Kinetic Modeling of Microwave-Assisted Chemoenzymatic Epoxidation of Styrene

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Novozym 435 (Candida antarctica lipase B immobilized on polyacrylic resin) is used to generate perlauric acid directly from lauric acid and hydrogen peroxide. This generated perlauric acid was then applied for both in situ and ex situ epoxidation of styrene to styrene oxide. The reactions were carried out by using conventional heating as well as microwave heating. Various kinetic parameters affecting the conversion and initial rates of styrene to styrene oxide were studied including mass transfer, mechanism, kinetic modeling, and deactivation. Under microwave irradiation, there was an increase in the frequency factor resulting from enhanced collision of molecules, which can in turn be attributed to the increasing entropy of the system. The values of activation energy for the conventional and microwave heating are almost the same. The enzyme is deactivated by hydrogen peroxide and perlauric acid and also at high temperature under conventional heating. In the presence of microwaves, the deactivation of Novozym 435 was substantially reduced. © 2005 American Institute of Chemical Engineers *AIChE J,* 52: 1235–1247, 2006

Keywords: epoxidation, microwave irradiation, kinetic modeling, lipase deactivation, styrene oxide

Introduction

Lipases are the most widely used enzymes for synthesis of organic chemicals, mainly in aqueous media and in some cases nonaqueous media, because they are inexpensive, stable, and easy to recycle.[1,2] Lipases possess wide substrate specificity, have an ability to recognize chirality, and do not require labile cofactors.[3-6] Of late, lipases have been used to catalyze a number of reactions in nonaqueous media such as esterification, transesterification, amidation, hydrolysis, thioesterification, and transthioesterification.[5,7-19] The versatility of lipase catalysis in the synthesis of other groups of chemicals needs to be explored. For instance, there is no report on chemoenzymatic preparation of styrene oxide by lipase catalysis, including effect of microwave irradiation, kinetic modeling, and deactivation. Styrene oxide is an important and efficient intermediate in organic process industries, fragrance chemicals, and perfumery ingredients, to cite but a few.[20] The current work was therefore undertaken on synthesis of styrene oxide by a chemoenzymatic route using a suitable oxidant and enzyme.

Organic peroxy acids are widely used as oxidizing agents in organic synthesis because of their versatility, specificity, and excellent selectivity of desired product in a short time under mild conditions without the formation of by-products. Peroxy acids are used in many oxidation reactions such as converting a double bond to epoxide,[20] sulfides to sulfoxides and sulfones, tertiary amines to amine oxides, and ketones to esters or lactones.[21] The active oxygen in hydrogen peroxide is not readily available for most organic oxidation reactions, but it can be easily converted to the useful peroxy acid by using various chemical catalysts and enzymes.[16,22] This in situ–generated peroxy acid is continuously consumed in the presence of an oxidizable substance as follows:

$$RCO_2H + H_2O_2 \xrightarrow{H^+} RCO_3H + H_2O \quad (1)$$

$$RCO_3H + \text{oxidizable substance} \rightarrow RCO_2H + \text{oxidized product} \quad (2)$$

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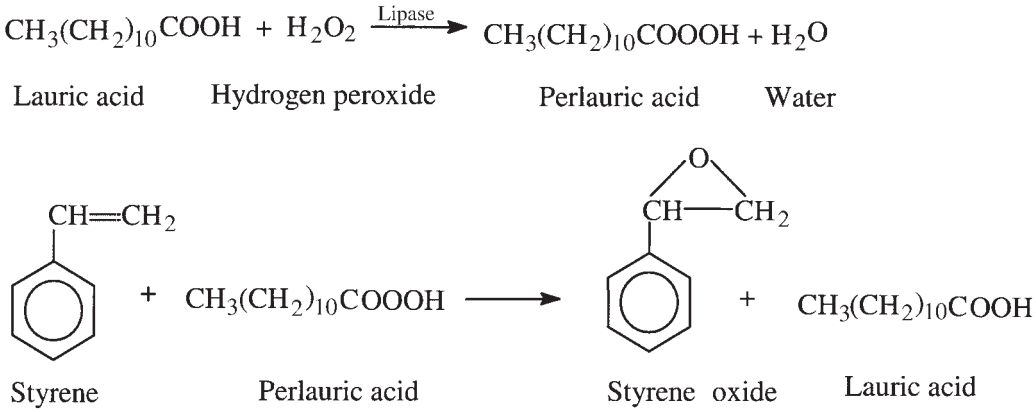


Scheme 1. Synthesis of styrene oxide by lipase-catalyzed formation of perlauric acid.

The usage rate of peroxy acid should be faster than the rate of peroxy acid formation; otherwise, peroxy acid will accumulate. In the current work, in situ–generated perlauric acid by an enzymatic route[16] was used as an oxidizing source for epoxidation of styrene oxide.

This work focuses on chemoenzymatic epoxidation of styrene oxide, in the presence of immobilized lipases, including kinetic modeling (Scheme 1). The first step is the generation of perlauric acid from lauric acid and hydrogen peroxide in the presence of *Candida antarctica* lipase B. This generated perlauric acid is allowed to react simultaneously with styrene to give styrene oxide. Because the enzyme was reported to deactivate by hydrogen peroxide,[16] special emphasis is made to increase stability and activity of the enzyme by using microwave irradiation. To date no literature reports are available on the epoxidation of styrene by lipases, including the stability of enzyme in the presence of microwave irradiation. Because lipase-catalyzed reactions are rather sluggish in nature, the synergism with microwave can be expected to enhance the rates of reactions. Such an exploration was previously conducted to witness rate enhancement in microwave-irradiated lipase-catalyzed reactions.[14,23-29] Thus, it was deemed worthwhile to study the preparation of styrene oxide, under synergism between microwave and enzyme catalysis including kinetic modeling.

Experimental Setup and Methods

Enzyme and chemicals

Novozym 435 (*C. antarctica* lipase B immobilized on macroporous polyacrylate resin beads) had the following characteristics: bead size, 0.3–0.9 mm; bulk density, 430 kg/m^3; water content, approximately 0.66% (w/w); activity, 7000 PLU/g (PLU is the ester synthesis activity expressed in "propyl laurate units"), and was received as a gift from Novo Nordisk (Copenhagen, Denmark). Lauric acid (LR grade) was obtained from Loba Chemie (Mumbai, India). Toluene, ceric sulfate, potassium iodide, sodium thiosulfate, 30 and 50% (w/v) aqueous H_2O_2, and sulfuric acid were all of AR grade and received from S. D. Fine Chemicals Ltd. (Mumbai, India).

Conventional heating

The experimental setup consisted of a fully baffled, mechanically agitated glass reactor (capacity, 50 cm^3; ID, 3 cm), equipped with four baffles and a six-bladed pitched-turbine impeller. The entire reactor assembly was immersed in a thermostatic water bath maintained at the desired temperature with an accuracy of ± 1°C.

In ex situ epoxidation, first the enzymatic step was carried out to form perlauric acid completely, after which styrene was added to the system and the reaction was monitored. In the in situ method all reagents and enzyme were added and the reaction was monitored. The in situ–generated perlauric acid, consumed in the reaction mass utilization of perlauric acid, was referred to as in situ epoxidation of styrene.

In a typical experiment, the reaction mixture contained 0.015 mol lauric acid, 0.01 mol styrene, 0.033 mol hydrogen peroxide (30% w/v aqueous solution), and 0.4 g *n*-decane as internal standard and the mixture was diluted to 25 mL with toluene as a solvent. The reaction mixture was agitated at 55°C for 15 min at a speed of 600 rpm and a known quantity of the enzyme (0.6% w/w) was then added to initiate the reaction.

Microwave reactor

The studies were carried out in a microwave reactor (Discover, model CEM-SP1245). The reactor was a fully baffled, cylindrical glass vessel (capacity, 120 mL; ID, 4.5 cm) with provision for mechanical stirring. A standard four-blade pitched turbine impeller (diameter, 1.5 cm) was used for agitation. However, the actual reactor volume exposed to the microwave irradiation was 45 mL with 5.5 cm height. There was no bubble formation. Temperature in the reactor was computer controlled.

The quantities of reactant and enzyme for reaction procedure were identical to those used for conventional heating.

Analytical methods

Aliquots (0.5 mL) of the organic phase from the reaction mixture were withdrawn periodically and analyzed, first for total active oxygen content by iodometry followed by cerimetric titration to determine the H_2O_2 content.[21,30]

Concentrations of styrene and styrene oxide were determined on a Chemito Gas chromatograph (Model 8510) equipped with a flame ionization detector. A 2 m $\times$ 3.8 mm SE-30 stainless steel column was used for analysis. The temperature of the column was 110°C for 6 min and then it was increased up to 270°C at a ramp rate of 20°C/min. The carrier

gas used was nitrogen at a flow rate of 1 mL/min. The injection and detection temperatures were set to 270 and 280°C, respectively. Synthetic mixtures of reaction were prepared from pure authentic components and calibration was done to quantify the collected data for conversions and rates of reactions. Styrene oxide was confirmed by gas chromatography–mass spectrometry (GC-MS).

Determination of initial rates

To determine the initial rates of enzymatic reaction, the concentrations of lauric acid were varied from 0.2 to 0.8 M for known concentrations of hydrogen peroxide (between 0.32 and 1.2 M). The total volume was made to 25 mL with toluene, after which 200 mg Novozym 435 was added to initiate the reaction and the reaction was continued until 30% conversion at 55°C. Similarly, initial rates based on concentrations of styrene were calculated in the range of 0.2–1.2 M.

Experimental Results

The purpose of doing the in situ and ex situ experiments was to analyze, optimize, and study kinetic modeling of the system.

Speed of agitation

The effect of speed of agitation was studied both for in situ and ex situ (preformed perlauric acid) epoxidation over the range of 100–800 rpm under conventional heating. The reaction rates and conversions in both cases were nearly independent of the speed (data not shown). At a speed beyond 600 rpm for in situ epoxidation reaction, there was a marginal decrease in conversion arising from attrition of particles, some of which were thrown out of the reaction mixture, sticking to the wall and also partly by shearing of enzymes. This type of behavior was also observed for Novozym 435 in other reactions.[16-19,31] Thus the optimum speed was taken as 600 rpm for further studies.

Evaluation of the contribution of external solid–liquid mass transfer resistance and intraparticle diffusion limitation was done by theoretical calculations. The liquid-phase diffusivity of hydrogen peroxide in toluene ($D_{S:B}$) at 55°C was calculated by using the Shiebel equation[32] as 2.26×10^{-9} m^2 s^{-1}. The value of solid–liquid mass transfer coefficient k_{SL} was calculated by assuming a limiting value of the Sherwood number, $Sh = k_{SL}d_p/D_S = 2$, for nonagitated systems. It should be noted that the actual Sherwood number, which is a function of Reynolds number and Schmidt number, would be much higher in well-agitated systems. However, for the sake of comparison and for orders-of-magnitude calculation, it is safe to take the lowest Sherwood number. Thus, $k_{SL:B}$ was calculated as 8.77×10^{-6} m s^{-1} for a particle size (d_p) of Novozym 435 of 0.06 cm. Similarly $D_{S:A}$ and $D_{S:C}$ were calculated as 1.15×10^{-9} and 1.5342×10^{-9} m^2 s^{-1}, respectively, for lauric acid (A) and styrene (C). The values of liquid side mass transfer coefficients, $k_{SL:B}$, $k_{SL:A}$, and $k_{SL:C}$, were obtained as 8.77×10^{-6}, 3.88×10^{-6}, and 1.34×10^{-6} m s^{-1}, respectively.

For solid (catalyst)–liquid reactions, the contribution of external mass transfer resistance was calculated in comparison with the reaction within the particle as given by Yadav and Krishnan.[31] By using these values, external mass resistance (R_D) and internal reaction resistance (R_r) for hydrogen peroxide were evaluated as $R_r = 378.5$ and $R_D = 36.67$, indicating $R_r > R_D$. Because $R_r > R_D$ there was no external mass transfer limitation. It was also verified by applying criteria for external mass transfer, as given by Bailey and Ollis.[33] [($\eta_B = 0.72$, $\eta_A = 0.058$, $\phi_B = 1.125$, $\phi_A = 17$, $Bi_B = 388$, $Bi_A = 164$), $(\eta\phi^2/Bi)_B = 0.0028 \ll 1$, $(\eta\phi^2/Bi)_A = 0.1 \ll 1$, which indicates no external mass transfer controlling.]

However, to rule out any intraparticle diffusion resistance, the Wiesz–Prater criterion[34] or modulus (CWP) was used in which CWP is less than one ($CWP = 0.082 \ll 1$), suggesting that there was no intraparticle diffusional resistance.

Thus, it was concluded that the rate of in situ and ex situ epoxidation of styrene was solely controlled by the intrinsic kinetics.

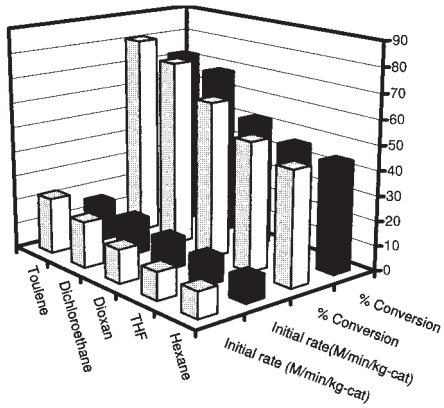


Figure 1. Solvent effect: irradiation time = 150 min at an imposed temperature to 55°C.

□ Microwave; ■ conventional.

Effect of different solvents

Microwave effects have to be considered according to reaction medium. Solvent effects are especially of great importance. If polar solvents are involved, either aprotic [such as dimethylformamide (DMF)] or protic (such as alcohols), the main absorption occurs between microwaves and the polar molecules of the solvent. In this case, energy transfer is from solvent to reaction mixtures and reactants. Consequently, the results should be nearly the same under classic heating.

More interesting could be the use of nonpolar solvents (such as xylene, toluene, hydrocarbons) because there is only a very weak solvent–microwave interaction (they can be viewed as transparent), thus allowing specific absorption by reactants. Energy transfer can occur from the reactants to the solvent, and the results could be different under microwave and conventional heating. It has thus been shown that the magnitude of specific microwave effects in solution is decreased when the polarity of solvent is increased, as advocated in some Diels–Alder reactions.[35]

Fewer microwave effects were observed in polar solvents (pentanol or DMF), whereas in nonpolar solvents (toluene) the conversion and initial rate are noticeably affected by the activation mode (Figure 1). A polar solvent can therefore be advantageously replaced by a nonpolar one, providing the use of microwave irradiation. The effect of solvent on the reaction rate is generally described by its log P value and it was found

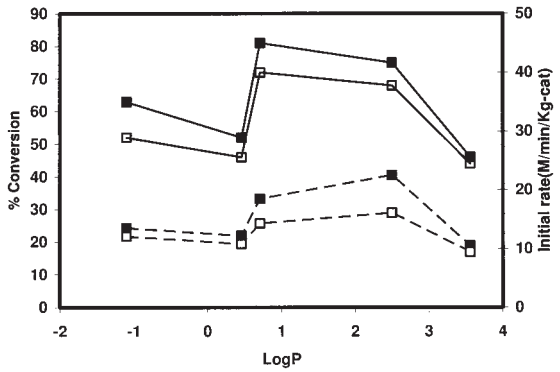


Figure 2. Effect of solvent log *P* on initial rate and final conversion of styrene.

Solid lines indicate microwave heating and dotted lines indicate conventional heating. ■ Microwave heating; □ conventional heating.

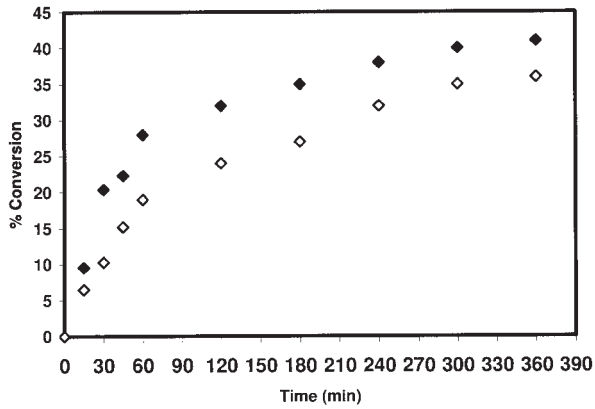


Figure 3. Effect of microwave irradiation on perlauric acid generation.

(◆) Microwave; (◇) conventional.

that at very low and high log *P* values there was less effect of microwave on the initial rate (Figure 2), which is attributed to the low solubility of reactant(s) and hydrophobic interaction of solvent with enzyme.

Effect of enzyme loading

The effect of enzyme concentration on the synthesis of styrene oxide was studied in conventional heating. As the concentration of the Novozym 435 increased, the amount of styrene oxide formed also increased. The highest conversion of styrene was observed after 3 h, when all hydrogen peroxide was added in the reaction mixture. For different concentrations of enzyme, the initial rate and final concentration were calculated. It was found that initial rate of reaction was sensitive to enzyme concentration as shown by the following power-law model equation:

$$\text{Initial rate} = 0.0016 \times [\text{E}]^{0.51} \tag{3}$$

Effect of temperature

Effect of Microwave Irradiation on Perlauric Acid Generation. Enzymatic perhydrolysis was performed in the presence of microwave (Figure 3). It was found that both the overall conversion and the rate of reaction were higher under microwave irradiation than under conventional heating, indicating that the effect may not be purely thermal. Controlled experiments in the absence of Novozym 435 did not show any conversion. Only microwave irradiation without the enzyme also did not initiate the reaction. Thus, there is a definite synergism between enzyme catalysis and microwave irradiation. Further, the rate enhancement arises from a combined effect of the microwave absorption properties of some liquid and solid materials, resulting from their polar and ionic characteristics, as suggested in "dipolar polarization mechanism," [36] and also the enzyme seems to behave slightly differently when heated with microwaves and becomes energized. The enzyme is in the immobilized form and there could be better accessibility of the enzyme to the reacting molecules. Altogether this significantly enhances the rate of chemical reactions when compared to traditional energy application (conventional heating) techniques.

Effect of Microwave Irradiation on Ex Situ (Preformed Perlauric Acid) Epoxidation of Styrene. Epoxidation of styrene with preformed perlauric acid was studied in which first perlauric acid was allowed to form by enzymatic step for 3 h, then the organic phase containing perlauric acid was separated and allowed to react with styrene. The effect of microwave was significant in enhancing the rates and overall conversions. The detailed analysis of microwave irradiation on the reaction rate constant is discussed later. Observation of this drastic change was explained by molecular heating.

Effect of Microwave Irradiation on In Situ Epoxidation of Styrene. The effect of temperature (conventional and microwave) was studied in the range of 40–60°C (Figure 4). Although the initial rate had increased with increasing temperature, the conversion was found to decrease beyond 60°C. At higher temperatures, the denaturation process was still faster in

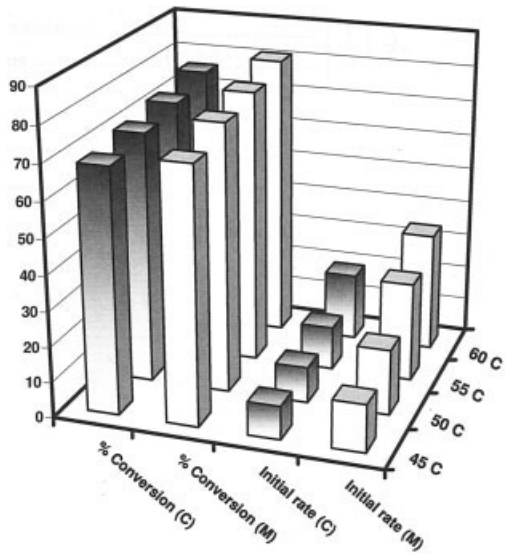


Figure 4. Temperature effect: irradiation time = 150 min imposed at different temperatures.

(□) Microwave; (■) conventional.

Table 1. Effect of Perlauric Acid on Novozym 435 Activity

Type of Heating	Fresh (% Conversion after 3 h)	First Reuse (% Conversion after 3 h)
In situ microwave	78	42
Ex situ microwave	73	30
In situ conventional	68	23
Ex situ conventional	63	18

conventional heating than that under microwave irradiation. In general, the activation energy (E_a) for enzyme catalysis falls within the range of 5–15 kcal/mol, whereas E_a for inactivation is generally much higher (50–150 kcal/mol). At low temperatures, the rate of denaturation is quite slow compared to the catalytic rate. Because E_a(denaturation) $>$ E_a(catalysis), the rate of denaturation increases more rapidly than the rate of catalysis as the temperature is increased.[18]

It was found that both the overall conversion and the rate of reaction were higher under microwave irradiation than under conventional heating.

Effect of concentration of substrates

Effect of Pregenerated Perlauric Acid on Enzyme Activity. Reusability of Novozym 435 in the case of ex situ epoxidation was found to be more than that in in situ epoxidation because of the continuous consumption of in situ–generated perlauric acid (Table 1). The above analysis indicates that perlauric acid plays a role in deactivation of the enzyme. The detailed mechanism and kinetics of deactivation will be discussed later.

Effect of Styrene Concentration on In Situ Epoxidation of Styrene. The effect of styrene concentration on in situ epoxidation was studied for fixed concentrations of other reactants. Styrene reacts with in situ–generated perlauric acid by a second-order kinetics (with respect to styrene and perlauric acid). The rate of utilization of styrene was sensitive to both styrene and perlauric acid concentrations. Therefore, it was found that both the initial rate and the final conversion had increased with increasing concentration of styrene (Figure 5).

Effect of Lauric Acid Concentration on In Situ Epoxidation of Styrene. When high concentrations of lauric acid were used ($>$0.5 M), there was no change in the conversion of styrene to styrene oxide (Figure 6). This probably arose from an inhibitory effect of the lauric acid concentration on the catalytic action of the enzyme. Higher formation of styrene oxide was observed when lower concentrations of lauric acid were used ($<$0.5 M).

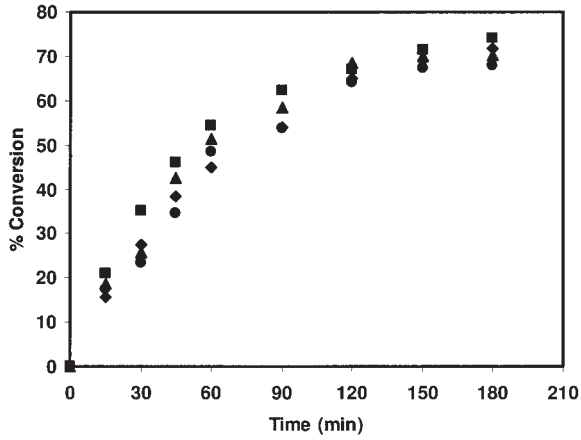


Figure 6. Effect of lauric acid concentration on in situ epoxidation of styrene.

(♦) 0.16 M; (■) 0.33 M; (▲) 0.5 M; (●) 0.66 M.

Effect of H_2O_2 Concentration on In Situ Epoxidation of Styrene. The concentration of hydrogen peroxide was found to be an important parameter in epoxide synthesis. A high conversion of styrene oxide was achieved when 1 M of H_2O_2 was used (Figure 7). The initial rate of reaction was affected by the concentration of H_2O_2 but beyond 1 M concentration, the initial rate was nearly constant as a result of the deactivation of the enzyme caused by high concentrations of H_2O_2.[16]

Effect of H_2O_2% (w/v) on In Situ Epoxidation of Styrene. The mole ratios of the reactants were kept constant, while changing the percentage of hydrogen peroxide. Changing the percentage was equivalent to adding water to the system. When the percentage of hydrogen peroxide was decreased from 50 to 30%, the reaction rate decreased (Figure 8). In a biphasic system, once the enzyme is well hydrated, it can display its maximum activity whatever the water concentration. The re-

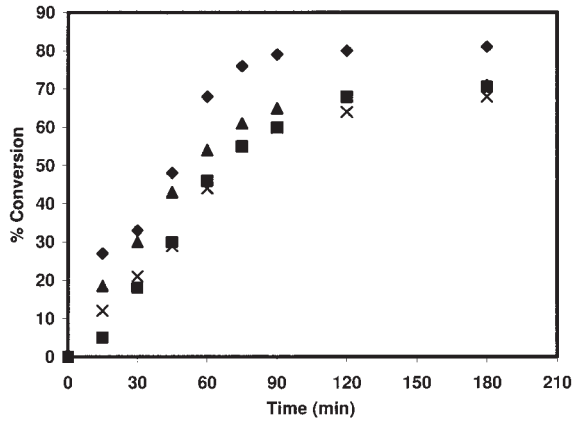


Figure 5. Effect of styrene concentration on in situ epoxidation of styrene.

(♦) 1 M; (■) 0.66 M; (▲) 0.5 M; (×) 0.33 M.

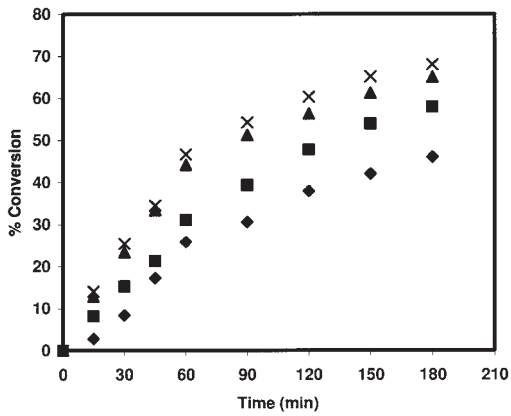


Figure 7. Effect of H_2O_2 concentration on in situ epoxidation of styrene.

(♦) 0.33 M; (■) 0.66 M; (▲) 1 M; (×) 1.33 M.

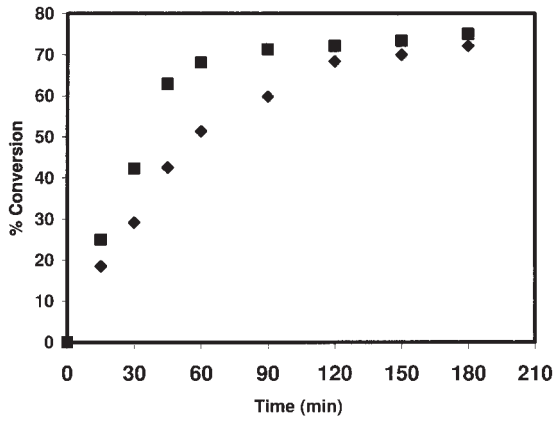


Figure 8. Effect of H_2O_2% (w/v) on in situ epoxidation of styrene.

(◆) 30% w/v H_2O_2 M; (■) 50% w/v H_2O_2 M.

action produces water as the coproduct, which separates out as a distinct phase and thus equilibrium is established. In all experiments, hydrogen peroxide was taken in excess, with a large excess of organic phase, which causes the hydrogen peroxide to be partitioned in the organic phase. In fact, it is possible to analyze it mathematically because

$$[H_2O_2]_{org} = K_{H_2O_2}[H_2O_2]_{aq}$$

$$nH_2O_{2\text{-org}}/V_{org} = K_{H_2O_2}nH_2O_{2\text{-aq}}/V_{aq}$$

and therefore

$$nH_2O_{2\text{-org}} = K_{H_2O_2}nH_2O_{2\text{-aq}}V_{org}/V_{aq}$$

In the present case, the initial volume ratio of the two phases $V_{org}/V_{aq} = 29.5/4.5 = 6.55$ and, even if the partition coefficient $K_{H_2O_2}$ is 0.1, there will be almost 0.022 mol of hydrogen peroxide in the organic phase to 0.011 mol in the aqueous phase, that is, about 66% in the organic phase. In the standard experiments, 0.033 mol of hydrogen peroxide was used per 0.015 mol of lauric acid.

The literature on solubility of hydrogen peroxide in water shows it is soluble in all proportions and is soluble in many polar solvents (such as low molecular alcohols, glycols, and ketone) and insoluble in benzene. The log *P* (octanol) value is −1.36, which would mean that the partition coefficient between octanol and water is 0.043 at 20°C. In our system, toluene was used as a solvent with styrene as the substrate, which forms a nonpolar system. Thus, our conservative estimate of partition coefficient of hydrogen peroxide at 55°C is perfectly justified.[37-40] Thus, hydrogen peroxide was always available in the organic phase. Because the amount of water was greater in the case of dilute hydrogen peroxide solution, it resulted in a decrease in the concentration of hydrogen peroxide in the vicinity of the enzyme, which in turn resulted in a decrease in the rate.

Effect of Rate of Addition of H_2O_2 on In Situ Epoxidation of Styrene. One of the most important parameters affecting the initial rate and conversion of styrene to styrene oxide is the rate of hydrogen peroxide addition. The highest conversions of styrene were obtained when the addition rate of hydrogen peroxide was 30 mmol/h up to 1 M final concentration of H_2O_2 (Figure 9). The initial rate of reaction was also affected by the rate of H_2O_2 addition but, beyond the addition rate of 20 mmol/h, the initial rate of reaction was nearly constant, which was attributed to the deactivation of enzyme by the high concentration of H_2O_2.[16]

Reusability of enzyme

The activity of lipase in the case of microwave irradiation decreased by almost 50% after first reuse and then it was gradually decreased during further reuses. Lipases are known to be deactivated by hydrogen peroxide. However, it was possible that lauric acid, styrene, styrene oxide, and toluene might also have caused denaturation. The detailed analysis with respect to deactivation arising from various factors in conventional and microwave heating is discussed in the section on kinetic modeling. The rate of deactivation in conventional heating was greater than that in microwave heating. The initial rates after each reuse were calculated and the rate is given by the following equations:

- In the presence of microwave irradiation

$$\text{Initial rate} = 0.0029e^{-0.4581(\text{Reuse})} \quad (4)$$

- In the presence of conventional heating

$$\text{Initial rate} = 0.0023e^{-1.0459(\text{Reuse})} \quad (5)$$

This would suggest that there is no change in mechanism arising from the mode of heating, although the rate constant is augmented in microwave heating because of the greater degree of collision frequency of reaction molecules.

Kinetic modeling

Kinetic Model Based on Initial Rate for Enzymatic Step. The initial rate measurements demonstrated that the rate increased with increasing quantity of lauric acid. The Lineweaver–Burk plot (Figure 10) demonstrates that the slope increases and the intercept decreases with increasing concentration of hydrogen peroxide, although the reverse is the case when the concentration of hydrogen peroxide is low, which implies that

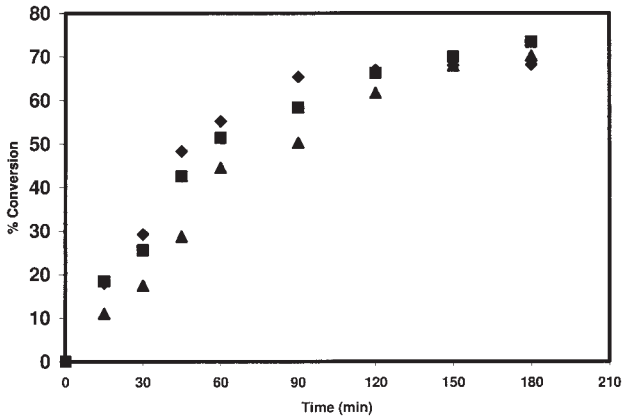


Figure 9. Effect of rate of addition of H_2O_2 on in situ epoxidation of styrene.

(◆) 30 mmol/h H_2O_2; (■) 20 mmol/h H_2O_2; (▲) 15 mmol/h H_2O_2.

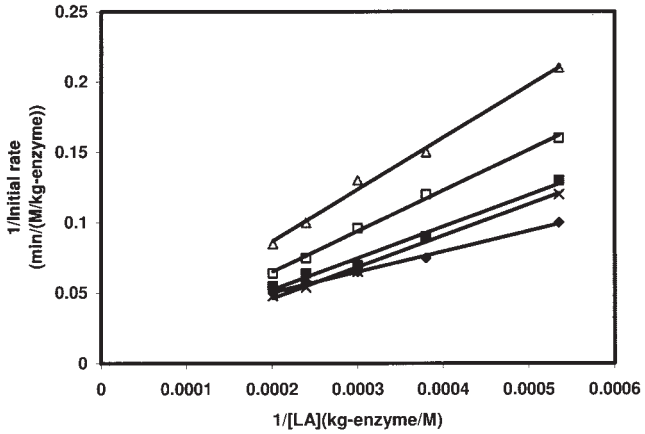


Figure 10. Initial rate^{-1} vs. lauric acid^{-1}.

Different quantities of lauric acid at various constant quantities of hydrogen peroxide. □ 3149.074 kmol H_2O_2/m^3/kg-catalyst, (△) 3811.518 kmol H_2O_2/m^3/kg-catalyst, (■) 4900.518 kmol H_2O_2/m^3/kg-catalyst, (×) 5989.518 kmol H_2O_2/m^3/kg-catalyst, (◆) 70789.518 kmol H_2O_2/m^3/kg-catalyst.

hydrogen peroxide acts as an inhibitor. Based together on the progress curve analysis, reusability studies, and deactivation studies, it is confirmed that hydrogen peroxide causes irreversible deactivation of the enzyme. However, for initial rate analysis it is assumed that hydrogen peroxide acts only as a dead-end inhibitor and the inhibition step is shown to be reversible. At lower quantities of lauric acid, the rate decreases with increasing quantity of hydrogen peroxide and at higher quantities of lauric acid, the rate increases with increasing quantities of hydrogen peroxide, in agreement with the observations made when progress curve analysis was performed for the same reaction. From the Lineweaver–Burk plot, it was observed that there were no parallel lines, ruling out the ping-pong bi–bi mechanism.[41] In fact, the lines were intersecting at a point suggesting a ternary complex mechanism. The formation of ternary complex can be depicted as follows:

$$\mathrm{A} + \mathrm{E} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \mathrm{AE} \tag{6}$$

$$\mathrm{AE} + \mathrm{B} \underset{k_4}{\overset{k_3}{\rightleftharpoons}} \mathrm{ABE} \tag{7}$$

$$\mathrm{ABE} \underset{k_6}{\overset{k_5}{\rightleftharpoons}} \mathrm{P} + \mathrm{EQ} \tag{8}$$

$$\mathrm{EQ} \underset{k_7}{\overset{k_8}{\rightleftharpoons}} \mathrm{E} + \mathrm{Q} \tag{9}$$

$$\mathrm{E} + \mathrm{B} \underset{k_{10}}{\overset{k_9}{\rightleftharpoons}} \mathrm{EB} \tag{10}$$

The rate equation obtained with the above mechanism is

$$V = \frac{V_{\max}[\mathrm{A}][\mathrm{B}]}{K_{\mathrm{iA}}K_{\mathrm{mB}}(1 + [\mathrm{B}]/K_i^1) + K_{\mathrm{mB}}[\mathrm{A}] + K_{\mathrm{mA}}[\mathrm{B}](1 + [\mathrm{B}]/K_i^1) + [\mathrm{A}][\mathrm{B}]} \tag{11}$$

To verify the application of the ternary complex mechanism, the same data were analyzed by ENCORA,[42] which uses the Nelder–Mead-modified simplex optimization method, and also by nonlinear regression using the software package Mathcad (Mathsoft Engineering & Education, Cambridge, MA). Because the computer program ENCORA was specially designed for parameter estimation of enzymatic reactions using progress curve analysis, it was found to be more accurate compared to results obtained using Mathcad. Thus, the results of ENCORA are given in the revised text (Table 2).

We have thoroughly studied the effect of microwave irradiation on the enzymatic step and it was observed that there was an increase in the initial rate and also in the final conversion over the same period without any change in reaction mechanism. In our previous work[14,23] it was also reported that there was no change in mechanism of the reaction but the rate constants were found to increase (Table 2).

Kinetic Model for Chemical Step. The rate of reaction of styrene with preformed perlauric acid was correlated by a typical second-order kinetic as follows:

$$\frac{d[\mathrm{C}]}{dt} = -k_{\mathrm{cat}}[\mathrm{C}][\mathrm{Q}] \tag{12}$$

By solving the above equation we obtain

$$\ln\left(\frac{[\mathrm{Q}][\mathrm{C}_0]}{[\mathrm{C}][\mathrm{Q}_0]}\right) = ([\mathrm{Q}_0] - [\mathrm{C}_0])k_{\mathrm{cat}}t \tag{13}$$

If the initial mole ratio of $[Q_0]/[C_0] = M$ and X_C = fractional conversion based on styrene, then

Table 2. Kinetic Parameters for the Synthesis of Perlauric Acid

Parameter	Conventional Heating		Microwave Irradiation
	ENCORA	SSE	
$V_{\max}$, kmol m^{-3} s^{-1} kg-cat^{-1}	14.73	1.98×10^{-4}	16.36
K_{mA}, kmol m^{-3} kg-cat^{-1}	2.67×10^3		2269
K_{mB}, kmol m^{-3} kg-cat^{-1}	3.09×10^4		24,720
K_{iA}, kmol m^{-3} kg-cat^{-1}	10.98		6.58
K_i^1	3.48×10^2		216

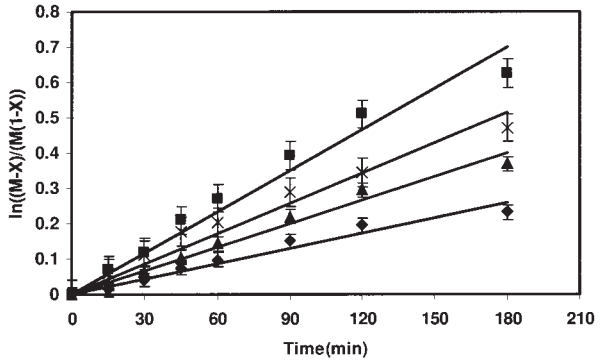


Figure 11. Effect of conventional heating on ex situ epoxidation of styrene.

(♦) 40°C; (▲) 50°C; (×) 55°C; (■) 60°C.

$$\ln\left[\frac{M - X_C}{M(1 - X_C)}\right] = [C_0](M - 1)k_{cat}t \tag{14}$$

Plots of $\ln\{(M - X_C)/[M(1 - X_C]\}$ vs. time were made at different temperatures in both microwave and conventional heating (Figures 11 and 12). It was found that at low temperature there was no significant change in reaction rate by microwave irradiation. However, at higher temperatures, in the range of 40–60°C, there is enhancement in rates under microwave irradiation over rates under thermal heating. The lines are almost parallel in Arrhenius plots. The values of activation energy for the conventional and microwave heating are almost the same (Figure 13), which also suggests that it is the frequency factor that results in higher rates and this can in turn be attributed to the increase in the entropy of the system. The value of activation energy also indicates that the reaction is kinetically controlled. The frequency factor was increased 150-fold in the presence of microwave irradiation over that of conventional heating (Table 3).

Kinetic Model for In Situ Epoxidation of Styrene. Because the reaction scheme is complicated, it is necessary to take a key step into consideration in which generation and utilization of perlauric acid are important. The general reaction is represented as

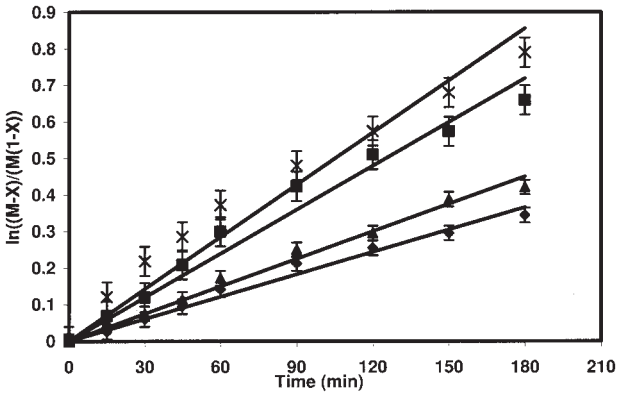


Figure 12. Effect of microwave heating on ex situ epoxidation of styrene.

(♦) 40°C; (▲) 50°C; (■) 55°C; (×) 60°C.

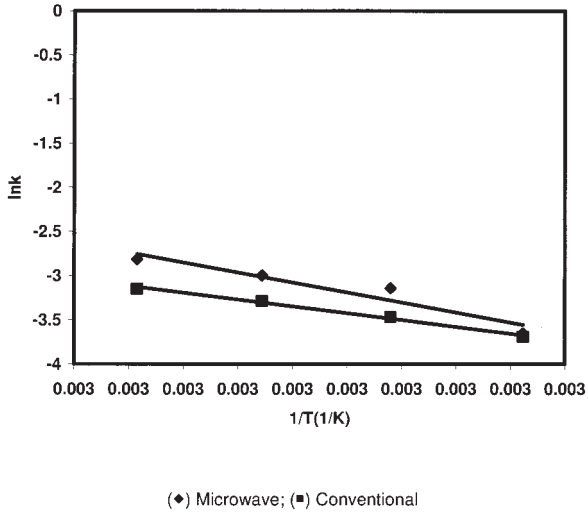


Figure 13. Arrhenius plot for ex situ epoxidation of styrene.

$$A + B \rightleftarrows P + Q + C \rightarrow R + A \tag{15}$$

Taking material balance for perlauric acid,

Rate of perlauric formation

= Rate of perlauric acid utilization

+ Rate of perlauric acid accumulation (16)

$$\text{Rate of formation of perlauric acid} = k_1[A][B] - k_{-1}[P][Q] \tag{17}$$

Because the rate of perlauric acid generation was found to be greater than its rate of utilization in epoxidation, the rate of accumulation was found to be first order:

$$\text{Rate of accumulation of perlauric acid} = k_2[Q] \tag{18}$$

From the concentration time profile, the rate of utilization of perlauric acid in epoxidation with styrene was

$$\text{Rate of perlauric acid utilization} = -k_{cat}[Q][C] \tag{19}$$

From Eqs. 16–19 we have

$$k_1[A][B] = k_{-1}[P][Q] + k_{cat}[Q][C] + k_2[Q] \tag{20}$$

Table 3. Activation Energy and Frequency Factor for Ex Situ Epoxidation

	Microwave Heating	Conventional Heating
Activation energy, kJ/mol	45.27	43.88
Frequency factor, $m^3\ kmol^{-1}\ s^{-1}$	3.936×10^9	2.652×10^7

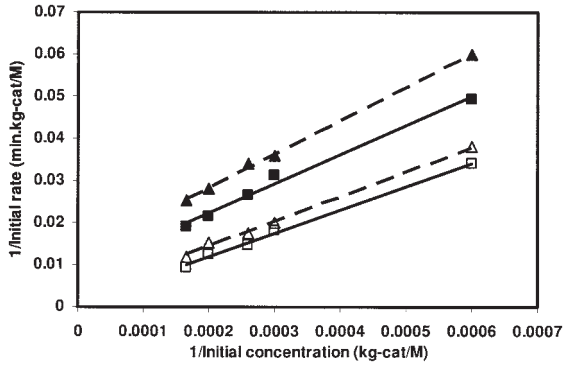
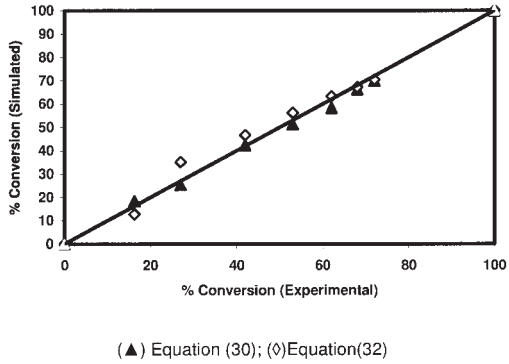


Figure 14. Initial rate^{-1} vs. initial concentration^{-1}.

Different quantities of one substrate at various constant quantities of other. Solid lines indicate microwave heating and dotted lines indicate conventional heating (▲, ■). For varying styrene and fixed 5000 M H_2O_2/kg-cat; (△, □). For varying H_2O_2 and fixed 5000 M styrene/kg-cat.



(▲) Equation (30); (◊)Equation(32)

Figure 15. Experimental and simulated values for in situ epoxidation of styrene.

There was 100% selectivity for perlauric acid with no diacyl peracid formation: lauric acid concentration = initial concentration − concentration of perlauric acid, or

$$[A] = [A_0] - [Q] \tag{21}$$

Therefore Eq. 20 will become

$$k_1\{[A_0] - [Q]\}[B] = k_{-1}[P][Q] + k_{cat}[Q][C] + k_2[Q] \tag{22}$$

After rearranging we obtain

$$[Q] = \frac{k_1[A_0][B]}{k_1[B] + k_{-1}[P] + k_{cat}[C] + k_2} \tag{23}$$

although the rate of epoxidation is second order with respect to styrene (C) and perlauric acid (Q):

$$v = -\frac{d[C]}{dt} = k_{cat}[Q][C] \tag{24}$$

By inserting [Q] into Eq. 24, we obtain

$$v = \frac{\{k_{cat}k_1[A_0]\}[B][C]}{k_1[B] + k_{cat}[C] + \{k_{-1}[P] + k_2\}} \tag{25}$$

$$v = \frac{M_1[B][C]}{M_2 + k_1[B] + k_{cat}[C]} \tag{26}$$

where

$$M_1 = k_{cat}k_1[A_0] \qquad \text{and} \qquad M_2 = k_{-1}[P] + k_2 \tag{27}$$

Taking the reciprocal of Eq. 26,

$$\frac{1}{v} = \left\{\frac{M_2}{M_1}\right\}\frac{1}{[B]}\frac{1}{[C]} + \left\{\frac{k_1}{M_1}\right\}\frac{1}{[C]} + \left\{\frac{k_{cat}}{M_1}\right\}\frac{1}{[B]} \tag{28}$$

When the reactions are carried out at constant [B], the equation is reduced to an equation of a straight line:

$$\frac{1}{v} = \left\{\frac{M_2}{M_1}\frac{1}{[B]} + \frac{k_1}{M_1}\right\}\frac{1}{[C]} + \left\{\frac{k_{cat}}{M_1}\frac{1}{[B]}\right\} \tag{29}$$

$$\frac{1}{v} = \frac{K_{MC}}{v_{MC}}\frac{1}{[C]} + \frac{1}{v_{MC}} \tag{30}$$

Also by changing the concentration of [B] at constant concentration of [C]:

$$\frac{1}{v} = \left\{\frac{M_2}{M_1}\frac{1}{[C]} + \frac{k_{cat}}{M_1}\right\}\frac{1}{[B]} + \left\{\frac{k_1}{M_1}\frac{1}{[C]}\right\} \tag{31}$$

$$\frac{1}{v} = \frac{K_{MB}}{v_{MB}}\frac{1}{[B]} + \frac{1}{v_{MB}} \tag{32}$$

Thus, Lineweaver–Burk double-reciprocal plots were plotted for the above equations (Eqs. 29 and 31) (Figure 14) in the presence of microwave irradiation and conventional heating. In both cases a linear relationship was found to have different slopes and intercepts. These models were used to simulate the conversion profiles, which are in good agreement with the experimental profiles (Figure 15). The kinetic parameters determined by the above method are given in Table 4.

Kinetic modeling for deactivation of Novozym 435

The kinetic model for deactivation of Novozym 435, based on the contribution of deactivation arising from temperature (unfolding of enzyme), agitation, different substrates, change of working media, and temperature, was studied extensively.

Table 4. Kinetic Parameters for In Situ Epoxidation of Styrene

Constant	Microwave Heating	Conventional Heating
v_{MC}, kmol m^{-3} min^{-1} kg-cat^{-1}	104.25	88.54
K_{MC}, kmol m^{-3} kg-cat^{-1}	6754	7091
v_{MB}, kmol m^{-3} min^{-1} kg-cat^{-1}	592	453
K_{MB}, kmol m^{-3} kg-cat^{-1}	147,925	184,906

Because the system contains many reactants, product, intermediate, solvent, and multiphase, it was complicated to identify the contribution of each to deactivation of the enzyme. The exact cause of deactivation in the enzymatic step was extensively studied by Yadav and Manjula Devi.[16] Novozym 435 was stirred with the solvent along with lauric acid and hydrogen peroxide individually for 1 h at 30°C and the reaction was initiated by adding the appropriate reactant; results showed that the enzyme was not deactivated by lauric acid but was first-orderly deactivated by hydrogen peroxide. The same sets of experiments were carried out to analyze whether the deactivation was a result of the other remaining reactants, intermediate, and product or temperature. It was found that in the presence of styrene and styrene oxide, Novozym 435 showed no loss in activity, although there was a decrease in activity arising from perlauric acid. The effect of in situ–generated perlauric acid on enzyme activity has already been discussed above to conclude that the enzyme is deactivated by accumulated perlauric acid.

Therefore the overall equation for deactivation of Novozym 435 by hydrogen peroxide, perlauric acid, and temperature is generally represented by Eq. 33, given below, in which E_1, E_2, U, and D represent the deactivated enzyme by hydrogen peroxide, perlauric acid, and temperature (unfolding), respectively. K_{ud} and k_{ud} are equilibrium constants for enzyme unfolding and deactivation constant attributed to unfolding.

$$\begin{array}{c} E_1 \\ \uparrow \\ E_0 \overset{K_{ud}}{\longleftrightarrow} U \overset{k_{ud}}{\longrightarrow} D \\ \downarrow \\ E_2 \end{array} \tag{33}$$

Deactivation of Novozym 435 was studied systematically by considering the contribution of individual parameters.

Deactivation Kinetics of Novozym 435 in the Presence of Temperature (Unfolding). The activity of enzyme was sensitive to change in temperature. The stability of this enzyme has also been studied by Rejasse et al.[43] in the presence of microwaves in organic medium to find that it is sensitive. Therefore, it was necessary to study the effect of temperature. Changes in temperature and agitation (shear) cause unfolding of the enzyme because of which the enzyme loses its activity. As the enzyme is immobilized within hard beads, there is no shearing (as discussed earlier). In this study, the enzyme was incubated at 55°C for different periods and then its activity was studied. It was found that the enzyme was deactivated by a first-order kinetics (Figure 16).

The equilibrium constant for protein folding is given as

$$K_{ud} = \frac{[U]}{[E]} \tag{34}$$

According to transition-state theory

$$k_{ud} = \frac{k_B T}{h} \tag{35}$$

Therefore

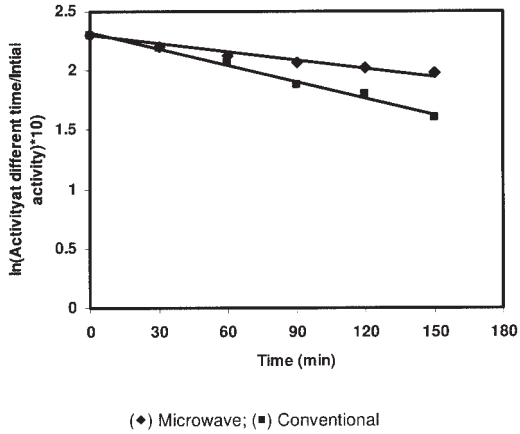


Figure 16. Effect on microwave irradiation on stability.

$$v_d = \frac{k_B T}{h}[U] \tag{36}$$

where k_B, h, and T are the Boltzmann constant, Planck's constant, and absolute temperature, respectively.

From the definition of equilibrium constant and the above equation, we obtain the rate of deactivation as a result of unfolding:

$$v_d = \frac{k_B T}{h} K_{ud}[E] \tag{37}$$

However, K_{ud} is given by

$$K_{ud} = e^{-\Delta G/RT} \tag{38}$$

Therefore Eq. 37 becomes

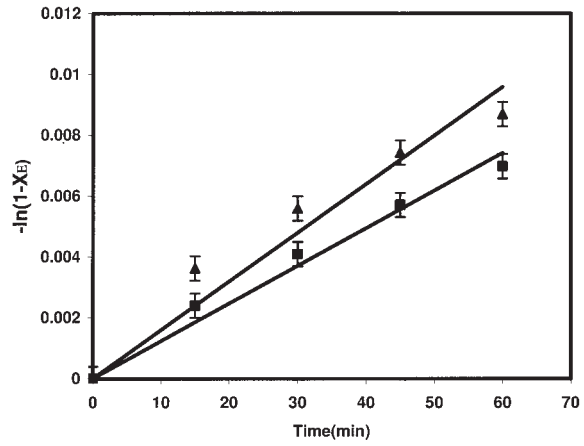
$$v_d = \frac{k_B T}{h} e^{-\Delta G/RT}[E] \tag{39}$$

$$k_{uD} = \frac{k_B T}{h} e^{-\Delta G/RT} \tag{40}$$

$$-\Delta G = RT \ln\left(\frac{k_{uD} h}{k_B T}\right) \tag{41}$$

The Gibbs free energy (ΔG) for deactivation was calculated as 112 and 105 kJ/mol for microwave heating and conventional heating, respectively. Because the entropy of the system increases in the presence of microwaves, there is less deactivation and the molecules are desorbed faster from the sites. Thus, these values should not be the same. Deactivation constants and half-life time were 0.0046 (min^{-1}), 0.0021 (min^{-1}), 150 min, and 330 min in conventional and microwave heating, respectively.

Deactivation Kinetics of Novozym 435 in the Presence of Hydrogen Peroxide. Because it was observed that there was deactivation of the enzyme by hydrogen peroxide, a systematic



(▲) Deactivation due to Hydrogen peroxide and perlauric acid

(■) Deactivation due to Hydrogen peroxide

Figure 17. Plot of $-\ln(1 - X_E)$ vs. time.

study was conducted.[16] The deactivation is represented by the following equation:

$$E_0 + B_0 \xrightarrow{k_{d_1}} E_1 + \mathrm{R} \tag{42}$$

where E_0, B_0, E_{d_1}, and k_{d_1} are initial concentration of enzyme, initial concentration of hydrogen peroxide, concentration of deactivated enzyme, and deactivation constant, respectively.

If X_E is the fraction of E_0 deactivated, then for $[B_0] > [E_0]$, the integrated form of the equation is

$$\ln(1 - X_{E_1}) = k_{d_1}[\mathrm{B}_0]t = k_{D_1}t \tag{43}$$

where

$$k_{D_1} = k_{d_1}[B_0] = \text{constant}$$

Novozym 435 was incubated in the presence of 1 M initial concentrations of hydrogen peroxide for different time periods and the enzyme was filtered off from the hydrogen peroxide solution and used for the reaction under otherwise similar conditions. The activities of the enzyme were measured based on the conversion of lauric acid to perlauric acid, from which the fraction of enzyme that had deactivated was calculated. A plot of $-\log(1 - X_E)$ vs. time produced a linear graph for different fixed amounts of hydrogen peroxide (Figure 17). From the slope of this line k_{d_1} was calculated and it was found to be 0.024 min^{-1}.

The enzyme is immobilized within the macroporous polyacrylic resin, which is hydrophilic, and therefore it may entrap hydrogen peroxide within the support and may form a film around the resin.[44] Analysis of a three-dimensional model of *Candida* lipase B based on the crystal structure clearly shows that the amino acid residue A281 is part of an α-helix (α10) located at the top of the substrate-binding pocket in a highly hydrophobic environment.[45] The glutamic acid side chain of A281 projects into the binding pocket, thereby creating steric hindrance in binding of hydrogen peroxide at the active site. Given that the active site pocket of Novozym 435 is hydrophilic and the environment around that pocket is hydrophobic, the concentration of hydrogen peroxide and its residence time at the active site are greater than those of lauric acid. Therefore, there is a deactivation of the enzyme. External mass transfer limitation was eliminated by stirring. The speed of agitation is very low and thus the force of interaction between support and hydrogen peroxide is greater than the shear created at the surface and there is no deactivation of the enzyme at the outer surface. When microwave irradiation was applied, there was a reduction in deactivation as a result of (1) reduction in constants K_{mA} and K_{iA}, and increase in rate constant leading to enhanced rate $V_{\max}$; and (2) an increase in molecular vibration of hydrogen peroxide and polar amino acids of enzyme by microwaves.

Deactivation of Novozym 435 with H_2O_2 and In Situ–Generated Perlauric Acid. It was found that the reusability of enzyme in the case of in situ microwave epoxidation was greater than that of ex situ epoxidation (Table 1), which indicated that in situ–generated perlauric would contribute to deactivation of the enzyme.

Perlauric acid contains a hydrophobic tail and a polar head. Because of the hydrophobic nature of the enzyme, there may be a hydrophobic–hydrophobic interaction between the enzyme and perlauric acid, which will increase the residence time of perlauric acid. As a result of the close contact with the neighboring hydrophobic residues, the carboxylate would have to be partially dehydrated, which might destabilize the native conformation of enzyme that acts as "molecular lubrication."[14] Desorption of perlauric acid from the active sites and transportation through pores are enhanced by microwave irradiation, leading to an increase in reaction rates.

Because the enzyme follows the ternary complex mechanism, it is difficult to explain adsorption–desorption rates of the substrate at the active sites. However, it is clear from the foregoing that increased residence time of the substrate in pores and its increased concentration at the active site are the major reasons for deactivation. Microwave irradiation up to a certain extent helps to improve the overall effect by reducing the residence time of substrate inside the pores.

The overall deactivation of enzyme by hydrogen peroxide plus perlauric acid is given by

$$E_0 \xrightarrow{k_d} E \tag{44}$$

$$-\ln(1 - X_E) = k_d[B_0]t = k_D t \tag{45}$$

To analyze the contribution of perlauric acid in deactivation of Novozym 435, a systematic study was carried out as follows. Experiments of generation of perlauric acid were carried out for different periods. The enzyme was filtered off from the solution and used in the reaction under otherwise similar conditions. The activities of the enzyme were measured based on the conversion of lauric acid to perlauric acid, from which the fraction of enzyme that had deactivated was calculated. A plot of $-\log(1 - X_E)$ vs. time produced a linear relation for the

fixed amount of hydrogen peroxide (Figure 17). From the slope of this line, the deactivation constant (k_d) was calculated and it was found to be 0.03 min^{-1}. Therefore, in this case it was also found to be a first-order deactivation with increased deactivation constant. To avoid deactivation of the enzyme arising from perlauric acid, it was in situ used for epoxidation.

Discussion

Because the deactivation was attributed to the substrate hydrogen peroxide, to avoid substrate inhibition/inactivation, it was necessary to keep its concentration low in the vicinity of active sites. A CSTR in the presence of microwave irradiation will be helpful to minimize this effect up to a certain extent. If product inhibition is dominant because of perlauric acid, a fixed bed reactor with microwave irradiation is suitable. This will allow greater driving force for the reaction (fresh enzyme is in contact with fresh substrate, provided that the substrate is not inhibiting and product is inhibiting). This type of operation will definitely increase production/kg enzyme than that in other cases. Ultrasonic irradiation is known to reduce mass transfer resistance by decreasing film thickness[46]; the synergism between microwave irradiation and ultrasonic irradiation would be interesting to study in improvement of recycling. To avoid excess concentration of hydrogen peroxide in the vicinity, first the preequilibration of hydrogen peroxide with organic phase was achieved and then only the organic phase was allowed to react separately in the presence of enzyme. The following advantages are offered:

(1) It will reduce hydrogen peroxide contribution to the deactivation rate.

(2) Because the hydrogen peroxide molecule is very small compared to lauric acid and perlauric acid, it can easily diffuse. On the other hand, lauric acid has to cross hydrophilic film around the enzyme and then move through the aqueous-phase–filled pores toward the active site of enzyme, which may reduce the reaction rate.

(3) Because the enzyme is frequently changing its microenvironment in a two-phase system (stirring), this may cause a decrease in rate.

(4) All substrates except hydrogen peroxide are in organic phase, which makes them easily accessible for the reaction in the organic phase.

(5) Novozym 435 is not interfacially activated and contains no lid or flap region that covers the active site.[47] Therefore, it can easily react in the organic phase.

Conclusions

This article has addressed an important issue of epoxidation of styrene to styrene oxide using both in situ and ex situ perlauric acid as the oxidizing agent from lauric acid and hydrogen peroxide with Novozym 435 as the catalyst at 55°C. The effects of conventional and microwave heating were studied. The generation of perlauric acid in situ was more effective. It was observed that ex situ epoxidation caused deactivation of the enzyme arising from perlauric acid concentration. The kinetics of deactivation was systematically studied. Microwave irradiation leads to an increase in the frequency factor as a result of enhanced collision of molecules and an increase in the entropy of the system. The values of activation energy for the conventional and microwave heating are almost the same. Only the rate constants were found to change as a result of change in frequency factor. The Michaelis constant and inhibition constants were reduced by the microwaves.

Acknowledgments

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Notation

A = lauric acid
ABE = ternary complex of the enzyme, lauric acid, and hydrogen peroxide
AE = enzyme–lauric acid complex
B = hydrogen peroxide
Bi = Biot number
C = styrene
$D_{SL:A}$ = liquid phase diffusivity of lauric acid, $m^2\ s^{-1}$
$D_{SL:B}$ = liquid phase diffusivity of hydrogen peroxide, $m^2\ s^{-1}$
$D_{SL:C}$ = liquid phase diffusivity of styrene, $m^2\ s^{-1}$
E = free enzyme
EQ = enzyme–perlauric acid complex
G = free energy of enzyme unfolding, kJ mol^{-1}
h = Plank's constant, $m^2\ kg^{-1}\ s^{-1}$
K_i^I = inhibition constant attributed to hydrogen peroxide
k_B = Boltzmann constant, $m^2\ kg^{-1}\ s^{-2}\ K^{-1}$
k_{cat} = rate constant in chemical step, $m^3\ kmol^{-1}\ s^{-1}$
k_d = deactivation constant attributed to hydrogen peroxide and perlauric acid, s^{-1}
k_D = deactivation constant in Eq. 45, s^{-1}
k_{d_1} = deactivation constant attributed to hydrogen peroxide, s^{-1}
k_{D1} = deactivation constant in Eq. 43 (s^{-1})
$K_{H_2O_2}$ = partition coefficient for hydrogen peroxide
K_{iA} = dissociation constant for enzyme–lauric acid complex
K_{mA} = Michaelis constant for lauric acid, kmol m^{-3} kg-cat^{-1}
K_{MB} = constant in Eq. 32, kmol m^{-3} kg-cat^{-1}
K_{mB} = Michaelis constant for hydrogen peroxide, kmol m^{-3} kg-cat^{-1}
K_{MC} = constant in Eq. 30, kmol m^{-3} kg-cat^{-1}
$k_{SL:A}$ = liquid side mass transfer coefficient for lauric acid, m s^{-1}
$k_{SL:B}$ = liquid side mass transfer coefficient for hydrogen peroxide, m s^{-1}
k_{ud} = deactivation constant attributed to temperature in Eq. 33, s^{-1}
k_{uD} = deactivation constant attributed to temperature in Eq. 40, s^{-1}
K_{ud} = equilibrium constant for enzyme unfolding
M = mole ratio of perlauric acid to styrene
M_1 = constant in Eq. 27
M_2 = constant in Eq. 27
$n_{H_2O_2}$ = moles of hydrogen peroxide
P = water
Q = perlauric acid
R = gas constant, kJ $mol^{-1}\ K^{-1}$
R = styrene oxide
R_D = external mass transfer resistance
R_r = internal reaction resistance
U = unfolded enzyme
V_{aq} = volume of aqueous phase, m^3
v = rate of reaction in in situ epoxidation, kmol m^{-3} min^{-1} kg-cat^{-1}
v_d = rate of deactivation of enzyme attributed to temperature, kg active cat m^{-3} s^{-1}
V_{max} = maximum velocity in enzymatic step, kmol m^{-3} min^{-1} kg-cat^{-1}
v_{MB} = constant in Eq. 32, kmol m^{-3} min^{-1} kg-cat^{-1}
v_{MC} = constant in Eq. 30, kmol m^{-3} min^{-1} kg-cat^{-1}
V_{org} = volume of organic phase, m^3
X = fractional conversion

Greek letters

η = effectiveness factor
ϕ = Thiele modulus

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