J Am Oil Chem Soc (2010) 87:1027–1034
DOI 10.1007/s11746-010-1593-y

ORIGINAL PAPER

Biodiesel (FAME) Productivity, Catalytic Efficiency and Thermal Stability of Lipozyme TL IM for Crude Palm Oil Transesterification with Methanol

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Received: 23 June 2009 / Revised: 7 March 2010 / Accepted: 15 April 2010 / Published online: 6 May 2010

Abstract Crude palm oil (CPO) transesterification with methanol at room temperature is an important factor for optimizing biodiesel processing costs with respect to energy input; in addition, good stability of expensive lipase activity was ensured and is reported in this study. The enzyme loading, agitation speed and reaction time at a constant operating temperature of 30 °C were studied to find favourable operational conditions using a factorial design. Statistical analysis was used to assist the enzymatic transesterification so that a reduced mass transfer effect was achieved to obtain high FAME yields. The combination of optimum enzyme loading of 6.67 wt% and 150 rpm agitation speed for the system at 30 °C gave 81.73% FAME yield at 4 h and a production rate of 85.86% FAME yield/h. The high viscosity of CPO observed at 30 °C compared to 40 °C hindered the achievement of 96.15% FAME yield at room temperature. It was found that an increase of 10 °C invariably deactivated the lipase, but was compensated by the enhanced FAME production rate with 96.15% FAME yield after only 4 h reaction time. Thus, 40 °C was considered the most suitable operating temperature for lipozyme TL IM to catalyze CPO transesterification.

Keywords Transesterification · Fatty acid methyl ester (FAME) · Lipase · Temperature · Statistical analysis · Biodiesel · Palm oil · Mass transfer

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Introduction

The use of refined edible vegetable oil as a feedstock for biodiesel production allows for a simple conversion method and promises the highest conversion rate through the transesterification reaction. The sustainable cultivation of edible vegetable oil guarantees that the oil supply is sufficient for food consumption, consumer products as well as biodiesel production. Crude palm oil (CPO; *Elaeis guineensis*) is currently one of the most attractive feedstocks for renewable energy production. This is mainly because it is the least expensive of the vegetable oils and has a high yield of CPO, with 4.2 tonnes per hectare produced annually. Malaysia is well known as the world's largest producer and exporter of palm oil, with CPO production increasing dramatically from 8.3 million tonnes in 1998 to 15.8 million tonnes in 2009 [1]. The large availability of palm oil is attributed to the high oil palm yield per area of land, with the palm oil possessing the highest fossil energy balance and the lowest production costs relative to other energy crops [2]. For biodiesel to be commercially viable, the cost of the feedstocks must be inexpensive. It is therefore logical to use cheap CPO in the transesterification reaction as it conforms to the process requirement of low production costs.

Refined palm oil has given better results than CPO in terms of biodiesel production. However, in addition to the free fatty acid and water content, refined palm oil and CPO also differ in their phospholipid contents. Phospholipids as the main component of oil gum actually contribute to the high viscosity in CPO and thus lead to a significant mass transfer effect [3]. The methanolysis of refined palm oil without phospholipids progresses faster with Novozym 435, 4.65 (g biodiesel/h/g lipase), by more than half compared to unrefined palm oil, 2.02 (g biodiesel/h/g lipase)

[3]. However, the addition of tert-butanol dilutes the phospholipids and thus reduces the CPO viscosity and increases the activity of Novozym 435 in CPO [4, 5]. With the tert-butanol aided dissolution, the reactants become homogeneous in the reaction mixture and thus overcome internal and external mass transfer limitations. In addition, the tert-butanol forces the residual water to stay around the lipase and thus helps to activate and stabilize the immobilized lipases [6].

As mentioned earlier, the presence of phospholipids resulted in high viscosity and thus reduced the mass transfer [3]. Hence, if the process were to be conducted at room temperature, the high viscosity would hinder the reactant diffusion to the immobilized lipase and result in low productivity. On the other hand, increasing the temperature increases the collision frequency between reactants and lipase, but will simultaneously affect the enzyme stability. Enzyme, especially lipozyme TL IM, has been shown to be deactivated easily when subjected to heat treatments as low as 40 °C [7].

The aim of this study was to report the most desirable reaction temperature for CPO transesterification between an optimum temperature of 40 °C (to ensure high enzyme activity) and a lower temperature of 30 °C (to help preserve enzyme stability). The CPO transesterification mediated by immobilised lipase, lipozyme TL IM, at room temperature was studied. Optimisation of process parameters like agitation speed, enzyme loading and reaction time at 30 °C was studied with the aid of factorial design combined with response surface methodology (RSM). The selection of reaction temperature for CPO transesterification relies on factors like the enzyme stability and FAME production rate. The FAME productivity in terms of FAME yield, initial reaction rate and lipase specific activity as well as the measurements of thermodynamic stability like the deactivation coefficient and half-life time was evaluated at 30 and 40 °C to determine the most suitable operating temperature for transesterification using CPO.

Materials and Methods

Biocatalyst and Chemicals in Transesterification

Lipase *Thermomyces lanuginosus* immobilised on silica gel (lipozyme TL IM) with catalytic activity of 170 IUN/gl was purchased from Novozymes (Bagsvaerd, Denmark); 170 IUN is defined as 170 mmol of enzyme converting 0.01% tristearin/min under standard assay conditions. The CPO was procured from M.P. Mathew Palm Oil Mill Sdn Bhd located in Sungai Bakap, Malaysia. The chemical properties of CPO, compositions of total fatty acids in CPO, acylglycerols compositions and phospholipid content are tabulated in Table 1. Analytical grade tert-butanol and methanol in 99.0% purity were obtained from J.T. Baker and Merck, respectively. Methyl ester of lauric acid, palmitic acid, stearic acid, oleic acid, linoleic acid and heptadecanoic acid were of chromatographic grade and were purchased from Sigma.

Crude Palm Oil Transesterification

Batch reactions with varied enzyme loading, agitation speed and reaction time were conducted for the optimization process with minimum mass transfer effects. The pre-treatment process on CPO was carried out to decant the impurities and the gummy residue that were present. CPO was first centrifuged at a relative centrifugal force (RCF) of $2{,}173 \times g$ at 32 °C for 10 min, and two layers of liquid and solid phases were formed. The supernatant of CPO was collected as a triglyceride substrate, while the residue solid content was decanted. Based on the preliminary study, the transesterification process was initiated at optimum mixture blends of 0.37, 0.10 and 0.53 proportions for CPO, methanol and tert-butanol, respectively [8]. Lipozyme TL IM with a range of 2.5–10.0% wt lipase/wt CPO as per the factorial design matrix was used to initiate the transesterification reaction. A total volume of 32 ml of reaction mixture contained in a 100-ml conical flask was incubated in a water bath shaker at the designated agitation speed (150–225 rpm) so as to investigate the extent of mixing intensity. Constraint for agitation speed was adopted from the literature on

Table 1 Specifications of crude palm oil (CPO) obtained from M.P. Mathew Palm Oil Mill Sdn Bhd, Nibong Tebal, Malaysia

Description	Value
Chemical properties	
Iodine value	53.8 meq/kg
Acid value	6.4 mgKOH/g
Saponification value	192
Water content	0.105%wt/wt oil
Free fatty acid	2.91%wt/wt oil
Gum (as phospholipids)	50 ppm
Fatty acid compositions of total fatty acids in CPO (%)	
Myristic acid, C14:0	1.46
Palmitic acid, C16:0	38.8
Steric acid, C18:0	4.00
Oleic acid, C18:1	43.8
Linoleic acid, C18:2	11.9
Acylglycerol compositions (%wt/wt oil)	
Monoglyceride	0.38
Diglyceride	6.58
Triglyceride	89.5

transesterification reactions with solvent systems [9, 10]. The agitation speeds used were ±5% deviation from the actual stirring speed. For statistical evaluation, 50 µl aliquots of the sample were withdrawn at 5, 10, 15 and 30 min for the initial reaction rate determination, and the sampling time was extended to 2, 4 and 6 h to detect the equilibrium yield of FAME. The product FAME achieved at various process conditions was analysed by gas chromatography. Each experimental run was performed in duplicate, and the results were expressed as mean values ± standard deviations. The standard deviation for FAME was ±5% of the mean value.

Measurement of Dynamic Viscosity

The dynamic viscosity of the reaction mixture and product was measured using the Programmable Rheometer, Model DV-III, version 3.3 (Brookfield). Approximately 20 ml of sample was placed in the sample tube with a cylindrical spindle rotating inside an accurately machined tube. Measurements were performed in the temperature range of 30–60 °C in steps of 10 °C. Viscosity measurements were recorded using Rheocalc software, version 2.3, with the spindle speed maintained at 150 rpm.

FAME Determination with Gas Chromatography

A 50-µl sample taken at a specified time was centrifuged to obtain the upper layer. Heptadecanoic acid methyl ester as an internal standard was mixed with the upper layer and dissolved in tert-butanol. To quantify the FAME produced, a 0.4-µl sample was injected into a Perkin Elmer Clarus 500 gas chromatographer equipped with a programmed split/splitless injector (PSS) and flame ionisation detector (FID). The split ratio was defined as 20:1. A Nukol™ fused silica capillary column with dimensions of 0.53 mm i.d. × 15 m length × 0.50 µm film thickness (Supelco, USA) was used. The injector and detector temperatures were 220 and 250 °C, respectively [10]. The column temperature was maintained at 110 °C for 0.5 min, increased to 200 °C at 10 °C/min and maintained at this temperature for 10 min. Helium was employed as a carrier gas with a flow rate of 1.2 ml/min. Product expressed as % FAME yield was calculated using Eq. (1).

$$\text{FAME yield}\ (\%) = \text{DF} \times \frac{M_{\text{M}} + M_{\text{P}} + M_{\text{S}} + M_{\text{O}} + M_{\text{L}}}{3 \times M_{\text{CPO}}} \times 100\% \quad (1)$$

where *DF* is the dilution factor for the sample.

M_{M}, M_{P}, M_{S}, M_{O} and M_{L} represent moles of methyl myristate, methyl palmitate, methyl stearate, methyl oleate and methyl linoleate, respectively.

M_{CPO} indicates moles of total fatty acids in CPO and is equal to the molecular weight of CPO, 847 g/mol.

Statistical Modeling

Factorial design analysed by response surface methodology (RSM) was selected for the system development and process enhancement for obtaining high FAME yield at 30 °C [11, 12]. In the study, agitation speed, enzyme loading and reaction time, each coded with four study levels, were considered to be significantly affecting the process, and an effective transesterification reaction was evaluated on the basis of the FAME yield and initial reaction rate. Consequently, the four level-three factor factorial design formulates the design matrix layout prior to execution on the optimization study. The Design-Expert (version 6.06, Stat-Easy Inc., Minneapolis, MN) statistical tool uses a second order polynomial equation for fitting the experimental results from the factorial design. The experimental work with factorial design facilitates the investigation of individual and collective effects from all process parameters simultaneously on responses.

Results and Discussion

Effect of Agitation Speed, Enzyme Loading and Reaction Time on the CPO Transesterification at 30 °C

Experimental results collected from the factorial design matrix were categorized into four phases based on the ranges of FAME yield attained at agitation speeds of 150, 175, 200 and 225 rpm regardless of enzyme loading (Table 2). Meanwhile, the FAME yields produced from the promising transesterification reaction with the corresponding reaction time and agitation speed imposed were cited to evaluate the enzyme efficiency at room temperature operations of CPO transesterification. Both minimum (33.06%) and maximum (83.02%) FAME yields were achieved in the system under variation of enzyme loading at a constant 150 rpm compared to other agitation speeds. Since the transesterification reaction at 150 rpm covers the minimum and maximum FAME yield, the effect of agitation speed is less important. When CPO transesterification was initiated at 150 rpm, a reduction in 30% FAME yield was observed with 2.5% enzyme loading instead of 7.5% loading. The results show that the variability in FAME productivity catalyzed by lipozyme TL IM was mainly attributed to enzyme loading variation. An agitation speed of 150 rpm was optimal to provide mechanical stirring for effective external mass transfer, and a further increase of agitation speed of more than 150 rpm did not improve

Table 2 Comparison of FAME yield achieved at different reaction conditions in the present study with previous studies on enzymatic transesterification mediated by lipozyme TL IM

Reaction conditions			FAME yield		Remarks
Agitation speed (rpm)	Reaction time (h)	Enzyme loading (wt%)	Minimum (%)	Maximum (%)	
150	2–6	2.50–10.0	33.06	83.02	Present study
175	2–6	2.50–10.0	40.34	71.99	Present study
200	2–6	2.50–10.0	35.39	73.47	Present study
225	2–6	2.50–10.0	37.39	77.49	Present study
200	30	10.0	>60		Soumanou and Bornscheuer [8]
130[a]	12	4.0	95		Li et al. [5]

[a] Combined use of 3 wt% TL IM and 1 wt% N435 for the transesterification reaction

FAME yield. This was revealed by the maximum FAME yield of 83.02% that was achieved at 150 rpm, and a subsequent increase in agitation speed from 175 to 225 rpm resulted in a FAME yield reduction where only 71.99–77.49% FAME yield was achieved (Table 2). The higher agitation speed employed of more than 150 rpm did not improve the FAME yield, which may be due to the substantial biocatalyst particles that were dispersed and stuck to the wall. The low agitation speed required in the system is due to the utilisation of a homogeneous reaction mixture aided by tert-butanol solvent, which reduced the viscosity and thus increased the mass transfer of reactants from the stagnant film to lipase.

When the transesterification system operated at higher agitation speeds of 175 rpm and above, the maximum FAME yield achieved at 6 h was less than 78%. The drop in the FAME yield resulting from the actual amount of enzyme loading was less than the enzyme loading used in the system. The system encountered difficulties in obtaining uniform enzyme suspensions in the reaction medium where substantial immobilized lipase particles immersed in the reaction mixture were dispersed randomly outside the liquid phase and stuck to the wall as a result of strong mechanical repulsion provided at the orbital shaking speeds of 175, 200 and 225 rpm, respectively. Similar results are reported where the FAME yield dropped from 97 to 69% when 180 rpm was used as agitation speed instead of the optimal 150 rpm [9]. Consequently, the agitation mode for magnetic stirring is preferable for promoting vigorous stirring in homogeneous reaction mixtures. As seen in Table 2, the study done by Soumanou and Bornscheuer [13] showed that transesterification with 10 wt% of TL IM achieved 60% FAME yield after 30 h reaction time, whereas the combined use of 3 wt% TL IM with 1 wt% Novozyme 435 as biocatalyst and agitated at 130 rpm was able to attain 95% FAME yield after 12 h as observed by Li et al. [6]. Thus, within 130–200 rpm agitation speed, both types of lipase used as biocatalyst gave unsatisfactory catalytic efficiency [6, 13]. In comparison, 150 rpm was optimal in the current study to accelerate reactants to diffuse from bulk liquid to the external surface of the catalyst with 82.69% FAME yield at 6 h.

Process Optimisation via the Statistical Approach

The correlation among the independent variables, agitation speed, enzyme loading and reaction time, to FAME yield and initial reaction rate as dependent variables was expressed as an empirical model (Eq. 2) developed in response surface methodology (RSM) [11, 12].

$$\begin{aligned}\text{FAME yield} = {} & +118.49 - 1.37 \times \text{agitation speed} \\ & + 11.65 \times \text{enzyme loading} \\ & + 12.12 \times \text{reaction time} + 3.62E-003 \times \text{agitation speed}^2 \\ & - 0.54 \times \text{enzyme loading}^2 - 0.59 \times \text{reaction time}^2 \\ & - 0.58 \times \text{enzyme loading} \times \text{reaction time} \end{aligned} \quad (2)$$

The initial reaction rate is represented in Eq. (3), which is linearly dependent on the enzyme loading.

$$\text{Initial reaction rate} = +7.13 + 10.84 \times \text{enzyme loading} \quad (3)$$

In contrast to the system at 30 °C, both agitation speed and enzyme loading showed a significant effect on the initial reaction rate when operated at 40 °C in the previous study [8]. The insignificant effect of agitation speed at low temperatures of operation was probably due to the high viscosity of the reaction mixture, 6.30 mPas compared to 4.20 mPas at 40 °C. Viscosity of the mixture was temperature sensitive, and both variables are antagonistic to each other. Therefore, high viscosity of the reaction mixture largely diminished the importance of agitation speed in promoting the rapid mass transfer rate. Moreover, the parameter was considered as the determining step to suppress high FAME yield attainment as was produced at 40 °C. The FAME yields for 30 and 40 °C were 81.73 and 96.15%, respectively. A reduction of 14.42% in FAME yield was observed at 30 °C when compared to 40 °C.

Through numerical optimisation in RSM, the optimal condition for 30 °C was obtained at 6.76% enzyme loading, and 150 rpm agitation speed with 81.73% FAME yield was obtained at 6 h with an initial reaction rate at 85.86% FAME yield/h.

Comparison in CPO Transesterification at 30 and 40 °C

FAME production was carried out at constant room temperature, 30 °C, to elucidate the lipase catalyzing performance in comparison to 40 °C with 96.15% FAME yield. Figure 1a shows FAME yield variation with enzyme loading obtained at two different temperatures. The FAME yield at 40 °C was higher than the FAME yield obtained at 30 °C. For the entire enzyme loading process at 40 °C with 96.15% equilibrium, the FAME yield was superior to that at 30 °C with 84.62% equilibrium FAME yield at 24 h. When the system operated at 40 °C, 5.0% enzyme loading gave the same equilibrium FAME yield as with 10.0% enzyme loading, whereas methanolysis at 30 °C required higher enzyme loading of 7.5% to obtain the maximum FAME yield, and 10.0% enzyme loading resulted in intraparticle mass transfer limitations, which were responsible for the low FAME yield. The biodiesel (FAME) produced at 30 and 40 °C was not influenced by the agitation speed variation, and 150 rpm was the optimum value for both temperatures. The time sequential of FAME yield between 30 and 40 °C under optimum operating conditions is displayed in Fig. 2. The transesterification initiated at room temperature of 30 °C instead of 40 °C gave a 17.3% reduction in equilibrium FAME yield and an extended equilibrium reaction time from 4 to 10 h. The 10 °C temperature difference influenced the equilibrium FAME yield significantly. The two process parameters of the viscosity and denaturation constant, which are temperature sensitive, were presumed to be the limiting factors in the FAME yield variation.

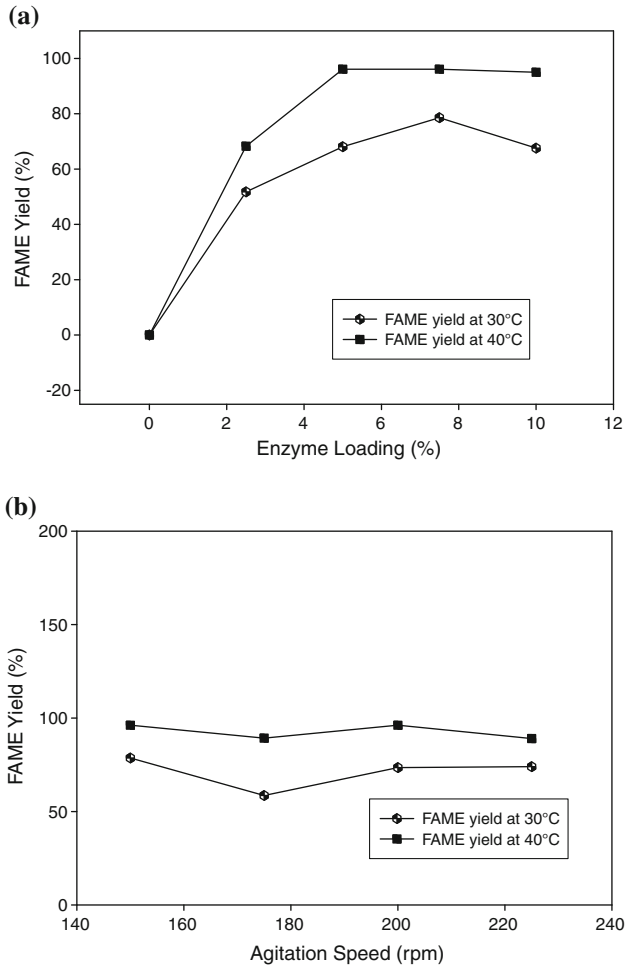

Fig. 1 Comparison of FAME yield between 30 and 40 °C under **a** variation of enzyme loading at 150 rpm, 4 h reaction time, and **b** variation of agitation speed at 7.5% enzyme loading, 4 h reaction time

Effect of Temperature Variations on Dynamic Viscosity

Dynamic viscosity is a measure of the internal friction of the oil molecules, and the parameter decreased exponentially with the increase of temperature [14]. For instance, the differences in dynamic viscosity obtained between 30 and 40 °C were substantially large. However, the effect of dynamic viscosity due to temperature variation gradually diminished at higher temperatures of 50 and 60 °C (Fig. 3). It can be said that the molecular movements of the fuels constructed by a polyatomic molecule move energetically with the rising of fuel temperature [14]. Since transesterification at 40 °C achieved 96.15% FAME yield, 4.2 mPas was considered to be the optimum reaction mixture viscosity. Moreover, the dynamic viscosity of the reaction

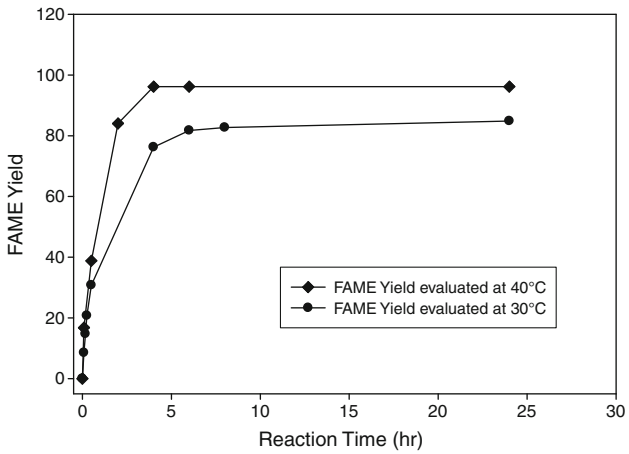

Fig. 2 Time course of FAME yield under optimum conditions at 30 and 40 °C

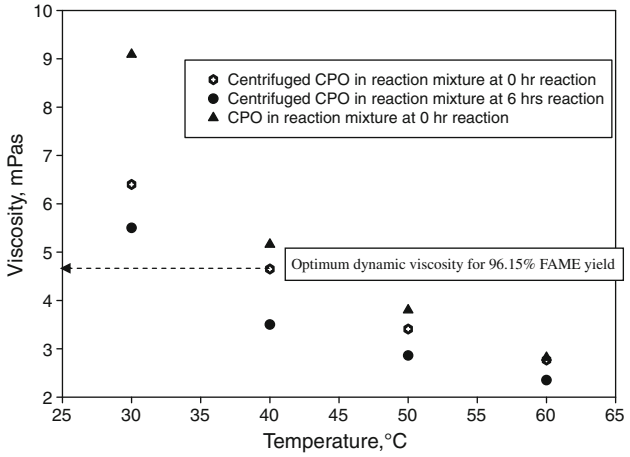

Fig. 3 Dynamic viscosity as a function of temperature of centrifuged crude palm oil plus reaction mixture and unrefined crude palm oil plus reaction mixture at different reaction times

mixture plus FAME product at 6 h reaction time was determined at 30 °C, and the value of 5.40 mPas was observed to be higher than the defined optimum dynamic viscosity, 4.2 mPas. As a result, 84.62% equilibrium FAME yield was attained in 24 h at 30 °C in contrast to 96.15% FAME yield being produced in 4 h at 40 °C. The FAME production as time progressed significantly reduced the mixture viscosity as the plot for the reaction mixture at 6 h remained at the lowest dynamic viscosity value regardless of the temperature studied. CPO without centrifuging together with the reaction mixture presented a significantly higher viscosity at 30 °C, and the value surged to 3.80 mPas (lower than 4.2 mPas) when operated at 50 °C. Thus, the CPO without centrifuging should be reacted at 50 °C for 96.15% FAME yield. Both plots for CPO without centrifuging plus the reaction mixture and for the centrifuged CPO plus the reaction mixture eventually attained similar dynamic viscosity at 60 °C.

Comparison of Thermal Activity and Stability of Lipozyme TL IM at 30 and 40 °C

Although a reaction temperature of 40 °C gave a 96.15% FAME yield compared to 30 °C, the thermal stability and activity, being dependent on the temperature, were compared before establishing the most suitable operational temperature for an enzymatic reactor. From the perspective of FAME productivity, transesterification at 40 °C energetically activated lipase catalyzing behaviour to produce 96.15 and 2.66% FAME yield/min, which is nearly 1.90 higher than the initial reaction rate of 1.43% FAME yield/min observed at 30 °C (Table 3). In addition, lipozyme TL IM exhibited different catalytic efficiencies at two temperatures with the specific enzyme activity being determined as 11.47% FAME yield/h/g lipase at 30 °C and 33.73% FAME yield/h/g lipase at 40 °C, respectively.

Thermal stability in terms of the denaturation constant, k_d, which relies on deactivation energy, was compared at 30 and 40 °C because the most suitable operational temperature is a compromise between high enzyme activity and good thermal stability. The activation energy for transesterification at optimum conditions was 22.00 kJ/mol, which indicated that the energy barrier required for the reaction was considerably small compared to the deactivation energy, 45.18 kJ/mol. Ettalbi and Baratty [15] also reported a similar activation energy value (26 kJ/mol) for

Table 3 Comparison of FAME productivity and thermal stability for transesterification at 30 and 40 °C

	Process parameters at 30 °C	Process parameters at 40 °C	Net gain or net loss for reaction at 30 °C compared to 40 °C (%)
Comparison of FAME productivity			
FAME yield at equilibrium phase	84.84% FAME yield	92.79% FAME yield	−8.57
Time period for equilibrium FAME	10 h	4 h	–
Initial reaction rate	1.43% FAME yield/min	2.66% FAME yield/min	−46.21
Specific enzyme activity	11.47% FAME yield/h/g lipase	33.73% FAME yield/h/g lipase	−66.0
Comparison of thermodynamic parameters			
Denaturation constant, k_d at deactivation energy, $E_d = 45.18$ kJ/mol	6.14×10^{-3} h^{-1}	1.10×10^{-2} h^{-1}	−44.18
Half life, $\tau = \frac{0.69}{k_d}$	112.37 h	63.23 h	+43.98
Residue lipase activity after 1 h (%), $\left(\frac{E}{E_0} = Exp^{-k_d t}\right) \times 100\%$	99.39	98.91	+0.48
Residue lipase activity after 24 h (%), $\left(\frac{E}{E_0} = Exp^{-k_d t}\right) \times 100\%$	86.30	76.87	+10.93

immobilised inulinase from *Aspergillus niger*. The denaturation constant, k_d, estimated at 40 °C was 1.10×10^{-2} h^{-1}, and it was 1.79-fold higher than the k_d value estimated at 30 °C, 6.14×10^{-3} h^{-1}. Consequently, the half life time was prolonged from 63.23 to 112.87 h when operated at room temperature instead of 40 °C. When comparing lipase activity on a 1-h basis, the decrease in enzyme stability with temperature was not significant. However, lipase was able to retain 86.30% of its original activity after 24 h of continuous room temperature operation, whereas 76.87% lipase remained viable at 40 °C thermal treatment. The utilization of higher enzyme loading in the reaction medium can help to circumvent the low productivity due to the subsequent enzyme denaturation with time [16].

The net gain and net loss in FAME productivity and lipase stability for temperatures of 30 and 40 °C are calculated and tabulated in Table 3. The discussion of the net gain or net loss in lipase activity and stability due to 30 and 40 °C operating temperatures was compared to show the advantages and disadvantages of 30 and 40 °C operating temperatures. According to Table 3, the catalytic efficiency for 30 and 40 °C was 11.47% FAME yield/h/g lipase and 33.73% FAME yield/h/g lipase, respectively. This means that CPO transesterification at 40 °C has successfully enhanced the catalytic efficiency to 66.0% higher than the catalytic efficiency achieved at 30 °C. CPO transesterification at 40 °C with 96.15% FAME yield was accomplished in 4 h, which was 6 h shorter than the reaction time required for 30 °C. In terms of lipase stability, the deactivation rate at 30 °C was 44.18% lower than at 40 °C. The lower deactivation rate observed at 30 °C means that it successfully retained a 43.98% longer half life time for lipase compared to 40 °C. The loss in lipase stability of 44.0% at 40 °C was overcome by the 60.0% gain in lipase activation. Consequently, 40 °C was determined to be the most desirable working temperature for lipozyme TL IM.

Conclusion

CPO transesterification at room temperature operation is feasible because the process gave a reasonable FAME yield of more than 80% and an initial reaction rate of 85.86% FAME yield/h. Among the independent variables, enzyme loading was the most influential process parameter for responses like FAME yield and initial reaction rate when the transesterification was operated at 30 °C. The failure of the reaction at 30 °C to attain a high equilibrium FAME yield of 96.15%, as was observed at 40 °C, was mainly attributed to the heavy viscosity of the reaction mixture, which was temperature dependent. The enzyme denaturation constant at 40 °C was 1.79-fold greater than at 30 °C, but the low yield due to enzyme instability was circumvented by the high production rate observed at 40 °C. Besides, the thermal effect at 40 °C that caused the enzyme to denature was only pronounced after the reaction mixture and the lipozyme TL IM were preincubated at 40 °C for at least 1 day prior to the transesterification reaction. For the lipozyme TL IM used in the study, 40 °C was defined as the optimum temperature, resulting in a 96.15% FAME yield and a moderate enzyme deactivation rate.

Acknowledgments The authors express their gratitude to the Ministry of Science, Technology and Innovation (MOSTI), Malaysia, for their financial support of the postgraduate study and project funding through the National Science Fellowship and Science Fund (project no. 02-01-05-SF0122). The research university grant (project no. 1001/PJKIMIA/814004) and research university postgraduate research grant scheme (project no. 1001/PJKIMIA/8031036) awarded by Universiti Sains Malaysia are also appreciated. Khor Guat Kheng from the School of Chemical Engineering, Universiti Sains Malaysia (USM), is kindly acknowledged for her technical support.

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