Journal of Molecular Catalysis B: Enzymatic 67 (2010) 1–9

Contents lists available at ScienceDirect

Journal of Molecular Catalysis B: Enzymatic

journal homepage: www.elsevier.com/locate/molcatb

Enzymatic hydrolysis of oil in a spray column

Virendra K. Rathod, Aniruddha B. Pandit*

Department of Chemical Engineering, Institute of Chemical Technology, Matunga, Mumbai 400019, Maharashtra, India

ARTICLE INFO

Article history:
Received 19 September 2009
Received in revised form 19 June 2010
Accepted 24 June 2010
Available online 1 July 2010

Keywords:
Enzymatic hydrolysis
Spray column
Drop size distribution
Castor oil
Interfacial area
Batch reactor

ABSTRACT

Free enzyme lipolase 100 T from *Thermomyces lanuginosus* has been investigated as a hydrolyzing catalyst in a spray extraction column. The aqueous buffer (pH 7.0) enzyme solution as continuous phase (batch-wise) and the oil as dispersed phase have been used. Various operating parameters which affect the drop size in a spray column, i.e. nozzle diameter, oil flow rate, etc. have been studied. Using the optimized flow rate and nozzle diameter, the effect of various other operating parameters such as concentration of enzyme, enzyme solution height (oil residence time) on the hydrolysis reaction has been evaluated. The highest conversion is obtained for the optimum flow rate of 3.8 ml/min and the nozzle diameter of 0.75 mm. The experimental result showed that the extent of hydrolysis of castor oil is 0.16% in a column reactor due to drop coalescing is almost equal to the hydrolysis due to drop residence time of 1 min in the column. It was also observed that the enzyme activity does not show any appreciable change up to a maximum of 44 passes in the spray column. The contributions of various hydrodynamic phenomena, such as drop formation, rise and coalesce of drops, to the overall hydrolysis rate have been estimated and discussed. Experimental data obtained for the spray column have been compared with that of an agitated batch reactor.

1. Introduction

Fatty acids produced by hydrolysis of triglycerides are the basic raw material for a wide range of chemicals. The usual methods for hydrolysis of triglycerides to fatty acids and glycerol employ high temperature and pressure [1] and therefore, are not suitable for the hydrolysis of heat sensitive oils such as castor oil. Enzymatic hydrolysis is a good alternative for such heat sensitive oils since the use of enzyme for the hydrolysis (carried out at room temperature) not only gives colorless pure product but also reduces the byproduct formation due to enzyme specificity. The main disadvantage of enzymatic hydrolysis is slow reaction rate and high cost of enzyme. Due to reduction in the cost of enzymes in recent times, the enzymatic fat-splitting and other similar enzymatic reactions have shown a renewed interest [2]. The industrial use of lipase for splitting of lipids as energy saving process has been cited in the literature, especially for value-added products and heat sensitive fatty acids [3].

Usually, enzymatic hydrolysis is carried out in a batch reactor. Various reactor configurations have been reported using immobilized lipases and a comprehensive review of the literature has been carried out by Malcata et al. [4], Prazeres and Cabral [5], and Balcao et al. [6]. In our earlier work on optimization of the enzymatic hydrolysis of the castor oil in a batch reactor, it was shown that the rate of reaction shows a significant decrease after 1 h [7]. Similarly, different modifications in the processes aimed at increasing the hydrolysis rate were not successful [8]. In addition, our study concluded that the decrease in the rate of hydrolysis was because of the replacement of enzyme from the oil/aqueous phase interface by the product fatty acid due to latter's higher affinity for the oil–water interface. Thus, it was thought desirable to develop a continuous process for the hydrolysis of castor oil with continuous removal of the product (fatty acid) resulting into enhanced hydrolysis rates. Stirred tank reactor (STR) operated in a batch and continuous manner (CSTR) [4,8], packed bed reactor (PBR) [9,10–15], fluidized bed reactor (FBR) [16] and membrane reactor [17,18–21], etc. have been reported to carry out different enzyme catalyzed reactions. All the work reported has mostly used an immobilized enzyme. The immobilization of enzyme on certain material makes the system heterogeneous. Packed bed reactor is an option in such situation. Even though the enzyme can be recovered and reused due to heterogeneity, intraparticle diffusion retards the reaction significantly. These disadvantages of heterogeneous system can be overcome using enzyme in a soluble form. In a spray column operation, column is filled with continuous phase while the other phase is dispersed using a nozzle either from top or bottom of the column depending upon the density of the dispersed phase. Usually, spray columns are used for the liquid–liquid extraction; but it can also be used for enzyme catalyzed reactions especially

* Corresponding author. Tel.: +91 22 24145616; fax: +91 22 24145614.
E-mail addresses: ab.pandit@ictmumbai.edu.in, abp@ictmumbai.edu.in (A.B. Pandit).

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doi:10.1016/j.molcatb.2010.06.010

Nomenclature

n	number of coalesce events (drop/s)
A	disappearance of the area (m^2)
m	mass of single oil drop (kg)
v_∞	rise of single oil drop (m/s)
N_p	power number
d	diameter of the impeller (m)
N	speed of impeller (rev/s)
d_P	diameter of the oil drop (m)
v_∞	terminal rise velocity of the drop (m/s)
H	hydrolysis of oil (%)
t	time (min)
a	interfacial area (m^2/m^3)
c	concentration of the enzyme in buffer solution (mg/l)

Greek characters

σ	interfacial tension (N/m)
ρ	density of the mixture (kg/m^3)
$\Delta\rho$	density difference between dispersed and continuous phase (kg/m^3)

when enzyme is in a solubilized form and is present as a separate phase.

In the present work, enzyme has been selected as a bio-catalyst for the hydrolysis of castor oil. Lipases are ubiquitous enzymes whose biological function is to catalyze the hydrolysis of triacylglycerols. Due to high specificity and stereo-selectivity, lipases have been used as catalyst for various reactions such as hydrolysis, esterification, and transesterification. Roberto [22] has reviewed various properties and uses of *Thermomyces lanuginosus*. The presence of water–oil interface is known to enhance the hydrolytic activity of lipases. The first confirmation of the structural rearrangement was provided by the crystal structure for small R. *michei* lipase [23,24]. The active sites of lipases are found to be covered by surface loop called a lid or flap. On contact with the interface, the lid opens out and the active sites are accessible to the substrate.

In a spray column reactor, the formation of the oil drop, its size, and frequency which decides the interfacial area between the oil and continuous enzyme buffer phase is dependent on the nozzle diameter, flow rate of oil feed, etc. The usual high temperature high pressure process of manufacturing fatty acids are not suitable for castor oil hydrolysis because of intermolecular esterification of ricinoleic acid resulting in the formation of estolide [1]. Thus, enzymatic hydrolysis of castor oil was investigated. The effect of geometric parameters such as nozzle diameter, continuous phase height and operating parameters such as oil flow rate, residence time and enzyme concentration on the extent of reaction was studied. The effect of interfacial area, interfacial agitation, and concentration of enzyme solution on the rate of hydrolysis has been correlated. The data obtained from the spray column study have been compared with the batch stirred reactor data for energy efficacy of the hydrolysis process.

2. Materials and methods

2.1. Enzymes and chemicals

The substrates, tributyrin and castor oil, were purchased from Himedia Laboratories Ltd. (Mumbai, India) and IPCA Chemicals and Cosmetics Ltd. (Mumbai, India), respectively. The enzyme lipase from *T. lanuginosus* was gifted by Novo Nordisk Ltd. (Bagsvaerd, Denmark). As per the specifications provided by the manufacturers, the enzyme promoted the hydrolysis of a wide variety of triglycerides with 1,3-specificity. Eudragit L-100 used for immobilization was obtained from Rohm Haas, Germany. The cross-linker 1-ethyl-3-(3-dimethylaminopropyl) carbodi-imide (EDC) was purchased from Sigma Chemical Co., Germany. All the other chemicals used were of laboratory reagent grade.

2.2. Analytical methods

2.2.1. Lipase assay with tributyrin as a substrate

Lipase assay was performed with tributyrin as a substrate. Tributyrin (1 ml) was incubated with the conjugated lipase (1 ml) in the presence of phosphate buffer (pH 7) for 10 min [25]. At the end of incubation, the reaction was terminated by the addition of 20 ml methanol and the contents were titrated against NaOH (2 M, in methanol) using phenolphthalein as an indicator. The blank contained the same constituents as the test except the enzyme. *One unit of Lipase activity was defined as the amount of enzyme necessary to hydrolyze 1 μmol of ester bond per minute under assay conditions.*

2.2.2. Determination of the fatty acid concentration

The concentration of fatty acid was determined by titrimetric method as discussed in earlier work [7].

2.2.3. Measurement of drop size and residence time

The drop size of the castor oil, i.e. dispersed phase in a column reactor is required to estimate the interfacial area available for the enzymatic hydrolysis of the oil. The castor oil was sparged continuously in the form of individual oil drops through a nozzle of known diameter into a column (batch-wise) filled with the enzyme solution and single drop was formed at the nozzle tip. The volumetric flow rate of the dispersed phase (oil) was monitored with the help of a peristaltic pump by determining the time required for a measured quantity of castor oil to flow through the pump. The number of drops was manually measured over a required period of time and the diameter of the drop was estimated by volume balance using formula.

$$d_o = \left(\frac{6Tv}{\pi N}\right)^{(1/3)} \quad (1)$$

Similarly, the time of rise of drop from bottom up to top level of the enzyme solution (i.e. residence time of the drop in column) for one pass was measured three times, and the average drop residence time in the column of the enzyme solution of a specific height was estimated.

2.3. Enzyme preparation

The enzyme was dissolved in 0.2 M sodium phosphate buffer solution of pH 7. The enzyme solution was kept overnight to settle the binder added in the solid enzyme. Next day, the enzyme solution was centrifuged and clear enzyme solution obtained was used for the experiments after measuring its activity.

2.4. The process of conjugation of lipolase to Eudragit L-100

The conjugation method developed by Kulkarni [25] has been adopted. According to the procedure, conjugation was carried out by cross-linking the enzyme to the Eudragit L-100 polymer using the bifunctional cross-linking agent 1-ethyl-3-(3-dimethylaminopropyl) carbodi-imide (EDC).

2.5. Hydrolysis of oil

The spray column reactor consists of a glass column of 25 mm inner diameter and 2 m height (Fig. 1). The bottom of the column

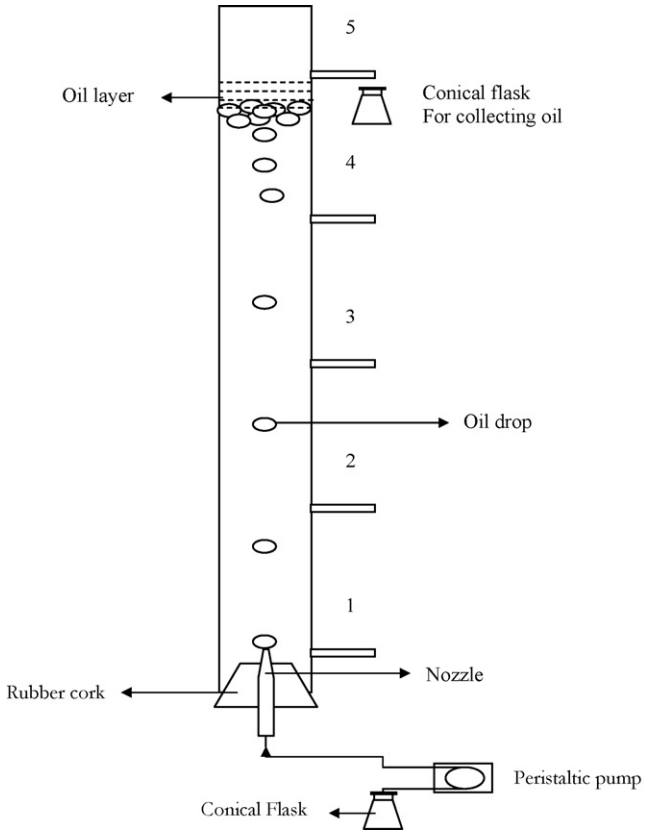

Fig. 1. Experimental set-up for enzymatic hydrolysis of oil in spray column.

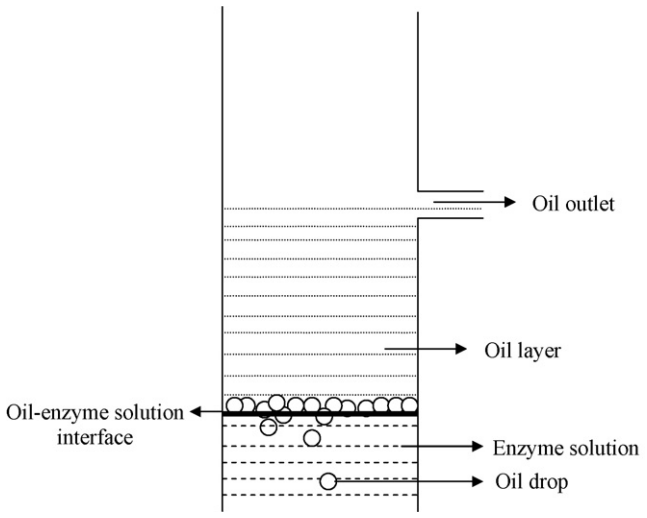

Fig. 2. Enlarge view of oil–enzyme solution at the top of spray column.

was packed using a rubber cork provided with a single glass nozzle of 0.75 mm inner bore diameter. Five side ports were provided to the column at the interval of 470 mm each for the continuous removal of partially hydrolyzed oil from a predecided flight path (liquid column height of the enzyme solution). The provision of the ports helps to check the activity of the enzyme at any time intervals from the different locations and also provides the effect of the height of the enzyme solution (oil drop residence time) on the rate of the castor oil hydrolysis. The continuous phase, i.e. lipolase enzyme in a buffer solution (pH 7) was placed in the glass column in such a way that there was a gap of 40 mm from the predecided outlet. Thus, there was always 40 mm oil layer at the top of the oil–enzyme solution interface. This arrangement prevents the entrainment of the aqueous solution (enzyme loss) along with the oil during the collection of the oil drop after every pass of the dispersed oil phase. Thus, the oil gets added to the lower part of the oil layer and is removed from the upper section (Fig. 1). The expanded view of the oil–enzyme solution interface at the top of column has been shown in Fig. 2. This arrangement also promoted some enhancement in the hydrolysis rate due to the coalescence of drops with the oil layer. The contribution of this has been discussed later in detail. The dispersed phase, i.e. oil was pumped though the nozzle at a known flow rate using a peristaltic pump. The partially hydrolyzed oil was collected from the predecided outlet side port. The hydrolyzed oil was analyzed for the acid value to assess the percentage of hydrolysis (extent of reaction). The column was operated at a room temperature (35–40 °C) for all the experiments.

The effect of various operational and design parameters were studied and the results are described below.

2.5.1. Effect of oil flow rate

Flow rate of oil is an important parameter in this study as it decides the size of the oil drop formed with a specific nozzle size as well as the average disperse phase hold-up and the available aqueous-oil interfacial area (drops and the top layer interface). Experiments were carried out in a spray column with four different oil flow rates. The free enzyme solution with a concentration of 10 g/l (activity 10 unit/ml and protein content 0.37 mg/ml) was filled in a glass column up to height of 1800 mm. Using nozzle diameter of 0.75 mm, the oil was passed though the free enzyme solution at a flow rate of 1.7 ml/min and the partially hydrolyzed oil was collected at the top and was analyzed after every two passes. The process was continued for a total of 10 passes. The procedure was repeated for three other oil flow rates, i.e. 1.3, 3.8, and 4.4 ml/min. The height of the enzyme solution (1800 mm), concentration of the enzyme in the aqueous buffer solution, and the nozzle diameter (0.75 mm) were kept constant for all these oil flow rates.

2.5.2. Effect of nozzle diameter

Second set of experiment was carried out using three different nozzle diameters. Lipolase enzyme solution (concentration 10 g/l) was filled in a glass column up to a height of 1800 mm. Castor oil was pumped at a flow rate of 3.8 ml/min with a nozzle diameter of 1 mm. The partially hydrolyzed oil was collected from the outlet was again recycled. This procedure was continued for 10 such cycles (passes) and the samples were collected after every two cycles (passes) for the determination of the acid value to assess the extent of hydrolysis. This procedure was repeated using 1.5 mm diameter nozzle while the flow rate and the height of the enzyme solution were kept constant.

2.5.3. Effect of enzyme concentration

Lipase-based enzymatic catalytic reactions are interfacial reactions; hence the concentration of the enzyme at the interface plays an important role in deciding the overall rate of the hydrolysis of the oil. In order to check the effect of enzyme concentration on the rate of hydrolysis in a spray column, three different enzyme concentrations, i.e. 2.5 g/l (activity 6.16 unit/ml and protein content 0.215 mg/ml), 5 g/l (activity 7.3 unit/ml and protein content 0.266 mg/ml), and 10 g/l (activity 10 unit/ml and protein content 0.37 mg/ml) were tested in a spray column. The height of the enzyme solution, flow rate of the oil, and the nozzle diameter were 1800 mm, 3.8 ml/min and 0.75 mm, respectively, for all the studies of the enzyme concentration.

2.5.4. Effect of drop residence time

The residence time of a single drop in a column was changed by changing the height of the enzyme solution. Two sets of experiments were carried out in a spray column. In set I, the enzyme solution was filled up to a height of 1800 mm. The oil was pumped through the enzyme solution and the partially hydrolyzed oil was analyzed after every two passes. The residence of the single drop in a column was measured after each pass. The totals of 36 passes were given. In set II, the enzyme solution was filled up to a height of 900 mm with a 40 mm space below the outlet in each of the cases. The procedure as discussed for set I was repeated. In order to maintain the total residence time equal to 36 passes for 1800 mm height in the enzyme solution, 72 passes were made for 900 mm column height of the enzyme solution. The enzyme concentration (10 g/l), flow rate of oil (3.8 ml/min) and the nozzle diameter (0.75 mm) were kept constant for these sets of experiments.

2.5.5. Comparison of spray column with agitated batch reactor

Various batch experiments were performed for the different time intervals, i.e. 2, 4, 6, 8, 10, 12, and 14 min (equivalent to the total residence time in the spray column) in a 100 ml glass reactor. Oil-to-free enzyme solution ratio and the stirring speed were 3:1 and 2500 rpm, respectively, which were optimized earlier [7]. Every time, the experiments were performed in separate batches without sampling. The concentration of the free enzyme solution used for all these experiments was 10 g/l (activity 10 unit/ml and protein content 0.37 mg/ml), same as that used in the spray column experiments.

2.5.6. Comparison of free enzyme with conjugated enzyme

In the present study, the conjugation of the enzyme was carried out using the method reported by Kulkarni [25]. As the enzyme obtained after the conjugation is in the soluble form, the spray column is the best equipment for carrying out the hydrolysis of the reaction. In order to compare the performance of the conjugated enzyme in a spray column with that of the free enzyme, the same experimental procedure was repeated for the free enzyme solution experimental runs. All the experimental runs using conjugated enzyme were carried out at room temperature (35–40 °C).

3. Results and discussion

3.1. Effect of oil flow rate

The effect of oil flow rate on the extent of castor oil hydrolysis (expressed as percentage) with respect to the number of passes is shown in Table 1 for a nozzle diameter of 0.75 mm. It was found that with an increase in the oil flow rate, the percentage hydrolysis of oil increases up to an optimum flow rate of 3.8 ml/min after which it decreases. The percentage hydrolysis obtained for an oil flow rate of 3.8 ml/min after 10 passes (equivalent residence time of 5 min) was 4.52% while it was 3.0% for an oil flow rate of 4.4 ml/min. It was observed that at higher oil flow rate, the distribution of oil drops was uneven and also the preceding drop disturbed flight and the rise velocity of the subsequent drop. At higher flow rate of 4.4 ml/min, pressure drop across the nozzle is very high and rather than forming a single drop, the oil phase forms a jet which subsequently breaks up randomly to give uneven distribution of drop size. This uneven drop distribution may have given rise to higher average drop diameter resulting in lower interfacial area and lower extent of hydrolysis. Up to a flow rate of 3.8 ml/min, with an increase in the oil flow rate, the oil drop size was found to decrease resulting in higher interfacial area and higher dispersed phase residence time as well as oil phase hold-up (lower rise velocity due to smaller drop size) resulting in higher extent of hydrolysis. Here, it was found that the change in drop size and terminal rise velocity with oil flow rate was marginal. For oil flow rates of 1.3, 1.7, and 3.8 ml/min, the sizes of drops formed were 4.2, 4.12, and 3.92 mm, respectively. The interfacial area formed was higher for higher flow rates. It was found that the interfacial area formed for the oil flow rate of 3.8 ml/min was 2.9 m^2/m^3 while it was 0.941 and 1.27 m^2/m^3 for the oil flow rate 1.3 and 1.7 ml/min, respectively. Thus, with an increase in the dispersed phase flow rate, interfacial area also increases and hence the percentage hydrolysis was also found to increase. In jetting region, the drop size is controlled by Rayleigh–Taylor type of instability, whereas buoyancy and interfacial tension decide the drop size in the drop regime. A relation between the hydrolysis rates and the interfacial area for a given concentration of the enzyme solution has been empirically correlated as follows:

$$\frac{dH}{dt} = 0.37(a)^{0.52} \quad (2)$$

This correlation was found to be valid only up to an oil flow rate, where individual drop formation occurs at the nozzle. This correlation does not explain the behavior at a higher oil flow rate where a liquid jet is issued through the nozzle rather than individual drops. The percentage hydrolysis can be obtained by integrating Eq. (2) over the drop residence time for the desired number of passes.

Table 1
Effect of flow rate on percentage hydrolysis of oil (nozzle diameter = 0.75 mm).

Number of passes	% hydrolysis			
	1.3 (ml/min)	1.7 (ml/min)	3.8 (ml/min)	4.4 (ml/min)
0	0	0	0	0
2	0.37	0.35	0.67	0.42
4	0.77	0.71	1.36	0.9
6	1.13	1.4	1.9	1.3
8	1.49	1.86	2.7	1.6
10	1.9	2.4	3.42	1.9

3.2. Effect of nozzle diameter

Fig. 3 shows the effect of the nozzle diameter on the hydrolysis of oil with respect to the number of passes for the flow rate of 3.8 ml/min. It shows that the percentage hydrolysis of the oil increases linearly with the number of passes for all the nozzle

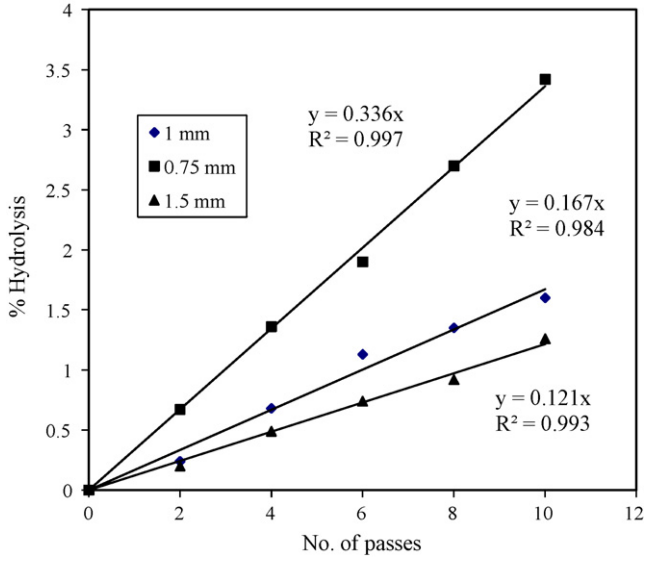

Fig. 3. Effect of nozzle diameter on the percentage hydrolysis of castor oil (flow rate – 3.8 ml/min and enzyme solution concentration – 10 gm/l).

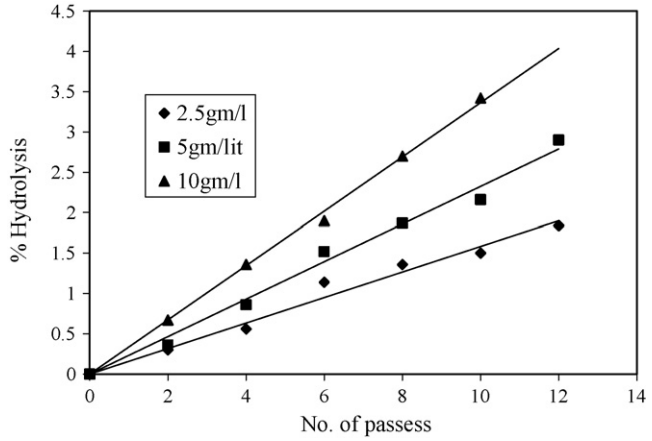

Fig. 4. Effect of enzyme concentration on the percentage hydrolysis of castor oil (flow rate of 3.8 ml/min, nozzle diameter of 0.75 mm and enzyme solution concentration – 10 g/l).

diameters studied in this work. However, the percentage hydrolysis of oil decreases with an increase in the nozzle diameter for the flow rate of 3.8 ml/min. The percentage hydrolysis obtained after 10 passes for the nozzle diameter of 0.75 mm at a flow rate of 3.8 ml/min was 3.42% as compared 1.6 and 1.26% for the nozzle diameters of 1 and 1.5 mm, respectively (i.e. 37 and 54% higher than that for 1 and 1.5 mm nozzle diameter, respectively) for the same flow rate (3.8 ml/min). This is mainly due to formation of very small drops with 0.75 mm nozzle diameter, i.e. 3.92 mm diameter of the drop at a flow rate of 3.8 ml/min. The drop sizes obtained with 1 mm diameter and 1.5 mm nozzle diameter were 4.6 and 5.78 mm, respectively. Similarly, interfacial areas formed (due to smaller drop size and higher dispersed phase hold-up) for 1 and 1.5 mm nozzle diameter were 2.28 and 1.45 m^2/m^3, respectively, as against 2.9 m^2/m^3 for 0.75 mm diameter nozzle.

3.3. Effect of enzyme concentration

Fig. 4 gives the effect of the enzyme concentration on the % hydrolysis of oil. It can be seen that with an increase in the concentration of the enzyme from 2.5 g/l (activity 6.16 unit/ml and protein content 0.215 mg/ml) to 10 g/l (activity 10 unit/ml and protein content 0.37 mg/ml), the percentage hydrolysis also increases. For a total residence time of 5 min in the spray column (i.e. 10 passes), the percentage hydrolysis obtained for enzyme solution concentration of 2.5, 5 and 10 g/l was 1.5, 2.16 and 3.42%, respectively. The experimental data also shows that the increase in the hydrolysis with time (for all concentrations of enzyme solution) was linear but not directly proportional. This indicates that as the concentration of the enzyme is doubled, the extent of hydrolysis increases only by 30% (from 1.5 to 2.16% after 10 passes). As the reaction take place at the interface, an increase in the bulk enzyme concentration leads to an increase in the enzyme concentration at the interface, and hence, percentage hydrolysis of oil increases. Nevertheless, for given hydrodynamic parameters, the extent of hydrolysis is likely to increase with an increase in the enzyme concentration till the interface is saturated with the enzyme. Once the interface is saturated with the enzyme, any further increase in the enzyme concentration may not necessarily lead to a further increase in the hydrolysis rate. Similarly, it is also reported that the enzyme has tendency to form aggregates at higher concentration [26], and this could be another reason for no appreciable change in the extent of hydrolysis with an increase in the enzyme solution concentration. An empirical correlation showing the dependence of interfacial area and enzyme concentration on the hydrolysis rate has been developed, and is

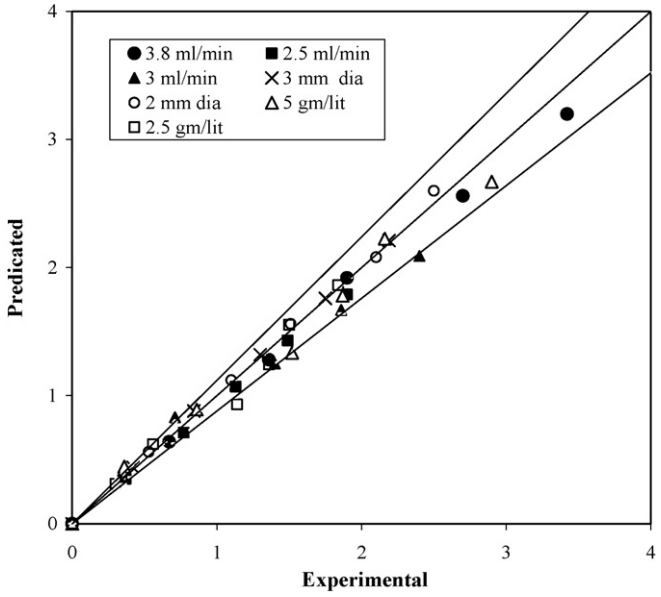

Fig. 5. Parity plot for the rate of hydrolysis of oil.

given by:

$$\frac{dH}{dt} = 0.112(a)^{0.51}(c)^{0.52} \quad (3)$$

The exponents over interfacial area and concentration of the enzyme in buffer solution are almost same, which shows an equal dependence of the interfacial area and the enzyme concentration on the rate of hydrolysis. The percentage hydrolysis could also be predicted using Eq. (3) for different operational parameters, i.e. flow rate and nozzle diameter. For the purpose of comparison, these results have also been plotted in Fig. 5. The parity plot shows that Eq. (3) is able to correlate all the experimental data within ±15%.

3.4. Effect of drop residence time

Fig. 6 shows the effect of drop residence time during its flight in a spray column with respect to the number of passes for differ-

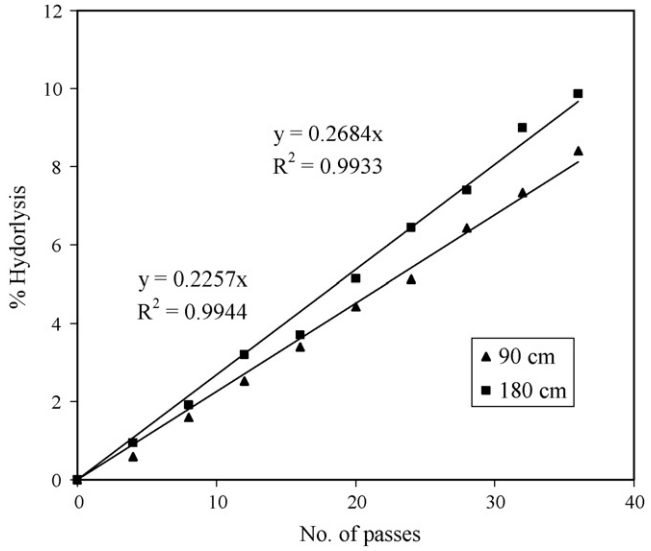

Fig. 6. Effect of number of passes on the percentage hydrolysis of oil for different height of enzyme solution (flow rate – 3.8 ml/min, nozzle diameter – 0.75 mm and enzyme solution concentration – 10 g/l).

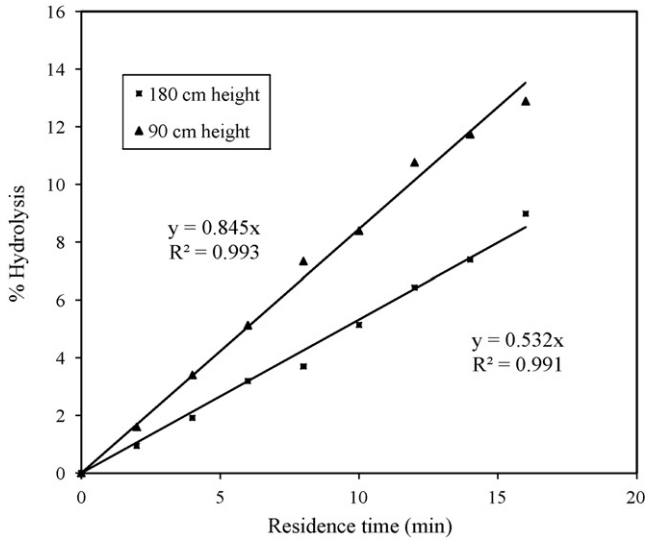

Fig. 7. Effect of drop residence time on % hydrolysis (combined) for two different height of enzyme solution.

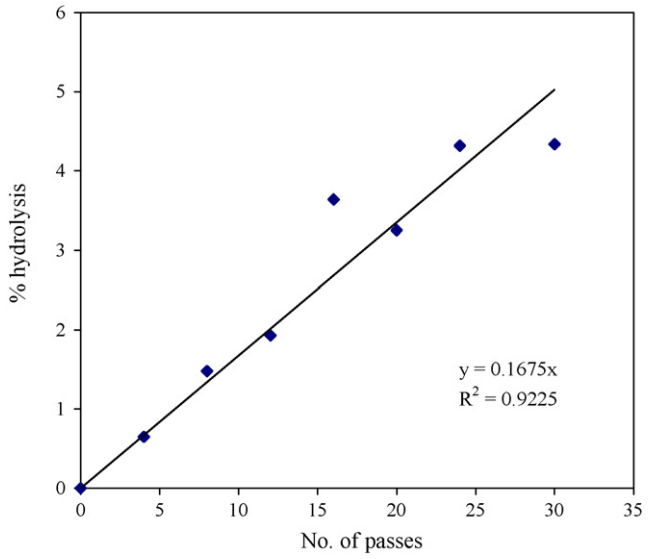

Fig. 8. Effect of number of passes on the percentage hydrolysis obtained due to coalescence only.

ent heights. As the height of the enzyme solution was increased from 900 to 1800 mm, the percent hydrolysis of oil did not increase proportionally as expected. It was expected that the percentage hydrolysis obtained for 1800 mm enzyme solution height would be double that of 900 mm enzyme solution height for equivalent mean drop residence time. The effect of enzyme solution height on the percentage hydrolysis of oil can be seen by plotting residence time of oil drop against the enzyme solution height (Fig. 7). Fig. 7 shows that the difference in the percentage hydrolysis of the oil increases linearly with an increase in the residence time. For 16 min of mean drop residence time, the percentage hydrolysis obtained was 12.5 and 8.99% for 900 and 1800 mm height of enzyme solution, respectively. This unexpected behavior is due to the contribution from the coalescence of the oil drops at the oil–enzyme solution interface at the top of the column disturbing and renewing the interface, which must be contributing to the overall hydrolysis. Due to the coalescence of the oil drops in the top oil layer, the interface gets disturbed, and possibly gets renewed, exposing it to the fresh enzyme solution entrained by the rising oil drop, thus prompting further hydrolysis. To get the same oil drop residence time in an enzyme solution height of 900 mm as against 1800 mm, the number of passes needs to be doubled. This also increases the contribution to hydrolysis from the coalescence phenomenon as the numbers of coalescence events are also doubled. The percentage hydrolysis obtained after 2 min of residence time for 1800 mm enzyme solution height was 0.95% while it was 1.6% for the same residence time (2 min) for 900 mm enzyme solution height. However, the numbers of passes for 2 min residence time for 1800 mm enzyme solution height were 4, and these were 8 for 900 mm enzyme solution height. Thus, the difference in the extent of hydrolysis for the same mean drop residence time was 0.65%. This difference gives the extent of hydrolysis due to the increased number of coalescence events of the oil drops (four additional passes and hence coalescence events). Fig. 8 gives the effect of number of passes on actual percentage hydrolysis of oil only due to the coalescence of drop with the top oil layer and the interfacial disturbance. The percentage hydrolysis due to coalescence increases at a constant rate with an increase in the number of passes. Similarly, the percentage hydrolysis obtained because of mean drop residence time of drop (drop flight time) in enzyme solution was estimated (Appendix) by subtracting the effect of coalescence events (per cycle or pass) from the total extent of hydrolysis obtained and the results can be seen in

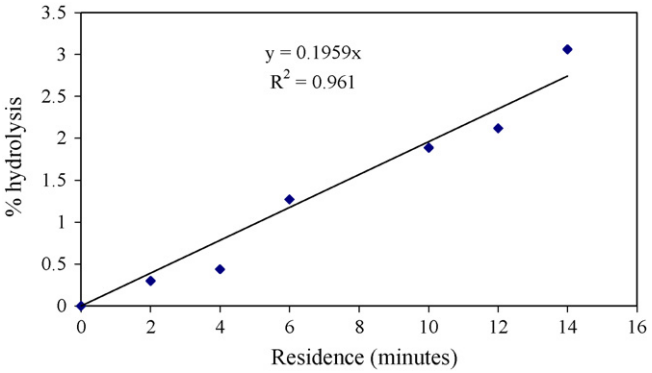

Fig. 9. Effect of residence time of drop on the percentage hydrolysis obtained by residence time of drop only.

Fig. 9. It is found that the extent of hydrolysis obtained only by the coalescence of oil drop with the top oil layer for four pass (i.e. four set of coalescence events) is two times higher than the contribution of the moving oil drop mean residence time (drop flight time). The total extent of hydrolysis obtained with an enzyme solution height of 1800 mm after four passes was 0.95% with the balance hydrolysis of 0.30% coming from the drop rising phenomenon (i.e. for residence time of 2 min) for four passes. This suggests that, just by increasing the liquid height (residence time) is not an efficient way to increase the hydrolysis of the oil in a spray column type of apparatus, but having intermediate sieve plates, where the drop coalesce and redispersion occurs may be a better choice. The energy efficiency of these two modes (flight/drop rise and coalescence) of reaction has been presented in Table 2. The detailed calculation can be seen in Appendix.

3.5. Comparison of spray column with agitated batch reactor

The results obtained from different batch experiments are compared with the spray column study and are shown in Fig. 10. The hydrolysis obtained in spray column by considering only 14 min of the mean oil drop residence time in the enzyme solution is only 3.06% as against 14.19% in the batch stirred reactor. In addition, the hydrolysis obtained after combining the effect of coalescence (25 coalesces event due to 25 passes) and drop rising phenomenon

Table 2
Comparison of energy dissipation for different modes and batch reactor.

Mode of hydrolysis	Drop flight	Drop coalesce at interface	Batch reactor
Energy dissipation rate (W)	3.96×10^{-7}	5.822×10^{-7}	0.188
% hydrolysis	0.3	0.1615	1.93
Time/coalesce	2 min	Singe coalesce	2 min
% hydrolysis/energy dissipated (J)	6.313×10^{3}	5.54×10^{5}	0.0855

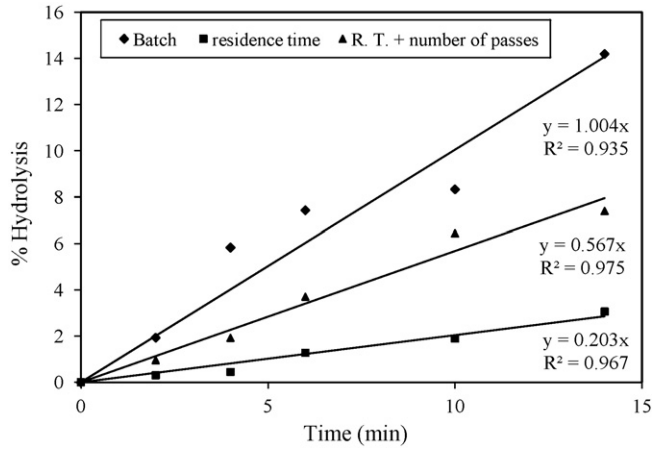

Fig. 10. Comparison of the batch experiments (oil: enzyme solution – 3:1, speed of impeller – 2500 rpm and enzyme concentration – 10 g/l) with spray column (flow rate – 3.8 ml/min, nozzle diameter – 0.75 mm and enzyme concentration – 10 g/l).

(14 min of residence time) is 50% when compared with batch hydrolysis for 14 min of reaction period. When oil drop coalesce, there is a release of energy due to decrease in the surface area and the dissipation of the kinetic energy due to the rising drop dissipating its momentum at the interface and interfacial energy released due to the reduction in the interfacial area. Thus, the energy dissipation rate during the coalescence of oil drop at the interface of enzyme solution and oil has been calculated using Eq. (4) (Appendix):

$$E_C = [n \times A \times \sigma] + \left[\frac{1}{2} \times n \times m \times v_\infty^2\right] \quad (4)$$

Also, the energy dissipation rate for the batch process was calculated by the following equation:

$$E_B = N_p d^5 N^3 \rho \quad (5)$$

Similarly, the energy dissipation rate during the drop fight time in spray column was calculated by the following equation:

$$E_R = \frac{\pi}{6} d_p^3 \Delta\rho v_\infty \quad (6)$$

It has been found that the energy dissipation rate during the coalescence of the oil drops at the interface, during drop flight for one pass, and in the batch reactor was 5.822×10^{-7}, 6.8×10^{-5} and 0.188 J/s respectively (Table 2). Thus, the energy dissipation rate is very high in a batch reactor as compared to energy dissipation rate at the interface in a spray column reactor. Similarly, the percentage hydrolysis obtained for a single set of coalescence events (for a drop flight time of 2 min and for a 2-min batch reaction) was also calculated. Finally, the extent of hydrolysis per unit energy dissipated was estimated for a single drop, drop flight time, and for batch reactor. The extent of hydrolysis obtained per unit energy dissipated due to drop flight time and coalescence phenomenon was found to be 3.6×10^4 and 6.4×10^5 times higher as compared to that for a batch reactor (solution). On the other hand, the extent of hydrolysis per unit energy dissipated due to coalescence phenomenon is 175 times higher than that for the drop flight time. In a batch reactor, the energy used in agitation is also used for the agitation of the bulk continuous phase. This energy is not fully utilized; only a fraction of it is utilized in renewing the oil–enzyme solution interface. This indicates that the energy dissipation due to the coalescence phenomenon in a spray column is much more effective in promoting the hydrolysis as compared to the stirred reactor. A very small fraction (<1%) of the total energy supplied by the agitator is utilized in generating the interfacial area and for an increase in the interfacial mass transfer as compared to the spray column with coalescence at the top. Coalescence and redispersion phenomena occurring at the interface, and the energy dissipation due to the same appears to be much more effective for interfacial transport and interfacial reaction phenomenon.

3.6. Comparison of free and immobilized enzyme

Fig. 11 gives the comparison between the percentage hydrolysis obtained for different number of passes in spray column for free enzyme and conjugated enzyme. It was observed that the percentage hydrolysis increases linearly with an increase in the number of passes for conjugated enzyme. However, the rate of reaction for the free enzyme solution was marginally higher than conjugated enzyme solution. It was found that the percentage hydrolysis obtained after 44 passes which equals 22 min of residence time in a spray column for free enzyme was 12% more than obtained for immobilized enzyme solution. This difference in the extent of hydrolysis is attributed to the variation in the activity of the two forms of the enzyme. The activity of the conjugated enzyme and free enzyme solution used for the reaction was 10.5 and 10.8 units/ml (at 10 g/l concentration), respectively. It was also observed that the activity of the enzyme solution after 44 passes remains unchanged. This clearly shows that there was no deactivation of enzyme in the spray column operation. Although conjugated enzyme gives lower conversion as compared to the free enzyme solution, the conjugated enzyme can be continuously used in the spray column for

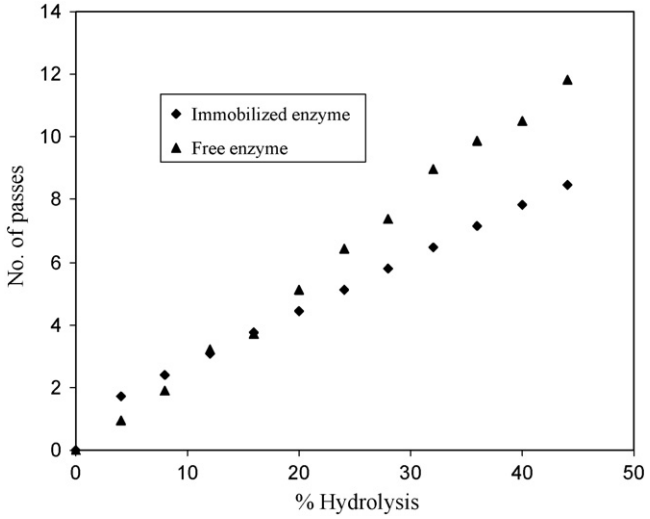

Fig. 11. Comparison of free enzyme with conjugated enzyme.

a continuous process as it can be separated, recovered, and recycled. Also, it has been proved that the conjugated enzyme used in the present work can be successively reused up to 10 times in a batch reactor for a reaction time of 1 h each time, indicating more than 10 h of stability, which is a main requirement in an industry [7].

4. Conclusions

Various operating parameters optimized in a spray column for enzymatic hydrolysis of castor oil illustrated that a higher yield can be obtained at optimum conditions of flow rate and nozzle diameter, without encountering the deactivation of the enzyme. The study revealed that the coalescence of the drops at the top of the spray column plays an important role in the oil hydrolysis. More the number of passes more will be the coalescence events at the column top and more will be the extent of hydrolysis. A sieve plate type of column will be more useful to carry out enzyme catalyzed reactions. A correlation to predict the rate and the extent (combined) of hydrolysis has been developed as a function of the operational parameters (interfacial area and the enzyme solution concentration). This correlation was found to be in good agreement with the experimental results obtained. The data reported in the present work can be used to design a spray column for an enzymatic reaction. The required oil drop mean residence time, number of coalescence event (number of partitioning sieve plates) can be designed/accommodated into a spray column design to achieve a desired degree of hydrolysis.

Appendix A.

A.1. Calculation of percentage hydrolysis due drop coalesce and its flight time in spray column

Total hydrolysis obtained for 2 min of oil drop residence time.

	% hydrolysis	Number of passes
a. For 1800 mm enzyme solution height	0.95	4
b. For 900 mm enzyme solution height	1.6	8

Hydrolysis obtained due to the coalescence phenomenon for four passes = (1.6 − 0.95) = 0.65%.

Similarly, hydrolysis obtained due to 2 min residence time of the oil drop only = (0.95 − 0.65) = 0.3%.

Thus, hydrolysis due to coalescence phenomenon and residence time was calculated separately.

A.2. Calculation of energy dissipation

A.2.1. Determination of energy dissipation rate due to coalesce of the oil drop at the interface (E_C)

$$E_C = [n \times A \times \sigma] + [(1/2) \times n \times m \times v_\infty^2]$$

$$\text{Number of coalesce events}\,(n) = \frac{\text{no. of drops}}{\text{unit time}} = \frac{10}{5} = 2$$

$$\text{Diameter of oil drop}\,(d_P) = 3.92 \times 10^{-3}\ \text{m}$$

$$\text{Disappearance of the area} = \frac{\pi d_P^2}{4} = 4.82 \times 10^{-5}\ \text{m}^2$$

$$\text{Volume of single drop}\,(V) = \frac{\pi d_p^3}{6} = \frac{3.14 \times (3.92 \times 10^{-2})^3}{6} = 31.53 \times 10^{-9}\ \text{m}^3$$

$$\text{Mass of the single drop}\,(m) = V \times \rho = 31.53 \times 10^{-9} \times 815 = 2.56 \times 10^{-5}\ \text{kg}$$

Thus, energy dissipation rate at the interface (E) is given by

$$E_C = [n \times A \times \sigma] + \left[\frac{1}{2} \times n \times m \times u^2\right]$$

$$E_C = [2 \times 4.82 \times 10^{-5} \times 4.8 \times 10^{-3}] + \left[\frac{1}{2} \times 2 \times 0.256 \times 10^{-4} \times 0.052^2\right]$$

$$E_C = 5.822x10^{-7}\ \text{J/s}$$

Now, hydrolysis obtained due to drop flight time of 2 min = 0.3%.

$$\%\ \text{hydrolysis/energy dissipate} = \frac{0.1615 \times 2}{5.822 \times 10^{-7} \times 120} = 5.54 \times 10^5\%\ \text{hydrolysis/J}.$$

A.2.2. Energy dissipation due to the drop flight time in column (E_R)

$$E_R = \frac{\pi}{6} d_p^3 \Delta\rho v_\infty$$

$$E_R = \frac{\pi \times (3.92 \times 10^{-3})^3 \times (1000 - 815) \times (6.8 \times 10^{-3})}{6}$$

$$E_R = 3.96 \times 10^{-7}\ \text{J/s}$$

Therefore, % hydrolysis per unit energy dissipated

$$= 0.3/(5.822 \times 10^{-7} \times 120) = 6.13 \times 10^3 \infty\ \%\ \text{hydrolysis/J}$$

A.3. Energy dissipation in batch reactor (E_B)

$$E_B = N_p d^5 N^3 \rho$$

$$E_B = 1 \times \left(\frac{2500}{60}\right)^3 \left(\frac{2}{100}\right)^5 \times 815$$

$$E_B = 0.188\ \text{J/s}$$

Percentage hydrolysis per unit energy dissipated

$$= \frac{1.93}{0.188 \times 120} = 0.0855\%\ \text{hydrolysis/J}.$$

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