA molar ratio = 3:1, temperature = 80°C, catalyst = 2 g. Figures 9 and 10 show contour plots (with two variables at a time) which support the optimum conditions of the present work.

CONCLUSION

The present study revealed that WCO can be successfully hydrolyzed with \textit{Candida rugosa} lipase to get FFA at an enzyme concentration of 1 g L\(^{-1}\) for 30 h. The resulting FFA can be esterified with higher alcohol (octanol) using Amberlyst 15H to produce environment-friendly biolubricant. Temperature, catalyst amount, reactant molar ratio (alcohol : FFA) were found to have significant effects on the reaction rate. The maximum conversion can be achieved in minimum time with the use of an appropriate desiccant to remove the water produced during the esterification reaction. We can conclude from the experiment that the most favourable conditions for maximum (100%) conversion in minimum time are, octanol : FFA molar ratio = 3 : 1, temperature = 80°C, catalyst = 2 g, and desiccant (preferably silica gel powder) = 50% weight of FFA. A key attribute of the two-step process developed is its ability to utilize waste oil to provide a value added, environment-friendly product, i.e. biolubricant.
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REFERENCES

Synthesis of Biolubricant Components from Waste Cooking Oil Using a Biocatalytic Route

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The call for “sustainable development” has laid special emphasis on the “go green” concept and thus countries worldwide are taking initiatives to procure successful “clean and green technologies”. In this milieu the ungenial conventional mineral-oil based lubricants, causing adverse environmental impacts, can efficaciously be replaced by biodegradable vegetable oil based biolubricants. This article presents successful utilization of waste cooking oil (WCO) to synthesize biolubricant using a biocatalytic route. The developed methodology includes a two-step process of enzymatic (Candida rugosa) hydrolysis of WCO to free fatty acids (FFA), followed by biocatalytic (Novozyme 435) esterification of FFA with octanol in a solvent-free system to produce the octyl esters (desired biolubricant components). A classical method of parametric study was employed to explore the effect of different physico-chemical parameters on the esterification reaction. The reaction conditions to achieve maximum conversion (95%) in minimum time were: initial water content = 0.5 wt % of FFA; octanol: FFA molar ratio = 3:1; catalyst = 5 wt % of FFA and temperature = 60°C. Fourier Transform Infrared Spectroscopy confirmed that the product formed was ester. WCO thus serves as an alternative feedstock for biolubricant synthesis and additionally aids in skillful waste minimization and reuse. © 2013 American Institute of Chemical Engineers Environ Prog., 00: 000-000, 2013.

Keywords: waste cooking oil, biocatalysis, hydrolysis, esterification, biolubricant

INTRODUCTION

World demand for lubricants is growing at an alarming rate coupled with increasing technological advancement. Most lubricants are formulated products consisting of 70–90% base oils mixed with functional additives to modify their properties. The base oil can be mineral, vegetable, or synthetic. Conventional lubricants are prepared from mineral oil, which is a nonrenewable resource. An irrevocable decrease in mineral oil reserves with increasing crude oil prices has highlighted the pressing need to manufacture nonmineral oil-based lubricants. Thus, it is high time to explore the natural raw materials to formulate “biolubricants”. Biolubricants must be used in priority for applications where total loss lubricants (TLL) are employed as in two-stroke engines, chainsaw bars and chains, railway switch gears, aquatic machinery and equipment, etc. and in hydraulic fluids and greases where partial loss lubricants (PLL) are required. Biolubricant applications are liable to minimize the risk of environmental contamination and bio-degradability loss as against conventional petroleum based lubricants. European countries, especially Germany and Austria, have already led efforts in this region [1].

Vegetable oils (VOs) represent promising alternatives as a lubricant feedstock to other synthetic and mineral-oils due to some of their specific functional attributes such as high viscosity index, high lubricity, high flash point, very low volatility, bio-degradability, etc. [2,3] The poor thermo-oxidative stability, low pour point and poor low temperature properties of VOs can capably be minimized by various chemical modifications to produce an effectual biolubricant. These eco-friendly lubricants have been successfully formulated from various edible and nonedible plant oils such as rapeseed [4], sunflower [2], palm [5], jatropha [6], castor [7], etc. Production of biolubricants by direct transesterification of VOs with alcohols has been reported in literature [8]. It is also evident that both chemical (acid/alkali) and enzymatic catalysts can bring about effective biolubricant synthesis [9–11].

In this study waste cooking oil (WCO) has been chosen as the lubricant base stock which serves a dual purpose. Being an inexpensive feedstock it reduces production cost and additionally its utilization aids for the waste minimization and reuse. A novel approach of biolubricant synthesis has already been established by the authors in the recent literature [12]. In this work the authors have undertaken a similar approach of biolubricant synthesis, but the significance lies in adopting a complete biocatalytic route, which has not been attempted earlier as per the reported literature. The present study includes a two-step process of Candida rugosa lipase mediated hydrolysis of WCO to free fatty acids (FFA) followed by enzymatic esterification of FFA with octanol to produce biolubricant components using Novozyme 435 as the biocatalyst in a solvent free system. A classical method of parametric study was employed to explore the effect of various physico-chemical parameters like initial water content, octanol: FFA molar ratio, catalyst amount and temperature on the enzymatic esterification reactions. Octanol being cheapest among the higher alcohols was chosen as the working alcohol. Feedstock flexibility i.e., acceptance of feedstock with any percentages of FFA and water is the main advantage of this two-step process developed over single-step alkali/acid or enzymatic transesterification [13]. Additionally, the authors have presented a brief comparison between the present enzymatic (Novozyme 435) esterification and their previous work on chemical esterification (using Amberlyst 15H) process.
EXPERIMENTAL

Materials and Methods

The WCO was provided by a local restaurant with an acid value of 6 mg of KOH g$^{-1}$ of WCO. Candida rugosa lipase (powder) was purchased from from Sigma Aldrich, USA to undergo hydrolysis of WCO. Novozyme 435 (lipase from Candida antarctica immobilized on macroanoric anionic resin) was obtained from Novozymes South Asia, Bangalore, India. The saponification value of WCO was determined following ASTM D5558 and was found to be 236–240 mg of KOH g$^{-1}$. The water content in the WCO was 0.45 wt % (determined following ASTM D959-99). 1-Octanol (99% pure) was obtained from Merck. All the other chemicals used in the analysis were of analytical grade and purchased from Merck.

The esterification reaction was carried out in a specialized batch reactor (1.5 L) made of stainless steel in respective operating conditions. The schematic diagram of the reactor has been provided in the recently published work of the authors [12].

Hydrolysis of WCO and Separation of FFA

WCO was hydrolzyed using 1.0 g L$^{-1}$ C. rugosa lipase (powder) for 30 h at 30°C with 250 rpm in a shaker incubator. The water to WCO ratio (w/v) was kept at 4:1 with a conversion of 92% (determined in our previous study) [12]. After specified reaction time of 30 h an excess of n-hexane was added to the reaction mixture. At first the mixture was centrifuged at 4000 rpm and 30°C for 10 min when the upper layer of free fatty acids in hexane was separated. A final centrifugation of 5 min ensured complete separation. Anhydrous sodium sulphate was added to the fatty acid solvent phase to remove any trace of water. Finally the hexane part was removed by distillation and the FFA components were recovered. FFAs from various flasks were taken and their initial acid value was determined. To measure the FFA concentration 20 mL of ethanol-acetone solution (1:1, v/v) was added to 5 mL of FFA and titrated against 0.2N KOH using phenolphthalein indicator to get the acid value of FFA. The percentage hydrolysis was calculated using the following formula [14]:

$$\text{Percentage Hydrolysis} = \frac{\text{Acid value} \times 100}{\text{Saponification value}}$$

Esterification of FFA

Fatty acids (500 mL) obtained from several batch experiments of hydrolysis were esterified in the batch reactor with a varying octanol to free fatty-acid molar ratio of 1:1 to 4:1. FFA and Novozyme 435 were taken in the reactor vessel and the reaction was initiated with the addition of octanol. The effect of initial water content on the conversion efficiency of Novozyme 435 was investigated at a range of 0.25 -1 wt % of FFA. The amount of catalyst was varied from 1.5 to 10 wt % of FFA and reaction temperature was varied from 40°C to 70°C. In all sets of experiments the agitation speed was kept constant at 250 rpm (determined from preliminary experiments).

Analysis of Feed and Products

The composition of fatty acids present in the WCO were determined using a Gas Chromatograph (Agilent 6890, version N.05.05) equipped with FID detector. The analysis was performed using a DB-23 (Agilent) column (60 m × 320 nm I.D.). The carrier gas used was nitrogen and the flow rate was maintained at 1.5 mL min$^{-1}$. Inlet and detector temperatures were kept at 250°C and the oven temperature was programmed as 150–190–230°C, with initial increase of 15°C min$^{-1}$ to 190°C and held for 5 min. Then at 4°C min$^{-1}$ up to 230°C and held for 10 min. The constituent fatty acids of the WCO were found to be oleic acid, palmitic acid, linoleic acid, stearic acid alongwith traces of myristic and erucic acids. The average molecular weight of the total FFA from WCO was 286.67 g mol$^{-1}$.

The esters produced from the reaction with FFA from WCO and octanol is the desired biolubricant (octyl esters). The acid values of the reaction mixture were determined by titration as described in the previous work of the authors [12]. Fourier transform infrared spectroscopy (FTIR) was employed to the final product to confirm that the product formed is ester. FTIR scans were carried out in a Jasco-670Plus FTIR fitted with a DLATGS detector.

Statistical Analysis of Data

All experiments were completed in triplicate unless mentioned otherwise and the results were presented as mean ± standard deviation. Graphs were plotted with the mean values including the error bars. Statistical differences of mean values were analyzed (for esterification reactions) using student’s t-test by using STATISTICA software. Differences were considered significant when probability value $P < 0.05$.

RESULTS AND DISCUSSION

Effect of Initial Water Content

Initial water content has a profound impact on the enzyme activity. Figure 1 shows the ester conversion in the reaction as a function of initial water content. Initial water content up to 0.5 wt % of FFA has considerably increased the conversion, whereas a further increase of water content negatively affected the conversion. Too low (0.1 wt %) and too high (1 wt %) water content retards the reaction rate. Therefore the optimal water content (0.5 wt % in this study) is essential for the immobilized enzyme to hydrate and work proficiently [15]. The decrease in the conversion rate with increasing water content may be attributed to the fact that at high water content the enzyme particles tend to agglomerate leading to diffusional limitation or it may favor the backward hydrolysis reaction resulting in reduced ester conversion rate[16].

Figure 1 depicts percentage conversion are significantly different in all 0.5–3 h reaction time intervals for each initial water content with respect to percentage conversion just before starting of the reaction i.e., $t = 0$ h, at the level of $P < 0.01$. Percentage conversions are also compared with respect to the control i.e., samples without any initial water (other conditions remaining constant), at a particular reaction time at 0.5-h intervals. Values showed significant enhancement of percentage conversion with the increment of initial water content in the reaction mixture, at the level of $P < 0.01$ (except ** 0.05 > $P > 0.01$ and * $P > 0.05$).

Effect of Molar Ratio of Octanol: FFA on Esterification

The substrate molar ratio is an important parameter to be optimized in a chemical reaction. The octanol: FFA (from WCO) molar ratio was varied to explore their effect on the esterification reaction. The reaction temperature was set at 60°C, agitation speed was maintained at 250 rpm and 2.5 wt % of Novozyme 435 was employed. The conversion increased significantly with the increase in the octanol: FFA molar ratio (Figure 2). Similar results have been reported in the literature [16,17]. Such increase in conversion with increasing octanol: FFA molar ratio is due to the fact that higher amount of alcohol shifts the esterification equilibrium towards right and as a result the ester yield is magnified [13,17]. Moreover the rate of ester formation also increased
with time at higher molar ratios. The maximum conversion was found at the octanol: FFA ratio of 3:1 in 4.5 h time. Further increase in the alcohol: FFA molar ratio to 4:1 did not show any significant increase either in the ester conversion percentage or in the rate of conversion. Thus, considering the limited use of alcohol, 3:1 was chosen as the optimum molar ratio.

Figure 2 shows the percentage conversion that are significantly different in all 0.5–5 h reaction time intervals for each molar ratio with respect to percentage conversion just before starting of the reaction i.e., t = 0 h, at the level of P < 0.01. Percentage conversions are also compared with respect to the control i.e., samples containing only fatty acids without octanol (not shown in figure since no reaction occurred in...
absence of alcohol), at a particular reaction time i.e., 0.5–5 h at 0.5-h intervals (other conditions remaining constant). Values showed significant enhancement of percentage conversion with the increment of octanol in the reaction mixture, at the level of $P < 0.01$ (except $^* P > 0.01$ and $^* P > 0.05$).

**Effect of Catalyst Amount on Esterification**

The effect of varying amounts of biocatalyst (Novozyme 435) was investigated for the esterification reaction with octanol: FFA ratio of 3:1 at 60°C and 250 rpm. Figure 3 shows the esterification profile with varying amounts of Novozyme 435. The rate of conversion significantly increased with the increase in catalyst amount. 1.25 wt % catalyst gave a maximum conversion of 83.93% after 5.5 h of esterification, while 5 wt % of catalyst brought 95% conversion in just 2.5 h time. Therefore it is evident that esterification reaction is very much dependent on the amount of catalyst loading. More catalyst reveals more active sites which participate in the reaction and catalyse the production of lubricant [13]. A further increase in Novozyme 435 amount to 10 wt % although slightly increased the rate of conversion of FFA to ester, but the final conversion percentage remained unchanged. Excess of enzyme present in the reaction medium does not further boost the conversion due to diffusional limitation [18–20]. The enzyme molecules would agglomerate, limiting the entrance of substrates inside the particles and as a consequence the overall conversion does not enhance [21]. The final conversion was not found to increase beyond 95% due to the accumulation of water as a by-product of the reaction. The accrued water is liable to shift the reaction equilibrium towards hydrolysis, ceasing further conversion of FFA to octyl esters. In this study majority of sets of experiments have been conducted with 2.5 wt % catalysts (which also presented satisfying conversion of 95% in 4 h time) in order to reduce costly biocatalyst consumption.

Figure 3 indicates percentage conversion are significantly different in all 0.5–5.5 h reaction time intervals for catalyst amount with respect to percentage conversion just before starting of the reaction i.e., $t = 0$ h, at the level of $P < 0.01$ (except $^* P > 0.05$). Percentage conversions are also compared with respect to the control i.e., samples without catalyst (other conditions remaining constant), at a particular reaction time i.e., 0.5–5.5 h at 0.5 h intervals. Values showed significant enhancement of percentage conversion with the increment of catalyst dosage, at the level of $P < 0.01$ (except $^* P > 0.01$ and $^* P > 0.05$).

**Effect of Temperature on Esterification**

The effect of temperature on the production of biolubricant was observed at a varying range of temperatures from 40°C to 70°C (Figure 4). The reported literatures are in support for the endothermic nature of the esterification reaction i.e., increasing temperature results in an increase in the conversion of FFA to esters [18,22]. This is because temperature rise might cause a reduction in the mixture viscosity of the substrates, thereby enhancing mutual solubility and diffusion process of substrates, thus reducing mass transfer limitations and favoring FFA conversion [20,25]. Similar findings have been found in the present study upto 60°C. A further increase in the reaction temperature did not show any significant increase in conversion. This may be attributed to the fact that high temperatures are liable to cause thermal denaturation of the biocatalyst. It is reported that for Novozyme 435 the optimal working condition is between 40 and 65°C [17,24]. Maximum ester (~95% conversion) was obtained at 60°C in 4.5 h. Thus, 60°C was chosen as the optimal reaction temperature.

Figure 4 shows percentage conversion are significantly different in all 0.5–5.5 h reaction time intervals for each temperature with respect to percentage conversion just before starting of the reaction i.e., $t = 0$ h, at the level of $P < 0.01$ (except $^* P > 0.05$). Percentage conversions are also compared with respect to the control i.e., samples at room temperature of 35°C (other conditions remaining constant), at a particular reaction time i.e., 0.5–5.5 h at 0.5-h intervals. Values showed significant enhancement of percentage conversion with the increment of temperature in the reaction.
mixture, at the level of $P < 0.01$ (except ** $0.05 > P > 0.01$ and * $P > 0.05$).

**Product Analysis**

The FTIR analysis of the final octyl esters, presented in Figure 5, confirmed that the product formed is ester. Before subjecting to spectroscopic analysis the final reaction product was distilled in a rotary vacuum evaporator to remove the excess water and octanol. Figure 5 illustrates the typical changes that were found in the absorption peak of the product (ester) formed compared to the FFA absorption data. The characteristic C=O stretching for carboxylic acid of FFA gave a peak at 1709 cm$^{-1}$ that shifted to 1738 cm$^{-1}$ for the ester. A characteristic CO bond peak was seen in the ester produced at 1056 cm$^{-1}$ wavelength [6,12].

**Figure 4.** Esterification reaction with varying temperature, octanol: FFA 3:1, initial water content 0.5 wt % of FFA, catalyst 2.5 wt % of FFA, 250 rpm ($n = 3$, mean ± S.D).

**Figure 5.** FTIR absorption spectra for FFA and product ester (biolubricant).

**Figure 6.** Contour plot showing the effect on % conversion with the variation of molar ratio and catalyst weight.
Optimization of Reaction Parameters

The present experimental design was done following the classical approach of parametric study (one parameter at a time) [12]. The optimum values of the reaction parameters to achieve maximum conversion (~95%) in minimum time were found to be octanol: FFA molar ratio = 3:1, catalyst = 5 wt % and temperature = 60°C. Figures 6-8 are the contour plots (with two variables at a time) which also support the optimum conditions of the present investigation.

Comparison Between Novozyme 435 and Amberlyst 15H

In a recent publication the authors have reported a successful esterification of FFA (from WCO) and octanol via chemical catalyst Amberlyst 15H that resulted in a decent conversion of about 98% [13]. The reaction conditions to achieve maximum conversion were 80°C, 10 wt % (of FFA) catalyst and 3:1 Octanol: FFA molar ratio. Chemical esterification with Amberlyst 15H is an energy intensive process requiring about 80°C temperature for the catalyst to work efficiently in the present reaction medium, while only 60°C is required for Novozyme 435 catalyzed esterification. The biocatalyst was found to be more active than the chemical catalyst since 0.06 moles of biolubricant was produced per gram of Novozyme 435 per hour which is thrice that of produced by Amberlyst 15H (0.02 moles g⁻¹ of Amberlyst 15H h⁻¹). Therefore higher amount of Amberlyst 15H is required to achieve maximum conversion compared to Novozyme 435. The initial water content was found to affect both the chemical and enzymatic esterification reaction of FFA with octanol. Figure 9 reveals that an increase in the water content inhibits the activity of Amberlyst 15H and the conversion drops to 82% from 98%. Amberlyst 15H being hygroscopic is likely to adsorb water on its surface thereby inhibiting the access of hydrophobic substrate [25]. On the other hand, as already mentioned, for Novozyme 435 an initial water content of 0.5 wt % (of FFA) is essential to bring about maximum conversion, but with the subsequent increase in water content the conversion decreased to 84% from 95% due to diffusional limitation. Thus Novozyme 435 was found relatively more active than Amberlyst 15H in terms of catalytic activity and requires less temperature aiding in less energy consumption. However, the high price of the enzyme (~16.7 USD g⁻¹) could limit its economic usage for large scale biolubricant production subduing its beneficial attributes. On the contrary, Amberlyst 15H costing around 0.258 USD g⁻¹ could be a better choice of catalyst for synthesizing biolubricant due to its cost-effectiveness and higher conversion percentage.

CONCLUSIONS

In the present study a novel route of lipase (C. rugosa) catalyzed hydrolysis followed by Novozyme 435 (C. antarctica) catalyzed esterification reaction in a solvent free system has been established. FFA was successfully generated from WCO through hydrolysis with Candida rugosa lipase at enzyme concentration of 1.0 g L⁻¹ for 30 h. The resulting FFA was esterified with higher alcohol (octanol) using Novozyme 435 to produce environment friendly biolubricant. Initial water content was found to significantly affect the efficiency of the biocatalyst (Novozyme 435) functioning. The physico-chemical parameters like reactant molar ratio (alcohol: FFA), catalyst amount and temperature were found to have substantial effects on the esterification reaction. It can be concluded from the investigated experiment that the optimized parametric values to get a maximum yield of ester (biolubricant) are initial water content 0.5 wt%, octanol: FFA molar ratio = 3:1, catalyst = 5 wt% and temperature = 60°C. The developed two-step process not only promises a cost-effective way of biolubricant synthesis from cheap feedstock, but also provides a successful way for waste utilization. A comparison between Novozyme 435 and Amberlyst 15H catalyzed esterification revealed that both the catalysts can be effectively used for biolubricant production from FFA (derived from WCO). However the results suggest that despite of being less active than Novozyme 435, Amberlyst...
15H is more economical to be used as a catalyst for biolubricant synthesis.

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Optimization of the production parameters of octyl ester biolubricant using Taguchi’s design method and physico-chemical characterization of the product

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The enzymatic esterification of free fatty acids (FFA) from waste cooking oil (WCO) and octanol in a solvent free medium has been investigated. A statistical experimental design method (Taguchi L9 orthogonal array) was implemented to optimize the experimental conditions to maximize conversion of FFA to the corresponding octyl esters. The optimum conditions inferred from the Taguchi analyses were: temperature = 60°C, Novozyme 435 = 5 wt% of FFA, molar ratio of octanol:FFA = 2.5:1 and reaction time = 3 h. The product octyl ester was characterized by Fourier transform infrared spectroscopy (FT-IR) and Nuclear magnetic resonance (1 H-NMR and 13 C-NMR). The physico-chemical properties of the waste cooking oil and the product ester (developed from WCO) were determined following standard methods. The results revealed that the developed octyl esters have improved viscosity index, pour point, flash point and oxidation stability when compared to that of the raw material (WCO). Moreover the product is biodegradable (>90% biodegradability). Thus the synthesized octyl esters have shown potential to be used as an environment-friendly biolubricant base-oil.

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1. Introduction

The esterification reaction between an alcohol and a carboxylic acid finds considerable industrial importance. The product esters are frequently used in manufacturing lubricants, plasticizers, paints, solvents, flavors, pharmaceuticals, cosmetics, and liquid fuels (Gryglewicz et al., 2006). Esters resulting from the reaction of long-chain acids (12–20 carbon atoms) and long-chain alcohols (C8–C14) are used to produce lubricating oils for high-precision machinery (Malca et al., 1990; Budnick, 2013). The chemically catalyzed esterification reaction possesses various short comings such as high energy consumption, undesirable by-product formation and corrosion of equipment (Demirkol et al., 2006). Enzyme catalyzed esterification reactions are being studied extensively in recent years due to their milder operating conditions, degree of purity of the obtained products, lower energy consumption and environment friendly nature (Rocha et al., 1999; Foresti and Ferreira, 2005; Richetti et al., 2010). Novozyme 435 has been widely used to catalyze such esterification reactions (Duan et al., 2010; Richetti et al., 2010; Tamayo et al., 2012).

Synthesis of a product should be accompanied by process optimization to maximize yield. Previously, optimization was carried out using classical approach varying one variable at a time keeping other variables constant. Such methods are time consuming, require large sets of experimental data and fall short to show interaction between process variables (like temperature, substrate concentration, etc.) (Beg et al., 2003). Statistical experimental design techniques are useful in simultaneous optimization of multiple variables (independent factors) to achieve the best response (dependent factor) with minimum number of observations. Taguchi’s orthogonal array (Taguchi, 1986) method is a statistical technique of designing experiments that can provide sufficient information about the undergoing process, requiring minimum experimental trials (Houng et al., 2006). The fundamental principle of this method serves as screening filters where various process parameters can be studied at a time and those with major effects are easily identified (Dasu et al., 2003). Taguchi design is considered to be a milestone in the robust design methodology (Rao et al., 2006). A robust design is the one that is insensitive to uncontrollable or noise factors (Mohan et al., 2005). The advantages of using Taguchi method has been reported by various authors (Yang and Taring, 1998; Yazdian et al., 2005; Yasotha et al., 2006; Salehzadeh et al., 2007; Ballantyne et al., 2008; Chen and Kitts, 2008; Rao et al., 2008; Zarei et al., 2010) which are summarized as follows:

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• Orthogonal arrays (OAs) reduces the number of experiments compared to the factorial design.
• The use of the signal-to-noise (S/N) ratio to analyze the results reduces the sensitivity of the system to sources of variation, thus resulting in good performance.
• The use of such design enhances robustness of the process, reducing time and cost.

Very recently, it has been employed for the optimization of biochemical techniques (Cobb and Clarkson, 1994) and bioprocess applications (Mohan et al., 2005).

The objective of the present study was to optimize the various process variables (temperature, catalyst dosage, molar ratio of substrates and time) for the production of octyl esters using bio-catalytic (Novozyme 435) esterification reaction of free fatty acids (FFA) and octanol applying Taguchi L9 orthogonal design. The product characterization and its physico-chemical characteristics have shown that the produced octyl esters from waste cooking oil possess high potentiality to be used as an eco-friendly lubricant.

2. Experimental

2.1. Materials

The waste cooking oil (WCO) for the present study was provided by a local restaurant with an acid value of 6 mg of KOH g⁻¹ of WCO. Candida rugosa lipase (powder) was purchased from Sigma Aldrich, USA. Novozyme 435 (lipase from Candida antarctica immobilized on macroporous anionic resin) was obtained from Novozymes South Asia Pvt. Ltd., Bangalore, India. 1-Octanol (99% pure) was obtained from Merck. All the other chemicals used in the analysis were of analytical grade and purchased from Merck.

2.2. Hydrolysis of WCO

The FFA for esterification were obtained by hydrolyzing the WCO using candida rugosus lipase. Prior to enzymatic hydrolysis the WCO was filtered to remove all suspended impurities. The reaction was initiated by adding 1.0 g L⁻¹ of lipase solution in 500 ml of glass stoppered bottles containing WCO. The temperature and reaction time of the shaker incubator were maintained at 30 °C and 30 h respectively (Chowdhury et al., 2013).

2.3. Taguchi orthogonal array design

The execution of Taguchi design method has been represented in the form of a flow diagram in Fig. 1 (Jahanshahi et al., 2008; Yusoff et al., 2011). The primary objective of the Taguchi method is to determine the optimum settings of the input parameters (Sivarao et al., 2010). Kondapalli et al. (2013), has recently published a detailed review report on Taguchi designs concluding that Taguchi analysis can provide definitive information mainly for single response systems. Thus for the present study the standard orthogonal array of L9 (Roy, 2001) was employed to examine the four factors at three levels (all experiments were performed in triplicate) in order to maximize % conversion (single response) of octyl ester. The L and the subscript 9 represent the Latin square and the number of experimental runs, respectively.

Instead of conducting 81 experiments, for general factorial experimental design involving four parameters, only 9 experimental runs were required in the Taguchi’s design method to optimize the parameter settings for the present study. Table 1 enlists the four independent process factors (temperature, enzyme amount, molar ratio of octanol: fatty acids and time) and their corresponding levels for the present study. The range of the parameters was specified based on preliminary laboratory experiments. The software MINITAB-16 (Minitab Inc. USA for Windows?) has been employed to undertake the Taguchi design method. Table 2 depicts the experimental design matrix along with the mean conversion values and the calculated S/N ratios. The S/N ratio values corresponding to the conversions were calculated, using the ‘larger-the-better’ characteristics, since the aim of the work was to maximize the response (FFA conversion). The S/N ratio for each run was calculated according to the following equation (Roy, 2001):

$$\frac{S}{N} = -10 \log \left( \frac{1}{n} \sum_{i=1}^{n} \frac{1}{yi} \right) \quad (1)$$

where $y$ is the FFA conversion for corresponding run, $i$ is the number of replicate and $n$ is the number of trial experiments performed in any particular parametric combinations as per Table 2. The predicted S/N ratio at the optimal process conditions for achieving maximum conversion was estimated from the following equation (Taguchi, 1986; Kaladhar et al., 2011; Chakraborty and RoyChowdhury, 2013):

$$\frac{S}{N}_{\text{predicted}} = \frac{S}{N} + \sum_{j=1}^{n} \left( \frac{S}{N} \right)$$

(2)

where $S/N$ is the mean of all $S/N$ ratios. $S/N_j$ is the S/N ratio at optimal level for each parameter and $n$ is the number of the process parameters that significantly affect the process.

2.4. Esterification reaction

The esterification reaction was carried out in a laboratory batch reactor (capacity 1.5 L) made up of stainless steel. The reactor was equipped with the following features: a jacketed reaction vessel containing thermic fluid that served as the heating medium, an electrically driven stirrer with a digital display of rpm, a thermostat

<table>
<thead>
<tr>
<th>Process parameter</th>
<th>Temperature (°C)</th>
<th>Novozyme 435 (wt% of FFA)</th>
<th>Molar ratio</th>
<th>Time (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1(L1)</td>
<td>40</td>
<td>1.25</td>
<td>1.0</td>
<td>1.5</td>
</tr>
<tr>
<td>Level 2(L2)</td>
<td>50</td>
<td>2.5</td>
<td>2.5</td>
<td>3.0</td>
</tr>
<tr>
<td>Level 3(L3)</td>
<td>60</td>
<td>5.0</td>
<td>4.0</td>
<td>4.5</td>
</tr>
</tbody>
</table>
to maintain the desired temperature (digital display), allowing temperature variation within ±0.5 °C; a sample inlet duct and a sampling point equipped with a valve (Chowdhury et al., 2013). The free fatty acids (500 mL) were esterified in this reactor with octanol. In all sets of experiments the agitator speed was kept constant at 250 rpm (determined from preliminary experiments).

2.5. Product analysis and characterization

The esters produced from the reaction with FFA and octanol was the desired biolubricant (octyl esters). The product was withdrawn from the sampling point time to time and tested for its acid value. The acid values were determined by titrating the product sample with an alkali (0.02 N KOH). The percentage conversion of FFA to ester is given by (Marchetti and Errazu, 2008):

\[
\text{Conversion(\%)} = \frac{AV_0 - AV_t}{AV_0} \times 100
\]

where \(AV_0\) is the initial acid value of the sample and \(AV_t\) is the acid value of the sample at time \(t\). Fourier transform infrared spectroscopy (FT-IR) and Nuclear Magnetic Resonance (NMR) analyses were conducted to confirm that the product formed is ester. FT-IR scans were carried out in a Jasco-670Plus FT-IR fitted with a DLTGS detector. \(^1\)H-NMR and \(^1\)C-NMR spectra were recorded in the Bruker AVANCE 600 MHz spectrometer with TCI cryoprobe using CDCl3 solvent.

2.6. Physico-chemical attributes of the biolubricant

The physico-chemical properties of the product were determined following standard methods to ensure its usability as a lubricant. The kinematic viscosity and viscosity index were measured using ASTM methods D 445 and D 2270 respectively (ASTM Standards, 2005). The pour point was determined following ASTM D 97 (ASTM Standards, 2005) standard method. The flash point and oxidation stability of the product were obtained following ASTM D 92 (ASTM Standards, 2005) and IP 48 (IP Standards, 2006) respectively. The relative biodegradability of the ester (biolubricant) was determined according to the most commonly used standard test CEC-L-33-A-93 (CEC, 1993). All the above mentioned tests were run in triplicate and the average values were reported.

3. Result and discussion

3.1. Taguchi design analysis and prediction of optimal conditions

The ANOVA (Analysis of Variance) results for the L9 orthogonal array are shown in Table 3. The results indicated that out of the four process-control factors studied, temperature and catalyst amount have significant effect on the esterification reaction with their \(P\) values less than 0.05. The highest \(F\) value for the temperature indicates its highest influence on the reaction followed by catalyst amount, time and temperature. The \(S/N\) ratio was calculated using “the larger the better” criterion, as the key aim was to maximize the FFA conversion. Table 4 presents the ranking of the parameters based on the delta values (the difference of the \(S/N\) ratio values between the highest and the lowest levels of process factors). The higher the delta value of each factor, the higher is its effect on the conversion of FFA to ester. Thus, the results (Table 4) once again corroborate, that the factor with the maximum effect on Novozyme 435 catalyzed FFA esterification with octanol is temperature followed by enzyme amount, molar ratio and time. Fig. 2 shows the plots for the \(S/N\) ratios and each of the control parameter. The highest \(S/N\) ratio corresponding to the highest FFA conversion suggested the best level for each of the significant parameters viz. temperature (L3) and Novozyme 435 (L3). Although molar ratio and reaction time have insignificant effect on the response according to ANOVA analysis, molar ratio at L2 and time at L2 have been optimized (Fig. 2) to maximize the ester yield. The predicted optimal \(S/N\) ratio for the optimized conditions was computed from equation (2) (Taguchi, 1986) and was found to be 39.7 db (corresponding predicted conversion = 96.18%). In order to validate the optimal conditions confirmatory runs (triplicate) were conducted that gave 95.19% conversion with 39.35 db.

Esterification of FFA with alcohol being an endothermic reaction, temperature has an imperative effect in raising the reaction kinetics and thereby bringing substantial conversion. Conversion increased at higher temperatures being maximum at 60 °C (optimal). This might be due to the fact that high temperatures increased the kinetic energy of the system resulting in better interactions between enzyme particles and substrates thereby increasing the rate of reaction (Soo et al., 2004). Furthermore, an increase in temperature reduces the system viscosity, enhance mutual solubility and improve diffusion process of substrates, thus reducing
It has also been reported that the optimal working temperature value of Novozyme 435 is between 40 and 65 °C (Novo Nordisk, 1992). The increase in conversion with increasing amount of Novozyme 435 (1.25–5 wt% of FFA) is probably due to the availability of more active sites (Talukder et al., 2010) while additional increase might have caused diffusional limitation which could not significantly enhance the conversion any further.

The molar ratio of 2.5:1 (octanol:FFA) kept the reaction equilibrium towards product side retarding backward hydrolysis. Though there was negligible difference in conversion with changing times from 1.5 to 4 h (Fig. 2), a reaction period of 3 h was optimized by Taguchi analysis. Temperature and catalyst amount have been found to affect the esterification reaction most significantly. This evidently indicates that the heterogeneous reaction under study is kinetically controlled.

3.2. Interaction among process variables

Fig. 3 demonstrates the interaction of various parameters studied in the esterification of FFA with octanol. The interactions between two variables were evaluated keeping the other two variables at their optimal levels. The interaction between temperature and Novozyme 435 amount showed that at all levels of temperature, an increase in enzyme amount could enhance the conversion of FFA to octyl ester. At the lowest level of temperature there was a monotonic increase in conversion with an increase in molar ratio, while maximum conversion could be achieved at the highest level of temperature and intermediate level of molar ratio. Similarly, temperature and time interactions revealed that maximum conversion could be obtained at the highest level of temperature in minimum time of 1.5 h keeping the enzyme amount and molar ratio at their optimal levels. It is significant to note here that maximum conversion could only be obtained at the highest level of temperature.
temperature for all other combinations of parametric values studied. Keeping temperature and reaction time constant, maximum conversion was achieved at the highest level of Novozyme 435 and intermediate level of molar ratio. Whereas for interaction between enzyme amount and time, the optimal level of Novozyme 435 could bring about maximum conversion in minimum time. Finally keeping temperature and the enzyme amount fixed at the optimum level, the conversion was found to gradually increase with increase in reaction time from 1.5 to 4.5 h for the minimum molar ratio. However, maximum conversion could be obtained at 1.5 h with the intermediate level of molar ratio.

3.3. Product characterization and physico-chemical property testing

The product was characterized by spectroscopic analysis (FT-IR and NMR). Prior to the analysis the final reaction product was subjected to vacuum distillation in order to remove the excess water and octanol. Fig. 4 illustrates the distinctive changes that were found in the absorption peak of the product (ester) formed compared to the corresponding FFA absorption data. The characteristic peak at 1709 cm\(^{-1}\) for the stretching of carboxylic acid (FFA) shifted to 1738 cm\(^{-1}\) for the ester. Another characteristic CO bond peak for the ester was seen at 1056 cm\(^{-1}\) wavelength (Arbain and Salmon, 2010; Chowdhury et al., 2013).

The structure of the synthesized product was further verified with \(^1\)H-NMR and \(^13\)C-NMR spectroscopy and is presented in Figs. 5 and 6 respectively. \(^1\)H-NMR spectra shows the significant proton signals at 0.89–0.90 ppm due to the terminal methylene groups (–CH\(_2\)) and at 1.19–2.78 ppm due to aliphatic –CH\(_3\) groups. The characteristic signals for protons attached to ester group are prominent at 2.0–2.78 ppm and 4.04–4.07 ppm. Furthermore, the signal at 5.31–5.39 ppm signifies the protons attached to olefinic carbons (–C=CH\(_2\)). The \(^1\)H spectra show a singlet at 7.29 ppm which is due to the –COOH group (a minimum amount of fatty acid was left in the final product). Fig. 6 (\(^13\)C-NMR) indicates similar findings showing significant band at 173.95–173.98 which exhibit characteristic signal attributed to ester groups (Silverstien et al., 2005; Salih and Salmon, 2010; Salih et al., 2011). The bands at 127.8–130.16 and 22.55–29.75 of \(^13\)C spectra refers to the olefinic carbons and aliphatic carbons respectively. A distinctive signal at 64.37 signifies existence of methine carbons (Hwang, 2006).

![Fig. 4. FT-IR spectra for FFA and product ester.](image)

![Fig. 5. \(^1\)H-NMR spectra for product ester.](image)

| Table 5 | Various physico-chemical properties of the product biolubricant (octyl ester). |
|---------|-----------------------------|-----------------------------|
| Property | WCO | Product ester |
| Viscosity (mm\(^2\)/s, 40 °C) | 46.13 | 32.35 |
| Flash point (°C) | 13 | +1 |
| Iodine value (mg I\(_2\)/g oil) | 36 | 35 |
| Oxidation stability (viscosity at 1.5% ratio) | 2.93 | 1.18 |
| Biodegradability (%) | >95 | >90 |

Physico-chemical properties of the product are summarised in Table 5. Viscosities are important in classifying lubricants since this property directly corresponds to the ability of the lubricant in reducing friction and wear. As reported in Table 5, the kinematic viscosity of the octyl ester is higher than the WCO. This is possibly because viscosity index (VI) increases with the increasing linearity of the molecule (Wagner et al., 2001). An efficient lubricant should always show a high viscosity index (VI) owing to limited change of viscosity with change in temperature. The product has presented a sufficiently high VI as desirable. The low temperature property of a lubricant is another vital attribute to be taken in concern when formulating one. Plant oils usually form macro-crystalline structures at low temperature through the uniform
stability of the developed octyl ester is much higher in comparison to the raw material used. This result is also in agreement with other studies; oxidation stability increases with increasing chain length of the esterified FFA (Kubouchi et al., 2002; Salimon et al., 2011). The priority of using vegetable oils for producing biolubricant is mainly due to their ability of rapid biodegradation causing minimal environmental damage. A lubricant formulation must also show similar biodegradation to be certified as an efficient biolubricant. The overall biodegradability of the product was determined by the standard test method of Co-ordinating European Counsel (CEC) and the test results have proven that both WCO and produced octyl esters are biodegradable.

4. Conclusions

Novoenzyme 435 catalyzed esterification reaction of FFA and octanol to produce octyl esters was designed and analyzed via Taguchi L₉ robust design matrix. Temperature and enzyme amount were found to be the two most significant parameters affecting the esterification reaction. High response (conversion of 95.13%) was obtained at the optimized levels of the process controlling factors viz. temperature 60 °C, N435 amount 5 wt% of FFA, molar ratio of octanol:FFA 2.5:1 and reaction time of 1.5h. The FT-IR and NMR scans confirmed the ester structure of the product. A comparative analysis of the physico-chemical properties of the raw material (WCO) and the biolubricant revealed that the developed ester has ameliorated its properties compared to that of WCO. The produced ester can thus be used as an API grade V base oil as per required specifications.

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