Conceptual study of transesterification of vegetable oils in the continuous-stirred-tank reactor at unsteady-state and isothermal conditions

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Abstract The continuous-stirred-tank reactor (CSTR) is favorable for bi-phasic enzymatic reaction due to ease of operation, cost-effective and low downtime. The lack of study on the enzymatic reaction in the CSTR has disfavor this type of reactor compared to batch and packed bed. The purpose of this study is to prove the feasibility of enzyme-catalyzed production of biodiesel in the CSTR using the mathematical model and simulation. The model incorporated the effect of the kinetic, thermal, and operating parameters. Then, it was solved using Polymath 6.1 software. Then, suitable enzyme characteristics and operating conditions are suggested. A high conversion could be achieved by choosing an appropriate lipase and operating conditions. The lipase with $K_i > 100$ mol/L, $K_m < 0.15$ mol/L, and $\Delta G_{\text{inac}} > 120$ kJ/mol were selected as they are resistant to methanol inhibition, showing a high affinity for the oil substrate and thermally stable. For a thermally sensitive enzyme, the operating temperature should remain constant at its optimal level to avoid over denaturation. In addition, the residence time for the triglycerides is prolonged (>20 hr) whereas for the methanol is shortened since it can increase the conversion. This can be achieved by reducing the volumetric flow rate of the triglycerides over the methanol. However, it must not be too low to prevent lower productivity. This article highlights that the production of biodiesel using enzyme-catalyzed reaction can be feasible if an appropriate lipase and operating conditions are employed.

Keywords: Enzymatic, CSTR, Transesterification, Biodiesel, Lipase.

Introduction

The approach of biodiesel synthesis from vegetable oils via transesterification with alcohol imparted issues on the sustainability of the process especially its impacts on the environment and net production cost. The synthesis can either be readily carried out using acid, base or enzyme catalyst. The pros and cons of each method have been reviewed elsewhere [1]. Generally, the enzyme-catalyzed process is more sustainable than other approaches (Figure 1). This is because it requires mild reaction temperature, without side reaction, leads to simple downstream purification and recovery of other high-valued products. Nevertheless, the enzymatic approach possesses a high risk from the perspective of an investor or process engineer. The concerning issues are on the cost, reusability and stability of the catalyst. Due to this reason, a chemical catalyst has been used despite corrosion issues and the high volume of wastewater generated.

An enzyme in its native form exhibits the highest activity. However, they are prone to deactivation and
recovery which are impossible with nano size molecules. Currently, various methods are available to immobilize enzymes on organic and inorganic polymeric supports to ease the recovery process [2,3]. The reusability and the stability of the enzyme have been significantly improved [4]. In addition, if the enzyme is produced and prepared onsite it can further reduce the cost of the entire process.

![Diagram of Lipase-Catalyzed Synthesis](image)

**Figure 1.** Lipase-catalyzed synthesis of fatty acid methyl ester or biodiesel from triglycerides.

The remaining issue is on the biocatalytic reaction occurring in the reactor that is apparently not well understood. An enzyme lipase for instance is prone to inhibition by alkyl donor of methanol for biodiesel synthesis and sensitive to the change of temperature [5,6]. The activity of lipase might reduce in the presence of methanol which act as inhibitor and permanently deactivates or denatures when exposed to high temperature. Another alkyl donor has been proposed to eliminate the inhibitor such as the introduction of methyl acetate and ethanol [7,8]. Due to the exceptionally high cost of solvents, methanol is more preferable compared to other solvents. Alternatively, the intermittent feed of methanol was proposed such that the inhibitory effect could be reduced [9].

There are various options available to conduct biocatalytic reaction for biodiesel production such as batch, continuous-stirred-tank (CSTR) and packed-bed (PBR) reactors [10-13]. Until today, batch and PBR have been the most applied reactors for bench-scale studies due to their ease of operation in a small scale. Meanwhile, the CSTR is completely disfavoured. Technically, several criteria should be considered before choosing the appropriate reactor for any chemical reaction. The comparison between these reactors based on various criteria is listed in Table 1. Based on the comparison, CSTR is a good option for the mass production of biodiesel with minimum drawbacks compared to the PBR. For instance, a high initial concentration of alcohol substrate fed into the PBR might increase the risk of lipase inhibition. Meanwhile, an uneven rate of reaction along the PBR causes the biocatalyst close to the reactor inlet denatured or deactivated faster compared to the one at the reactor outlet. Besides, the pressure drop and surface contact are the main issues especially when two-phase liquid exists due to a large density difference between the alcohol and oil.

| Criterion                  | Batch            | CSTR             | Packed Bed    |
|----------------------------|------------------|------------------|---------------|
| **Mode of operation**      | Batch            | Continuous       | Continuous    |
| **Reaction Phase**         | Single/dual      | Single/dual      | Single        |
| **Initial Substrate**      | High             | Low              | High          |
| **concentration**          |                  |                  |               |
| **Exposure to inhibitor**  | High             | Low              | Low           |
| **Downtime**               | High             | Negligible       | Negligible    |
| **Reverse reaction**       | Most affected    | Less affected    | Less affected |
| **Dispersion of biocatalyst** | Good            | Good             | Not applicable |
| **Pressure drop**          | Not applicable   | Not applicable   | High          |
| **Feed velocity**          | Not applicable   | Flexible         | Limited by pressure drop |
| **Rate of reaction**       | Homogeneous      | Homogeneous      | Gradient      |

Table 1. Brief comparison between batch, stirred-tank, and packed-bed reactor for a continuous enzyme-catalyzed reaction.
The transesterification of triglycerides and methanol by enzyme-catalyzed reaction can be done in a CSTR since it allows good dispersion and wide range of feed flow rates. It is also possible to carry out the biphasic reaction with an additional small amount of water to enhance the catalytic activity. However, various reactions and operating parameters could affect the output parameters such as conversion, degree of inhibition, catalyst activity, the rate of reaction and others [14-16]. The combined effects of multiple input parameters make the analysis more complicated. For example, a decreased conversion could be due to the inhibition, enzyme deactivation, shorter residence time and reaction temperature. In order to achieve a sustainable process, the CSTR must exhibit a constant conversion, prolong the half-life of the biocatalyst and operate at lower reaction temperatures.

In the present work, a conceptual study of an unsteady-state and isothermal enzymatic reaction of biodiesel synthesis is carried out in a CSTR. It used to prove the feasibility and sustainability of the process. Kinetics and operating parameters were varied to simulate the actual case scenario and the process outcomes. Subsequently, appropriate enzyme characteristics and operating conditions were suggested.

Model development

Unsteady-state mols balance in the CSTR

Triglycerides and methanol are the key substrates of biodiesel synthesis. Ideally, these two substrates are fed separately into the reactor to provide better control on its concentration. The mols balance of any compound in the CSTR is given by;

\[
\begin{align*}
\frac{\text{Rate of accumulation of comp. } i}{\text{molar flow rate of comp. } i} &= \frac{\text{inlet molar flow rate of comp. } i}{\text{molar flow rate of comp. } i} - \frac{\text{Outlet molar flow rate of comp. } i}{\text{molar flow rate of comp. } i} + \frac{\text{Rate of generation of comp. } i}{\text{molar flow rate of comp. } i}
\end{align*}
\]

(1)

It can be written in an ordinary differential equation as;

\[
\frac{dN_i}{dt} = F_{i,0} - F_i + r_i V
\]

(2)

Where, \( F_i \) is the molar flow rate of the species \( i \) (mol/h), \( r_i \) is the reaction rate of species \( i \) (mol/L.h), \( V \) is the volume of the CSTR and \( N_i \) is the mole of species \( i \). The molar flow rate is also defined as the species molar concentration \( (C_i) \) multiplied by the fluid volumetric flow rate \( (v_o) \) which is given as

\[
F_i = v_o C_i
\]

(3)

The concentration of each compound at time, \( t \) can be solved through the derivation of Equation 2 to get the following equations;

\[
\frac{dC_{\text{oil}}}{dt} = \left( v_{\text{oil,0}} C_{\text{oil,0}} - v_{\text{out,0}} C_{\text{oil}} \right) \frac{1}{V} + r_{\text{oil}}
\]

(4)

\[
\frac{dC_{\text{met}}}{dt} = \left( v_{\text{met,0}} C_{\text{met,0}} - v_{\text{out,0}} C_{\text{met}} \right) \frac{1}{V} + r_{\text{met}}
\]

(5)

\[
\frac{dC_{\text{bio}}}{dt} = C_{\text{bio}} \frac{v_{\text{out}}}{V} + r_{\text{bio}}
\]

(6)

\[
\frac{dC_{\text{gly}}}{dt} = C_{\text{gly}} \frac{v_{\text{out}}}{V} + r_{\text{gly}}
\]

(7)

Where, the total outlet volumetric flow rate \( (v_{\text{out}}) \) is equivalent to the total inlet volumetric flow rate of triglyceride oil and methanol which feed in separately. This setup is adequate to control the feed ratio of the substrate. Hence \( v_{\text{out}} \) is given as;
\[ v_{\text{out}} = v_{\text{oil,0}} + v_{\text{met,0}} \]  

Whereas, the rate of formation and consumption of the products \( r_{\text{bio}} \) and \( r_{\text{gly}} \) and substrates \( r_{\text{oil}} \) and \( r_{\text{met}} \) are given in the following subsection. The Equation 4 to Equation 7 will be solved simultaneously using Polymath 6.1 software.

There is a various factor which could lead to the unsteady-state behavior in the CSTR, the most notable is due to the accumulation of excess mass or energy within the reactor system. However, in the present work, the unsteady-state behavior is due to the accumulation of component \( i \). Based on Equation 2 the accumulation of the substrate may occur due to the rate consumption of the substrate is less than the feeding rate or vice-versa. This is because the enzymatic rate reaction could vary depending on the real-time concentration of the substrates and the inhibitors. The reaction environment (pH, temperature) and operating conditions (agitation rate, type of solvent) can be the contributing factors as well.

**Rate of enzymatic reaction**
The general formula of an enzyme-catalyzed reaction between triglycerides and methanol is shown in Figure 1. The reaction is best described by ping-pong bi-bi mechanism of bi substrate reaction with the following rate of reaction;

\[ r_{\text{oil}} = -\frac{E_0 k_{\text{cat}} C_{\text{oil}} C_{\text{met}}}{K_{M,\text{oil}} C_{\text{oil}} + K_{M,\text{met}} C_{\text{met}} + C_{\text{oil}} C_{\text{met}}} \]  

It has been reported that methanol is known to be the inhibitor during the reaction. Hence, the rate consumption of the substrate is given as;

\[ r_{\text{oil}} = -\frac{E_0 k_{\text{cat}} C_{\text{oil}} C_{\text{met}}}{K_{M,\text{met}} C_{\text{oil}} + K_{M,\text{oil}} C_{\text{met}} \left(1 + \frac{C_{\text{met}}}{K_{I,\text{met}}} \right) + C_{\text{oil}} C_{\text{met}}} \]  

Whereas, the rate consumption of the substrates and rate formation of the products can be correlated as in the following stoichiometric ratio;

\[ \frac{r_{\text{oil}}}{\frac{1}{3}} = \frac{r_{\text{met}}}{\frac{3}{3}} = \frac{r_{\text{bio}}}{\frac{3}{3}} = \frac{r_{\text{gly}}}{\frac{1}{1}} \]  

**Effect of temperature on the enzyme**

Most of the enzymatic reactions show a gradual increase in conversion when the temperature is raised to their optimum level before it declines due to the denaturation. The mechanism was described in a two-step process by Daniel and co-workers [17]. The following equation is used to estimate the reaction rate velocity.

\[ V_{\text{max}} = \frac{k_{\text{cat}} E_0 e^{-\frac{K_{I,\text{cat}}}{K_{eq}}}}{1 + K_{eq}} \]  

Where the values of the catalytic turnover, inactivation constant and equilibrium constant are affected by the temperature according to the following equations [17];
\[ k_{\text{cat}} = \frac{k_BT}{h} e^{-\left(\frac{\Delta G_{\text{cat}}^\circ}{R}T\right)} \quad (13) \]

\[ k_{\text{inact}} = \frac{k_BT}{h} e^{-\left(\frac{\Delta G_{\text{max}}^\circ}{R}T\right)} \quad (14) \]

\[ K_{eq} = e^{-\frac{\Delta H_{eq}}{R} \left(\frac{1}{T_{eq}} - \frac{1}{T}\right)} \quad (15) \]

Meanwhile, the effect of temperature on the Michaelis and inhibition constants are given as;

\[ K_m = K_{m,0} e^{\frac{\Delta H_m}{RT}} \quad (16) \]

\[ K_{I,met} = K_{I,0} e^{\frac{\Delta H_I}{RT}} \quad (17) \]

**Scope of the study**

The simulation study was carried out for lipase-catalyzed transesterification of triglycerides with alcohol. It was assumed that the reaction was carried out in an isothermal condition and the loss of lipase activity was only due to deactivation or denaturation. A new biocatalyst was not added to the reactor throughout the reaction period and it was also assumed that the biocatalyst exiting the reactor was quickly recycled by appropriate means (i.e., decantation or physical filtration), hence the lipase concentration in the reactor remains constant.

The enzyme kinetic data from the literature shown in Table 2 were used as a basis for the simulation. The values reported seem inconsistent, however, this finding is understandable since it highly depends on the reaction and operating conditions during evaluation. The same type of enzyme does not necessarily give the same kinetic values; therefore, every batch of enzyme exhibits different performance. Even though they are having a similar unit activity, their response towards the change of temperature might be different. A similar phenomenon occurs when a kinetic investigation was carried out at the different reaction media, temperature and ionic strength (pH). These uncertainties disfavor enzymatic reaction besides the lack of understanding of process. Hence, a standardized method has to be applied to benchmark the enzyme before further use.

**Table 2. The kinetic parameters of lipase in the transesterification of oil.**

| Lipase            | \(V_{\text{max}}\) (mol/L.min) | \(K_{m,oil}\) (mol/L) | \(K_{m,alc}\) (mol/L) | \(K_{I,alc}\) (mol/L) | Ref. |
|-------------------|---------------------------------|------------------------|------------------------|------------------------|------|
| Novozym 435       | 0.018                           | 397                    | 1030                   | 1815                   | [18] |
| Novozym 435       | 2.34                            | 2.61                   | 10.25                  | 1.6                    | [10] |
| R. miehei         | 0.00022                         | 0.0067                 | 2.212                  | 0.042                  | [13] |
| C. rugosa         | 0.170                           | 0.137                  | 1.081                  | 0.826                  | [19] |
| Novozym 435       | 0.000613                        | 0.0438                 | 0.0008                 | 1.0761                 | [20] |
| *Burkholderia sp.*| 0.61                            | 7.93                   | 1.01                   | 0.24                   | [21] |
| *Candida rugosa*  | 0.0000155                       | 0.1083                 | 0.1612                 | 0.1083                 | [12] |
| *B. cepacia*      | 0.006823                        | 0.0837                 | 0.159                  | 0.00821                | [22] |
| *Burkholderia sp.*| 0.0294                          | 0.163                  | 0.105                  | 116.4                  | [23] |
In the present work, the values of the kinetic, thermal, and operating parameters will be varied to investigate their effects on the reaction performance. The range of parameters used in this study is summarized in Table 3. The simulation was carried out by using Polymath 6.1 software.

Table 3. The range of parameters and constants used.

| Parameters or Constants                      | Values (unit) | Ref. |
|---------------------------------------------|---------------|------|
| Volume of reactor ($V$)                     | 600 L         |      |
| Reaction Temperature ($T$)                   | 20 - 60°C     |      |
| Volumetric flow rate ($v_o$)                | 2.5 – 45 L/h  |      |
| Triglyceride feed concentration ($C_{oil,o}$) | 0.1 – 1.0 mol/L |      |
| Methanol feed concentration ($C_{met,o}$)    | 0.5 – 16 mol/L |      |
| Initial Enzyme Concentration ($E_o$)         | 1 g/L         |      |
| Gibbs energy of catalysis ($\Delta G^1_{cat}$) | 77 kJ/mol     | [17] |
| Gibbs energy of thermal inactivation ($\Delta G^2_{inac}$) | 110 – 120 kJ/mol | [17] |
| Enthalpy of enzyme transition ($\Delta H_{eq}$) | 150 kJ/mol   | [17] |
| Enthalpy of enzyme inhibition ($\Delta H_i$)  | 6 kJ/mol      | [24] |
| Enthalpy of enzyme-substrate adsorption ($\Delta H_m$) | 5 kJ/mol     | [24] |
| Boltzmann's Constant ($k_B$)                 | $1.38064852 \times 10^{-23} \text{ m}^2\text{kg/(s}^2\text{K}$) |      |
| Planck Constant                              | $6.62607004 \times 10^{-34} \text{ m}^2\text{kg/s}$ |      |
| Gas constant                                 | 0.008314 kJ/(mol.K) |      |
| Average molecular weight of lipase           | 40,000 g/mol  | [25] |

* The values are indicative of the parameters and with the same order of magnitude than that reported in the literatures.

Results and discussion

Validation of the model

The mathematical model of the simulation was initially validated using the data reported by Ilmi and co-worker [11]. The experimental data were obtained during the transesterification of sunflower oil with methanol in a continuous-stirred-tank reactor. The enzyme was a commercial lipase with a trade name TransZyme A. The estimation of the kinetic parameters is based on the rule of thumbs of enzyme catalysis. The values of the Michaelis constants ($K_m$) has to be in the same order of magnitude of the substrate concentration. Whereas, the value of inhibition constant ($K_i$) has to be be less or higher than the concentration of inhibitor presence in the media for highly inhibited and non-inhibited enzyme respectively. The magnitude of the values of Gibbs energy and the change of enthalpies are based on the common enzymes reported in the literature. Figure 2 shows the comparison between the simulated and the actual data for FAME production in a continuous CSTR with a $R^2 = 0.97$. In general, the model is able to predict the actual concentration of FAME produced. The only limitation of the model is unable to accurately predict the lag phase at the early period of the reaction process. Based on the experimental data, it could be observed in few hours that is required by the CSTR before its performance reached a steady-state. The stability of the reaction performance correlates with heat and mass flow rate as well as the homogeneity of the reaction mixture. In the current work, the isothermal and perfect homogeneous mixture was assumed.
Effect of methanol inhibitor
The concentration of the substrates is usually manipulated to give the desired rate of reaction and to reach the intended productivity. The maximum concentration of the oil is limited by its density. The average density of plant oils is about 920 g/L with an average molecular weight of 840 g/mol. Hence, its maximum molar density or concentration is about 1.1 mol/L. Due to considerably high viscosity (≈40 cP) of vegetable oil, a solvent is usually added to ease the operation and this action reduces its concentration. Solvents such as butanol and isooctane could also be added to the reaction mixture. Besides that, an excess amount of methanol also contributes to lower viscosity of the reaction media, on the other hand, it also causes deactivation of the enzyme [26]. Due to the immiscibility of methanol and oil, butanol could be added as a co-solvent to form a homogeneous liquid mixture. The inhibition effect of methanol to the lipase is observed based on the value of the inhibitory constant ($K_i$). The effect of inhibitor was investigated by increasing the methanol composition at a fixed oil content, while the value of $K_i$ was remained constant (Table 3). The set value was indicative to the specific lipase. It should be understood that the value varies according to the type of inhibitors, media and the origin of the lipases. Table 4 shows that the conversion of vegetable oil at $K_i=100$ mol/L increases when the methanol ratio was increased from 1:1 to 1:16. However, this trend starts to change when the value of $K_i$ approaching 0.1 mol/L where the conversion starts to decrease at higher methanol contents. Adding an excess of methanol does not necessarily give a better conversion since the lipase is already inhibited. However, it is not too severe as expected if the $K_i$ value is significantly large. Notably, the inhibition is not significant when the values of $K_i$ significantly larger than the concentration of the inhibitor and vice-versa. The performance of the reaction is very sensitive to the change of inhibitor concentration when the inhibition effect is very significant ($K_i < 1$ mol/L).

Table 4. The effect of inhibition constant values and methanol content on the maximum conversion in the CSTR.

| Oil/Methanol Molar Ratio | Methanol (mol/L) | $K_i=100$ | $K_i=10$ | $K_i=1$ | $K_i=0.1$ |
|------------------------|-----------------|-----------|----------|---------|-----------|
| 1:1                    | 0.5             | 0.24      | 0.24     | 0.24    | 0.23      |
| 1:2                    | 1.0             | 0.44      | 0.44     | 0.43    | 0.29      |
| 1:4                    | 2.0             | 0.63      | 0.62     | 0.52    | 0.19      |
| 1:8                    | 4.0             | 0.70      | 0.66     | 0.44    | 0.10      |
| 1:16                   | 8.0             | 0.72      | 0.64     | 0.32    | 0.06      |

* The unit of $K_i$ is mol/L
Effect of $K_m$ ratio

The value of $K_m$ is very useful to describe the affinity of enzyme and the substrate. For a bi-substrate reaction, the values of $K_m$ can be compared to investigate the degree of affinity of the substrates [27]. Later, this would give an impact on the CSTR performance. In the present work, the affinity of oil to enzyme and methanol to the acyl-enzyme complex is represented by $K_{m,\text{oil}}$ and $K_{m,\text{met}}$, respectively.

The reaction proceeds in a two-step mechanism where at first the oil binds to the enzyme to form an acyl-enzyme complex and then followed by the methanol binding to the acyl-enzyme complex before the product formation. Lower value of $K_m$ means a better affinity or a formation of the complexes and vice-versa. $K_{m,\text{oil}}$ could be lower or greater than the value of $K_{m,\text{met}}$ for lipase-catalyzed biodiesel production as these values are unique for a specific lipase. The comparison of the effect of $K_m$ ratio on the biodiesel production is shown in Figure 3. Higher production is obtained when the $K_{m,\text{oil}} < K_{m,\text{met}} (K_{m,\text{oil}}=0.15 \text{ mol/L and } K_{m,\text{met}}=0.70 \text{ mol/L})$. Lower production for $K_{m,\text{oil}} > K_{m,\text{met}} (K_{m,\text{oil}}=0.70 \text{ mol/L and } K_{m,\text{met}}=0.15 \text{ mol/L})$ indicates that the production of FAME is limited by the formation of the acyl-enzyme complex. When the value of $K_{m,\text{oil}}$ is large, it reduces the formation of acyl-enzyme for the subsequent step of methanol binding. At $K_{m,\text{oil}} = K_{m,\text{met}} = 0.35 \text{ mol/L}$, it is usual to expect moderate performance. However, it is recommended to use lipase enzyme with lower $K_{m,\text{oil}}$ and $K_{m,\text{met}}$ values for the best performance. The reaction is expected to proceed at a faster rate due to a better affinity for both substrates. The output of the reaction (i.e., conversion, yield and concentrations) gives a sluggish response when the $K_m$ values are larger, thus take a longer period to reach a steady-state during the continuous operation.

![Figure 3. The effect of $K_m$ on the productivity of FAME in the CSTR. (○) $K_{m,\text{oil}} < K_{m,\text{met}}$, (□) $K_{m,\text{oil}} > K_{m,\text{met}}$ and (△) $K_{m,\text{oil}} = K_{m,\text{met}}$.](image)

Effect of feed flow rate and concentration

The performance of reaction in the CSTR is highly dependent on the residence time. The longer the substrate retains in the reactor, the higher the conversion [28]. In contrast with the chemical-catalyzed reaction, there is no side reaction when using a long residence time in the CSTR for an enzyme-catalyzed reaction. The residence time in the reactor is prolonged with the slower volumetric flowrate or larger reaction volume ($t_{\text{res}} = V/v_f$). In this study, the feed volumetric flow rate of oil (Figure 4(a)) and methanol (Figure 4(b)) were varied with a reactor volume of 600 L. Whereas, the feed concentrations of oil and methanol were fixed at 0.5 and 2 mol/L, respectively. Increasing the feed volumetric flow rate of oil (5 to 100 L/hr) has reduced the conversion because of the shorter retention time. The actual period of reaction between the substrate and the enzyme is limited. Hence, most substrates flow out of the reactor without undergoing a transesterification reaction despite a larger molar flow rate. A slower feed volumetric flow rate of oil may give a higher conversion. However, it must not be too low to meet the desired rate of the production as the molar flow rate decreases. For example, the calculated rate of production of FAME at $v_{o,\text{oil}}=100 \text{ L/hr}$ is about 28.6 mol/hr whereas...
only 17.5 mol/h at \( v_{o,\text{oil}} = 5 \text{ L/hr} \).

Increasing the feed volumetric flow rate of methanol (5 to 100 L/hr) does not significantly change the conversion. It slightly increases by 0.015 at 25 L/hr before gradually decline at a faster speed (Figure 4(b)). There is no clear evidence for this behavior which might be due to the present of methanol being the inhibitor. The faster volumetric flow rate would definitely increase the amount of methanol in the CSTR to reach the necessary amount or a so-called optimum reaction. However, methanol causes inhibition hence the lower conversation. In addition, a faster flow rate reduces the residence time which causes a further decrease in the conversion.

It must be noted that the amount of oil and methanol in the CSTR are affected by the inlet concentration and volumetric flow rate. Thus, a similar trend was observed on the conversion when the initial concentration of oil (Figure 4(c)) and methanol (Figure 4(d)) in the feed were increased. An appropriate balance must be estimated in order to keep the ideal molar ratio between the substrates while retaining them for an optimum conversion. Based on the current work, a residence time which above 20 hours is required to achieve oil conversion above 80%. However, an appropriate optimization has to be carried out in a specific reaction condition with the consideration of the operating parameters constraints. In order to improve the conversion of triglycerides, it is suggested that both substrates have to be fed individually with a volumetric flow rate of methanol which is faster than the oil. This setup is able to reduce the residence time for methanol inhibitor while increasing the residence time for the oil.

\[ \text{(a)} \quad C_{\text{CH}_3\text{OH}} = 2 \text{ mol/L}, \quad C_{\text{oil}} = 0.5 \text{ mol/L} \]
\[ v_{o,\text{CH}_3\text{OH}} = 25 \text{ L/hr} \]
\[ \text{(b)} \quad C_{\text{CH}_3\text{OH}} = 2 \text{ mol/L}, \quad C_{\text{oil}} = 0.5 \text{ mol/L} \]
\[ v_{o,\text{oil}} = 25 \text{ L/hr} \]
\[ \text{(c)} \quad C_{\text{CH}_3\text{OH}} = 2 \text{ mol/L} \]
\[ v_{o,\text{oil}} = v_{o,\text{CH}_3\text{OH}} = 25 \text{ L/hr} \]
\[ \text{(d)} \quad C_{\text{oil}} = 0.5 \text{ mol/L} \]
\[ v_{o,\text{oil}} = v_{o,\text{CH}_3\text{OH}} = 25 \text{ L/hr} \]

**Figure 4.** The effect of (a) volumetric flow rate of oil, (b) volumetric flowrate of methanol, (c) oil feed concentration and (d) methanol feed concentration.
Effect of temperature
Even though a high temperature rarely leads to a runaway reaction, it does affect the activity of the enzyme. Maintaining optimum temperature is crucial to the lipase-catalyzed reaction as a slight change of temperature within ±5 °C could significantly change the reaction rate. Moreover, the half-life of the enzyme is also dependent on the operating temperature. Research has shown that an enzyme operated at high temperatures would denature faster [29]. In this work, the effect of temperature (between 20 to 60 °C) was investigated. The mathematical model employed considers that the denaturation of lipase occurs at a high temperature and the activation of lipase occurs when the temperature raised to an optimum value [17]. Figure 5 shows that the highest conversion of oil occurs at a temperature of 40 °C. This result agrees with the experimental data reported in the literature of a typical lipase-catalyzed reaction [30,31]. Enzymes usually exhibit activation behavior when the temperature is raised to the optimum level before the denaturation occurs and lose their activities at elevated temperatures. Loss of lipase activities was indicated at a temperature beyond 50 °C as the conversion of oil is depleted after reaching the highest peak as shown in Figure 5.

Effect of thermal inactivation constant
A thermal resistant enzyme is an enzyme that can resist high temperature without being denatured. The enzyme can be used for a number of the demanding reaction processes. These types of enzymes can be obtained by isolating from bacterial species growing in a harsh environment [32]. Otherwise, the thermal properties of the enzymes could be also improved via directed mutagenesis or directed evolution [33,34]. The enzyme usually lose their shape and activity with a prolonged use. This phenomenon disfavors an enzyme catalysis for a continuous process. Moreover, the downtime period may increase when the deactivated enzyme is replaced with an active enzyme in the reactor. The life span of the enzyme is indicated by its half-life which means a period taken to halve its initial activity. The half-life of an enzyme depends on its thermal resistance which is represented by the value of thermal inactivation constant.

The impact of the thermally unstable lipase on the biodiesel production in the CSTR was investigated by varying the value of thermal inactivation constant ($\Delta G_{\text{inac}}$) as shown in Figure 6. The value of $\Delta G_{\text{inac}}$ was increased from 110 to 120 kJ/mol where the larger value indicates a thermally stable lipase. The reaction profile was evaluated at 30°C and 60°C with varying $\Delta G_{\text{inac}}$ values. These temperatures were selected because the enzymatic reaction is usually carried out within this range. The reaction profile at both temperatures shows that the thermal resistance enzyme ($\Delta G_{\text{inac}}$=120 kJ/mol) gives a better performance for higher conversions. Overall, the lipase at a higher temperature considerably lose their activities thus, the conversion remains low and the concentration of the substrate remains at the feed concentration. The reaction profile at 30°C and $\Delta G_{\text{inac}}$=120 kJ/mol is the most ideal for a CSTR configuration. The conversion and concentration of the substrates remain constant at most of the 20 days period which indicate a minimal denaturation of the lipase. Regardless of $\Delta G_{\text{inac}}$ values, thermal denaturation of lipase shall take place for a prolonged period of the reaction. The decision to change or add the fresh lipase has to be made at the appropriate time to prevent underperformance of the reaction. The half-life of the enzyme is the best indicator which shows that the other operating parameters remain unchanged.
Figure 5. The effect of temperature on the conversion of oil in the CSTR.
Figure 6. Profile of reactions at temperatures 30°C and 60°C for lipase with thermal inactivation constants ($\Delta G_{\text{inac}}$) of (a) 110, (b) 115 and (c) 120 kJ/mol. The legends are; (○) oil, (x) methanol, (∆) FAME, and (□) conversion.
Conclusions

The feasibility and sustainability of the lipase-catalyzed synthesis of FAME in the CSTR was evaluated using mathematical modeling \((R^2 = 0.97)\) and simulation. The simulation process emphasized the kinetic, thermal, and operating parameters that need to be considered for an enzyme-catalyzed reaction. Lipase with \(K_m < 0.15 \text{ mol/L}, \Delta G_{\text{mec}} > 120 \text{ kJ/mol} \) and \(K_{\text{inhib}} > 100 \text{ mol/L} \) is an ideal characteristic that gives a fast reaction rate, good thermal stability, and resistance to inhibition. The reaction process is more sustainable if Lipase that meets this criterion is employed. The operating parameters such as temperature and molar flow rate should be manipulated to control the course of the reaction. For a thermally sensitive enzyme, the temperature should remain constant to avoid over-denaturation. The molar flow rate is proportional to the volumetric flow rate and concentration of the feed. The slower volumetric flow rate shall increase the conversion due to longer retention time, however, it must not too low such that to cause lower productivity. Further, the feasibility of the process should be evaluated based on the cost-benefit analysis.

Data availability

The simulation data and the programming code of this study are available from the corresponding author upon request.

Conflicts of interest

The authors declares that there is no conflict of interest regarding the publication of this paper.

Funding statement

A part of this work was supported by Universiti Sains Malaysia through the Short-Term Grant [Ref No. 2019/0566].

Acknowledgments

The authors would like to acknowledge the Universiti Sains Malaysia for providing the research facilities. The technical support and assistance by the academic and technical staff of the school of chemical engineering are also acknowledged.

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