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Simultaneous reaction and separation in enzymatic hydrolysis of high oleate sunflower oil – evaluation of ultrafiltration performance and process synergy

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Abstract

An experimental study has been carried out on an integrated reaction system consisting of a stirred tank reactor, a de-emulsifier and an ultrafiltration (UF) unit for simultaneous reaction and product separation during the enzymatic hydrolysis of sun flower oil. The study focuses primarily on how UF performance and enzymatic activity are affected by different components in the reaction products and by the operating parameters of the integrated system. An evaluation of the system productivity and synergistic relationships between the kinetics of the hydrolytic reaction, de-emulsification, and product separation is presented. The reaction system comprised a heterogeneous oil/water system with the free enzyme functioning at the liquid–liquid interface. In contrast to theoretical expectations, no significant gain in productivity and yield was observed in the operation of reaction and separation simultaneously. The continuous removal of the glycerol produced, surprisingly, resulted in only marginal improvements in overall reaction yield. This may be explained by the complex mass transfer processes occurring at the membrane/liquid interface and due to unsuccessful separation of the produced free fatty acids from both the de-emulsified aqueous and oil phases. The reactor operating parameters were also recorded including oil/water molar ratio and impeller stirring rate and their effect upon the UF flux performance and the stability of the lipase activity determined.

Keywords: Ultrafiltration; Integrated system; Simultaneous reaction and separation

1. Introduction

Recent developments in applied biocatalysis and recombinant technology offer scope for new biotransformation and biosynthesis. In contrast, the advances in biological sciences have not been matched by comparable developments in process/reaction engineering to accommodate different modes of expression of biological activities in reaction systems. New process design by integrating reaction and simultaneous separation in a more compact and flexible processing system ought to improve yield and system productivity, allow regulated control of biological catalyst function, and offer opportunities for process acceleration, intensification, and simplification.

Enzymatic modification of fats and oils (tri-glycerides) provides ambient reaction conditions unmatched in many chemical processes, for production of high-value products from cheap and plentiful raw material. Lipase catalysed hydrolysis, ester synthesis and inter-esterification reactions can produce commodity chemicals with immense scope of application in the food, cosmetic, detergent, explosive and pharmaceutical industries. Current industrial hydrolysis of oils/fats employs alkaline high pressure steam splitting. This involves high energy utilisation and yields a product requiring costly purification. The oxidation and polymerisation of high value unsaturated fatty acids are inevitable under these process conditions. Hence it is unsuitable for the splitting of sensitive tri-glycerides or high value polyunsaturated oils. On the other hand, the applications of enzymes to oil/fat hydrolysis may create significantly less burden on the environment in terms of energy consumption and waste produced.

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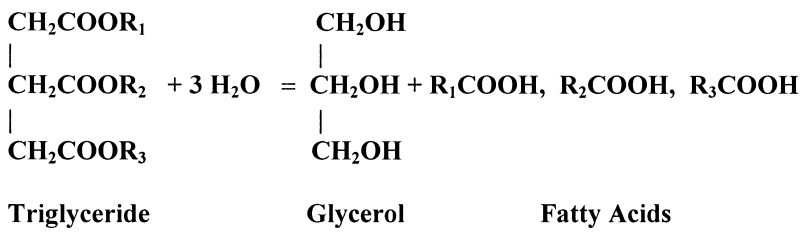
Stoichiometry of the enzymatic oil hydrolysis reaction



Two principal methods of conducting these enzymatic reactions are either as a liquid–liquid contact with the lipase freely acting at the interface or, with the enzyme immobilised onto a solid support with solid–liquid contact between an oil/water emulsion and the solid phase. Both of these techniques have limitations but in particular both would require a discrete separation process for the continuous removal of reaction products in order to achieve high degrees of equilibrium conversion and to alleviate product induced enzyme inhibition.

Application of membrane technology in the processing of fats and oils is widespread in the areas of lipid refining, separation, decolouring, and decontamination [1–4]. There is also an increasing interest in using membranes for constructing combined reaction and separation systems. These systems can be divided into those in which there are one [5]or two liquid phases [6,7] or an emulsion phase [8]. They can also be divided into those in which the lipase is immobilised either onto or within the membrane [9–11] and those in which the lipase is at the interface of an emulsion [7–12]. In most immobilised systems, the reaction kinetics are not limited by the intrinsic enzyme catalytic power but by the slower mass transfer step which is directly linked to the availability of accessible interfacial area. The complex rheology involved in two-phase flow is also problematic. In emulsion systems with free enzymes functioning at the oil/water interface, mass transfer can be enhanced by high intensity mixing. This is not normally available in packed column reactors containing immobilised enzymes. In most systems, phase separation is necessary before product recovery by ultrafiltration (UF)/micro-filtration of either the oil or the aqueous phase can be achieved. In an integrated system for simultaneous reaction and separation, an effective continuous de-emulsification and phase separation process is required between the reactor and separator for effective product removal. Otherwise, severe membrane fouling may occur as a consequence of pore blockage by the emulsion. In some cases the emulsion may partly separate and cover the entire membrane surface[13,14].

Past studies of lipase kinetics in membrane reactors have largely concentrated on the selection of the enzymes and the support materials, including solid particles and membranes [9,15]. The studies were less focused on the mass transfer of the hydrolysates within the two-phase heterogeneous systems and across the membranes. Historically, reaction and separation were studied separately and there are only sporadic data reported on the evaluation of the process synergy existing between the reaction and separation processes. Using simple expressions for the rate of UF and reaction, Wang et al.[16] and Closset et al. [17] developed mathematical models assuming laminar flow and first-order reaction kinetics. Recently Prazeres et al. [18] presented a model for the hydrolysis of olive oil in a membrane-emulsion reactor system. The kinetic model employed a second-order dependence on substrate concentration and a third-order inhibition by fatty acids. These were combined with a simple reactor dynamics model and a model for the transport of the olive oil and oleic acid across the membrane. The influence of cross-flow hydrodynamics and separation efficiency has not been sufficiently accounted for in all the reported models. In addition there has been little evidence of attempts to validate such models especially in systems involving concentration polarisation and membrane fouling.

The work described here is principally an experimental study employing a stirred batch tank reactor, a continuous de-emulsifier, and a Millipore Pellicon Cassette ultrafiltration unit fitted with PLCC hydrophilic UF membranes. The paper focuses primarily on (i) how UF performance and the enzyme's activity are affected by different constituents in the aqueous oil hydrolysis product and operating parameters of the integrated system, and (ii) an evaluation of the system productivity and process synergy existing between the kinetics of the hydrolytic reaction, de-emulsification, and product separation. The system studied was based on a two phase heterogeneous liquid–liquid system with the free state enzyme functioning at the interface of the oil/water emulsion. The effects of oil/water molar ratio, enzyme concentration, mixing regime and UF transmembrane pressure upon system productivity and separation efficiency were systematically studied.

2. Experimental materials and methods

Lipase extracted from *Candida Cylindracea* (Amana Pharmaceutical Company) was used with an activity of 30 000 unit/gram 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

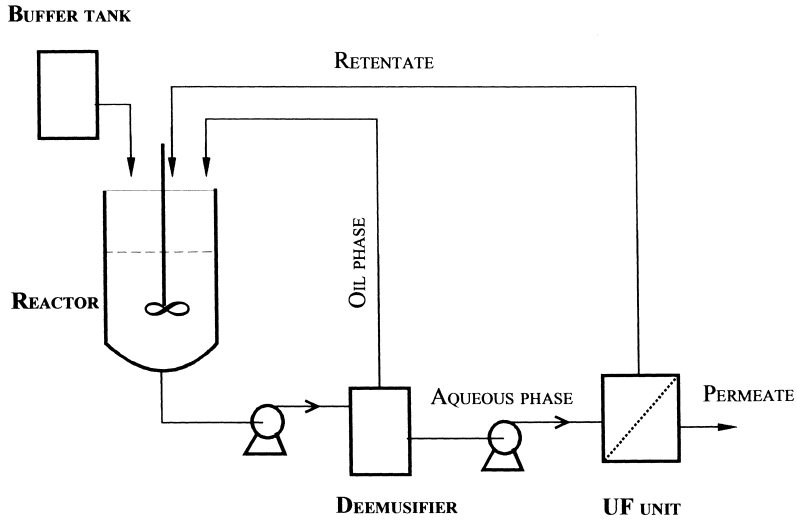


Fig. 1. Schematic diagram of the combined stirred tank reactor and UF system.

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 UF module (Millipore Limited). Analysis of lipase activity, concentrations of glycerol, fatty acids, and protein in the feed, retentate and permeate over various filtration times was carried out. The glycerol concentration was determined using a Glycerol Assay kit (Bohringer and Mannheim UK Limited, 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 Bailie [19]. Composition of the fatty acids produced was determined by gas chromatography (column BP21 was supplied by SGE UK Limited). 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, varied from 300 to 850 rpm. The sunflower oil/water volume fraction in the system varied from 0.0833 to 0.833 corresponding to oil/water molar ratios in the range of 0.01–0.1. Table 1 summarises the operating conditions, including the lipase concentration and UF transmembrane pressures which were used. The reaction mixture was maintained at pH 6 using a buffer. A de-emulsifier was built in between the reactor and ultra-filter to break the oil/water emulsion comprising the reactor's effluent stream to yield an oil phase, which was recycled directly back to the reactor. The aqueous phase was pumped to the UF system for separation of glycerol and dissolved fatty acids. A partition was built into the de-emulsifier to facilitate continuous break-up of the oil/water emulsion in the effluent stream

Table 1
Conditions in operating the integrated reactor and UF system

Impeller stirring rate in the reactor (rpm)	321	462	663	803
Lipase concentration ($g\,l^{-1}$)	0.02	0.05	0.10	
Ultrafiltration transmembrane pressure (bar)	0.65	0.95	1.15	
Oil/water volumetric ratio	0.083	0.500	0.833	
Corresponding oil/water molar ratio	0.010	0.066	0.100	

out of the reactor. The separated aqueous phase, containing mostly glycerol, proteins and a small proportion of dissolved fatty acids, was fed to the UF unit. The drain of aqueous permeate from the UF unit was compensated by balanced addition of buffer during the experiments. The cross-flow rate was based on maintenance of the predefined transmembrane pressure difference. Fig. 2 shows a schematic diagram of the Pellicon Minicassette membrane system.

3. Results and discussion

3.1. Ultrafiltration of aqueous oil hydrolysis product

3.1.1. De-emulsification of the oil/water emulsion

The de-emulsification process aimed to facilitate product separation and to protect the UF membrane from oil contamination which can cause serious flux decline [13,14]. Partitioning of soluble hydrolysates (glycerol and soluble fatty acids) during the de-emulsification into the aqueous phase was an essential prerequisite for their separation through the UF unit. The partition of the enzyme between the oil and water phase was also an important factor influencing the UF performance and the enzymatic activity in the system. The de-emulsifier worked well and allowed continuous operation of the system with recycle. After going

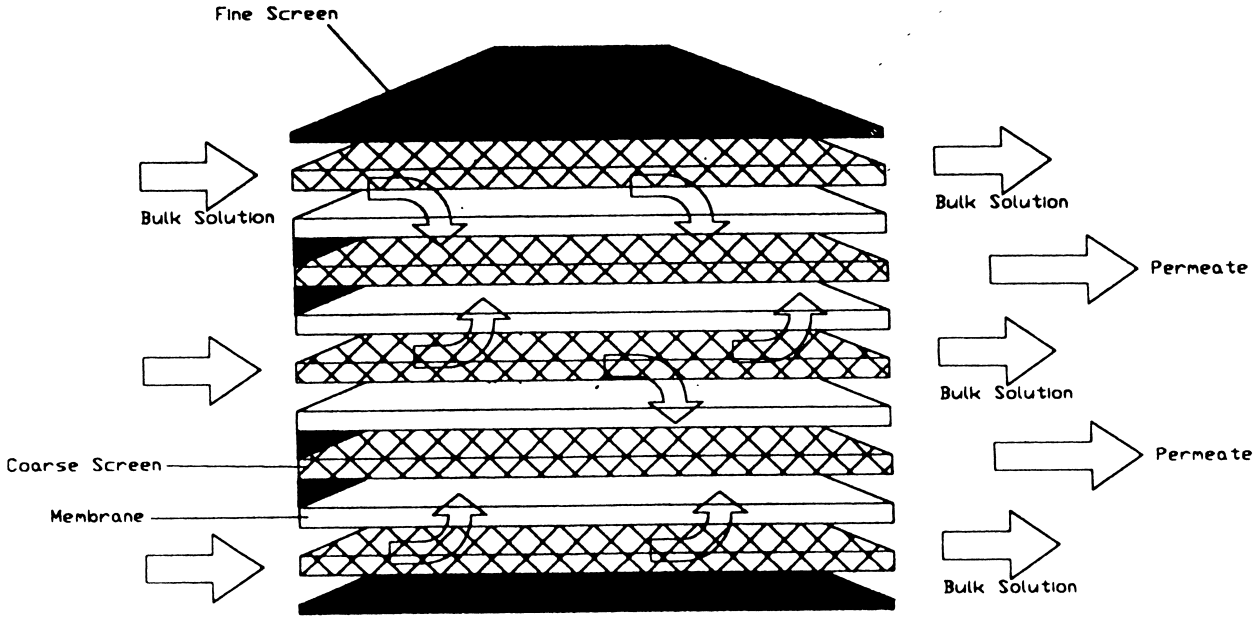


Fig. 2. Schematic diagram of the Pellicon Minicassette crossflow UF system.

Table 2
Equilibrium partitioning of glycerol, fatty acids, and the lipase between aqueous and oil phase after de-emulsification

Constituents	Partitioning factor between aqueous/oil phase
Glycerol	99:1
Fatty acids	23:77
Lipase	93:7

Table 3
Glycerol, fatty acids, and lipase concentration in UF retentate and permeate

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

through the UF process, the aqueous phase from the UF retentate was recycled back to the reactor to remix with the separated oil phase.

Table 2 shows the mass partitioning ratio of glycerol, fatty acids, and the lipase between the oil and aqueous phase in the integrated system at steady state (after the partitioning reached equilibrium at t>2 h). The results were obtained by measuring the respective constituent concentrations in samples withdrawn from the oil and aqueous stream coming out of the de-emulsifier during continuous operation. Glycerol is water soluble and stayed almost 100% in the aqueous phase. The lipase concentration in the aqueous phase started with a low concentration and gradually increased with time until it reached an equilibrium partitioning at steady state.

3.1.2. Membrane selectivity of hydrolysis products and flux performance

Concentrations of glycerol, fatty acids, and the lipase in the UF retentate and permeate were measured at various time intervals during continuous operation of the integrated process. The results are presented in Table 3. Permeate analysis showed 100% glycerol transmission and a complete retention of the lipase by the membrane. Surprisingly, no fatty acids were detected in the UF permeate by the titration method.

Total passage of glycerol through the PLCC membrane with a molecular weight cut-off of 5000 is expected since a glycerol molecule has a molecular mass of only 92, and is entirely soluble in water. The fatty acids produced from the hydrolysis of the sun flower oil (oleic, linoleic, stearic acids, etc.) have typically a molecular mass in the region of 300, which at first impression should penetrate through the membrane without difficulty. The failure of the fatty acid transmission can be attributed to a number of factors. The critical one could be the formation of micelles or aggregation by the 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 [20].

Another important observation is the continuous decrease of glycerol concentration with time. This reflects a decrease

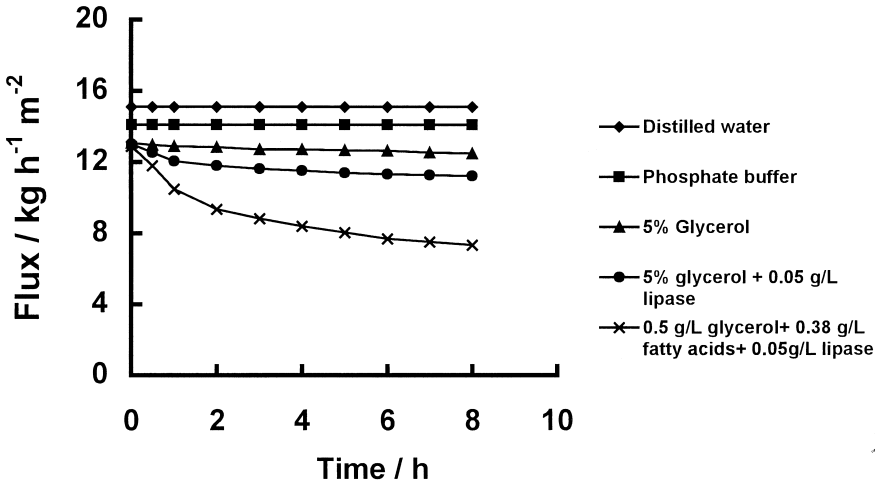


Fig. 3. Ultrafiltration fluxes of the aqueous solutions consisting different constituents of the oil hydrolysis products. T=35°C, TMP=1.15 bar, pH 6.

in reaction rate and one important cause of this is the accumulation of fatty acid product, which may inhibit lipase activity. In addition since the reaction here is reversible, the rate of forward reaction would be reduced at higher product concentrations. Therefore, with progressively reduced rate of glycerol production, its concentration in the system was continuously diluted by the addition of the buffer for compensating the loss of aqueous fluid in the form of the UF permeate.

Ultrafiltration fluxes of (i) distilled water, (ii) a phosphate buffer of pH 6, (iii) a 0.5 g l^{-1} glycerol buffer solution, (iv) a constituted glycerol (0.5 g l^{-1}) and lipase (0.05 g l^{-1}) buffer solution, and (v) a buffer solution containing 0.5 g l^{-1} glycerol, 0.34 g l^{-1} fatty acids, and 0.05 g l^{-1} lipase are presented in Fig. 3. The flux of the 0.5 g l^{-1} glycerol buffer solution was about 18% lower than the pure water flux, but was consistent over the 10 h filtration. This suggests that glycerol molecules did not cause irreversible membrane fouling upon their passage through the membrane pores. The flux reduction could be attributable to drag forces acting on the fluid during its passage through the very small membrane pores. The presence of the lipase molecules in the filtrate had a marked effect on the flux decline, but it appeared to be the presence of the fatty acids which exerted the greatest negative influence on the flux. The direct deposition of fatty acids on to the membrane surface may reduce the hydrophilicity of the membrane surface with a consequence of successive multi-layer adsorption and gel formation.

The fouling of the membrane is clearly a central issue in determining the feasibility of the technique in the context of oil hydrolysis. The PLCC membranes used in this study were made from regenerated cellulose, but it is believed that this was chemically modified by the manufacturer to render the surface of the membrane more hydrophilic. Overall it is difficult to avoid membrane fouling even using hydrophilic materials, indeed protein adsorption is an established cause of fouling in many biofiltration systems.

3.1.3. *The effect of shear and adsorption on the lipase activity in the integrated system*

Efficient product separation by UF often requires high velocity cross-flow conditions, which may deactivate the lipase through shear induced deformation of the enzyme molecules. The loss of enzyme activity in the combined system could arise under two circumstances: (i) the existence of a shear stress field in the reactor due to intensive mixing, and in the UF unit due to a high cross-flow velocity, and (ii) loss of active enzyme molecules due to adsorption onto the membrane surface. Other work in the literature [21] concerning the micro-filtration of alcohol de-hydrogenase (YADH) showed that no significant loss in enzymatic activity occurred when the enzyme was in a stable solution environment. A gradual loss in activity occurred when the YADH was kept in prolonged recurring contact (re-circulation of the enzyme solution) with the micro-filtration membrane surface which was exposed to a shear field. The propensity of the loss of an enzyme's activity in a shear field has been related to the 'mass average shear', the product of the average shear stress (τ) and exposure time (t), which for cylindrical capillaries of radius (r) and length (L) is given by [22]

$$\langle \tau t \rangle = \frac{8\mu L}{3r}$$

Analysis of the lipase activity within the integrated system showed around 20% fall over an operating time span of 96 h (Fig. 4). More than 15% activity loss occurred during the first hour. The activity level then stabilised with only a marginal further decrease over the remaining 95 h of the run. This indicated that the large initial activity reduction is likely caused by loss of the active lipase molecules through adsorption onto the fresh membrane surface. Further shear deactivation of the enzyme was insignificant as the lipase was stable in the buffer solution at pH 6. This is in contrast to Bowen and Gan's work on the micro-filtration of YADH [21]. The reason could be that there was no shear induced

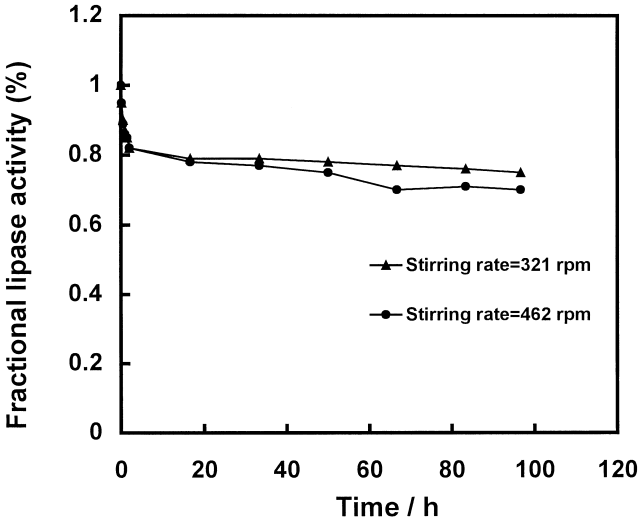


Fig. 4. The loss of lipase activity during a simultaneous reaction and UF operation in the integrated system. T=35°C, TMP=1.15 bar, pH 6, oil/water molar ratio=0.066, lipase concentration=0.05 g l^{-1}.

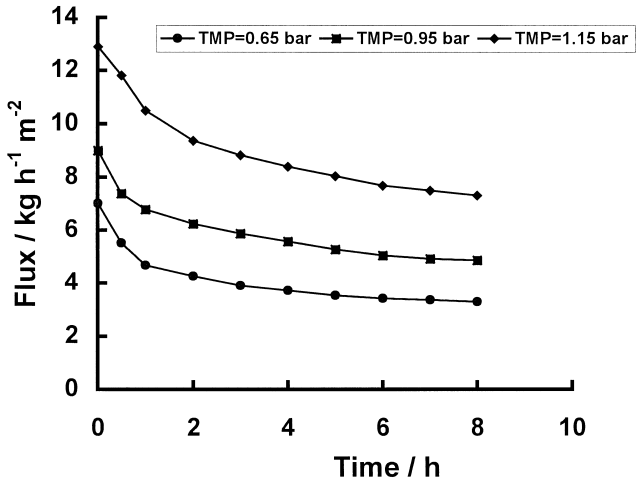


Fig. 5. Effect of transmembrane pressure on the flux performance of an aqueous oil hydrolysis products consisting 0.5 g l^{-1} glycerol, 0.38 g l^{-1} free fatty acids, and 0.05 g l^{-1} lipase. T=35°C, pH 6.

deformation of the enzymatic proteins inside membrane pores since the 5000 MWCO PLCC UF membrane did not allow the passage of the protein molecules.

3.1.4. Effect of transmembrane pressure

The variation of transmembrane pressure during the UF was investigated, see Fig. 5. Since the UF process involves the fractionation of different constituents of the aqueous phase, a complex surface and in-pore flux reduction and fouling mechanism is involved [13,23]. Nonetheless, the increased driving force at higher transmembrane pressures had almost a proportional effect on the increase of the flux level, though all fluxes showed continuous decline. The absence of a greater flux decline with increased transmembrane pressure, which was observed in many reported

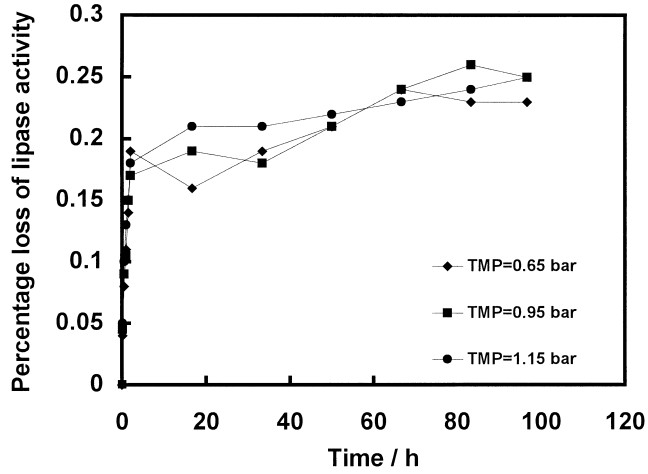


Fig. 6. Effect transmembrane pressure on the loss of lipase activity in the integrated system. T=35°C, pH 6, oil/water molar ratio=0.066, impeller stirring rate=462 rpm.

studies [24–26], could be due to a less complex in-pore fouling mechanism because of the total exclusion of free fatty acids and the enzymatic protein molecules.

The effect of increased transmembrane pressure on the lipase activity was also studied, see Fig. 6 and it was concluded that the magnitude of transmembrane pressure within the operating range had no significant impact on lipase activity.

3.2. Process synergy between reaction and separation

Process synergy exists between two or more discrete (unit) operations involved in one complete process if the conditions of one operation have direct or indirect influence on the outcome of the next operation, and vice versa. This synergy can have important implications on overall process design, operation and optimisation. In the previous section, the UF performance was examined separately without simultaneous operation of the hydrolysis reaction. Although the experiments allowed the assessment of the effect of different constituents in the aqueous phase and UF operating parameters upon UF performance and membrane fouling, an evaluation of the process synergy has to be based on simultaneous operation of both the reaction and separation processes. The results of the evaluation are now presented.

3.2.1. Fatty acids production in the integrated system

In the presence of excess water and active lipase, triglycerides could be completely hydrolysed to free fatty acids and glycerol. Fig. 7 compares fatty acids production in the conventional stirred batch tank reactor (without operating the de-emulsifier and UF unit) with that in the integrated system with oil/water molar ratio fixed at 0.01.

Compared with the conventional stirred tank reactor, the continuous separation of one reaction product (glycerol) and re-circulation of lipase in the combined system delivered only limited gains on the yield of fatty acids, and an almost

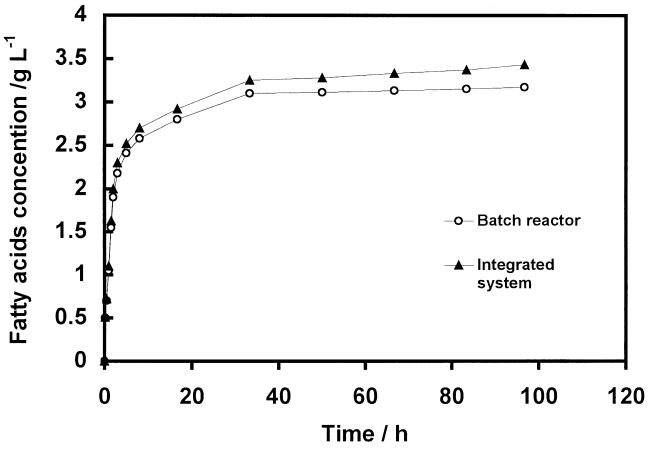


Fig. 7. Production of fatty acids in the conventional batch reactor and the integrated reaction/separation system. T=35°C, TMP=1.15 bar, pH 6, oil/water molar ratio=0.066, impeller stirring rate=462 rpm, lipase concentration=0.05 g l^{-1}.

insignificant increase in reaction kinetics. The continuous removal of glycerol from the integrated operation may have contributed to the small increase of the fatty acids yield.

The smaller than expected improvement in yield in the integrated system is possibly attributable to slow mass transfer of fatty acids from the oil/water emulsion interfaces, where the reaction is actually occurring. The surfactant properties of the free fatty acids give them a natural tendency of attachment at the oil/water interfaces. The failure to remove the dissolved free fatty acid from the aqueous phase by the membrane may have also further depress product yield.

The surfactant property of fatty acids can be best illustrated in Fig. 8 which shows the change of oil/water interfacial tension of the oil/water emulsions during the operation of the integrated system. The interfacial tension was measured using a surface tension balance (Surface and Interfacial Tension Torsion Balance model OS, White

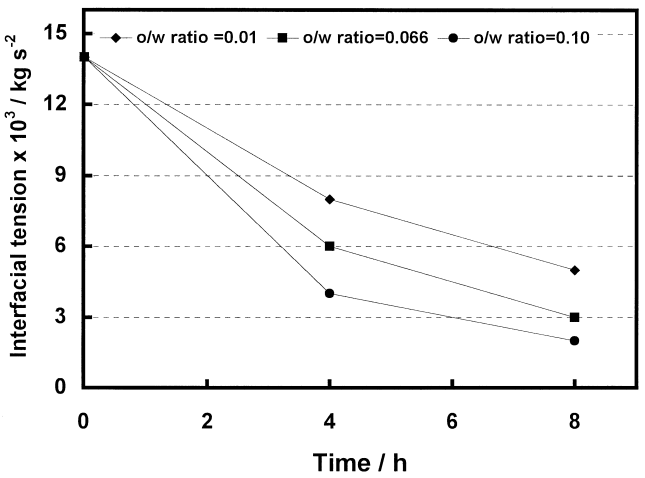


Fig. 8. Change of the oil/water interfacial tension of the emulsions during the operation of the integrated system. T=35°C, TMP=1.15 bar, pH 6, lipase concentration=0.05 g l^{-1}, impeller stirring rate=462 rpm.

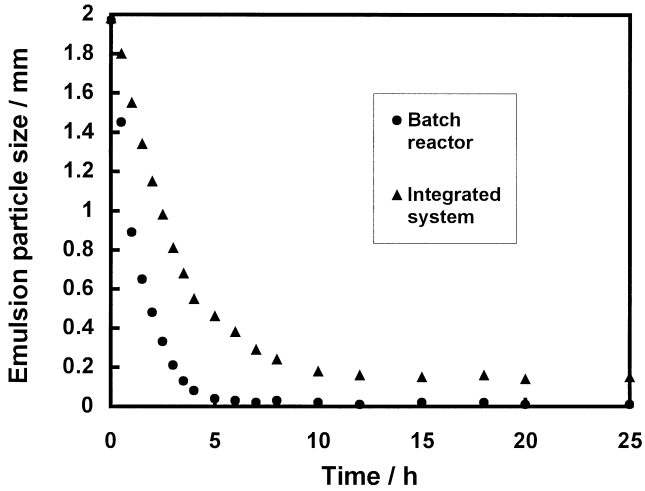


Fig. 9. Change of the average oil/water emulsion size in the stirred batch reactor and the integrated system. T=35°C, TMP=1.15 bar, pH 6, lipase concentration=0.05 g l^{-1}, oil/water molar ratio=0.066, impeller stirring rate=462 rpm.

Electrical Instrument Company Limited). The decrease of the interfacial tension is more pronounced at higher oil/water molar ratios. The attachment of the free fatty acids at the interface may have had a stabilising effect on the emulsion, resulting in a continuous decrease of the emulsion drop sizes. Efficient phase separation is critical for the success of the integrated system and continual size reduction and stabilisation of the emulsion particles may render the de-emulsification process ineffective at long run times.

Fig. 9 compares the change of average emulsion size in the conventional stirred batch reactor (with de-emulsification and UF closed) with that in the integrated system. The average emulsion size was measured using a Malvern Particle Mastersizer (3600E) in the integrated system. The average values observed were notably larger because of the continuous recycling of the oil phase and droplets back to the reactor after the de-emulsification. Whilst the larger oil/water emulsion size in the integrated system could have benefited the de-emulsification process, the smaller overall interfacial area could be another important factor which hindered the achievement of higher fatty acids productivity.

Glycerol and fatty acids have very different chemicophysical properties and separation characteristics. The separation of glycerol is relatively easy, but its separation had a very limited effect on the improvement of system kinetics and yield. The PLCC UF membrane was found to be incapable of separating fatty acids in the integrated system. This was a significant factor in explaining the disappointing yields obtained. Poor removal of fatty acids would also reduce lipase activity and thus further reduce yield. Removal of the fatty acids from the system by a separate technique would require quite an elegant separation to remove them because of their highly surfactant nature.

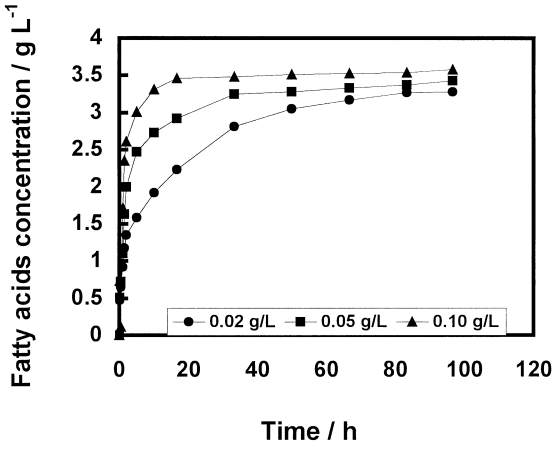


Fig. 10. Effect of increased lipase concentration on the production rate of fatty acids in the integrated system. T=35°C, TMP=1.15 bar, pH 6, oil/water molar ratio=0.066, impeller stirring rate=462 rpm.

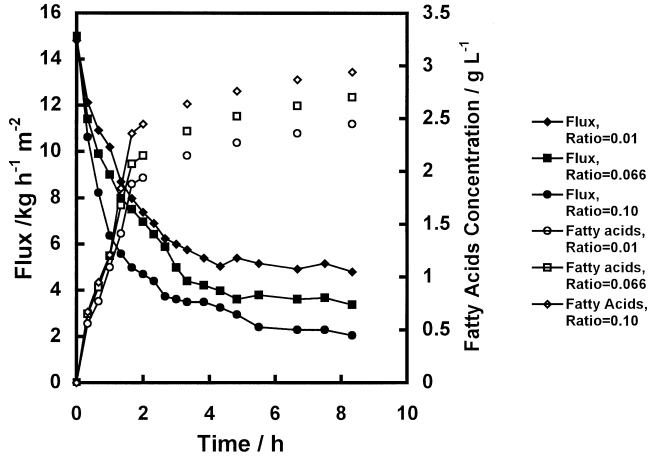


Fig. 12. Effect of oil/water molar ratio on fatty acids production and UF flux. T=35°C, TMP=1.15 bar, pH 6, lipase concentration=0.05 g l^{-1}, impeller stirring rate=462 rpm.

3.2.2. *Effect of system lipase concentration on fatty acids production and UF flux*

The total lipase concentration in the integrated system had a direct influence on reaction kinetics and UF performance. Fig. 10 shows that increase in lipase concentration had a marked beneficial effect on the initial rate of fatty acid production. The effect on final yield is less pronounced. The UF flux was however, reduced at higher lipase concentrations in the aqueous phase, see Fig. 11. This reduction would be exarcebated by the higher fatty acid concentrations associated with higher initial reaction rates.

3.2.3. *Effect of oil/water molar ratio on fatty acids production and UF flux*

A low oil/water ratio is desirable for high equilibrium conversion of valuable oils. The ratio also affects the quality of the oil/water emulsions and thus mass transfer rate, subsequent de-emulsification, and UF efficiency. According to the stoichiometry of the reaction complete conversion of 1 mole tri-glyceride requires 3 mole of water. Intermediates such as mono-glycerides and di-glycerides are also produced during the hydrolytic reaction. The surfactant nature of these intermediates may also contribute to slow mass transfer and phase separation. The effect of oil/water ratio on the fatty acids production and on the UF flux performance is shown in Fig. 12. More fatty acid was produced at higher oil/water ratios but UF fluxes were reduced.

The final fatty acid concentration in the system increased with increasing oil/water ratio. However, this does not necessarily mean a high oil conversion rate or yield of the free fatty acids, see Fig. 13. This shows the molar oil molar conversion rate after 96 h hydrolysis reaction at different starting values of oil/water ratio. The rates are very poor at high oil/water ratios, these are most likely explained by slow mass transfer which limits overall reaction kinetics and final equilibrium. The molar oil/water ratio

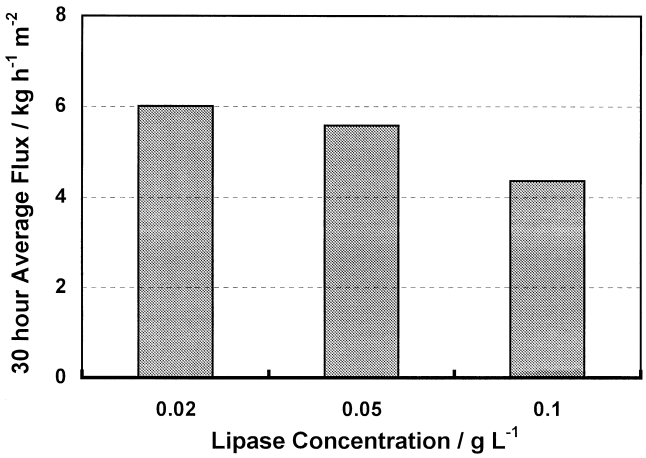


Fig. 11. Effect of increased lipase concentration on the performance of UF. T=35°C, TMP=1.15 bar, pH 6, oil/water molar ratio=0.066, impeller stirring rate=462 rpm.

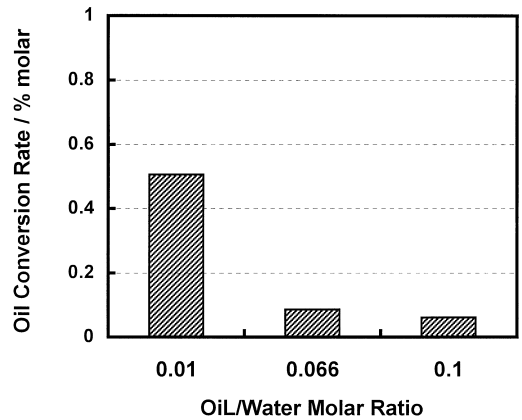


Fig. 13. Effect of oil/water molar ratio on the oil conversion rate. T=350°C, TMP=1.15 bar, pH 6, lipase concentration=0.05 g l^{-1}, impeller stirring rate=462 rpm.

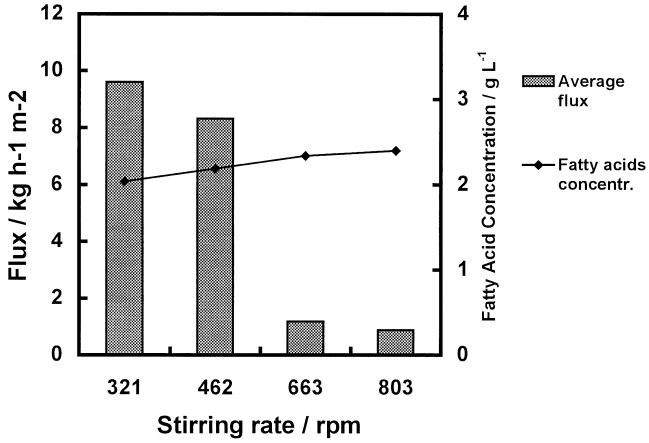


Fig. 14. Effect of impeller stirring rate on fatty acids production and UF flux in the integrated system. T=35°C, TMP=1.15 bar, pH 6, lipase concentration=0.05 g l^{-1}, oil/water molar ratio=0.066.

of 0.1 is equivalent to a volumetric oil/water ratio of 0.833, which may result in an emulsion system of relatively high viscosity thus exhibiting slow mass transfer. Low conversion rates at high oil/water ratios such as are seen here would have serious implications for the hydrolysis of highly valuable oils.

3.2.4. *Effect of impeller stirring rate on fatty acid production and UF flux*

The reactions here were conducted in an immiscible two-phase system and thus intensive mixing is necessary in order to maximise oil/water interfacial areas for optimal reaction kinetics and mass transfer. However intensive mixing generates smaller emulsion particles which could be difficult to separate, and their presence would be detrimental to efficient membrane UF. Shear stress also increases with the mixing intensity and this may affect stability of the lipase (Fig. 14).

The effect of stirring rate on fatty acid production and on the average UF flux after 16 h operation is shown in Fig. 14. At stirring rates greater than 660 rpm, de-emulsification of the effluent emulsion stream coming out of the reactor became very slow with a consequential rapid reduction in UF flux due to oil contamination of the membrane surface. On the other hand, increasing stirring rate benefited oil conversion in the system as indicated by the small increase of fatty acid concentration in the reactor.

4. Conclusions

The PLCC membrane with a 5000 MW cut-off value showed complete retention of lipase and fatty acids, and almost 100% transmission of glycerol. The membrane was seriously fouled by surface adsorption/deposition of fatty acids and their micelles, and by subsequent gel formation. It is difficult from a single study to make a conclusive recommendation on the suitability of the PLCC membrane for this particular lipase separation. Future work might focus on the fouling mechanism, especially to determine whether the adsorption is chemical or physical in nature. Another aspect would be to determine the contribution which concentration polarisation on the membrane surface makes to loss of activity via denaturation.

Compared to the conventional stirred tank reactor, the continuous separation of one reaction product (glycerol) and re-circulation of free state lipase in the integrated system delivered only limited gain on the yield of free fatty acids, and a very small improvement in overall reaction kinetics. The operating conditions in the reactor, including oil/water ratio, enzyme concentration, and impeller stirring rate, had a significant impact on downstream UF performance, which subsequently affected overall reaction yield and kinetics. Thus a significant synergy between the reaction and membrane separation process was confirmed and this could be used beneficially in the future for optimisation purposes.

Approximately 20% loss of enzymatic activity was observed in the early stages of operation. This figure is high and suggests a fairly significant adsorption of protein onto the clean surface of the membrane during the early stages of contact, with the remaining enzymatic activity remaining relatively stable. Surface scanning of the membrane was not conducted in this study but should be considered in the future as it would reveal the extent of deposition of protein on the membrane surface.

Compared with the conventional stirred batch tank reactor, the continuous separation of one reaction product (glycerol) and re-circulation of lipase in the combined system delivered very limited gain on the productivity of free fatty acids. The cause of the deviation from theoretical expectation is complex and not clearly understood. The most likely explanation centres on the mass transfer and distribution of free fatty acids between the oil and aqueous phases and their subsequent removal. The reactor operating parameters had a significant influence on the UF performance. The magnitude of the water/oil molar ratio was especially important. With a high proportion (>75%) of produced fatty acids remaining in the oil phase after de-emulsification and partition, the proportion remaining in the de-emulsified aqueous phase played a dominant role in fouling the hydrophilic PLCC membrane. Approximately 20% loss of the enzymatic activity in the integrated system was caused by initial loss of the protein molecules by adsorption on the membrane surface. The remaining enzymatic activity appeared to be was relatively stable.

The mass transfer of substrate and reaction products to and from the interface appeared to be the most important factor in determining overall system kinetics and yield. It appeared to be more significant than the UF efficiency.

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