Chemical Engineering Journal 103 (2004) 7–11

Chemical Engineering Journal

www.elsevier.com/locate/cej

High enzyme concentration model for the kinetics of hydrolysis of oils by lipase

Sulaiman Al-Zuhair, K.B. Ramachandran*, Masitah Hasan

Department of Chemical Engineering, University of Malaya, 50603 Kuala Lumpur, Malaysia

Received 22 December 2003; received in revised form 29 June 2004; accepted 5 July 2004

Abstract

A mathematical model to predict the hydrolysis rate of oils by lipase, from a proposed kinetic mechanism of the reaction is derived. The model predicts interfacial saturation at high enzyme concentration. It is used to determine, under different operating conditions, the critical enzyme concentration, i.e., the enzyme concentration at which the interface between the oil and the aqueous phase containing the enzyme is saturated. To verify the model predictions, experimental results of the hydrolysis of palm oil and sunflower oil are used. The model predictions closely agree with the experimental results for sun flower oil. The sensitivity of the model predictions to the values of the kinetic parameters is also investigated.

Keywords: Lipase; Oil hydrolysis; Interfacial saturation; Kinetic model; Palm oil; Sunflower oil

1. Introduction

The applications, importance and significance of lipase in oleochemical industry have been thoroughly demonstrated in literature [1–8]. The most important among these applications is the use of lipase for the production of fatty acids from oils. It is recently attempted as an energy-saving method, especially for producing high value-added products or heat sensitive fatty acids [1,2].

Lipase catalysed reactions take place at the interface between the aqueous phase containing the enzyme and the oil phase, where the enzyme has to penetrate the interface as a first step in the reaction [1,4,5]. In a stirred bioreactor, the interfacial area is affected by agitation speed, substrate concentration, and temperature [1,4,6]. At any particular operating condition, the total free interfacial area is limited. Although an increase in the bulk enzyme concentration is assumed to increase the rate of reaction, there would be a critical enzyme concentration at which the interfacial area would be saturated with the penetrated enzyme. Beyond this point, any increase in the enzyme concentration in the bulk will not enhance the reaction rate. This phenomenon of interfacial area saturation with enzyme has been demonstrated experimentally by Al-Zuhair et al. [1] for the hydrolysis of palm oil and by Albasi et al. [6] for the hydrolysis of sunflower oil. To use the enzyme effectively, the bulk enzyme used should not exceed the critical concentration.

In order to predict interfacial saturation, a mathematical model applicable at high enzyme concentration is needed. In literature, several mathematical models have been developed from hydrolysis reaction mechanism, but are mostly applicable to low enzyme concentration region, where it is assumed that the area occupied by the enzyme is small compared to the available interfacial area [3–4]. Only the models developed by Al-Zuhair et al. [1] and Tsai and Chang [2] avoided this assumption. Although Al-Zuhair et al. [1] derived a general model to predict the behaviour of hydrolysis reaction; they later simplified the model equations to low enzyme concentration regions to compare the model predictions with the experimental results. It can be seen from their study that the simplified model predictions agreed with the experimental results at low enzyme concentrations but diverged at high enzyme concentrations. It is clear that the simplified model

* Corresponding author. Tel.: +60 3 7967 5293; fax: +60 3 7967 5319.
E-mail address: kbram@um.edu.my (K.B. Ramachandran).

1385-8947/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.cej.2004.07.001

Nomenclature

a	specific free interfacial area (m^{-1})
a_t	specific total interfacial area (m^{-1})
A_m	enzyme molar area ($m^2\ mol^{-1}$)
$(A_m)_m$	enzyme mass area ($g\ mol^{-1}$)
C	proportionality constant defined by Eq. (8)
E	free enzyme (mole/total reactor volume) ($mol\ m^{-3}$)
E^*	penetrated enzyme (mole/total interfacial area) ($mol\ m^{-2}$)
E^*S	enzyme–substrate complex (mole/total interfacial area) ($mol\ m^{-2}$)
E_t	total active enzyme ($mol\ m^{-3}$)
$(E_t)_m$	total enzyme mass concentration ($g\ m^{-3}$)
k_{cat}	catalytic rate constant (min^{-1})
k_d	desorption rate constant (min^{-1})
k_p	adsorption rate constant ($m^2\ min^{-1}$)
k_1	reaction rate constant ($m^3\ mol^{-1}\ min^{-1}$)
k_{-1}	reaction rate constant (min^{-1})
K_e	equilibrium constant of E^*S ($mol\ m^{-3}$)
P^*	interface product concentration (mole/total interfacial area) ($mol\ m^{-2}$)
P	bulk product concentration (mole/total reactor volume) ($mol\ m^{-3}$)
S	bulk substrate concentration (mole/total reactor volume) ($mol\ m^{-3}$)
t	time (min)
T	temperature (K)
W_m	molecular weight of the enzyme ($g\ mol^{-1}$)

Greek letters

ϕ	volume fraction of oil in the reaction mixture
υ	reaction rate ($mol\ m^{-3}\ min^{-1}$)
ω	agitation speed (rpm)

equations are not applicable to high enzyme concentration regions and hence could not predict the interfacial area saturation with the enzyme. Recently, Straathof [9] had attempted to model enzymatic reaction at any enzyme concentration to predict the critical enzyme concentration. However, his model was based on the assumption that the reaction rate follows Michaelis–Menten kinetics and the interfacial enzyme concentration obeys Langmuir adsorption model. By adopting Michaelis–Menten model, Straathof [9] has assumed that the apparent Michaelis–Menten constant is independent of the interfacial area. This is not true as shown by the experimental results of Mukataka et al. [3], Tsai et al. [4] and by the model equations and results of Al-Zuhair et al. [1]. Further, the model equations developed by Straathof [9] are not based on the mechanism of the hydrolysis reaction.

The objective of this work is to develop a general mathematical model, avoiding the assumption of low enzyme concentration in the bulk, to predict the hydrolysis rate of lipids at different enzyme concentration and to analyse the model sensitivity to variations in the values of rate constants. This work also includes using the model to determine critical enzyme concentration at different operating conditions and to verify it with the experimental results for the hydrolysis palm oil and sunflower oil.

2. The kinetic model

The mathematical model proposed to describe the action of lipase for the hydrolysis of oil is similar to the one proposed by Tsai and Chang [2]. The first step is the reversible adsorption of a water-soluble enzyme at the interface to produce a penetrated enzyme, E^*. In order to develop the model equations, the adsorption rate is assumed to be proportional to the free enzyme concentration, E, and the specific free interfacial area, a [1,2]. The free substrate, S, then binds to the adsorbed enzyme giving an interfacial enzyme–substrate complex, E^*S [1,2,5]. This complex then generates the product, P^*, at the interface, while regenerating the enzyme in the form of E^*. The product, P^*, then desorbs from the interface in to the organic phase to give rise to the product, P. The steps up to the production of the product, P^*, are illustrated in Eqs. (1)–(3).

$$E + a \underset{k_d}{\overset{k_p}{\rightleftarrows}} E^* \tag{1}$$

$$E^* + S \underset{k_d}{\overset{k_1}{\rightleftarrows}} E^*S \tag{2}$$

$$E^*S \xrightarrow{k_{cat}} E^* + P^* \tag{3}$$

The concentration of the enzyme–substrate complex and the adsorbed enzyme are both assumed constant (quasi–steady state) [1,2,5] and the interfacial product concentration, P^*, is assumed to be proportional to the free product concentration, P [1,2]. It is also assumed that the interfacial product P^* is rapidly desorbed from the interface in to the organic phase, and hence, it occupies negligible fraction of the total interfacial area. With the above mechanism and assumptions, the model equations can be written as:

$$k_p E a - (k_d + k_1 S)(E^*) + (k_{-1} + k_{cat})(E^*S) = 0 \tag{4}$$

$$k_1(E^*)(S) - (k_{-1} + k_{cat})(E^*S) = 0 \tag{5}$$

$$a_t = a + A_m[(E^*) + (E^*S)]a_t \tag{6}$$

$$E_t = E + a_t[(E^*) + (E^*S)] \tag{7}$$

The penetrated product concentration, P^*, is assumed to be proportional to the free product concentration, P, according to Eq. (8)

$$P^* = \frac{CP}{a_t} \tag{8}$$

The rate of product formation can now be expressed as:

$$\upsilon = \frac{\mathrm{d}P}{\mathrm{d}t} = \frac{a_\mathrm{t}}{C}\frac{\mathrm{d}P^*}{\mathrm{d}t} = \frac{a_\mathrm{t}}{C}k_\mathrm{cat}(E^*S) \quad (9)$$

From the above equations, the rate of hydrolysis can be expressed as,

$$\upsilon = \frac{(k^*_\mathrm{cat}a_\mathrm{t}/(A_\mathrm{m})_\mathrm{m})S}{K_\mathrm{e}+S}(G_1 - G_2) \quad (10)$$

where,

$$K_\mathrm{e} = \frac{k_\mathrm{cat}+k_{-1}}{k_1} \quad (11)$$

$$k^*_\mathrm{cat} = \frac{k_\mathrm{cat}}{2CW_\mathrm{m}} \quad (12)$$

$$G_1 = \frac{(k_\mathrm{d}/k_\mathrm{p})}{a_\mathrm{t}^2\left[1+(S/K_\mathrm{e})\right]} + 1 + \left(\frac{(A_\mathrm{m})_\mathrm{m}}{a_\mathrm{t}}\right)(E_\mathrm{t})_\mathrm{m} \quad (13)$$

and

$$G_2 = \left(G_1^2 - \frac{4(A_\mathrm{m})_\mathrm{m}(E_\mathrm{t})_\mathrm{m}}{a_\mathrm{t}}\right)^{0.5} \quad (14)$$

The Eqs. (10)–(14) are applicable for predicting the hydrolysis rate of oils by lipase at any enzyme concentration.

Eq. (10) can be simplified at low enzyme concentrations to

$$\upsilon = \frac{k^*_\mathrm{cat}(E_\mathrm{t})_\mathrm{m}S}{K_\mathrm{e}[(k_\mathrm{d}/k_\mathrm{p}a_\mathrm{t}^2)+1]+S} \quad (15)$$

3. Results and discussions

3.1. Model verification

First, the experimental results from our previous work [1] for the hydrolysis of palm oil in well-agitated bioreactor are used to validate the proposed model equations. The enzyme used was a solid powder of lipase Type VII from *Candida rugosa*, obtained from Sigma Chemical Co., Japan. Measurements of the total specific interfacial area and initial rate of palm oil hydrolysis in aqueous solution were reported. The measurements were done at different oil volume fractions (0.05–0.5), temperatures (35–65 °C), enzyme concentrations (25–250 g m^{-3}) and stirrer speeds (500–1300 rpm). Other experimental details can be found else were [1]. The values of the rate constants and the expression for total specific interfacial area, listed in Table 1, were obtained from the experimental results by multiple regression method using MATLAB. The high-enzyme concentration equations (Eqs. (10)–(14)) and low enzyme concentration model (Eq. (15)) were solved by MATLAB, using the rate constants and the total interfacial area equation given in Table 1. The specific conditions used for the model validation were substrate concentration of 660.7 mol m^{-3}, which is equivalent to an oil volume fraction of 0.2, temperature of 318 K and two agitation speeds, namely, 800 and 1000 rpm. The results are shown in Figs. 1 and 2 for an rpm of 800 and 1000, respectively. It can be seen that the high enzyme concentration model has indeed followed the trend of the experimental data and showed the effect of interfacial area saturation. The relative standard deviation between the high enzyme model prediction and the experimental results is ±0.354. The low enzyme model curve deviates from the experimental data at high enzyme concentrations and also did not predict the interfacial area saturation.

Table 1
Reaction rate constants and an expression of the total specific interfacial area reported by Al-Zuhair et al. [1]

Parameter	Value/expression
k^*_cat	1.8×10^{-3} min^{-1}
K_e	5.65 mol m^{-3}
$k_\mathrm{d}/k_\mathrm{p}$	7.7×10^{7} m^{-2}
a_t	$0.024\omega^{0.6}T^{1.7}\phi/(1+3\phi)$

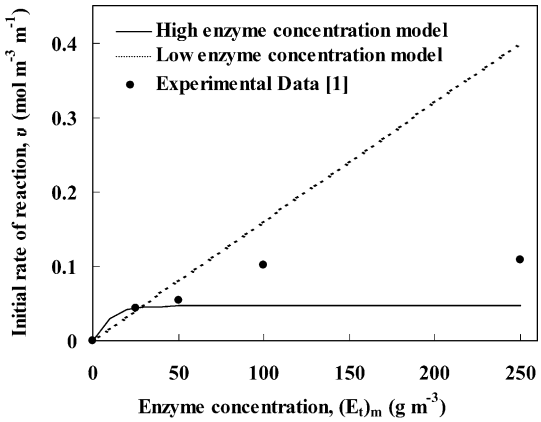

Fig. 1. Comparison between high enzyme and low enzyme model predictions (ω = 800 rpm, T = 318 K and S = 660.7 mol m^{-3}).

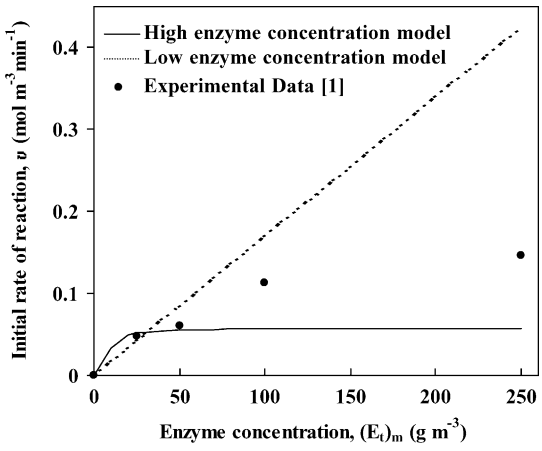

Fig. 2. Comparison between high enzyme and low enzyme model predictions (ω = 1000 rpm, T = 318 K and S = 660.7 mol m^{-3}).

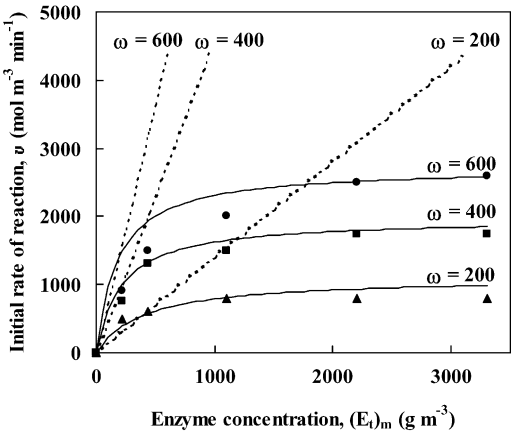

Fig. 3. Comparison between the model prediction and the experimental results of Albasi et al. [6] for sunflower oil. (—) High enzyme model; (- - -) low enzyme model; experimental results at: (▲) = 200 rpm; (■) = 400 rpm; (●) = 600 rpm.

The experimental results of Albasi et al. [6] for the hydrolysis of sunflower oil in well-agitated bioreactor are also used to further validate the proposed model equations. The enzyme used by them was *Candida cylindracea* lipase obtained from Meito Sangyo, France. The simulation studies were done using MATLAB. Fig. 3 shows the initial rate of sunflower oil hydrolysis as predicted by the low enzyme concentration model and the proposed high enzyme concentration model at 310 K and at a substrate concentration of 1578 mol m^{-3}. The kinetic parameters used in the simulation were k_d/k_p = 2 × 10^{10} m^{-2}, K_e = 10 mol m^{-3} and k^*_{cat} = 110 min^{-1}. The comparison is made at three different agitation speeds of 200, 400 and 600. Again, it can be seen that the high enzyme concentration model closely agrees with the experimental results and indeed predicts interfacial saturation. The relative standard deviation between the experimental results and the high enzyme model prediction is ±0.210. The low enzyme model again deviates considerably from the experimental results.

3.2. Effect of model parameters

The adsorption and desorption of the enzyme at the interface are the most important steps that affect the interface saturation with the enzyme. Fig. 4 shows the effect of the ratio of desorption to adsorption constants (k_d/k_p) on the initial rate of palm oil hydrolysis as determined by the proposed high enzyme concentration model at the agitation speed of 800 rpm, temperature of 318 K and substrate concentration of 660.7 mol m^{-3}. It can be seen that as the ratio increases (i.e., desorption constant increases and/or adsorption constant decreases), the critical enzyme concentration, which causes saturation of the interfacial area, increases. This can be explained by realising that, at certain enzyme concentration, the amount of enzyme that can penetrate the interface reduces as the desorption constant increases and/or the adsorption constant decreases. Hence, higher enzyme concentration in the bulk is needed to totally saturate the available interfacial area.

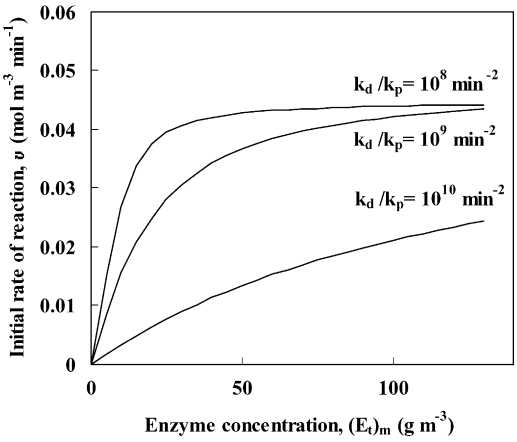

Fig. 4. Effect of k_d/k_p on the initial rate of hydrolysis of palm oil at different enzyme concentrations (ω = 800 rpm, T = 310 K and S = 660.7 mol m^{-3}).

Fig. 5 shows the effect of model parameter K_e on the initial rate of palm oil hydrolysis at different bulk enzyme concentrations. The parameter K_e is the equilibrium constant of E^*S and it can be seen from the figure that the enzyme concentration at which the interface gets saturated is dependant on it. As the numerical value of K_e is increased, the bulk enzyme concentration needed to saturate the interface also increases. K_e represents the ratio of rate constants involved in the break down of E^*S to that involved in its formation. High value of K_e means that the enzyme exists mostly as E^* rather than as E^*S. E^* molecule is smaller than E^*S molecule, and hence, it covers less area of the interface. Further, E^* may desorb from the interface, whereas, E^*S can only transform into another adsorbed form. Therefore, the bulk enzyme concentration needed to saturate the interface increases when the enzyme exists as E^* rather than as E^*S, which is the case when K_e has higher values, as shown in Fig. 5.

Fig. 6 shows the effect of k^*_{cat} on the initial rate of palm oil hydrolysis at different bulk enzyme concentrations. It can be seen from the figure, the model predicts the initial rate

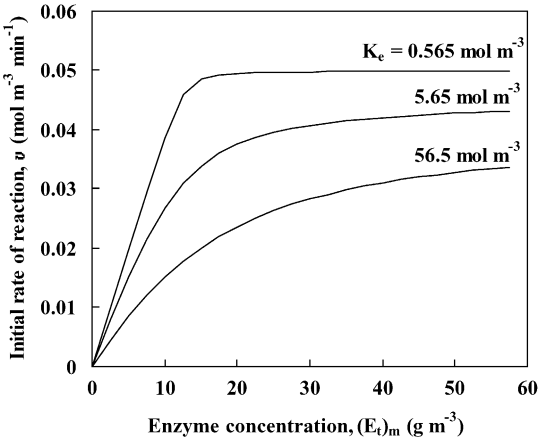

Fig. 5. Effect of K_e on the initial rate of hydrolysis of palm oil at different enzyme concentrations (ω = 800 rpm, T = 318 K and S = 660.7 mol m^{-3}).

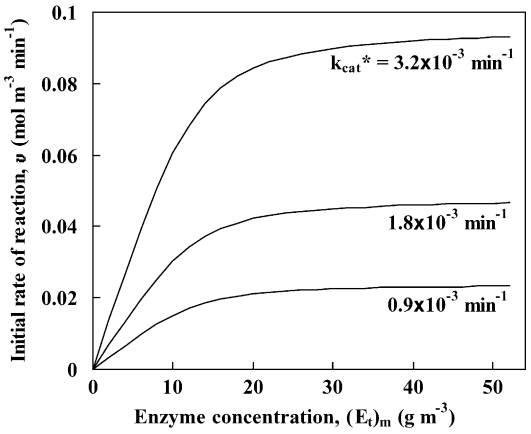

Fig. 6. Effect of k^*_{cat} on the initial rate of hydrolysis of palm oil at different enzyme concentrations (ω = 800 rpm, T = 318 K and S = 660.7 mol m^{-3}).

of reaction to increase with increasing k^*_{cat}, but the enzyme concentration at which the interface is saturated is independent on it. This result is expected as the value of k^*_{cat} does not influence the equilibrium concentrations of the penetrated molecules, E^* and E^*S.

4. Conclusion

The interfacial area saturation with lipase enzyme molecules has been investigated. This phenomenon has been observed in experimental results at high lipase enzyme concentrations. From the viewpoint of efficient usage of lipase, the maximum amount of enzyme used should not exceed the critical concentration, since, the added enzyme, beyond the critical concentration does not enhance the reaction rate. A kinetic model has been developed to predict the behaviour of hydrolysis reaction at high enzyme concentration regions and it is used to determine the critical enzyme concentration. The model predicts interfacial saturation at high enzyme concentration. The validate the model, the experimental results of palm oil and sunflower oil hydrolysis are compared with the model predictions. For sunflower oil, the model predictions closely agreed with the experimental results. Model simulations also showed that as the ratio of desorption to adsorption constants increases, the bulk concentration of the enzyme required to saturate the interface increases. It is also found that the critical enzyme concentration is sensitive to the equilibrium constant for E^*S, i.e. on K_e of the enzyme and not sensitive to the catalytic rate constant, k^*_{cat}.

References

[1] S. Al-Zuhair, M. Hasan, K.B. Ramachandran, Kinetic hydrolysis of palm oil using lipase, Proc. Biochem. 38 (2003) 1155–1163.
[2] S.W. Tsai, C.S. Chang, Kinetics of lipase-catalysed hydrolysis of lipids in biphasic organic–aqueous systems, J. Chem. Tech. Biotechnol. 57 (1993) 147–154.
[3] S. Mukataka, K. Tetsuo, T. Joji, Kinetics of enzymatic hydrolysis of lipids in biphasic organic–aqueous systems, J. Ferment. Technol. 63 (5) (1985) 461–466.
[4] S.W. Tsai, G.H. Wu, C.L. Chiang, Kinetics of enzymatic hydrolysis of olive oil in biphasic organic–aqueous systems, Biotechol. Bioeng. 38 (7) (1991) 761–766.
[5] R. Verger, C.E.M. Maria, H.D. Gerard, Action of phospholipase A at interfaces, J. Biol. Chem. 248 (11) (1973) 4023–4034.
[6] C. Albasi, N. Bertrand, J.P. Riba, Enzymatic hydrolysis of sunflower oil in a standardized agitated tank reactor, Bioprocess. Eng. 20 (1999) 77–81.
[7] Z.D. Knezevic, S.S. Siler-Marinkovic, L.V. Mojovic, Kinetics of lipase-catalysed hydrolysis of palm oil in lecithin/isooctane reversed micelles, Appl. Microbiol. Biotechnol. 49 (1998) 267–271.
[8] Y.J. Wang, J.Y. Sheu, F.F. Wang, J.F. Shaw, Lipase catalyse oil hydrolysis in the absence of added emulsifier, Biotechnol. Bioeng. 31 (1988) 628.
[9] A.J.J. Straathof, Enzymatic catalysis via liquid–liquid interfaces, Biotechnol. Bioeng. 83 (2003) 371–375.