ELSEVIER

Chemical Engineering Journal 135 (2008) 25–32

Chemical Engineering Journal

www.elsevier.com/locate/cej

Enzymatic catalysis and electrostatic process intensification for processing of natural oils

Laurence R. Weatherley [a,*], David Rooney [b]

[a] Department of Chemical and Petroleum Engineering, The University of Kansas, Lawrence, KS 66045, USA

[b] School of Chemistry and Chemical Engineering, The Queens University of Belfast, Northern Ireland BT9 5AG, United Kingdom

Received 5 December 2006; received in revised form 9 February 2007; accepted 13 February 2007

Abstract

The work described here is concerned with the enhancement of liquid–liquid contact of oil/water mixtures using high voltage electrical fields. The enzymatic hydrolysis of high oleate sunflower oil was studied in an electrically enhanced liquid–liquid reactor. The kinetics of the reaction were measured at a range of applied electrical field strengths. The effect of electrical field upon liquid hold-up was also determined. The performance of a laboratory scale electrically enhanced enzymatic reactor was evaluated in batch mode, in recycle mode and in continuous mode of operation. The results of the study showed that the rate of reaction was significantly enhanced as the magnitude of the applied electrical field was increased. The dispersed phase hold-up in the recycle reactor was reduced at increased electrical field strength and the rate of phase separation enhanced. The enhanced interfacial area generated by electrostatic dispersion was one factor in the observed increase in the reaction rate. However the specific rates of reaction (rate of conversion per unit interfacial area) for electrical dispersions showed a very large increase when compared with dispersions of similar quality which were generated by mechanical agitation. A clear difference in the rate of reaction for a negative electrical field was also observed when compared with a positive electrical field.

© 2007 Elsevier B.V. All rights reserved.

Keywords: Liquid–liquid systems; Electrostatic enhancement; Biocatalysis; Hydrolysis

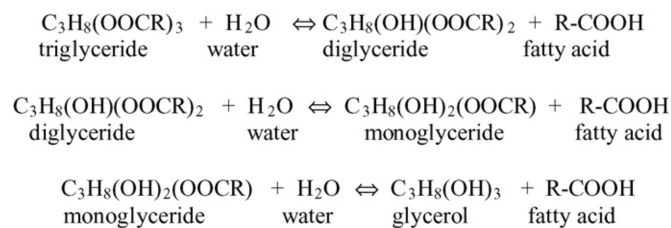
1. Introduction

The hydrolytic splitting of tri-glyceride esters to yield free fatty acids and glycerol – Scheme 1 – is a very important group of chemical reactions relevant to the industrial processing of natural oils and fats. Current large scale processing for this conversion involves high temperature, high pressure counter-current contact of molten fat, water and steam typically at 75 bar and a temperatures in the region of 250 °C [1–3]. Other options for the hydrolysis under less rigorous physical conditions include catalysis by acid, alkali and in the presence of enzymes. Lipolytic fat splitting in the presence of enzymes has been studied extensively [4–8] and appears to operate most effectively in a heterogeneous environment at a defined interface [9]. This complements the physical requirement of interfacial area for mass transfer processes necessary for the supply of reaction substrate from the corresponding bulk phases. Verger and deHaas [9] and Verger [10] reviewed various physical oil–water models in the context of lipase function including emulsions, bilayers, and micelles. Here we are concerned with liquid–liquid emulsions of relatively low stability since product and enzyme recovery via phase separation is an important process requirement. In particular we are concerned with using electrostatic fields to intensify the dispersion of enzyme into the oil substrate and thus enhance overall rate of reaction.

High voltage electrical fields may be used for the dispersion of the aqueous phase into the oil phase in order to generate an unstable emulsion [11–13]. With reference to Fig. 1 in which a small reactor is shown on the right hand side of the diagram, conducting aqueous phase is fed through a conducting nozzle where it acquires a high intensity electrical charge resulting in an electrostatic spray into the oil phase [14]. Dispersed phases of higher conductivity acquire electrical charge more rapidly at the nozzle and thus detachment conditions are enhanced thus leading to easier spray formation and drop de-stabilisation [15]. The pH of the solution, the presence of buffer and salt concentration may affect the dispersion rate and also exert an important influence upon lipase activity [16–19]. It is also possible that

* Corresponding author. Tel.: +1 785 864 3553.
E-mail address: lweather@ku.edu (L.R. Weatherley).

1385-8947/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.cej.2007.02.005



Scheme 1. Hydrolysis of tri-glyceride ester.

Table 1
Fatty acid composition of high oleate sunflower oil [20]

Name	Short	% (w/w)	Formula	MW	MP (°C)
Palmitic	16:0	3.6	$CH_3(CH_2)_{14}COOH$	256.43	62.9
Stearic	18:0	3.8	$CH_3(CH_2)_{16}COOH$	284.48	70.1
Oleic	18:1	80.9	$C_{17}H_{33}COOH$	282.47	14.0
Linoleic	18:2	10.0	$C_{17}H_{31}COOH$	280.46	−5.0
Eicosanoic	20:0	0.7	$CH_3(CH_2)_{18}COOH$	312.54	76.1
Docosanoic	22:0	1.0	$CH_3(CH_2)_{20}COOH$	340.60	80.0

electrical spraying conditions influence lipase activity because of the charged nature of the protein molecule.

The reaction of tri-glyceride esters with water in the presence of lipase is fundamentally an interfacial reaction and the key to a successful reactor is the efficient generation of interface between the two phases. The method of generation should take into account enzyme stability, liquid–liquid stability, and product recovery. Earlier work by Rooney [20] and Jones [21] clearly demonstrated the ability to enhance rates of enzymatic hydrolysis of sunflower oil using electrical fields. Preliminary analysis of the reaction kinetics [22] confirmed other work by Verger and deHaas [9] and Verger [10] that a Michaelis–Menten model which incorporates concentration of interfacial area as a parameter which partially determines reaction kinetics offers a good refinement for two phase systems. This model showed particularly good application to lipolytic hydrolysis of tri-glyceride ester.

In this paper we are concerned with comparing options for mode of operation of an electrically enhanced bioreactor. Factors which affect rate of reaction and the equilibrium conversion are the main focus. Since lipase catalysed hydrolysis is an interfacial reaction, it is clear from earlier studies that drop size and the volumetric hold-up of the dispersed enzyme-rich aqueous phase are critical parameters in determining reactor performance.

The goals of the research described here are as follows:

(1) To demonstrate the intensification of the lipase catalysed hydrolysis of high oleate sunflower oil by electrostatic spraying and to compare the effectiveness of the technique with mechanical dispersion.
(2) To compare the performance of a batch reactor, a recycle reactor, and a semi-continuous reactor and to quantify the role of hold-up and drop-size in each case.

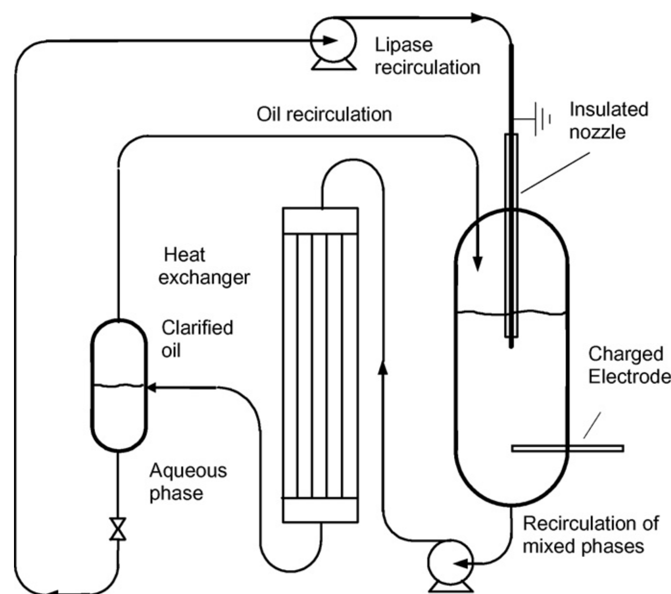


Fig. 1. Overall experimental arrangement of the reactor system—incorporating options for batch, recycle, or continuous operation [11].

2. Experimental

2.1. Materials

The tri-glyceride ester chosen for the study was high oleate sunflower oil which was chosen for its stability but also for its favorable physical properties for liquid–liquid studies in the presence of electrical fields, and because it met the criteria for efficient creation of electrostatic sprays [23]. The overall composition of the oil was 96.5% tri-glycerides; 2.5% diglycerides, 0.8% sterol esters and <0.3% free fatty acids. The fatty acid distribution of the oil is shown in Table 1. The mono-/di-/tri-glyceride compositions were measured by high performance liquid chromatography (HPLC) as described by Liu et al. [24].

The lipase used in the experiments was from Candida rugosa OF360 (Meito Sangyo) with specific activity of 115,000 LU g^{-1} based on a standard of olive oil hydrolysis at 30 °C.

2.2. Stirred batch reactor

Initial studies were conducted in a small mechanically stirred batch reactor and in an electrically enhanced batch bioreactor with a predetermined final oil:water ratio (weight basis) and enzyme concentration (also on a weight% basis) which were preset prior to the start of the reaction. Details of the reactor are described elsewhere [11]. The first experiment involved mechanical agitation of the reaction mixture, total volume 60 mL. The reaction under these conditions was conducted in a 100 mL conical flask using a mechanical stirrer (make IKAMAG® REO) used in conjunction with a 8 mm magnetic stirrer bar to effect mixing at a constant speed of 500 rpm. All reactions were carried out at ambient temperature (20–25 °C) and pressure. The reaction was followed by the periodic withdrawal of a small sample (0.5–1 g) to which was added approximately 50 mL of a 50:50 (v/v) mixture of acetone in ethanol to solubilize the oil and reaction products. An additional effect of adding the solvent is to denature the enzyme and stop any further reaction from occurring. This solvent mixture was observed to be the most effective

for the purpose. The mixture was then titrated against a 0.05N sodium hydroxide solution in the presence of phenolphthalein indicator. The back calculated value for the concentration of the sodium hydroxide solution was then used to determine the extent of oil hydrolysis. The percentage hydrolysis is defined as the percentage weight of free fatty acids in the sample divided by the maximum amount. In order to determine the extent of hydrolysis the equation below was used:

$$\%\text{hydrolysis} = \frac{\text{Na} \times 0.05 \times 10^{-3} \times 283.15}{Wt \times F_o} \times \frac{100}{1} \quad (1)$$

where Na is the volume of sodium hydroxide solution required during titrationl, Wt the weight of the sample taken and F_o is the fraction of oil at start of reaction.

The value of 283.15 used originated from the average molecular weight of fatty acids in the oil (see Table 1).

The data were curve fitted in the first instance using the Tablecurve 2D™ computer package to a simple exponential equation:

$$\%\text{hydrolysis} = (A - B) \times e^{(-C \times \text{time})} + B \quad (2)$$

where A is the initial free fatty acid content (0.3% from composition), B the equilibrium concentration and C is the overall rate of reaction. The determination of the maximum rate of reaction for kinetics analysis was by differentiation.

The electrically enhanced batch bioreactor consisted of a spray chamber constructed from a 50 mm internal diameter glass column with a central stainless steel electrode inserted into the side wall see Fig. 1. Silanisation of the column was conducted regularly to ensure a hydrophobic surface which reduced coalescence of the droplets on the reactor wall. Details of this procedure and the recycle reactor system are described in detail elsewhere [11,20].

For batch operation the reactor was filled with 200 g of high-oleate sunflower oil and 12.5 g of enzyme solution (0.375% enzyme solution by mass) was pumped into the oil using a peristaltic pump (Watson–Marlowe 101 U) at a flowrate of 5 g h^{-1}. The nozzle was charged to between 5 and 40 kV and the central electrode was connected to earth. The distance between the nozzle tip and the electrode was 50 mm. The coalesced aqueous phase could be drained from the bottom of the spray chamber for re-circulation, if required. The nozzle consisted of a 0.8 mm i.d. stainless steel tube which was insulated with silicone tubing to a distance 5 mm from the its tip. The central electrode was also insulated from where it passed through the wall of the spray chamber to within 5 mm of its tip.

In recycle mode, oil and coalesced aqueous phase were removed from the base of the reactor and recycled back via a multi-coil quickfit condenser (300 mm in length maintained at 50 °C) feeding into a glass separator constructed from two quickfit 24/29 connectors, see Fig. 1. The condenser was used to control the temperature of the recycle oil phase and to enhance the coalescence of the aqueous droplets. The aqueous phase was recovered from the base of the separator and pumped back to the reactor. Clarified oil was pumped back to the reactor at a rate of 200 g h^{-1}. In the recycle system the inter-electrode distance were increased to 70 mm to enhance the electrical stability of the system. Coalesced aqueous phase was recovered from the base of the separator and weighed before being returned to the main reservoir thus allowing calculation of dispersed phase hold-up. The fraction of the heavy phase in the recovered mixture was used together with a knowledge of the total initial inventory of heavy phase in the system to calculate the amount of heavy phase present in the reactor at any particular time.

For direct comparison with mechanically agitated systems, the electrostatic spray reactor was replaced by a 500 ml conical flask which was stirred at a speed of 300 rpm (magnetic stirrer bar). In this system, the enzyme solution was added at the same flowrate.

Continuous operation of the reactor was performed by drawing off the separated aqueous phase and pumping in fresh enzyme solution via the spray nozzle. Full oil recycle was maintained thus allowing almost 100% conversion to be achieved.

The determination of aqueous hold-up in the reactor was calculated by mass balance on the total aqueous phase fed to the system and amount recovered after phase separation. Thus hold-up of dispersion in retained in the reactor was thus calculated from the difference between the total weight fed, and the total collected after phase disengagement.

For all the experiments the mean drop sizes of the reaction mixtures were determine off-line either using a Malvern Mastersizer 2600C or photographically. The degree of natural coalescence induced by removing the emulsion from the reactor was found to be small. For larger drops the size data were recorded photographically using an optical microscope (Prior) equipped with a graticule, followed by digital image analysis (PC Image, Epson GT-6000 scanner).

3. Results and discussion

The preliminary results of the batch hydrolysis reaction, comparing the rate of reaction for in a mechanically agitated reactor with that in an electrostatic reactor are shown in Fig. 2, The rate of reaction and maximum conversion at −40 kV were just slightly less than that achieved in the stirred system. As expected visual observations, confirmed that in the electrostatic reactor high voltages rapid mixing and reduction in drop size smaller droplets were observed and qualitatively served to explain the similar rate of reaction when compared with the stirred system.

In the case of the recycle system, the results are presented in two different ways. Firstly the rate of reaction as percentage hydrolysis per hour was determined from the curve fitting procedure, see above, and subsequent differentiation of the experimental fatty acid—concentration time array of data. The rate of reaction is then shown as a function of reaction time, Fig. 3, and with respect to the concentration of the oil as unhydrolysed ester, see Fig. 4. Both these sets of data show clearly that in terms of gross rate of conversion that the stirred system reaches a maximum rate significantly earlier in the reaction than in the electrostatic system. This is consistent with the observed more rapid rate of phase break-up and mixing observed during the experiments in the case of the stirred system. Indeed the

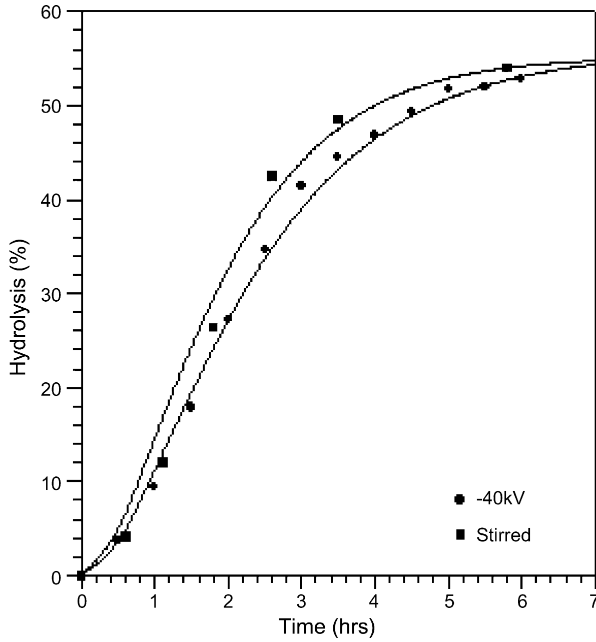


Fig. 2. Results of the batch enzymatic hydrolysis of sunflower oil – batch reactor – comparison of stirred contact with electrically sprayed contact.

consistent experience with the formation of electrostatic sprays is that in the case of lipase solutions sprayed into sunflower oil there is a significant transient start-up period. During this period the electrical field becomes established in the reactor, as space charge migration occurs and the electrical field responds accordingly. This is in line with theoretical predictions already determined by Petera et al. [25].

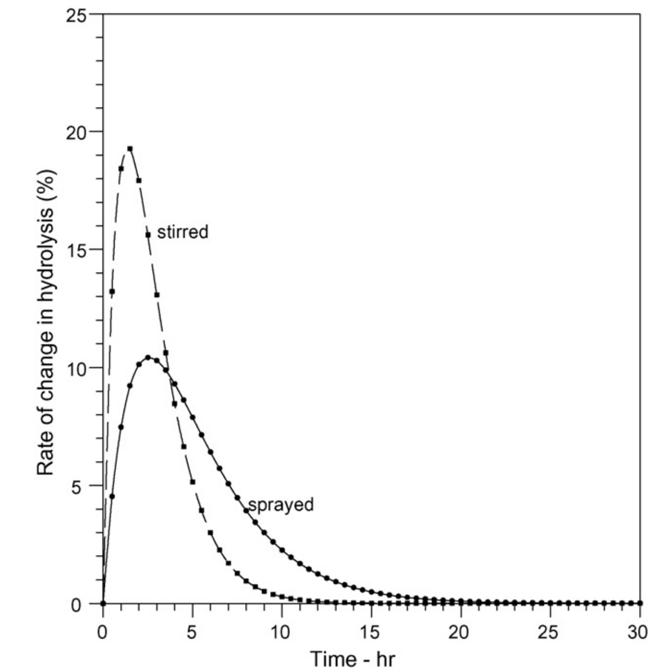


Fig. 3. Rate of hydrolysis in the recycle bioreactor (both phases recycled)—comparison of stirred contact with electrically sprayed contact (applied voltage of −15 kV).

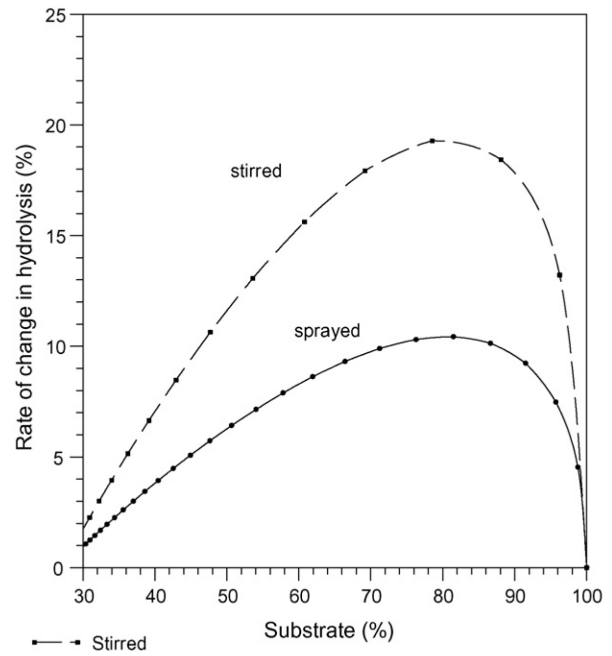


Fig. 4. Rate of hydrolysis in the recycle bioreactor (both phases recycled)—comparison of stirred contact with electrically sprayed contact (applied voltage of −15 kV).

The hold-up of the dispersed liquid in the reactor comprises the fraction of the reactor occupied by the droplets. If the mean drop size, density and hold-up are known this allowed calculation of the mean interfacial area which is available for reaction and mass transfer.

In Fig. 5 the volumetric hold-up data is used as a basis for comparison of the performance of the mechanically stirred and the electrostatic reactor. If it is assumed that the rate difference

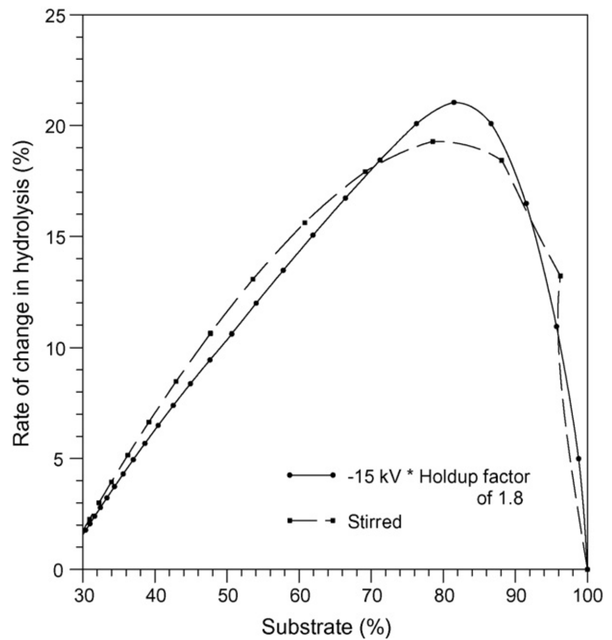


Fig. 5. Rate of hydrolysis in the recycle bioreactor (both phases recycled) – comparison of stirred contact with electrically sprayed contact – showing the effect of dispersed phase hold-up.

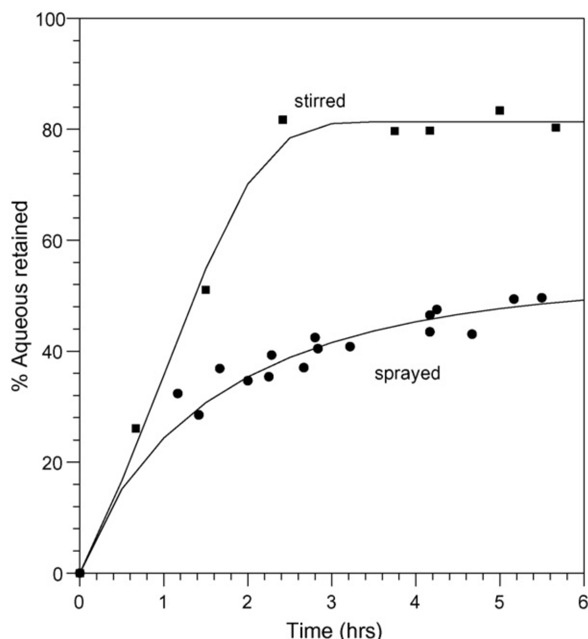


Fig. 6. Aqueous phase hold-up in the recycle—comparison of electrostatically sprayed with stirred (applied voltage of −15 kV).

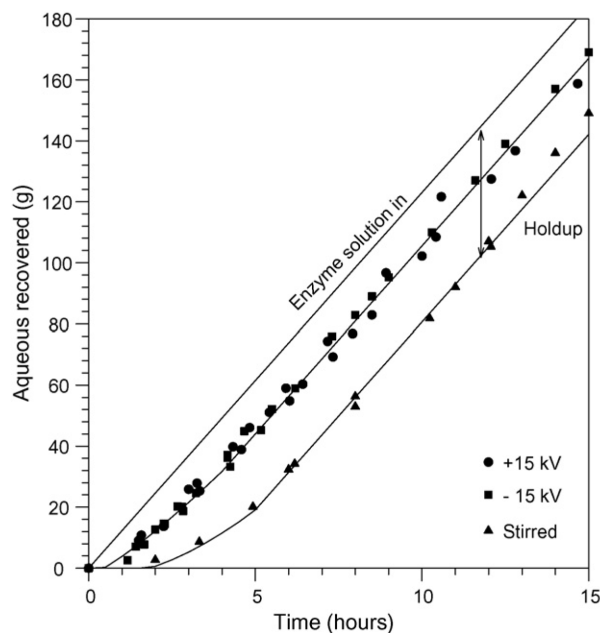


Fig. 7. Cumulative aqueous phase recovery from the reactor operating with continuous fresh AQ feed comparing the recovery with the total aqueous phase fed—comparison of electrostatically sprayed with stirred.

is only accounted for by a difference in the total interfacial area in the reactor, and the drop size remains the same, then differences in hold up alone would account for differences in overall reaction rate. In Fig. 5 the electrostatic reactor rate data have been multiplied by a factor of 1.8 to reflect the difference in hold-up and thus produce a match of the two data sets. It can be seen from this figure that this method is effective in explaining the differences between the curves. Therefore in this case the higher rate of conversion observed in the stirred reactor could be explained by higher hold-up (and hence available interfacial area).

The actual hold-up data for the recycle system are shown in Fig. 6 plotted as a percentage of the water retained by the reactor. The data clearly indicate that the hold-up in the mechanically agitated system is more than double that of the electrostatically sprayed reactor. When this is compared with the estimated hold-up difference inferred in Fig. 5, there is a small but significant difference. This gave a preliminary indication that perhaps volumetric hold-up does not alone explain the difference in observed reactor performance.

This was now investigated further in a comparison of reactor performance based on "once through" feeding of the aqueous enzyme solution. This allowed the reaction to proceed to almost complete hydrolysis (98%), yielding a product oil phase containing less than 5% water.

The aqueous phase separated from the reactor was collected and used to compare the aqueous phase hold-up for the stirred reactor with that of the electrostatic reactor. The cumulative amounts of aqueous phase exiting each reactor are shown in Fig. 7 along with the cumulative total of aqueous phase fed. The exit aqueous phase amounts for the electrostatic and stirred reactors settle out at constant values. The aqueous hold-up for each reactor is the difference between the amounts of aqueous phase fed and at the exit, as indicated in Fig. 7.

It is observed that the hold-up for the electrostatic systems was the same for negative and positive polarities. More significant is the large difference in hold-up for the stirred system compared with the electrostatic system, the former showing a volumetric hold-up of nearly 250% greater than in the case of the electrostatic system. This is significant when the differences in the observed rates of reaction are compared in Figs. 8 and 9.

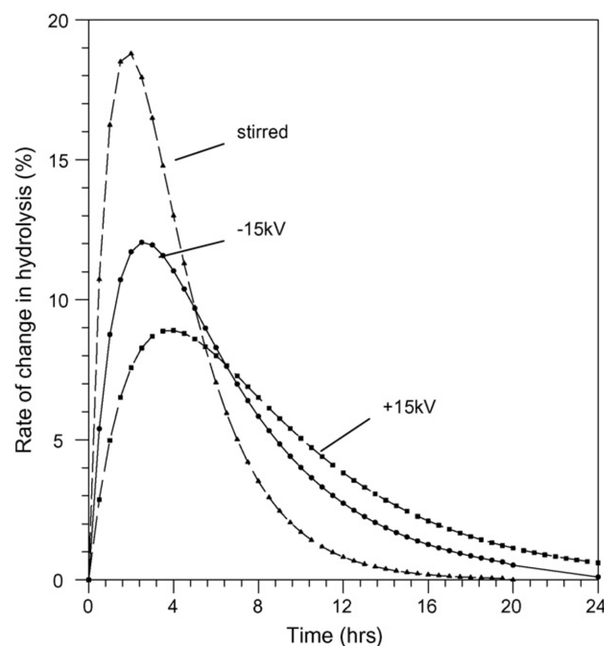


Fig. 8. Rate of hydrolysis vs. time in the continuous bioreactor (fresh enzyme feed – once through) – comparison of stirred contact with electrically sprayed contact.

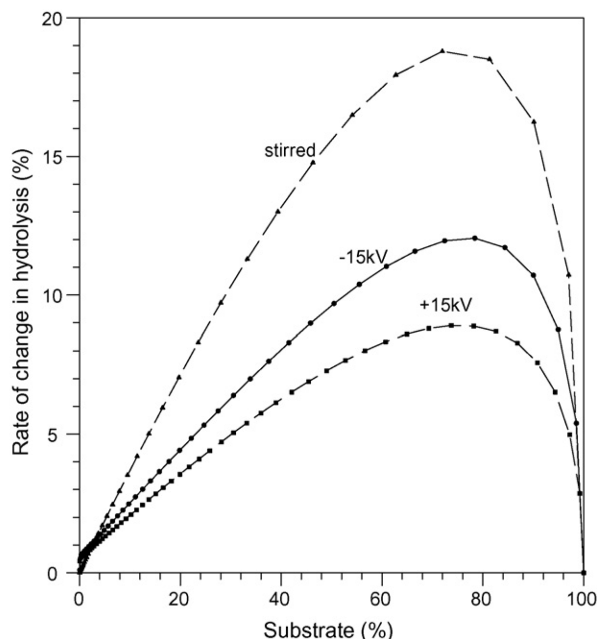


Fig. 9. Rate of hydrolysis vs. substrate concentration in the continuous bioreactor (fresh enzyme feed)—comparison of stirred contact with electrically sprayed contact.

For comparison, a procedure similar to that for the recycle reactor was adopted. The hold-up increase which would be required to bring the performance of the electrically enhanced reactor to coincide with that of the stirred reactor was calculated assuming no change in drop size. The comparison, illustrated in Fig. 10 shows that only 55% increase in hold-up would be required in order to match the performance of the stirred system. This compares with the actual difference actually measured (250% greater hold-up in the case of the stirred system), suggesting strongly that the rate of reaction on the basis of unit interfacial area is significantly greater in the case of the electrostatic reactor. This is true for both the positively and negatively charged modes of operation.

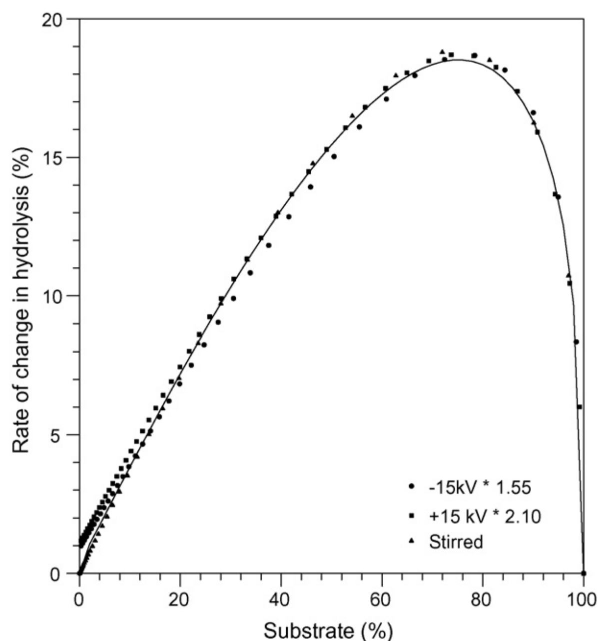


Fig. 10. Rate of hydrolysis in the continuous bioreactor (fresh enzyme feed – once through) – comparison of stirred contact with electrically sprayed contact – showing the effect of volumetric hold-up.

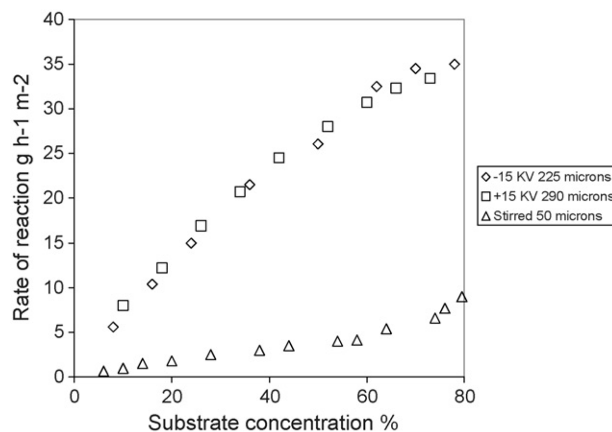


Fig. 11. Specific rate of reaction vs. substrate concentration continuous fresh AQ feed.

Another factor which would explain the significant difference between the two modes of operation is the more efficient phase disengagement apparent in the electrostatic system. A lower aqueous phase hold-up results in lower overall concentrations of glycerol within the reactor and thus more favourable conditions for the forward hydrolysis reaction.

The drop size is also important in determining the available interfacial area available for reaction. The further comparison of the stirred and electrostatic reactors is presented in Fig. 11. Here both the hold-up and mean drop size data were used to determine the rates of reaction per unit interfacial area which are plotted against substrate concentration. The difference between the two sets of data is very significant. It is clear that the specific rate of reaction, expressed as the rate of conversion on a per unit interfacial area basis is much greater in the case of the electrostatic reactor. The mean drop sizes were actually greater in the case of the electrostatic reactor. The hold-up values are significantly lower compared with the stirred reactor. The difference in observed reaction kinetics may be partially explained by the lower glycerol concentration which will be associated with lower aqueous phase hold-up, since the glycerol reaction product distributes into the aqueous phase. Lower glycerol levels were indeed confirmed by material balance calculation. The second possibility is that the enzyme activity is promoted by the nature of the electrical field at the liquid–liquid interface. This would be based on changes in enzyme orientation and enhanced binding at the interface leading to improved catalytic function.

It is also noteworthy that in the case of the electrostatic reactor, the kinetic data are virtually independent of the polarity of the applied field. The difference in mean drop size for the negative and positively charged systems is noted. This is not unexpected given that one of the key parameters affecting drop

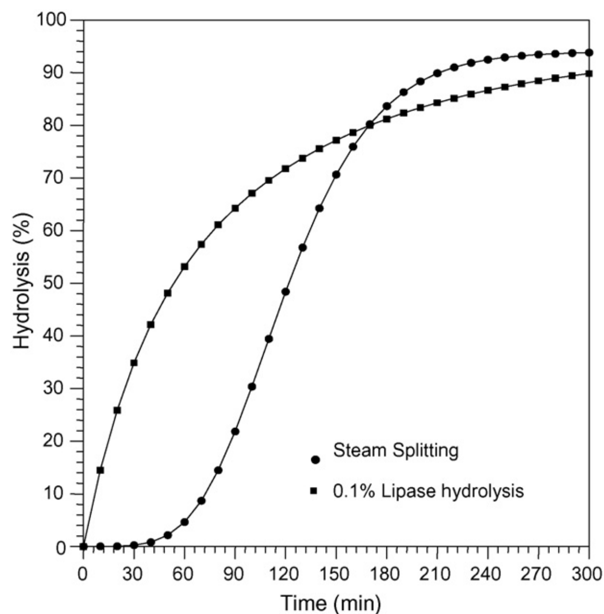


Fig. 12. Rates of hydrolysis of sunflower oil—comparison of lipase catalysed hydrolysis with a commercial batch steam splitting process performed at 240 °C and 33 bar.

size is likely to be the reduction in the interfacial tension due to the electrostatic charge on the surface of the forming drops. The reduction in interfacial tension is very possibly a function of the polarity.

Fig. 12 shows this reaction along with data on the rate of hydrolysis for a neutral fat performed at 240 °C and 33 bar in a commercial batch steam splitting process [2]. It can be seen that this reaction starts slowly, accelerates and then slows down as equilibrium is approached. This suggests that the reaction is autocatalytic and the presence of free fatty acids in the mixture assists in the hydrolysis reaction. This is essentially true as the mono- and diglycerides produced in the early stages of the reaction help to dissolve the aqueous. What this figure also shows is that even a 0.1% concentration the enzyme solution can outperform the steam splitting process, at least at the earlier stages of the reaction, and overall produces a reaction time similar to the steam based process. Although the substrates used in each of these two experiments were different, this figure does provide evidence that lipase catalysed hydrolysis could be scaled up successfully.

4. Conclusions

Comparable rates of overall reaction for the enzymatic hydrolysis of sunflower oil were achieved by mechanical agitation and by electrostatically spraying of aqueous enzyme with oil. In these experiments this was achieved at an applied voltage of 40 kV.

The volumetric hold-up of aqueous dispersion in the electrostatic reactors was consistently lower than in the case of the mechanically stirred reactor. The difference in hold-up and drop size was insufficient to explain the differences in overall reaction rate, giving rise to the possibility of another factor being involved in the enhanced rate of hydrolysis observed in the electrostatic systems.

It was concluded that two possibilities could explain the large difference. The first was based on the better rate of phase disengagement in the electrostatic reactor leading to reduced glycerol levels in the reactor and thus inhibition of the reverse esterification reaction. The second possibility is that the enzyme activity is enhanced in the presence of enhanced electrostatic charge at the liquid–liquid interface. This would be based on changes in enzyme orientation and binding at the interface leading to improved catalytic function. However further research is required to test this possibility.

References

[1] O. Acklesberg, Fat splitting, J. Am. Oil Chem. Soc. 35 (1958) 634–640.
[2] E. Woollatt, The Manufacture of Soaps, Other Detergents, and Glycerine, Wiley, 1985, pp. 194–236 (Chapter 5).
[3] N. Sonntag, in: D. Swern (Ed.), Bailey's Industrial Oils and Fat Products, 4th ed., Wiley, 1979 (Chapter 2).
[4] E.W. Lusas, K.C. Rhee, Animal and vegetable fats, oils and waxes, in: J.A. Kent (Ed.), Handbook of Industrial Chemistry, 1980, pp. 273–1030.
[5] A. Macrae, R. Hammond, Present and future applications of lipases, Biotechnol. Genet. Eng. 3 (1985) 193–217.
[6] P. Woolley, S.B. Petersen, Lipases—Their Structure, Biochemistry and Application, Cambridge University Press, 1994.
[7] K. Faber, Biotransformations in Organic Chemistry—A Textbook, Springer-Verlag, 1991.
[8] S. Okumura, M. Iwai, Y. Tsujisaka, Purification and properties of partial glyceride hydrolase of *Penicillium cyclopium* M1, J. Biochem. 87 (1980) 205–211.
[9] R. Verger, G.H. deHaas, Interfacial enzyme kinetics of lipolysis, Annu. Rev. Biophys. Bioeng. 5 (1976) 77–117.
[10] R. Verger, Enzyme kinetics in lipolysis, Methods Enzymol. 64 (1980) 340–392.
[11] L.R. Weatherley, D. Rooney, M.V. Niekerk, Clean synthesis of fatty acids in an intensive lipase catalysed bioreactor, J. Chem. Technol. Biotechnol. 68 (1997) 437–441.
[12] J.F. Hughes, I.D. Pavey, Electrostatic emulsification, J. Electrostat. 10 (1981) 45–55.
[13] T.C. Scott, Surface area generation and droplet size control using pulsed electric fields, AIChE J. 33 (1987) 1557–1559.
[14] G. Stewart, J.D. Thornton, Charge and velocity characteristics of electrically charged droplets. Part 1. Theoretical considerations, in: IChemE Symposium Series, No. 26, 1967, pp. 29–36.
[15] A. Cornish-Bowden, Fundamentals of enzyme kinetics, Revised ed., Portland Press, 1995.
[16] I. Arnott, L.R. Weatherley, The importance of the electrical properties of both phases in the electrically enhanced extraction of penicillin G, Trans. IChemE, Part C: Food Bioprod. Process. 70 (1992) 219–224.
[17] G. Benzonana, P. Desnuelle, Action of some effectors on the hydrolysis of long-chain triglycerides by pancreatic lipase, Biochim. Biophys. Acta 164 (1968) 47–58.
[18] A. Hoppe, R.R. Theimer, Titrimetric test for lipase activity using stabilized triolein emulsions, Phytochemistry 42 (4) (1996) 973–978.
[19] G. Venkataraman, K.K. Gleason, P.S. Kim, T.A. Hatton, Protein conformation at charged interfaces and water-restricted environments, in: Abstracts of Papers of the American Chemical Society 200 (AUG), 1990, 176 BIOT part 1.
[20] D. Rooney, Charge and polarity effects during natural oil hydrolysis in a novel enhanced bioreactor, Ph.D. Thesis, The Queens University of Belfast, UK, 1998.

[21] E. Jones, Electrostatic enhancement of lipase catalysed hydrolysis in a spray reactor, Ph.D. Thesis, The Queens University of Belfast, UK, 1997.
[22] E. Jones, L.R. Weatherley, Modelling the kinetics of lipase catalysed oil hydrolysis in an electrically enhanced liquid–liquid system, J. Chem. Technol. Biotechnol. 78 (2/3) (2003) 194–198.
[23] J.D. Thornton, Electrically enhanced liquid–liquid extraction, Birmingham Univ. Chem. Eng. J. (UK) (1976) 6–13.
[24] J. Liu, T. Lee, M. Bobik, M. Guzman-Harty, C. Hastilow, Quantitative determination of monoglycerides and diglycerides by high-performance liquid chromatography and evaporative light-scattering detection, J. Am. Oil Chem. Soc. 70 (1993) 343–347.
[25] J. Petera, D. Rooney, L.R. Weatherley, Particle and droplet trajectories in a non-linear electrical field, Chem. Eng. Sci. 53 (22) (1998) 3781–3792.