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# Analysis of a direct contact membrane reactor for lipase catalysed oil hydrolysis in a dynamic emulsion system

Q. Gan [a,*], F. Baykara [a], H. Rahmat [a], L.R. Weatherley [b]

[a] *Department of Chemical Engineering, The Queens University of Belfast, Belfast BT9 5AG, UK*

[b] *Department of Chemical and Process Engineering, The University of Canterbury, Christchurch, New Zealand*

**Abstract**

This paper describes the study of a continuous direct contact membrane reactor (CDCMR) for enzymatic catalysed oil hydrolysis. The reaction rate, kinetic stability, and product (fatty acids) yield of the CDCMR system are analysed and compared to a conventional stirred tank reactor to elucidate the potential advantages and optimal operating strategies of the combined reaction and separation process. The dynamism of the biphasic emulsion system and the constantly changing physicochemical properties of the organic–aqueous interface where the lipolytic reaction took place were studied for their effect on the overall system reaction rate and yield. The CDCMR performance was studied as a function of oil–water molar ratio, oil droplet size, interfacial tension, and feed stock flow rate. The effect of the presence of bovine serum albumin in the aqueous subphase for accelerated desorption of reaction product (fatty acids), and the effect of simultaneous separation of glycerol are highlighted.

This paper also proposes the application of a two-substrate reaction kinetic model with limited water accessibility to the active site of the enzyme molecules situated at the reaction interface. The validated model has been demonstrated and takes into account the complexity of the underlying reaction mechanism better than the theoretical hypotheses already existing in the research literature. 

*Keywords:* Continuous direct contact membrane reactor; Lipase catalysed oil hydrolysis; Dynamic emulsion system

## 1. Background

### *1.1. Enzymatic membrane reactors*

The basic concept of a membrane bioreactor employs a semi-permeable and selective membrane that separate the enzymes and products. A complete retention of the enzyme within the reactor is the prerequisite for continuous operation of a membrane reactor. This offers the great advantage of extensive and continuous use of enzymes. Membranes can also be used exclusively as an enzyme immobilisation matrix (direct membrane immobilisation) or an interface support between two distinct liquid phases. In a typical continuous membrane reactor where the enzyme is retained to one side of the membrane by size exclusion in a free soluble form, productivity gain and increased yield are the two anticipated benefits due to higher flow rate, lower pressure drop and simultaneous product separation during the reaction. Membrane reactors offer a few distinct advantages over conventional packed columns with immobilised enzymes or fluidised bed reactors [1,2]. The selective separation performance of membrane reactors is well suited to carry out complex enzymatic transformations involving, for instance,

* Corresponding author. Fax: +44-1232-381-753.
*E-mail address:* q.gan@qub.ac.uk (Q. Gan).

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several enzymes and cofactor regeneration [3,4]. Their unusual geometries also offer opportunities to promote interfacial contact between enzymes and substrates in complex organic–aqueous heterogeneous systems [5–8] and reversed micelles [9–11]. Therefore, membrane reactors presenting elaborate configurations for complex biocatalytic processes presents opportunities for new process design, integration and control in biocatalysis.

The unique advantages offered by these reactors, together with the wide variety of membrane shapes, modules, and materials commercially available at decreasing costs, have made them a more serious alternative to more conventional enzyme reactors.

### *1.2. Lipase catalysis in dynamic biphasic emulsion systems*

Most experimental approaches used so far dealing with model enzymatic membrane reactors are based on the combination of (a) an immobilised enzyme + soluble substrate or (b) a soluble enzyme + insoluble substrate. It comes as no surprise since most of the reported work based on approach (b) deals with lipolysis although heterogeneous systems might also be studied also using polyamino acids, polynucleotides, or polysaccharides as substrate in insoluble form. The fact that the substrate that forms an insoluble lipid–water interface makes lipolysis a very attractive system for studies of interfacial enzyme kinetics.

Emulsion systems provide an attractive option for lipase-mediated production of fine chemicals since emulsion reactors are low cost and easy to use. One of the most characteristic kinetic features of lipolytic enzymes in such heterogeneous emulsion systems is their "activation" by the organic–aqueous interfaces. Catalysis of oil hydrolysis requires an organic–aqueous interface to allow the enzyme to achieve an optimal configuration. The reaction mechanism involves adsorption of lipase and transport of the reacting substrates to the interface followed by catalysis at that interface. Stability, activity, and stereoselectivity are largely dependent on the physiochemical and kinetic parameters of the heterogeneous system. The theoretical description of this process has been developed over three decades but still remains largely incomplete [12,13]. Several kinetic mechanisms of lipase adsorption and catalysis in a heterogeneous oil/water emulsion system have been proposed, relating lipase activity to either interfacial quantity (interfacial area per unit volume) [14] or interfacial quality (physiochemical properties of the interface) [12]. The simplest kinetic model is based on the classic Michaelis–Menten mechanism as applied to emulsified oil–water systems [15,16]. The kinetic steps can be represented schematically as

$$\mathrm{E} + \mathrm{S} \underset{k_{-1}}{\rightarrow} \mathrm{ES} \overset{k_{\mathrm{cat}}}{\rightarrow} \mathrm{P} + \mathrm{E}$$

where E denotes the enzyme, S the substrate (triglyceride), ES the enzyme–glyceride complex, P the products (fatty acids and glycerol), and $k_1$, $k_{-1}$, $k_{\mathrm{cat}}$ the elementary reaction rate constants.

The rate of product formation per unit volume of reacting fluid $r_{\mathrm{v}}$ can be represented in terms of this mechanism as

$$r_{\mathrm{v}} = \frac{v_{\max}[\mathrm{S}]}{K_{\mathrm{m}} + [\mathrm{S}]}, \quad v_{\max} = k_{\mathrm{cat}}[\mathrm{E}]_{\mathrm{tot}},$$
$$K_{\mathrm{m}} = \frac{k_{\mathrm{cat}} + k_{-1}}{k_1}, \tag{1}$$

where $v_{\max}$ is the rate observed when the lipase is saturated with substrate, $K_{\mathrm{m}}$ is the Michaelis–Menten constant, and the subscript tot denotes the overall amount.

The attribution of a numerical value to $K_{\mathrm{m}}$ for an oil substrate in an emulsified state is subject to question on its physical meaning. This has been demonstrated by Benzonana and Desnuelle [17] who showed that certain physical parameters of the emulsion, such as the size of the emulsion droplet, strongly influence the $K_{\mathrm{m}}$ value. They compared the rates of lipolysis of coarse and fine emulsions of a substrate and found that the Michaelis constants differ and are not related to the weight/volume ratio of the substrate. If, however, substrate concentration is expressed as area/volume rather than as weight/volume, the Lineweaver–Burk plots for different emulsions of the same substrate coincide, and a single $K_{\mathrm{m}}$ value is obtained independent of the degree of dispersion of the substrate. The importance of the determination of an interfacial $K_{\mathrm{m}}$ having the dimensions of an area/volume instead of weight/volume has been questioned by Mattson et al. [18] and by Brockerhoff [19] who argued that

the orientation of the ester molecule at the oil–water interface and the specificity of the enzyme molecule for its substrate are the main factors determining the rate of hydrolysis. It becomes clear from either arguments that such an interfacial $K_m$ value is meaningless if the lipid–water interface behaves as an unspecified surface. The situation is further complicated in that the amount of lipolytic enzyme absorbed varies with different lipid–water interfaces.

Nury et al. [20] investigated the time dependence of the oil–water interfacial tension during enzymatic hydrolysis using the oil-drop and the Teflon-plunger method. By means of these qualitative techniques valuable information was obtained about the influence of substrate packing density on lipase adsorption, catalysis, and even on stereoselectivity. Mukataka et al. [21] proposed a kinetic model for lipolytic reactions in a two phase system by taking the interfacial area and the fraction of the organic phase into account. Wang et al. [22] studied the kinetics of oil hydrolysis by *Candida cylindracea* lipase using a Lewis cell, which provided a constant interfacial area.

Further complication arises from the dynamic change of interfacial area and quality during the course of the lipolytic reaction due to, for example, fatty acids formation. Consequently, the physiochemical properties of the emulsion system vary with time resulting in changing accessibility to the interface by the enzyme and fresh substrates. Due to these aforementioned complexities, the true kinetics of lipase in such a dynamics biphasic system are not fully understood.

### *1.3. Theory of two substrate limiting enzymatic catalysis*

We feel that a more serious determination of the interfacial $K_m$ value as proposed by Benzonana and Desnuelle [17] presents practical difficulties. First, it is not easy to determine accurately the surface area of emulsion droplets. Second, when surfactants are needed to stabilise the emulsion, or in the case where the lipolytic products (mono-, diglyceride, free fatty acids, etc.) adhere at the oil–water interface, the calculation of $K_m$ in terms of area/volume is questionable because the interfacial area occupied by the surfactants or products is unknown.

Therefore a more complete kinetic model of the lipolytic catalysis in a dynamic emulsion system ought to take account not only the surface/volume factor but also the surface quality and its accessibility to substrates including water. The authors believe that, in contrast to a single substrate (oil) limiting kinetics as being described by the Michaelis–Menten model, a two substrate (oil and water) limiting kinetic model with limited water accessibility to the reaction interface gives a more accurate representation of the underlying reaction mechanism. This model considers water as one of the reaction rate limiting substrates regardless of whether it is in a continuous phase or in a reversed micelle formation. The chief arguments are that (i) the active site of lipase, located at the interface, would be poorly hydrated in contrast to the heavily hydrated monomeric molecules moving freely in an aqueous solution [23]; (ii) the existence at the interface of an ordered array of lipid molecules in a high local concentration could create a situation where water access to the active site is highly restricted; (iii) the dynamism of the interfacial state profoundly affects the constant reshaping of the enzyme conformation, which would act unfavourably to water access under the influence of increasing surface tension; and (iv) the continuous production of fatty acids and their tendency of residing at the interface will also gradually reduce the accessible interfacial area.

The kinetic steps of the two substrate limiting reaction can be represented schematically as

$$\mathrm{E}+\mathrm{S}_1\rightarrow\mathrm{ES}_1 \qquad K_1$$
$$\mathrm{E}+\mathrm{S}_2\rightarrow\mathrm{ES}_2 \qquad K_2$$
$$\mathrm{ES}_1+\mathrm{S}_2\rightarrow\mathrm{ES}_1\mathrm{S}_2 \qquad K_{12}$$
$$\mathrm{ES}_2+\mathrm{S}_1\rightarrow\mathrm{ES}_1\mathrm{S}_2 \qquad K_{21}$$
$$\mathrm{ES}_1+\mathrm{S}_2\overset{k_{\mathrm{cat}}}{\rightarrow}\mathrm{P}_1+\mathrm{P}_2+\mathrm{E}$$

where E denotes the enzyme, $S_1$ and $S_2$ the substrate of water and triglyceride, respectively, $ES_1$ and $ES_2$ the enzyme substrate complex, $ES_1S_2$ the tertiary enzyme substrate complex, $P_1$ the complete or partially hydrolysed lipids (mono-, diglycerides, free fatty acids), $P_2$ the glycerol, $K_1$, $K_2$, $K_{12}$, $K_{21}$ the equilibrium constant of the corresponding reaction steps, and $k_{cat}$ the elementary reaction rate constant of the final reaction step.

The first four reversible reactions are fundamentally enzyme–substrate binding process. The $ES_1S_2$ complex is the effective state of the lipase–oil–water

formation at the oil–water interface which eventually leads to the production of fatty acids.

Assuming equilibria in the first four reversible reactions leads to the rate expression:

$$r_v = \frac{k[E]_{tot}}{1 + K_{21}/[S_1] + K_{12}/[S_2] + 0.5(K_2K_{21} + K_1K_{12})/[S_1][S_2]} \quad (2)$$

Equilibrium requires

$$K_1K_{12} = K_2K_{21}.$$

Rearrangement of Eq. (2) leads to the familiar Michaelis–Menten form

$$r_v = \frac{V^*_{max}[S_2]}{K^*_m + [S_2]} \quad (3)$$

where

$$V^*_{max} = \frac{v_{max}[S_1]}{[S_1] + K_{12}} = \frac{k_{cat}[E]_{tot}[S_1]}{[S_1] + K_{12}} \quad (4)$$

$$K^*_m = \frac{K_{21}[S_2] + K_1K_{12}}{[S_2] + K_{12}} \quad (5)$$

$V^*_{max}$ can be interpreted as the maximum reaction rate of the system when the oil–water interface is saturated with the lipase molecules and the enzyme is at its optimum interfacial conformation and orientation for substrate access and binding to its active site. Its value is governed by, in addition to the intrinsic value of $V_{max}$, the degree of hydration reflected by the local water concentration $S_1$, and the enzyme–water equilibrium binding constant $K_{12}$.

The value of $K^*_m$ is determined by the combination of the elementary equilibrium binding constants and the local water concentration. Eq. (5) takes consideration of both the interfacial water concentration and the degree of enzyme hydration. Since the values of the elementary equilibrium constants are influenced by the enzyme conformation and orientation at the interface, $K^*_m$ is therefore an indicator of the overall interfacial quality for water access to the active site of the enzyme located at the interface.

### *1.4. Lipase kinetics in combined reaction and separation systems*

While the free lipase catalysed reaction can be described by the aforementioned models, a more detailed model including the separation process is needed to describe simultaneous reaction and separation involving a CSTR/UF membrane reactor system. This is beyond the scope of this work where transport and separation efficiency are only qualitatively described but not quantified.

Much theoretical work exists in the literature of chemical engineering and heterogeneous catalysis concerning the coupling of reaction and transport process within porous solids along with other internal and external interactions. But few are related to combined reaction and separation in a membrane reactor system where the phenomenon of concentration polarisation/fouling together with the membrane permeability and rejection characteristics are distinctive which should be taken into account. Bowski et al. [24] and Ryu et al. [25] modelled the CSTR/UF membrane reactor system by considering the ultrafiltration membrane to be ideal; i.e., there is no concentration polarisation effect and that the membrane reject coefficient is 100%. But Bowski et al. [24] interpreted their experimental results in the light of the concentration polarisation effect. More detailed models for the effect of concentration polarisation in ultrafiltration/microfiltration processes have since been developed but they are yet to be extended to combined reaction and separation processes employing membrane units. In many practical systems, the permeation rate does not reach a true steady state. Further, the enzyme activity can also decline with time.

## 2. Experimental materials and methods

Lipase extracted from *Candida cylindracea* (Amana Pharmaceutical) was used with an activity of 30 000 unit/g at 37°C and pH 6. The enzyme has a molecular weight of 63 000 measured by gel electrophoresis (SDS method using a Sigma MW-SDS-200 kit). The sunflower oil (Power Import and Wholesale, Belfast) comprised 7%, 61%, and 31% saturated, monounsaturated, and polyunsaturated fat, respectively. Millipore PLCC hydrophilic UF membranes, effective area 0.0930 $m^2$ and with a molecular weight cut-off of 5000 were fitted into a Pellicon Cassette Ultrafiltration module (Millipore). Analysis of lipase activity, concentrations of glycerol, fatty acids, and protein in the feed, retentate

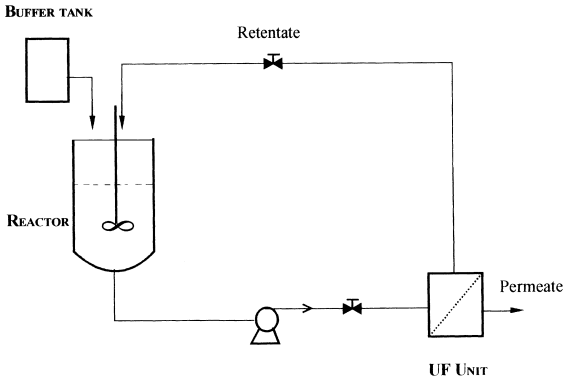


Fig. 1. Schematic diagram of the CDCMR system.

and permeate over various filtration times was carried out. The glycerol concentration was determined using a Glycerol Assay kit (Bohringer & Mannheim, UK, Cat. No. 148270). The protein concentration was determined using a Protein Assay kit (Cat. No. P5656) supplied by Sigma Pharmaceutical. Lipase activity was determined by NaOH titration using a standard procedure, see [26]. Composition of the fatty acids produced was determined by gas chromatography (column BP21 was supplied by SGE, UK). All other reagents and chemicals were of analytical grade.

A flow diagram of the experimental set-up is shown in Fig. 1. The unbaffled stirred tank reactor had a working volume of 3 l and was equipped with a heating/cooling jacket for maintenance of a constant temperature of 36°C. Mixing was achieved by means of an impeller with six flat blades. The stirring speed, measured by tachometer, was fixed at 426 rpm for all the experiments. The sunflower oil/water volume fraction in the system was varied from 0.0833 to 0.833 corresponding to oil/water molar ratios in the range from 0.014 to 0.094. Table 1 summarises the operating conditions, including the lipase concentration and ultrafiltration transmembrane pressures which were used. The reaction mixture was maintained at pH 6 using a buffer. The biphasic fluid within the CDCMR system was pumped and recirculated through the UF unit for continuous separation of glycerol and dissolved fatty acids. The drain of permeate from the UF unit was compensated by balanced addition of buffer during the experiments.

Table 1
Conditions in operating the CDCMR system

| | |
|---|---|
| Impeller stirring rate in the reactor (rpm) | 462 |
| Lipase concentration (g/l) | 0.05 |
| Ultrafiltration transmembrane pressure (bar) | 0.65, 0.95, 1.15 |
| Crossflow velocity (m/s) | 0.23, 0.57, 1.12 |
| Oil/water molar ratio | 0.014, 0.028, 0.042, 0.066, 0.080, 0.094 |

## 3. Results and discussion

### *3.1. Analysis of lipolytic kinetics in a conventional stirred tank reactor and the CDCMR system*

#### *3.1.1. Kinetic behaviour of the two systems*

The reaction rate, expressed as the rate of fatty acid formation per unit volume of reacting fluid, was measured at different oil/water molar ratios in both the conventional stirred tank reactor and the CDCMR system. Permeate was withdrawn simultaneously from the CDCMR system as the reaction started. Fig. 2 presents the observed relationship between the reaction rate at the beginning of each reaction and the oil/water molar ratio. Using the Lineweaver–Burk plot, the data were analysed to obtain the value of $V^*_{max}$ and $K^*_m$ which are presented in Table 2.

In comparison, there is only marginal difference in the calculated values of $V^*_{max}$ and $K^*_m$ between the two systems. As the lipase catalysis is largely governed by

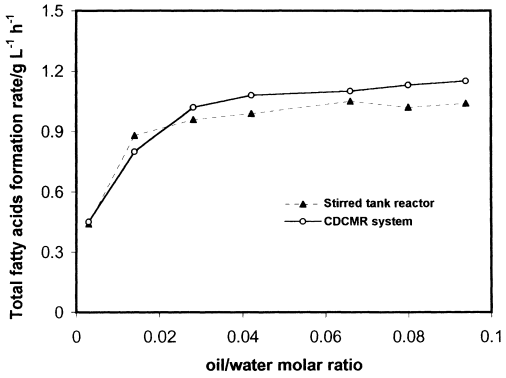


Fig. 2. Relationship between fatty acid formation rate and substrate oil/water molar ratio. $T = 35°C$, TMP = 1.15 bar, pH 6, impeller stirring rate = 462 rpm, lipase concentration = 0.05 g/l.

Table 2
Calculated $V^*_{max}$ and $K^*_m$ values using Lineweaver–Burk method

| | $V^*_{max}$ ($g\,l^{-1}\,h^{-1}$) | $K^*_m$ ($g\,l^{-1}$) |
|---|---|---|
| CDCMR system | 1.161 | 0.00455 |
| Stirred tank reactor | 1.111 | 0.00460 |

the enzyme–substrate–product interactions and transport at the oil/water interfaces, the little distinction of the kinetic performance between the two systems indicates that these dynamic interactions were only slightly affected by the two different reactor's geometric configurations. The stronger flow regime in the CDCMR system may have contributed to the slightly higher value of $V^*_{max}$ as the fluid dynamics may have promoted water transport and access at the reaction interface. However, the stronger fluid dynamics were not sufficiently vigorous to alter the interfacial enzyme conformation and the equilibrium formation of the $ES_1S_2$ complex, thereby an virtually unchanged $K^*_m$ value is expected.

Without proper knowledge of the local concentration of oil and water at the organic–aqueous interface, and in the absence of information regarding the elementary equilibrium constants ($K_1$, $K_2$, $K_{12}$, $K_{21}$), direct application of the two-substrate kinetic model is not possible. Nonetheless the model offers a clearer theoretical account of the complex reaction mechanism and the physical meanings of $V^*_{max}$ and $K^*_m$. Measurement of the interfacial substrate concentration and quantification of the elementary equilibrium constants are beyond the scope of this study. The hypothesis offered in this paper may serve as one alternative theoretical approach to complex interfacial lipolytic kinetics.

### *3.1.2. Kinetic stability of the CDCMR reactor system*

There are a few perceived problems associated with enzymatic membrane reactors where enzymes are in a soluble state. Performance can decrease during operation usually due to loss of catalytic and mass transfer efficiency [1,27]. When the enzyme is used in free form, its kinetic stability may be affected by unfavourable adsorption to the surface of the membrane and other materials in contact with the fluid leading to eventual alteration of the enzyme conformation and loss of activity. Enzymes are also susceptible to deactivation by shear forces and friction near the membrane walls in recirculating flow membrane reactors.

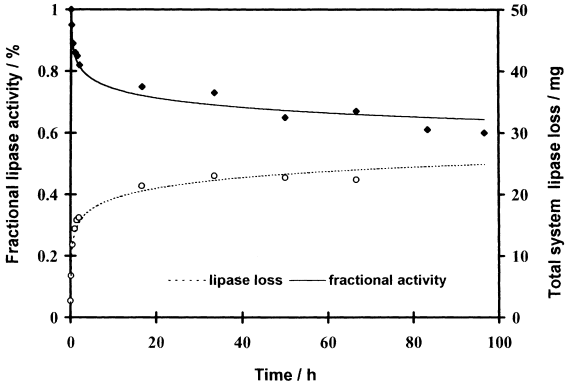


Fig. 3. Time course of total lipase concentration and catalytic activity in the CDCMR system. $T = 35^\circ$C, TMP = 1.15 bar, pH 6, oil/water molar ratio = 0.066, impeller stirring rate = 462 rpm, lipase concentration = 0.05 g/l.

To elucidate lipase deactivation within the CDCMR system due to the combined effect of protein adsorption, concentration polarisation, and shear stress, aqueous lipase solutions were circulated through the CDCMR system to allow measurement of the time dependence of lipase concentration and total lipolytic activity in the recirculating stream (Fig. 3). There is a marked reduction of lipase activity during the initial hour. This coincided with an intensive loss of protein which took place at a rapid rate during the first hour of operation. The total loss of protein was obtained by measuring the change of protein concentration in the recirculation fluid using a standard BCA assay (bicinichonic acid; Pierce and Warriner, UK). The lipase activity regained its relative kinetic stability after the rapid loss of almost 20% of kinetic power during the first hour.

To compensate for the loss of lipase activity in the CDCMR system, extra lipase was injected into the CDCMR by a stepwise way during the time course of the oil hydrolysis. The addition of fresh lipase to the CDCMR system is equivalent to an increased lipase concentration of 0.005 g/l in the system after each addition. So there was an accumulation of lipase within the system. The catalytic effect of the additional injection of lipase is shown in Fig. 4. The fractional catalytic power of the CDCMR system is based on the measurement of fatty acids production rate after each

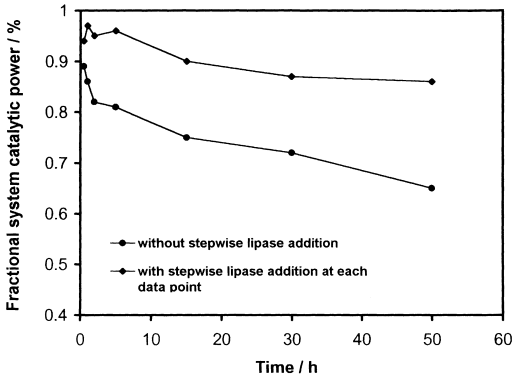


Fig. 4. Effect of stepwise lipase addition on the CDCMR system's kinetic performance. $T = 35^{\circ}$C, TMP = 1.15 bar, pH 6, oil/water molar ratio = 0.066, impeller stirring rate = 462 rpm, lipase concentration = 0.05 g/l, equivalent system lipase concentration of each stepwise addition = 0.005 g/l.

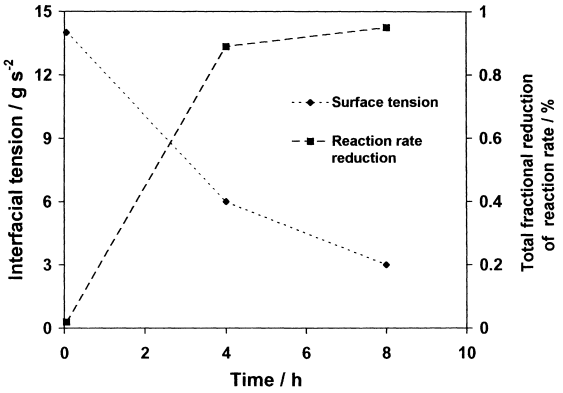


Fig. 5. Change of oil/water interfacial tension and fractional reduction of reaction rate during the time course of lipolytic reaction in the CDCMR system. $T = 35^{\circ}$C, TMP = 1.15 bar, pH 6, oil/water molar ratio = 0.066, impeller stirring rate = 462 rpm, lipase concentration = 0.05 g/l.

injection of the additional lipase. The extra lipase restored part of the lost production rate in a fashion that the recovery was more marked at the initial stage. But the subsequent addition of fresh lipase and its accumulation within the CDCMR system failed to effect a full recovery of the system's catalytic power. The system's kinetic performance continued to decline. The explanation for this behaviour could lie within the complex and dynamic lipase–oil–water interfacial interactions which influence the interfacial quality over the course of the reaction. The fresh lipase molecules introduced at later stages may have experienced difficulty in absorbing onto the oil–water interface because of saturation of the interface by the produced fatty acids. They may also experience inhibition by the fatty acids.

### *3.1.3. The influence of interface dynamics*

It has been acknowledged that lipase catalysis takes place in or near the lipid–water interface. It is also been suggested [28,29] that lipolytic products from the hydrolytic reaction immediately locate and spread throughout the interface, displacing substances with lower spreading pressure from the interface.

The formation of fatty acids, their adsorption and spreading over the oil–water interface will enhance the stability of the emulsion system. The effect of the dynamic change of the interface on lipolytic reaction rate was studied by examining the time dependence of the oil droplet size and the oil–water interfacial tension (or surface pressure) during the hydrolytic reaction. Fig. 5 shows the decrease of the oil/water interfacial tension during the continuous operation of the CDCMR system. The tension was measured using a surface tension balance (Surface and Interfacial Tension Torsion Balance model OS, White Electrical Instrument). As the hydrolytic products increasingly crowded the interface, the interfacial pressure decreased, hydrolysis slowed down, and substances with lower spreading pressures were further displaced from the interface (e.g., displacement of monoglycerides by oleic acids).

Fig. 6 shows the change of average oil droplet size during the time course of the lipolytic reaction in

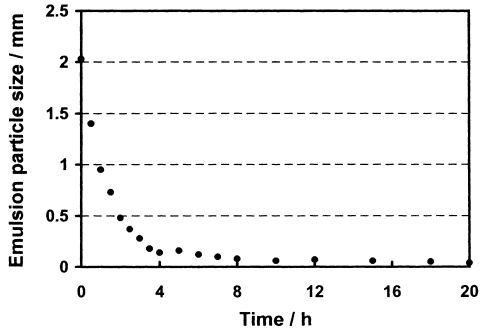


Fig. 6. Change of oil droplet size during the time course of lipolytic reaction in the CDCMR system. $T = 35^{\circ}$C, TMP = 1.15 bar, pH 6, oil/water molar ratio = 0.066, impeller stirring rate = 462 rpm, lipase concentration = 0.05 g/l.

the CDCMR system. The size was measured using a Malvern Particle Mastersizer (3600E). Although the overall interfacial areas increased with time as the larger oil droplets broke up under shear force to form a greater number of smaller ones which were stabilised by the formation and attachment of fatty acids at the emulsion interface, the presence of an increasing concentration of the lipolytic products at the interfaces might have, however, reduced the overall accessibility of the interfacial areas to fresh substrates. The change of the interfacial properties may have also altered the binding ability of the enzymes (thereby changing values of the equilibrium constants $K_1$, $K_2$, $K_{12}$, $K_{21}$), and led to enzyme inhibition.

In an attempt to improve the interfacial quality and product transport efficiency, bovine serum albumin (BSA) was introduced to the CDCMR system to facilitate the desorption of the lipolytic products from the oil–water interface. 1 g/l BSA solution was added to the system at the beginning of the CDCMR operation. The presence of the albumin in the subaqueous phase markedly improved reaction rate during the first four hour operation as demonstrated in Fig. 7. Scow et al. [30] suggested that when an albumin protein in aqueous phase was not contiguous with the site of hydrolysis, oleic acid and monoglyceride readily moved in the interface to the area of contact with albumin where they were desorbed from the interface. Although lipolytic products, composed of long chain acyl groups, have a strong tendency to remain in the interface, a small fraction of free fatty acids and monoglyceride desorb and diffuse to the aqueous subphase. Albumin in the aqueous subphase could therefore accelerate desorption of both fatty acids and monoglyceride from the interface and, thereby, enhances the hydrolysis.

### *3.1.4. Effect of crossflow velocity*

In the CDCMR reactor system, the emulsified substrate feed stock enwrapping the lipase molecules is continuously recycled from and to the batch vessel through the ultrafiltration unit. The accumulation of lipase on the membrane surface during the continuous separation of the aqueous phase could have resulted in increased lipase concentration at the membrane surface. The reduction in mass transfer efficiency due to the lipoprotein concentration polarisation effect could become a rate limiting factor [23,31,32]. It may also affect the kinetic stability of the reaction system.

High recycle crossflow rates through the UF unit were employed to minimise the concentration polarisation effect and to detect the possible loss of the system's kinetic stability due to the concentration polarisation effect. Fig. 8 shows the effect of increased crossflow rate on the kinetic performance of the CDCMR system. Despite a fivefold increase in crossflow velocity, the improvement in fatty acid production rate is minimal, suggesting a limited effect of the

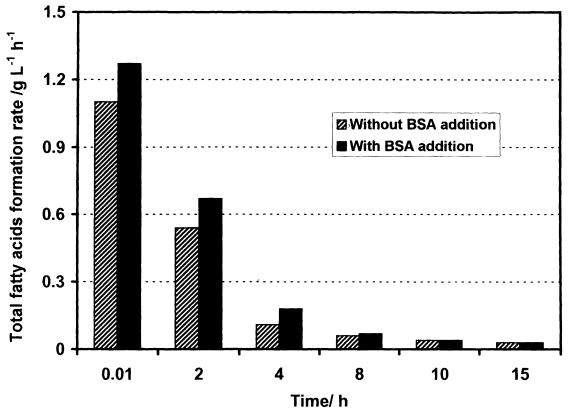


Fig. 7. Effect of BSA addition on total fatty acids formation rate in the CDCMR system. $T = 35^{\circ}$C, TMP = 1.15 bar, pH 6, oil/water molar ratio = 0.066, impeller stirring rate = 462 rpm, lipase concentration = 0.05 g/l, system BSA concentration = 1 g/l.

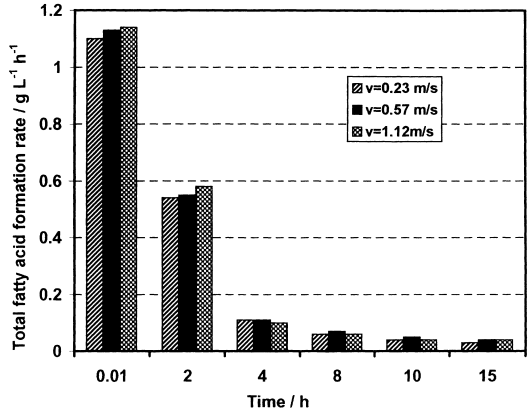


Fig. 8. Effect of flow velocity on total fatty acid formation rate in the CDCMR system. $T = 35^{\circ}$C, TMP = 1.15 bar, pH 6, oil/water molar ratio = 0.066, impeller stirring rate = 462 rpm, lipase concentration = 0.05 g/l.

Table 3
Final fatty acid yield and oil conversion rate at different oil/water molar ratios within the two systems

| Oil/water molar ratio | Final total fatty acid concentration (g/l) | | Comparable yield increase (%) | Oil molar conversion rate (%) |
|---|---|---|---|---|
| | Stirred tank reactor | CDCMR system | | |
| 0.14 | 2.45 | 2.56 | 4.5 | 0.44 |
| 0.66 | 2.72 | 2.88 | 5.9 | 0.085 |
| 0.94 | 2.96 | 3.04 | 6.1 | 0.061 |

increased fluid hydrodynamics in liberating polarised lipase within this velocity range. The highest velocity may have even contributed to the slight decrease of lipase catalytic power due to shear effect.

### 3.2. Analysis of yield in batch and CDCMR

If a membrane reactor is to be used in a commercial process it must offer some economical advantages over conventional reactors (packed columns with immobilised enzymes or fluidised bed reactors). There are two primary places where a membrane reactor is most likely to offer such advantages. First, the yield of the membrane reactor may be greater in equilibrium limited or inhibition constrained reactions by simultaneous product separation. Second, the membrane reactor may reduce the separation demand farther downstream in the process and thereby reduce costs.

Compared to the stirred tank batch reactor, the continuous separation of one reaction product (glycerol) delivered only limited gain on the yield of fatty acids as indicated by the final total fatty acids concentration after 50 h reaction time in the two reactor system (Table 3). The smaller than expected improvement in yield in the integrated CDCMR system could be attributed to slow desorption and transport of fatty acids at the oil–water interface, which are more dominant rate limiting factors than the retention of lipase and glycerol separation. The unsuccessful removal of the fatty acids from the system by the membrane unit may have also depressed yield.

In a theoretical analysis of defining conditions of improved yield in nine recycle porous membrane reactors, Reo et al. [33] concluded that for equilibrium reactions a porous membrane reactor can offer modest yield advantages (less than 20%) over a plug flow reactor network only if the reactor operates at relatively low conversions. The finding that the yield advantage is small suggests that a porous membrane reactor must also contribute significantly to the separation of reactants and products if it is to offer a clear advantage over more conventional reactors purely on the basis of operating economics.

When the BSA was added to the CDCMR system, the yield increased in the range 7.8–10.5% at different oil/water molar ratios (Table 4). This further illustrated the significance of facilitated desorption and transport of fatty acids away from the reaction interface, which enhanced both kinetic and yield performance. In contrast the pressure driven membrane process is not well suited for this intricate interfacial transport and separation task, especially in light of the fragility, mobility and dynamism of the biphasic system.

### 3.3. Analysis of combined reaction and separation

The performance of a membrane reactor can also be limited by the loss of mass transfer efficiency

Table 4
The effect of stepwise BSA addition on final fatty acid yield within the CDCMR system

| Oil/water molar ratio | Final total fatty acid concentration (g/l) | | Comparable yield increase (%) |
|---|---|---|---|
| | CDCMR system without BSA | CDCMR system with BSA present | |
| 0.14 | 2.56 | 2.83 | 10.5 |
| 0.66 | 2.88 | 3.16 | 9.7 |
| 0.94 | 3.04 | 3.27 | 7.8 |

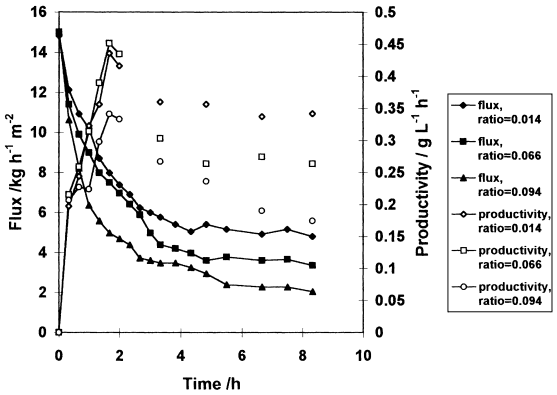


Fig. 9. Productivity and flux performance of the CDCMR system in combined reaction and separation operational mode. $T = 35^{\circ}C$, TMP = 1.15 bar, pH 6, oil/water molar ratio = 0.066, impeller stirring rate = 462 rpm, lipase concentration = 0.05 g/l.

(convection or diffusion) during the simultaneous product separation process. The reduction in membrane filtration capacity during an operation is usually caused by two distinctive phenomena: concentration polarisation and fouling. In many cases, concentration polarisation and fouling lead to an increase of the rejection capability of a careful preselected membrane system. An effective control over concentration polarisation and fouling effects is thus essential to maintain constant mass fluxes and productivity in continuous membrane reactors.

The results in Table 3 suggest that a low oil/water ratio is desirable for high equilibrium conversion of valuable oils. The ratio would also have an influence on the composition and structure of the oil–water emulsion which directly influence the ultrafiltration efficiency. The effect of oil/water molar ratio on UF flux performance and the productivity of the CDCMR system is shown in Fig. 9. The productivity of the CDCMR system is defined as

$$P = D \times C, \tag{6}$$

$$D = J/V, \tag{7}$$

where $P$ is the system productivity, $D$the system dilution rate, $C$ the system fatty acid concentration, $J$ the permeate flow rate, and $V$ is the total system volume.

The CDCMR system was operated with feed stream recycle and permeate withdraw. This configuration has been widely studied and reported in the literature [34–36]. In spite of the presence of a membrane module, studies on the residence time distribution in recycle reactors indicated that the whole system can be analysed as a single entity behaving like an ideal CSTR (continuous stirred tank reactor).

Table 5
Glycerol, fatty acids, and lipase concentration in ultrafiltration retentate and permeate

| | | Time (h) | | | | |
|---|---|---|---|---|---|---|
| | | 0.5 | 1 | 2 | 15 | 30 |
| Glycerol (g/l) | Retentate | 1.09 | 0.80 | 0.71 | 0.43 | 0.40 |
| | Permeate | 1.09 | 0.80 | 0.71 | 0.43 | 0.40 |
| Fatty acids (g/l) | Retentate | 0.51 | 0.62 | 0.70 | 0.74 | 0.73 |
| | Permeate | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Lipase (g/l) | Retentate | 0.09 | 0.18 | 0.19 | 0.19 | 0.19 |
| | Permeate | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |

The definition of productivity in Eq. (6) for the CDCMR system is a modified version of productivity for a conventional CSTR (continuous stirred tank reactor) since only a proportion of the hydrolytic products, the glycerol but not fatty acids, was present in the effluent stream (permeate flow). But the definition can be used as a reasonable representation of the true system productivity as long as the enzyme is not experiencing serious fatty acid inhibition.

Greater productivity was achieved at lower oil/water ratios. This is especially important for hydrolysis of valuable polyunsaturated oils. There appeared to be optimum dilution rates of 0.352, 0.331 and 0.308 $h^{-1}$ at oil/water molar ratios of 0.014, 0.066 and 0.094, respectively.

The selectivity of the PLCC membrane with an MWCO value of 5000 was analysed by measuring concentrations of glycerol, fatty acids, and the lipase in the ultrafiltration retentate and permeate flow at various time intervals during continuous operation of the CDCMR system. The results are presented in Table 5. The 100% glycerol transmission is expected since a glycerol molecule has a molecular mass of only 92, and is entirely soluble in water. A complete retention of the lipase by the membrane is also expected as it has a much greater molecular mass than the MWCO value of the membrane. Surprisingly, no fatty acids were detected in the UF permeate by the titration method.

The fatty acids produced from the hydrolysis of the sunflower oil (oleic, linoleic, stearic acids, etc.)

have typically a molecular mass in the region of 300, which at first impression should pass through the membrane. The underlying mechanism for the unsuccessful fatty acids transmission is complex. The critical factors could be (i) low aqueous solubility of the fatty acids; (ii) the adsorption of fatty acids at the oil/water interfaces which resulted in the retention of fatty acids together with the oil droplets which have much greater sizes, and (iii) the formation of micelles or aggregation by the dissolved free acid molecules in the aqueous phase since the fatty acids have typically a long hydrocarbon chain with a hydrophilic tip and a hydrophobic tail. Another plausible reason could be the adsorption of the fatty acids on to the membrane and the subsequent formation of a gel layer which acts as a second transmission barrier. Furthermore, unlike glycerol solutes, the elongated shape of the molecules could have hindered their transport through the membrane pores even if the molecules entered the pore [37].

The unsuccessful separation of fatty acids from the CDCMR system may lie at the heart of the somewhat indifferent kinetic as well as yield performance between the conventional batch reactor and the combined reaction and separation process in the CDCMR. It is therefore suggested that a membrane unit incorporated in a CDCMR configuration can offer higher separation efficiency and product selectivity and thus improvement in reaction rate, yield, and system productivity. However, this may be difficult to achieve in practice due to the complex interfacial interactions between the substrate, product, and enzyme.

## 4. Conclusions

The apparent reaction rates in the conventional batch reactor and the CDCMR reactor system are studied to draw comparison between the two systems' kinetic and product yield performance and associated phenomena. The addition of a small amount of BSA markedly improved the kinetic and yield performance in both conventional reactor and CDCMR configurations. The presence of the BSA molecule in the subaqueous phase accelerated the desorption and transport of fatty acids at the oil–water interface. The study also showed that the initial rapid loss of lipase protein molecules within the CDCMR system coincided with a large reduction of catalytic activity. Subsequent addition of the fresh lipoprotein did not fully restore the system's initial kinetic performance because of the dynamic change of the physicochemical properties of the biphasic fluid. An important factor here was the decrease in accessible interface due to the formation and accumulation of fatty acids at the interface. A further factor was the reduction in interfacial area reflected in the reduction in surface tension. The maximum productivity of the CDCMR system was achieved at the lowest value of oil/water molar ratio at an optimum dilution rate of $0.352\,h^{-1}$. The oil conversion rate was also higher at lower oil/water ratios.

These findings suggest that the performance of the CDCMR system will be strongly influenced by the selection of membranes (material, surface adsorption property, fouling tendency, etc.) and the performance of the UF unit (selectivity, flux, flow velocity, etc.). The relationship between productivity, flux, and oil conversion ratio clearly demonstrated the existence of a process synergy between the separation efficiency of the membrane system and the kinetic/yield performance of the CDCMR system when it was operated under a simultaneous reaction and filtration regime. The separation of glycerol alone did not produce anticipated improvement in the CDCMR system. Therefore, a continuous separation of fatty acids from the system seems to be a prerequisite to deliver a technical as well as economic edge to the CDCMR system. This will require a more efficient separation and transport of fatty acids from the oil–water interface. This is beyond the capability of a conventional ultrafiltration or microfiltration process. It is also clear that regardless of the reactor configuration, interfacial phenomena including adsorption, reaction, separation and transport would be the determining factors in deciding the catalytic performance in a dynamic emulsion system. A sensible approach to an integrated reaction–separation system would adopt facilitated adsorption, transport, and separation of the enzyme, substrate and product at the reaction interface. The separation process employed ought to be able to continuously extract fatty acids from the organic phase.

Lipase catalysis within the CDCMR system is mathematically modelled in a two-substrate kinetic model with limited interface accessibility. This model gives a clearer and more precise explanation of the physical

meaning of the $V^*_{max}$ and $K^*_m$. The apparent value of the $V^*_{max}$ is slightly greater in the CDCMR system than in the conventional stirred tank reactor while the $K^*_m$ value is virtually unchanged. The quantification of the interfacial substrate concentration and the primary binding equilibrium constants ($K_1, K_2, K_{12}, K_{21}$) are the prerequisite for the practical application of the two-substrate model. This is beyond the scope of the present study. Future kinetic modelling work should incorporate the consideration of the simultaneous separation and transport properties of the membrane system which will ultimately affect the dynamic interfacial composition and quality during the reaction.

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