

1-1-2008

Kinetic analysis of oleic acid esterification using lipolytic enzyme as catalyst

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Recommended Citation

Mahmud, Mohd S.; Ryan, James W.; Cooper, Cyrus; Safinski, Tomasz; Nelson, Mark I.; Sidhu, Harvinder S.; and Adesina, Soji A.: Kinetic analysis of oleic acid esterification using lipolytic enzyme as catalyst 2008, 1382-1392.

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Abstract

This paper deals with the esterification kinetics of oleic acid (OA) using ethanol (EtOH) over acrylic-supported lipase from *Aspergillus niger* (Novozym 435) as catalyst. The reaction was carried out in a stirred 500 mL Pyrex glass reactor at 45°C under conditions with negligible external and internal transport resistances. Reaction runs in a microaqueous medium employed OA: EtOH ratio, b , between 0.01 to 2.0 without initial addition of water. The rate dependency on reactant concentration has a maximum at $b=0.9$, just below the stoichiometric ratio, and was therefore described by a Michaelis-Menten kinetic expression implicating irreversible surface reaction between adsorbed OA and EtOH as the rate-controlling step. Ethanol inhibition was significant in this monophasic medium. Although there is no literature agreement on the optimum water content in the biphasic system, the existence of multiple peaks in the sinusoidally decaying rate-water content profile over a wider range (0 to 26.5wt%) than previously investigated is symptomatic of an autoregulatory behaviour between the organic and aqueous phases. This was ascribed to the dynamic migration of ethanol between the well-mixed water and organic pools leading to periodic states in enzyme revivification. The oscillatory kinetics was adequately fitted to under-damped sinusoidal expression with model parameters related to the resonant frequency of the open-close water-assisted activation of the lipase site.

Keywords

Kinetic, analysis, oleic, acid, esterification, using, lipolytic, enzyme, catalyst

Disciplines

Physical Sciences and Mathematics

Publication Details

Mahmud, M. S., Ryan, J. W., Cooper, C. G., Safinski, T., Nelson, M. I., Sidhu, H. S. & Adesina, A. A. (2008). Kinetic analysis of oleic acid esterification using lipolytic enzyme as catalyst. *Chemeca: Australasian Conference on Chemical Engineering* (pp. 1382-1392). Barton, ACT: Engineers, Australia.

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KINETIC ANALYSIS OF OLEIC ACID ESTERIFICATION USING LIPOLYTIC ENZYME AS CATALYST

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ABSTRACT

This paper deals with the esterification kinetics of oleic acid (OA) using ethanol (EtOH) over acrylic-supported lipase from *Aspergillus niger* (Novozym 435) as catalyst. The reaction was carried out in a stirred 500 mL Pyrex[®] glass reactor at 45°C under conditions with negligible external and internal transport resistances. Reaction runs in a microaqueous medium employed OA: EtOH ratio, β , between 0.01 to 2.0 without initial addition of water. The rate dependency on reactant concentration has a maximum at $\beta=0.9$, just below the stoichiometric ratio, and was therefore described by a Michaelis-Menten kinetic expression implicating irreversible surface reaction between adsorbed OA and EtOH as the rate-controlling step. Ethanol inhibition was significant in this monophasic medium. Although there is no literature agreement on the optimum water content in the biphasic system, the existence of multiple peaks in the sinusoidally-decaying rate-water content profile over a wider range (0 to 26.5wt%) than previously investigated is symptomatic of an autoregulatory behaviour between the organic and aqueous phases. This was ascribed to the dynamic migration of ethanol between the well-mixed water and organic pools leading to periodic states in enzyme revivification. The oscillatory kinetics was adequately fitted to under-damped sinusoidal expression with model parameters related to the resonant frequency of the open-close water-assisted activation of the lipase site.

INTRODUCTION

The replacement of fossil fuels by biomass derived equivalents has become increasingly important in the quest for sustainable and renewable energy supplies. Alkyl esters of high molecular weight fatty acids, are excellent alternatives to petrodiesel (Marchetti et al., 2007). Although the fatty acid esters are currently used in the manufacture of specialty chemicals, detergents and surfactants (Omota et al., 2003), the increased demand may be met by synthesis from plant sources since the fatty acid may be obtained from natural vegetable oils or fats and the alkyl donor from bioethanol. In this study, oleic acid (a key ingredient of spent cooking oils and ethanol from the fermentation of natural sugars) were used to synthesise ethyl oleate (biodiesel) in an esterification reaction over acrylic-supported lipase. Conventional alkali or acid catalysts yield products that may not comply with EN 14214 regulation since they may

corrode combustion engines (Demirbas, 2007). Immobilised lipase, however, has the added advantage of high conversions (75% - 95%) within relatively short period (2 - 8 hours), superior longevity (Garcia et al., 2000, Iso et al., 2001) and solvent free process (Linko et al., 1998, Selmi & Thomas, 1998) without losing its native attributes of high selectivity and low reaction temperature (40 - 60°C) (Koeller & Wong, 2001).

Water produced during ethyl oleate synthesis has detrimental effects on the quality of biodiesel and from a thermodynamic standpoint, the continuous removal of the co-product during reaction (e.g. via distillation) will improve ethyl oleate yield above equilibrium level while enhancing product quality. However, during enzymatic esterification, the lipase molecule is positioned at the oil-water interface so that the hydrophilic lid structure can undergo a conformational change responsible for its activation in catalysis (Lalonde et al., 1997). Fig. 1 illustrates the enzyme structure. This phenomenon suggests that an optimum water content is required in the reaction environment. In view of the complex behaviour of lipase-catalysed reaction, this investigation examines the kinetics of the esterification under microaqueous conditions and in biphasic (organic-water) media. In particular, lipase immobilisation on hydrophobic (acrylic) particle avoids complete miscibility with water and self-agglomeration thus maximising active surface exposed to the reaction media and separability (via filtration) for further re-use (Mateo et al., 2007). Many studies conducted in small-size (10ml) vials in either monophasic or biphasic media may not adequately capture the complex role that water plays in the enzymatic esterification reaction (Chulalaksananukul et al., 1990, Goddard & Al-Duri, 2000, Oliveira et al., 2001, Sandoval & Marty, 2002, Foresti et al., 2008) due to poor solid-liquid interphase transport in the absence of mechanical stirring.

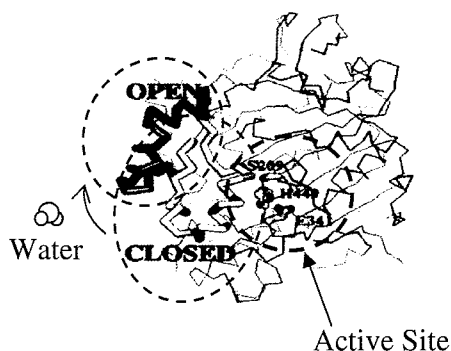


Fig. 1: Structure of *Candida regosa* lipase molecule at the oil/water interface (Lalonde et al., 1997).

EXPERIMENTAL

Material

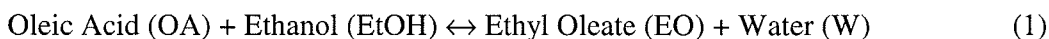
Immobilised lipase from *Aspergillus niger* (10000 U/ml), Novozym 435, was kindly supplied by Novozyme. Oleic acid (95%) and HPLC-grade methanol (99.5%) were purchased from Ajax Fine Chemicals, Sydney. Absolute ethanol (99%) was obtained from APS Chemicals, Sydney. Hydranal Composite 5 titrant, Hydranal

CompoSolvent and Hydranal Standard 5 for KF titration as well as analytical grade oleic acid (98%) and ethyl oleate (98%) for GC calibration were supplied by Sigma Aldrich.

Equipment and method

A 500-ml cylindrical Pyrex[®] glass reactor equipped with 5-hole flange lid, four equally-spaced baffles and a 6-bladed impeller driven by a Heidolph motor was used in all runs. The reactor, baffle and impeller dimensions were chosen to ensure optimum mixing for the viscous fluid mixture in accordance with the design specifications of an agitated vessel as detailed in Perry et al. (1997). Fig. 2 depicts the experimental set-up.

Esterification of ethanol (EtOH) and oleic acid (OA), described by Eq. (1), was carried out at 45°C using both monophasic and biphasic conditions. In the monophasic reaction, the oleic acid: ethanol ratio, β , was examined at 12 values for $0.01 \leq \beta \leq 2$ without adding water whereas in the biphasic reaction, the ratio was fixed at 0.9 and the initial water addition varied between 0 to 26.5wt% (on oleic acid weight basis).



The reactant mixture was charged with (1wt% for monophasic and 3wt% for biphasic runs) 320 μm particles of the supported-lipase. Preliminary hydrodynamic run confirmed that a stirring speed of 1200rpm was sufficient to eliminate mass transfer limitations.

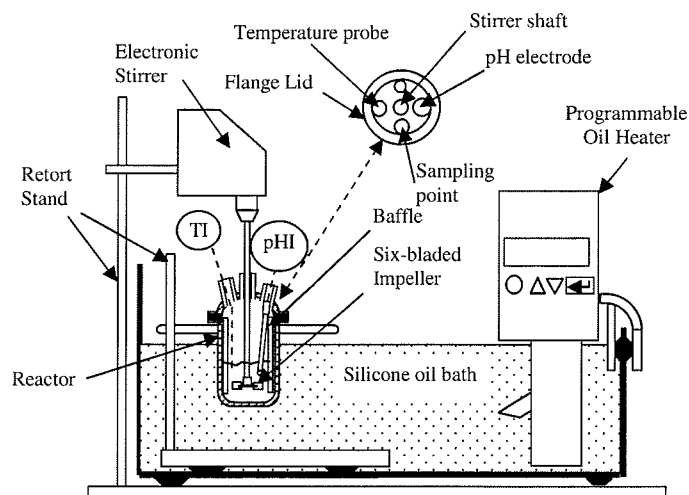


Fig. 2: Batch stirred reactor

Transient species concentration during reaction was monitored via gas chromatography on a Shimadzu GC-17A and Karl-Fischer (KF) titration of aliquots from filtered samples withdrawn at 5-minute to 1-hour intervals over a 10-hour period. About 200- μL of the homogenised aliquot was diluted with methanol to prepare 4000 ppm maximum from each sample to ensure reproducible measurements of OA, EtOH and ethyl oleate (EO) concentration from the GC analysis based on internal standard calibration. The GC employed a fused silica column (0.25mm x 30m Stabilwax[®]-DA). Water content was measured by the Karl-Fischer (KF) titration method on a Mettler Toledo autotitrator (model DL38).

RESULT AND DISCUSSION

Microaqueous Kinetic Analysis

The effect of oleic acid:ethanol ratio, β , on the reaction rate was investigated for $0.01 \leq \beta \leq 2$, in order to cover conditions above and below the stoichiometric ratio, $\beta = 1$. Reaction rate was practically negligible for $\beta < 0.005$ and $\beta > 5$. Experiments were carried out with no initial water present in the reactant mixture. However after about 1 hour, the clear light yellowish solution turned cloudy due to the formation of microaqueous emulsion phase. KF titration of the aliquots gave water content of 1.35 to 1.8 wt%. Initial reaction rate was computed from the derivative of the limiting reactant conversion-time profile at the 50-minute mark, as:

$$-r_{xn} = C_{A0} \left. \frac{dX}{dt} \right|_{t=50\text{min}} = C_{A0} X_{ss} \exp(-bt) \quad (2)$$

where the conversion-time profile has been adequately fitted to: $X = X_{ss}(1 - e^{-bt})$ as typified by Fig. 3 for various values of β (β_1 refers to $\beta = 1$).

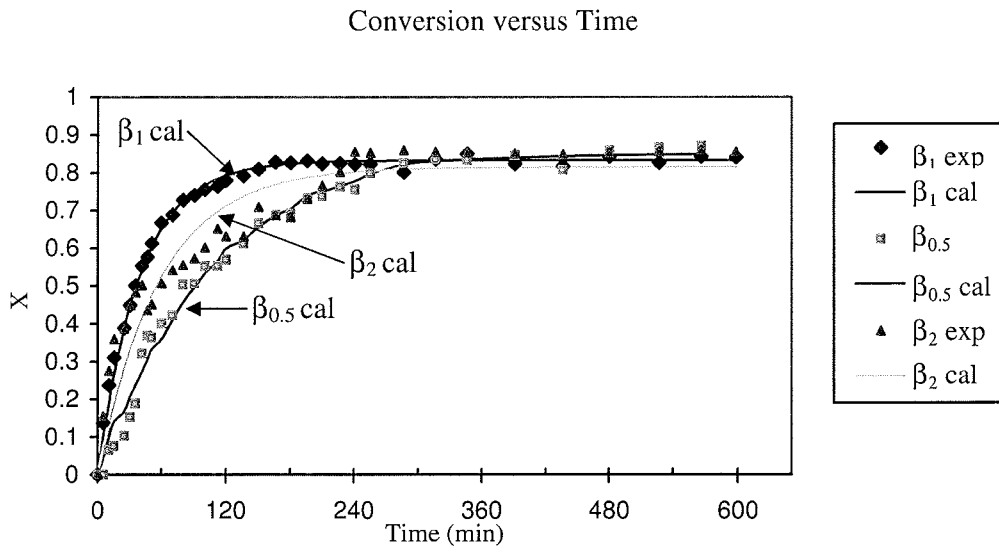


Fig. 3: Example of conversion-time profile

Fig. 4 shows the dependency of reaction rate on the reactant ratio, β . It is evident from this plot that above the stoichiometric ratio, ethanol has a deleterious effect on the reaction rate consistent with the findings of Foresti et al. (2008) and may therefore be explained by Ping-Pong-Bi-Bi mechanism involving competitive reactant inhibition. In the absence of any side-products such as dimethyl ether (undetected by GC analysis), the enzymatic esterification involving oleic acid and ethanol may be described by the sequence of elementary steps:





where eq. (4) and eq. (7) represent the transitory internal change of oleic acid and intramolecular reorganisation, respectively. After considering ethanol (competitive) inhibition, Foresti et al. (2008) derived

$$-r = \frac{v_{\max}^f v_{\max}^r \left(N_{OA} N_{EtOH} - \frac{N_{EO} N_W}{K_{eq}} \right)}{V \left\{ v_{\max}^f K_{m,EtOH} N_{OA} + v_{\max}^r K_{m,OA} \left(1 + \frac{N_{EtOH}}{K_{i,EtOH}} \right) \right\} + V \left(\frac{v_{\max}^f K_{EO}}{K_{eq}} N_W + \frac{v_{\max}^f K_{m,W}}{K_{eq}} N_{EO} \right)} + v_{\max}^f N_{OA} N_{EtOH} + \frac{v_{\max}^f K_{m,EO}}{K_{eq} K_{i,OA}} N_{OA} N_W + \frac{v_{\max}^f}{K_{eq}} N_W N_{EO} + \frac{v_{\max}^r K_{m,OA}}{K_{i,EO}} N_{EO} N_{EtOH} \quad (9)$$

Dividing through by V^2 and rearranging for monophasic ($C_W = 0$) condition, we have Model 1 as tabulated in Table 1 where $V_{\max}^{rf} = v_{\max}^r v_{\max}^f$, $K'_{m,EtOH} = v_{\max}^f K_{m,EtOH}$, $K'_{m,OA} = v_{\max}^r K_{m,OA}$, $K'_{m,EO} = v_{\max}^f K_{m,EO}$ and $K'_{m,W} = v_{\max}^f K_{m,W}$. As may be seen from the table, Model 1 gave the highest R-squared values. Other Ping-Pong models were similarly derived as shown in Table 1. Models 1 and 3 may be rejected on the grounds of the negative kinetic parameter estimates obtained, with Models 2 and 4 being the most feasible. However, Model 4 appears to be the better of the two since it has a higher R-squared value and also admits ethanol inhibition for the reaction as have been reported in other studies (Al-Zuhair et al. 2007).

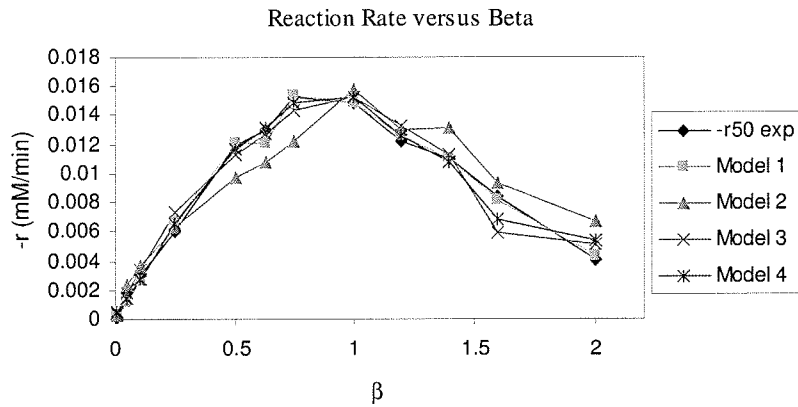


Fig. 4: Comparison between model prediction and experimental data for esterification rate as a function of OA: EtOH ratio

Tab.1: Microaqueous kinetic model

Mode	Equation	Variables	R ²
1	<p>Foresti-Pedernera-Ferreira-Bucala model (Foresti et al. 2008)</p> $-r = \frac{V_{\max}^{rf} (C_{OA} C_{EiOH})}{\left[K'_{m,EiOH} C_{OA} + K'_{m,OA} C_{EiOH} + \frac{K'_{m,OA} V}{K_{i,EiOH}} C_{EiOH}^2 + \frac{K'_{m,W}}{K_{eq}} C_{EO} + v_{\max}^r C_{OA} C_{EiOH} + \frac{K'_{m,OA}}{K_{i,EO}} C_{EO} C_{EiOH} \right]}$	<p>$V_{\max}^{rf} = 0.066$ mM/min $K'_{m,EiOH} = 8.009$ mM $K'_{m,OA} = -27.526$ mM $\frac{K'_{m,OA}}{K_{i,EO}} = 4.105$ $\frac{K'_{m,W}}{K_{eq}} = -7.075$ $v_{\max}^r = 3.318$ mM/min $\frac{K'_{m,OA}}{K_{i,EO}} = 20.353$</p>	0.993
2	<p>Paiva-Balcao-Malcata model (Paiva et al. 2000)</p> $-r = \frac{V_{\max} C_{EO}}{\left[K_{m,EO} \left(1 + \frac{C_{EO}}{K_{m,EO}} + \frac{C_{OA}}{K_{i,OA}} \right) \right]}$	<p>$V_{\max} = 0.032$ mM/min $K_{m,EO} = 0.635$ mM $K_{i,OA} = 0.983$ mM</p>	0.892
3	<p>Chulalaksananukul-Condoret-Delorme-Willemot model (Chulalaksananukul et al. 1990)</p> $V = \frac{V_{\max} C_{OA} C_{EiOH}}{\left[K_{m,OA} C_{EiOH} \left(1 + \frac{C_{EiOH}}{k_i} \right) + K_{m,EiOH} C_{OA} + C_{OA} C_{EiOH} \right]}$	<p>$V_{\max} = 0.019$ mM/min $K_{m,OA} = -1.181$ mM $K_{i,EiOH} = -2.358$ mM $K_{m,EiOH} = 0.948$ mM</p>	0.964
4	<p>This study</p> $V = \frac{V_{\max} C_{OA}^x C_{EiOH}^y}{\left[K_{m,OA} C_{EiOH} \left(1 + \frac{C_{EiOH}}{k_i} \right) + K_{m,EiOH} C_{OA} + C_{OA} C_{EiOH} \right]^{(x+y)}}$	<p>$V_{\max} = 0.916$ mM/min $K_{m,OA} = 2.382$ mM $K_{i,EiOH} = 1.643$ mM $K_{m,EiOH} = 13.159$ mM $x = 0.632$ $y = 0.785$</p>	0.984

Biphasic Reaction System

Although water is a co-product of the ethyl oleate synthesis and its removal from the reaction medium should shift equilibrium towards the right in favour of EO yield, lipase is more active in a suitably hydrated medium. Thus, a series of experiments were conducted to investigate reaction behaviour when water was added at different levels. Water addition introduces an interesting dimension to the reaction system since ethanol is also soluble in water. Thus, ethanol migration into the aqueous phase would set up a physical equilibrium between the organic and aqueous phase. The difference in solubility leads to a decrease in the reverse hydrolysis of the ester in the organic phase which in turn induces a shift of reaction equilibrium towards ester production (cf. Eq.

(1)) as pointed out by Foresti et al. (2008). Intuitively, this interaction between organic phase reaction and species transport into the aqueous phase may lead to steady-state reaction rate multiplicity (Omota et al., 2003, Adesina & Adewale, 1991). Foresti et al. (2008) have observed that although initial reaction rate in the biphasic system was higher, conversion was lower than in the monophasic medium due to ethanol storage in the aqueous medium in the former environment. Although not shown, monophasic run carried with $\beta = 0.9$ (optimum in Fig. 3) gave a final conversion of 0.87 while the same run in the biphasic media (with $\beta = 0.9$) yielded a final conversion of 0.79.

Fig. 5 shows that the reaction rate is a complex function of the water content with multiple resonant peaks typical of a sinusoidally decaying profile. In classical nonlinear process analysis, this behaviour is symptomatic of at least two interconnected first order stages with a feedback loop. In the present liquid-liquid-reaction system, the migration of ethanol between the organic and aqueous phases appears to be the communication 'loop' between the two phases (Fig 6). Whilst literature evidence suggests that different groups have observed varying optimum water concentrations, there has been no systematic change in the water content over the wide range (0 to 0.3) reported in the study. It would therefore seem that we have captured a phenomenon inadvertently overlooked in previous investigations (Foresti et al., 2007, Foresti et al., 2008, Iso et al., 2001, Oliveira et al., 2001, Sandoval & Marty, 2002).

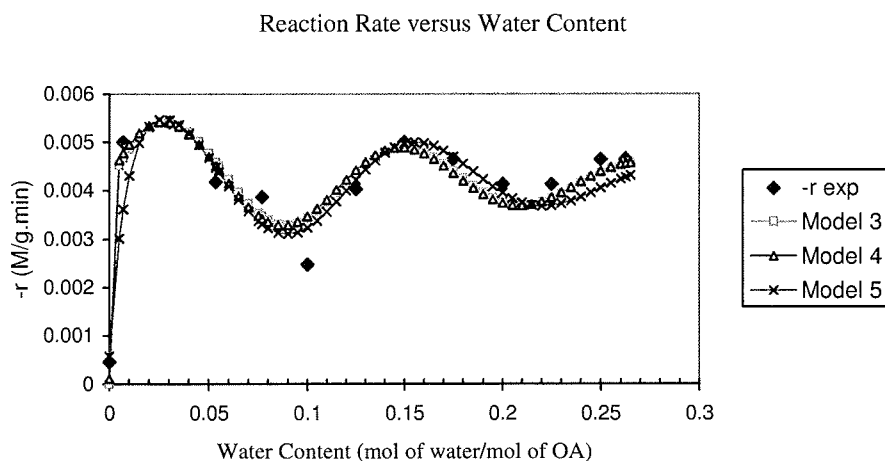


Fig. 5: Oscillatory kinetics during esterification in the biphasic system

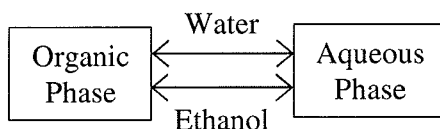


Fig. 6: Model for esterification of oleic acid with ethanol in a biphasic medium.

The first peak in Fig. 5 agrees with the data of Sandoval and Marty (2002) and Oliveira et al. (2001), which reported optimum water content in the window, 0.2 – 3 wt% while the second peak essentially coincides with the optimum water level observed by Foresti et al. (2008). We did not investigate water content higher than 30 wt% due to

significant dilution effects. Even so, the oscillatory nature of the reaction kinetics with respect to water addition is unmistakable. It may be posited that the occurrence of crests and troughs in the reaction rate profile is due to the dynamic migration of both ethanol and water across the organic phase and aqueous phase in order to restore phase equilibrium concentration while reaction is proceeding concurrently in the organic phase. This phenomenon in turn induces a periodic “opening and closing” of the hydrophilic lid structure of the lipase molecule and hence cyclical variation in the lipase activity with water content as the system seeks to attain an eventual ‘steady-state’ reaction rate as the water content increases. This behaviour will, however, terminate when the volume of water added exceeds that which allows a distinct thermodynamic identity for the organic phase. Consequently, phenomenological models (cf. Table 2) describing interacting well-mixed natural systems with recurrent feedback such as that found in ecosystems with symbiotic behaviour between species and autoregulatory physiological processes were examined (Namjoshi et al., 2003).

Tab. 2: Biphasic reaction model

Model	Variables	R ²
1. $-r_{rxn} = k \left[\frac{\exp\left(\frac{-w\zeta}{\tau}\right)}{\tau\sqrt{1-\zeta^2}} \sin\left(\frac{w\sqrt{1-\zeta^2}}{\tau}\right) \right]$	$k = 0.00406\text{M/g.min}$ $z = 0.15\text{M}$ $t = 0.0157\text{M}$	0.45
2. $-r_{rxn} = k \left[1 - \frac{\exp\left(\frac{-w\zeta}{\tau}\right)}{\sqrt{1-\zeta^2}} \sin\left(\frac{w\sqrt{1-\zeta^2}}{\tau} + \tan^{-1} \frac{\sqrt{1-\zeta^2}}{\zeta}\right) \right]$	$k = 0.0001\text{M/g.min}$ $z = 0.1\text{M}$ $t = 0.021\text{M}$	0.53
3. $-r_{rxn} = A_1 \exp\left(\frac{-w}{d}\right) \sin\left(\frac{-2\pi}{\lambda} w\right) + A_2 \left[1 - \exp\left(\frac{-w}{s}\right) \right]$	$A_1 = 0.0014\text{M/g.min}$ $A_2 = 0.0042\text{M/min}$ $d = 0.21\text{M}$ $\lambda = 0.121\text{M}$ $s = 0.001\text{M}$	0.899
4. $-r_{rxn} = A_1 \exp\left(\frac{-w}{d}\right) \sin\left(\frac{-2\pi}{\lambda} w + \theta\right) + A_2 \left[1 - \exp\left(\frac{-w}{s}\right) \right]$	$A_1 = 0.0014\text{M/g.min}$ $A_2 = 0.0042\text{M/g.min}$ $d = 0.21\text{M}$ $\lambda = 0.121\text{M}$ $s = 0.001\text{M}$ $\theta = 6.3616$	0.901
5. $-r_{rxn} = \left[A_1 \exp\left(\frac{-w}{d}\right) + C \right] \sin\left(\frac{2\pi w}{\lambda} + \theta\right) + A_2 \left[1 - \exp\left(\frac{-w}{s}\right) \right]$	$A_1 = 0.012\text{M/g.min}$ $A_2 = 0.004\text{M/g.min}$ $d = 2.5\text{M}$ $\lambda = 0.133\text{M}$ $s = 0.007\text{M}$ $\theta = 0.397$ $C = -0.01\text{M/g.min}$	0.918

It is apparent from the nonlinear regression analysis that model 5 is the best fit to the present data. The amplitudes A_1 and A_2 correspond to the maximum reaction rates in the two 'periodic states' of lipase activity, while λ is the natural wavelength of the water content (intervals at which water must be added to cause an oscillatory excitation of the 'opening and closing' of the lipase lid site with d being the natural frequency (in terms of water content) of the interaction between the organic and aqueous phase while s is the water damping factor for the sinusoidal exchange and the parameter C , accounts for the non-enzymatic transfer rate of the ethyl oleate to the aqueous phase and θ is the ratio of water content between the organic to aqueous phase under non-reacting conditions.

CONCLUSIONS

The kinetic behaviour of the esterification reaction between oleic acid and ethanol has been studied under both microaqueous and biphasic conditions. The reaction rate exhibited conventional Michaelis-Menten functionality in the absence of water (in the feed mixture). The lipase active sites appeared to be overwhelmingly covered by both ethanol and oleic acid with significant role in the rate-determining step. Under biphasic conditions, the reaction displayed a sinusoidally damped kinetics with respect to increase water content suggesting nonlinear interaction between the organic and aqueous phases. The oscillatory decaying rate was attributed to the dynamic exchange of water and ethanol between the two dominant phases coupled with the open-close characteristic of the lipase active site by critical water content.

The exponentially-decaying sinusoidal rate was adequately captured by phenomenological models known to describe autoregulatory physiological processes and natural ecosystems possessing symbiotic attributes. Since this behaviour has not been previously observed for non-enzymatic esterification, this study opens the horizon for further research into the nature of product or reactant-induced catalyst activation coupled with interphase transport in the evolution of natural oscillatory kinetics in reversible reaction systems such as esterification, fermentation, hydrogenation and reforming operations.

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BRIEF BIOGRAPHY OF PRESENTER

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Mohd Sabri Mahmud is currently a PhD student at UNSW. His doctoral thesis deals with the optimisation of a catalytic distillation reactor for biodiesel production. He is on study leave from Universiti Malaysia Pahang, Kuantan, Malaysia.

