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# KINETIC ANALYSIS AND SIMULATION STUDIES FOR LIPASE-CATALYSED RESOLUTION OF RACEMIC 2-METHYL-1-PENTANOL

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The lipase-catalysed optical resolution of a racemic mixture of 2-methyl-1-pentanol by transesterification using vinyl acetate as acyl donor has been studied experimentally. A mechanistic model has been developed for the double-substrate reaction sequence treating both enantiomers as competing substrates. The model is based upon a ping-pong mechanism with alternative substrates involving an acyl-enzyme intermediate. The kinetic constants of the model have been evaluated using initial rate experiments and nonlinear regression analysis. The model successfully predicts the evolution of the enantiomeric excess of substrate ($ee_R$) and the degree of conversion with time for batch experiments with various initial concentrations of vinyl acetate and (R,S)-2-methyl-1-pentanol. Furthermore, the rate equations have been used to theoretically study the dynamic progression of a continuous enzyme-catalysed resolution process. The enantiomeric excess as a function of conversion for different process configurations is discussed. It is found, that the maximum attainable $ee_R$ is strongly dependent on the residence time distribution of the continuous reactor and is rather low for a continuous stirred tank reactor (CSTR) due to competitive inhibition effects.

KEY WORDS Enzyme kinetics, mathematical modelling, optical resolution, lipase, 2-methyl-1-pentanol.

## LIST OF SYMBOLS

| | |
|---|---|
| Ald | abbreviation for acetaldehyde |
| c | conversion |
| ee | enantiomeric excess |
| $E$ | enantiomeric ratio |
| E | enzyme |
| $k_{cat}$ | turnover number, $min^{-1}$ |
| $K_m$ | Michaelis–Menten constant, $mmol \cdot l^{-1}$ |
| N | number of reactor stages |
| R | concentration of (R)-2-methyl-1-pentanol, $mmol \cdot l^{-1}$ |
| RAc | abbreviation for (R)-2-methyl-1-pentylacetate |
| S | concentration of (S)-2-methyl-1-pentanol, $mmol \cdot l^{-1}$ |
| SAc | abbreviation for (S)-2-methyl-1-pentylacetate |
| $t$ | time, h |

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$V$ specific reaction rate, mmol · (g · h)$^{-1}$
VAc concentration of vinylacetate, mmol · l$^{-1}$
$\tau$ mean residence time in the continuous reactor

*Subscripts*

$i$ index number
max maximal velocity
P product
R (R)-2-methyl-1-pentanol
S (S)-2-methyl-1-pentanol
VAc vinylacetate

## INTRODUCTION

In the last decade, increasing attention has been given to the use of enzymes in nonaqueous solvents. If water is replaced by nearly anhydrous organic media, hydrolytic reactions can be reversed towards esterification or transesterification thus providing serious alternatives to chemical synthesis. Various biocatalytic aspects of enzyme-catalysed processes in monophasic organic solvents and a number of potential industrial applications have been comprehensively reviewed (Dordick, 1989).

Among a wide class of enzymes that remain catalytically active at low water content, lipases (triacylglycerol hydrolase, EC 3.1.1.3) have played a major role in research activities due to their ability to catalyse stereo- and regioselective reactions. The production of optically pure chiral compounds is in high demand especially in pharmaceutical, food and environmental industries. Thus, lipases have been used for the stereoselective esterification of dl-menthol (Koshiro *et al.*, 1985), ester hydrolysis of emulsified substrates to obtain optically pure alcohols and fatty acids (Deleuze *et al.*, 1987) and resolution of racemic 2-chloropropionic acid by esterification in an organic solvent (Bodnár, Gubidcza and Szabó, 1990).

Optically active 2-methyl-1-alkanols are useful intermediates in the synthesis of insect pheromones and liquid crystals. Among these, (S)-2-methyl-1-decanol and (S)-2-methyl-1-hexanol have been used as chiral building blocks for the preparation of the pheromones of the european pine saw fly (Byström, Högberg and Norin, 1981) and the peach leafminer moth (Kato and Mori, 1985; Sonnet *et al.*, 1987). Using (R)-2-methyl-1-pentanol instead of (R)-3-methyl-1-hexanol as the chiral starting material could be an alternative in the synthesis of (R)-10-methyl-2-tridecanone, the pheromone of the southern corn root worm (Oppolzer *et al.*, 1985; Rossi, Carpita and Chini, 1985; Senda and Mori, 1985).

Because of the commercial interest in compounds of high enantiomeric excess and their production on a larger scale, the kinetic properties of lipase-catalysed optical resolution of enantiomers need to be studied in detail. Knowledge of reaction mechanisms and the corresponding kinetic constants is an essential step for the design and optimization of a continuous enzymatic resolution process. Chen *et al.* (1982) developed an elegant way to express the enantioselectivity of enzymes obeying Michaelis–Menten type kinetics for two competing substrate forms. They provided some useful equations to predict the enantiomeric excess of

both substrates and products as a function of reaction conversion by means of the newly defined enantiomeric ratio $E$, the ratio of the apparent (pseudo-second-order) rate constants $k_{cat}/K_m$ (Fersht, 1977). By assuming a simple three-step mechanism and neglecting the influence of additional nonchiral substrates, the ratio of the two partial reaction rates was given by

$$\frac{V_S}{V_R} = E \cdot \frac{[S]}{[R]} \tag{1}$$

with the enantiomeric ratio

$$E = \frac{V_{max\,S}/K_{mS}}{V_{max\,R}/K_{mR}} \tag{2}$$

and

$V_R$ reaction rate for conversion of R
$V_S$ reaction rate for conversion of S
$V_{max\,R}$ maximum reaction rate for conversion of R
$V_{max\,S}$ maximum reaction rate for conversion of S
$K_{mR}$ Michaelis–Menten constant for R
$K_{mS}$ Michaelis–Menten constant for S

The authors further showed that $E$ is independent of substrate concentrations and the relationship between the extent of conversion and the enantiomeric excess of the remaining substrate fraction is expressed by

$$\frac{\ln[(1-c)\cdot(1-ee_R)]}{\ln[(1-c)\cdot(1+ee_R)]} = E \tag{3}$$

with $ee_R$ and c being defined as follows:

$$ee_R = \frac{[R]-[S]}{[R]+[S]} \tag{4}$$

$$c = \frac{[R+S]_0 - [R+S]}{[R+S]_0} \tag{5}$$

Later, the basic concept was extended to reversible systems in order to describe enantioselective esterification reactions in biphasic water–organic media (Chen *et al.*, 1987). However, this kind of approach is mainly limited to low substrate concentrations where the reaction follows first-order kinetics and is generally not applicable to multi-substrate reaction sequences. Furthermore, it only provides a measure of enantiomeric specificity but is unable to predict the time courses of substrate concentrations and enantiomeric excess itself.

In a more detailed experimental investigation the kinetics of transesterification of a racemic mixture of phenylalanine by $\alpha$-chymotrypsin (EC 3.4.21.1) have been studied (Moresoli, Flaschel and Renken, 1992). A mathematical model has been proposed which successfully describes the evolution of both transesterification and hydrolysis reaction products with time. Enantioselective properties of

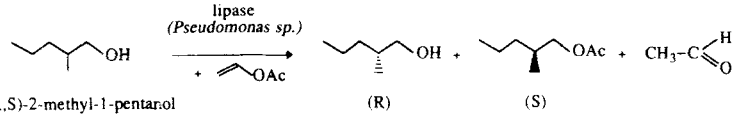


**Figure 1** Schematic representation of the reaction under study: stereoselective transesterification of (R, S)-2-methyl-1-pentanol with vinylacetate.

the enzyme were accounted for by considering the L-enantiomer as a non-reacting competitive inhibitor. The model is therefore limited to those enzyme-catalysed optical resolutions for which the extent of the undesired reaction may be neglected.

The most detailed kinetic study of an enzyme-mediated resolution process so far has been worked out on the separation of *O*-acetyl and *O*-formyl derivatives of several sterically hindered secondary alcohols (Bevinakatti *et al.*, 1991). Although this model was simplified to first order and second-order bimolecular rate equations by neglecting the formation of enzyme-substrate complexes as non-rate controlling steps, the time-dependent product concentrations and the final $ee_P$ (enantiomeric excess of the product) could be successfully predicted.

In this paper a kinetic model for the production of (R)-2-methyl-1-pentanol by lipase-catalysed optical resolution of a racemic mixture of 2-methyl-1-pentanol is proposed. The alcohol is stereoselectively converted into the acetate ester by transesterification with vinylacetate (Figure 1). Experiments have been performed to analyze the time-course of the enantiomeric excess of the remaining alcohol fraction as well as the time-dependent conversion of both the (R)- and (S)-enantiomer. The influence of substrate concentrations on the initial reaction rate of each enantiomer has been studied. The aim of this work was to develop a mechanistic model based on a sequence of enzyme-substrate intermediates to provide more information on how the enantiomeric concentrations affect the resolution process. By formulation of the appropriate rate equations for each enantiomer, quantitative simulation studies of various process configurations as well as the design and optimization of a continuous enzymatic separation process can be performed.

## MATERIALS AND METHODS

Lipase from *Pseudomonas* sp. (Amano PS, 30 units $\cdot$ $mg^{-1}$) was obtained from Amano Chemical Co. (R, S)-2-methyl-1-pentanol was purchased from Aldrich Chemical Co. All other chemicals were of analytical grade and distilled before use. Methylene chloride ($CH_2Cl_2$) was used as solvent and distilled over $CaH_2$.

The kinetic experiments were started by adding known amounts of crude lipase preparation (usually 2.5–10 g $\cdot$ $l^{-1}$) to a magnetically stirred solution of (R, S)-2-methyl-1-pentanol and vinyl acetate in $CH_2Cl_2$ in a stoppered 100 ml flask at 30°C. Samples (0.5 ml) were periodically withdrawn and the enzyme was removed by centrifugation.

The conversion was followed by gas chromatography (GC, Hewlett Packard 5700 A, OV 17 column, 2.3 m × 2 mm) using temperature programming (60°C → 8°C $\cdot$ $min^{-1}$ → 250°C). Nitrogen was used as carrier gas and compounds were detected with a flame ionization detector (FID).

The enantiomeric purity of the remaining alcohol fraction was determined on separate GC runs (Carlo Erba Fractovap 4160, temperature program 80°C→ 2°C · $min^{-1}$→ 200°C, carrier gas $H_2$ (0.5 bar), equipped with an FID) using a chiral capillary column (50 m × 0.3 mm) with a permethylated β-cyclodextrin phase (Fischer *et al.*, 1990, Mosandl *et al.*, 1990) after Jones-oxidation to the corresponding acid (2-methyl pentanoic acid) according to the method described by Sonnet (1987). A typical oxidation procedure was performed as follows: The reaction mixture of alcohol and acetate in $CH_2Cl_2$ was dissolved in 2 ml acetone and treated with Jones reagent (2.5 M $CrO_3$ in 36% $H_2SO_4$) with cooling for 10 min. 60 μl Jones reagent were used for 10 μl alcohol. After dilution with 5 ml water, the mixture was extracted with 5 ml ether and the ether solution was extracted with 10% NaOH (aq.). The alkaline solution was acidified with 10% HCl (aq.), extracted with 5 ml ether and filtered through a small silica gel column. The filtrate was analyzed by GC as previously described. The Jones oxidation occurs without racemization (Sonnet, 1987). Control oxidation experiments with optically active 2-methyl-1-alkanols without the acetate also indicated that no racemization occurred when this procedure was used.

## RESULTS AND DISCUSSION

### *Kinetic Model*

The proposed reaction mechanism for the lipase-catalysed optical resolution of (R, S)-2-methyl-1-pentanol by enantioselective transesterification with vinylacetate is illustrated in Figure 2. For simplification, the following abbreviations are defined:

| | |
|---|---|
| R | (R)-2-methyl-1-pentanol |
| S | (S)-2-methyl-1-pentanol |
| RAc | (R)-2-methyl-1-pentylacetate |
| SAc | (S)-2-methyl-1-pentylacetate |
| VAc | vinylacetate |
| Ald | acetaldehyde |
| E | enzyme |

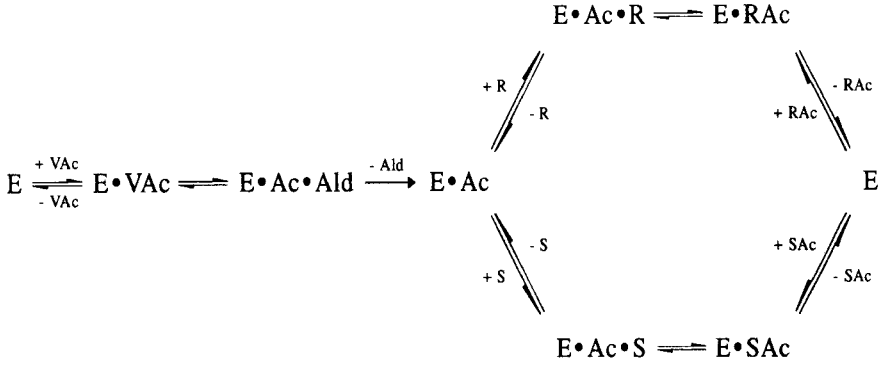


**Figure 2** Proposed reaction mechanism for the competitive transesterification of (R)- and (S)-2-methyl-1-pentanol with vinylacetate by lipase from *Pseudomonas* sp.

The model is based on the assumption that lipase-catalysed transesterification reactions proceed in a number of subsequent steps:

—covalent binding of the acyl donor with the enzyme

—formation of an acyl-enzyme intermediate followed by the release of alcohol

—attachment of the acyl acceptor (alcohol) to the acyl-enzyme complex

—final decomposition of the enzyme-substrate complex with the release of the transesterification product (ester)

The concept of lipase-catalysed acyl transfer can be summarized by a ping-pong bi-bi reaction mechanism and has been widely used in the past. It has been successfully applied to the interesterification of triglycerides (Miller, Prausnitz and Blanch, 1991), irreversible (Zaks and Klibanov, 1985) and reversible transesterification reactions (Rizzi *et al.*, 1992) and to ester synthesis performed in organic solvents as well as in supercritical fluids (Marty *et al.*, 1990). Additional experimental evidence for the formation of an acyl-enzyme intermediate has been given recently (Kawase, Sonomoto and Tanaka, 1992). If vinyl acetate is used as the acyl donor, the reaction becomes virtually irreversible because the resulting vinylalcohol tautomerizes completely to acetaldehyde (Degueil-Castaing *et al.*, 1987).

In the case of optical resolutions of racemic mixtures by transesterification the two enantiomers have to be considered as substrates competing for the same site on the enzyme. Consequently, two parallel pathways exist for the decomposition of the acyl-enzyme intermediate. (R)- and (S)-2-methyl-1-pentanol are alternatively bound to the complex with the subsequent formation of (R)- and (S)-2-methyl-1-pentylacetate, respectively. The rate equations for the conversion of a racemic mixture can be adapted from a ping-pong mechanism with alternative second substrate as defined by Segel (1975). The velocity equation for the transesterification of (R)-2-methyl-1-pentanol is

$$V_R = \frac{V_{\max R}[R][VAc]}{K_{mVAc}[R] + K_{mR}[VAc] + [VAc][R] + K_{mVAc}K_{mR}\frac{[S]}{K_{mS}} + [VAc]K_{mR}\frac{[S]}{K_{mS}}} \quad (6)$$

and similarily for the transesterification of (S)-2-methyl-1-pentanol

$$V_S = \frac{V_{\max S}[S][VAc]}{K_{mVAc}[S] + K_{mS}[VAc] + [VAc][S] + K_{mVAc}K_{mS}\frac{[R]}{K_{mR}} + [VAc]K_{mS}\frac{[R]}{K_{mR}}} \quad (7)$$

where $K_{mVAc}$ is the Michaelis–Menten constant for VAc.

Throughout this work, absolute concentrations of (R)- and (S)-2-methyl-1-pentanol were calculated from the $ee_R$- and c-values obtained from separate GC-analyses (see Methods) by transformation of equations (4) and (5):

$$[S] = \frac{[R+S]_0 \cdot (1-c) \cdot (1-ee_R)}{2} \quad (8)$$

$$[R] = [S] \cdot \frac{(1+ee_R)}{(1-ee_R)} \quad (9)$$

Equations (6) and (7) contain five kinetic constants which have to be estimated from initial rate experiments. Unfortunately, homochiral preparations of 2-methyl-1-pentanol were not commercially available, so initial rate experiments had to be performed with the original racemic mixture. Results of a typical batch transesterification run are illustrated in Figure 3 with initial concentrations $[VAc]_0 = 2000\ mmol \cdot l^{-1}$ and $[R + S]_0 = 1000\ mmol \cdot l^{-1}$.

It can be seen from the time courses that S was preferentially converted into SAc resulting in an increasing $ee_R$ for the remaining substrate fraction. However, the reaction rate of R increases with decreasing concentrations of S, as a result of the reduced inhibitory effect of S and therefore enhanced amount of R bound to

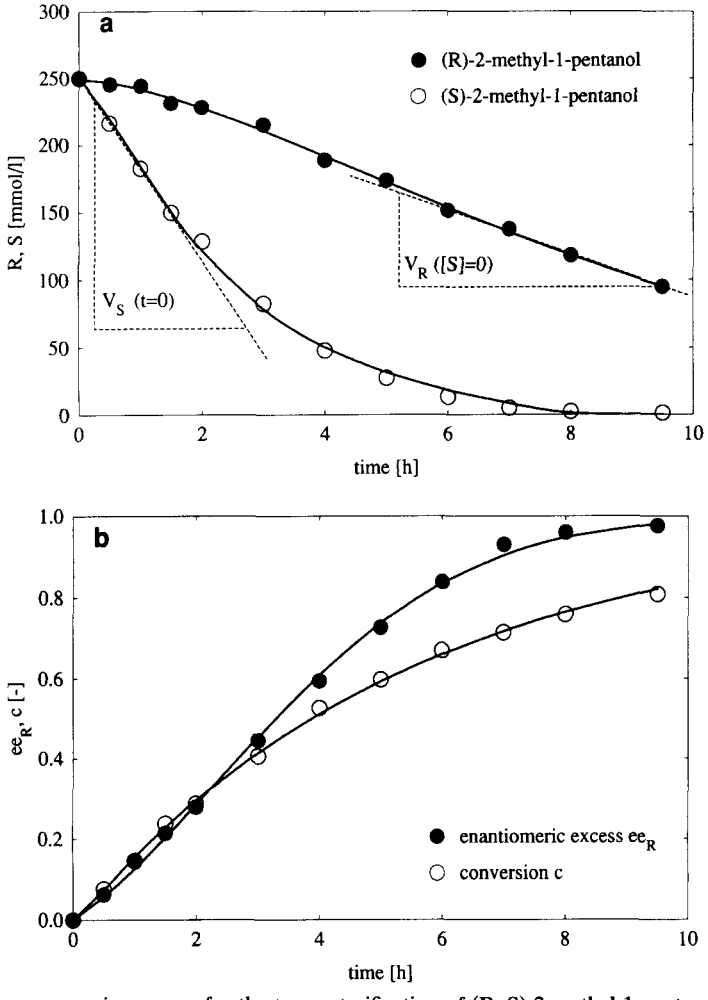


**Figure 3** Time progression curves for the transesterification of (R, S)-2-methyl-1-pentanol with initial concentrations $[R + S]_0 = 1000\ mmol \cdot l^{-1}$ and $[VAc]_0 = 2000\ mmol \cdot l^{-1}$ a) concentrations of R and S, dotted lines: estimation of reaction rates, b) enantiomeric excess and conversion c.

the acyl-enzyme complex. Considering the overall progress of the reaction, approximately 80% of the substrate must be converted before an $ee_R > 98\%$ is obtained. Similar experiments have been performed with various concentrations of VAc and (R, S)-2-methyl-1-pentanol. The concentration ranges chosen were 500–5000 mmol · $l^{-1}$ and 250–1000 mmol · $l^{-1}$ for vinylacetate and (R, S)-2-methyl-1-pentanol, respectively, in order to include a sufficiently high domain for reliable model predictions and the following theoretical process analysis. From the slope of the decrease of each enantiomeric concentration the reaction rates of R and S were calculated. Since most of the experiments had to be performed using equal concentrations of R and S (racemate), simultaneous estimation of the complete set of parameters from the initial rates could lead to incorrect values due to limited parameter sensitivities. Therefore, a stepwise parameter estimation procedure was chosen. First, the reaction rate of R for the homochiral transesterification reaction was computed from the slope of [R] at [S]→0 as schematically indicated in Figure 3. The reaction rates and the corresponding concentrations of VAc, R and E are summarized in Table 1.

If the concentration of S in the reaction mixture can be neglected, equation (6) simplifies to the following well-known expression for a double-substrate ping-*pong* reaction mechanism:

$$V_R = \frac{V_{\max R}[R][VAc]}{K_{mVAc}[R] + K_{mR}[VAc] + [VAc][R]} \tag{10}$$

Estimation of the kinetic constants $V_{\max R}$, $K_{mR}$ and $K_{mVAc}$ were performed by fitting equation (10) to the experimental results listed above. The sum of the residuals between measured and calculated reaction rates was minimized using an optimization algorithm based on a constrained simplex method. The kinetic parameters obtained are summarized in Table 2. Figure 4 shows a comparison between model predictions and experimental observations. As can be seen, acceptable descriptions of the observed data are given by the family of lines calculated according to the model.

**Table 1** Homochiral reaction rates of R for different concentrations of VAc and R estimated from the time-course of [R] at [S]→0.

| *[VAc]* *mmol · l⁻¹* | *[R]* *mmol · l⁻¹* | $[E]_0$ *g · l⁻¹* | $V_R$[a] *mmol · (g · h)⁻¹* |
|---|---|---|---|
| 285 | 35 | 2.5 | 1.5 |
| 1800 | 50 | 2.5 | 2.5 |
| 810 | 60 | 2.5 | 2.6 |
| 590 | 91 | 5.0 | 3.3 |
| 1594 | 91 | 5.0 | 4.0 |
| 1160 | 160 | 2.5 | 5.9 |
| 675 | 172 | 10.0 | 5.8 |
| 1170 | 172 | 10.0 | 6.3 |
| 200 | 207 | 10.0 | 3.7 |
| 4316 | 297 | 10.0 | 10.2 |

[a] Values calculated as specific reaction rates in terms of mmol substrate per gram enzyme preparation and hour.

**Table 2** Preliminary estimates of model parameters for the homochiral transesterification of (R)-2-methyl-1-pentanol with vinylacetate.

| *Parameter* | | | |
|---|---|---|---|
| $V_{\max R}$ | mmol · (g · h)$^{-1}$ | 30 | ±23%[a] |
| $K_{mR}$ | mmol · l$^{-1}$ | 541 | ±30%[a] |
| $K_{mVAc}$ | mmol · l$^{-1}$ | 972 | ±26%[a] |

[a] Confidence interval at a 95% confidence level.

The remaining model parameters $V_{\max S}$ and $K_{mS}$ were evaluated by fitting the rate equation of S (equation (7)) to the initial reaction rates obtained from the initial slope of the decrease of S (see Figure 3) under competitive conditions, with $V_{\max R}$, $K_{mR}$ and $K_{mVAc}$ taken from the preliminary estimates according to Table 2. The reaction rates for various initial concentrations of VAc and (R, S)-2-methyl-1-pentanol are listed in Table 3 with the results of the optimization procedure for $V_{\max S}$ and $K_{mS}$ listed in Table 4.

In Figure 5 the initial rates predicted by equation (7) are compared to the experimental observations. It is evident that the data can be satisfactorily described by the model in the concentration range of 500–2000 mmol · l$^{-1}$ for VAc. It should be noted, that in spite of the enlarged range of substrate concentrations no enzyme saturation could be observed with respect to VAc and consequently the reaction rates depend on the concentrations of both acyl donor and acceptor. This again shows the necessity to account for complex multi-

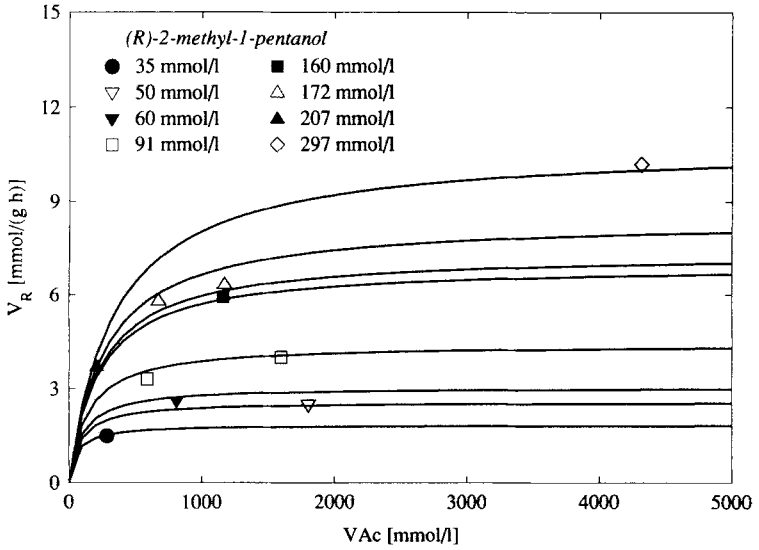


**Figure 4** Estimated velocities for the homochiral transesterification of (R)-2-methyl-1-pentanol and comparison with model predictions; for experimental conditions see Table 1.

**Table 3** Initial reaction rates of S for different initial concentrations of VAc and (R, S)-2-methyl-1-pentanol under competitive conditions.

| $[VAc]$ $mmol \cdot l^{-1}$ | $[R+S]_0$ $mmol \cdot l^{-1}$ | $[E]_0$ $g \cdot l^{-1}$ | $V_S$[a] $mmol \cdot (g \cdot h)^{-1}$ |
|---|---|---|---|
| 500 | 250 | 2.5 | 10.5 |
| 1000 | 250 | 2.5 | 14.4 |
| 2000 | 250 | 2.5 | 16.2 |
| 500 | 500 | 5.0 | 14.0 |
| 1000 | 500 | 5.0 | 16.0 |
| 2000 | 500 | 2.5 | 22.1 |
| 1000 | 1000 | 10.0 | 19.0 |
| 1500 | 1000 | 10.0 | 22.2 |
| 2000 | 1000 | 10.0 | 27.7 |
| 2000 | 1000[b] | 10.0 | 40.9 |

[a] Values calculated as specific reaction rates in terms of mmol substrate per gram enzyme preparation and hour.
[b] Substrate prepared with initial $(ee_S)_0 = 0.91$ ($[S]_0 = 955$ mmol $\cdot l^{-1}$, $[R]_0 = 45$ mmol $\cdot l^{-1}$).

**Table 4** Preliminary estimates of model parameters for the transesterification of (S)-2-methyl-1-pentanol with vinylacetate under competitive conditions.

| *Parameter* | | | |
|---|---|---|---|
| $V_{max\,S}$ | mmol $\cdot$ (g $\cdot$ h)$^{-1}$ | 73 | ±12%[a] |
| $K_{mS}$ | mmol $\cdot l^{-1}$ | 254 | ±30%[a] |

[a] Confidence interval at a 95% confidence level.

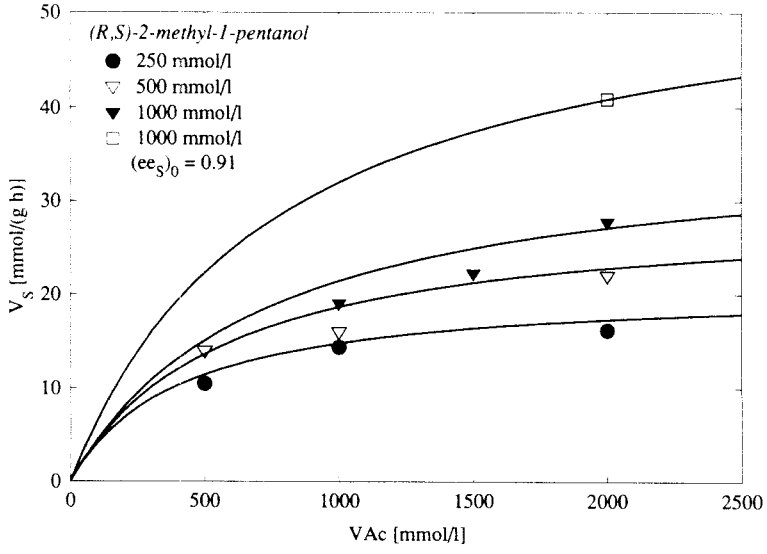


**Figure 5** Initial reaction rates for the transesterification of (S)-2-methyl-1-pentanol under competitive conditions and comparison with model predictions; for experimental conditions see Table 3.

substrate kinetic models in order to successfully predict the dynamic behaviour of the resolution process.

*Dynamic Simulation of the Resolution Process and Model Verification*

The model has been applied to the dynamic simulation of various batch transesterification runs in order to describe the progress of both the overall reaction conversion c and the enantiomeric excess $ee_R$ with time. Assuming that no product inhibition occurs and the reaction is irreversible, the consumption of R, S and VAc are represented by the following set of differential equations:

$$\frac{d[R]}{dt} = -[E]_0 \cdot V_R \tag{11}$$

$$\frac{d[S]}{dt} = -[E]_0 \cdot V_S \tag{12}$$

$$\frac{d[VAc]}{dt} = -[E]_0 \cdot V_R - [E]_0 \cdot V_S \tag{13}$$

Because of the nonlinearity of the model, the equations have to be solved numerically. It was found, that the enantioselectivity predicted by the estimated model parameters listed in Tables 3 and 4 was slightly low compared to the measured results. The limited amount of information contained in initial rate data obtained from experiments performed with the racemic mixture made it necessary to recalculate the preliminary estimates by considering the dynamic progress of all the transesterification experiments previously performed. To achieve an adequate representation of the experimental observations, average deviations between model predictions and the measured time courses of $ee_R$ and c were therefore minimized by slightly improved estimates of the kinetic constants. The adapted parameter values are summarized in Table 5.

In Figure 6, results are shown for two different initial concentrations of (R, S)-2-methyl-1-pentanol with the same concentration of $[VAc]_0 = 2000\ mmol \cdot l^{-1}$. Figure 7 illustrates the modelling of experiments with varying initial amounts of the acyl donor VAc where the initial content of (R, S)-2-methyl-1-pentanol is fixed at $[R + S]_0 = 1000\ mmol \cdot l^{-1}$. In each case, the evolution of the enantiomeric excess $ee_R$ and the overall conversion c are successfully predicted by the model. The order of magnitude of the remaining deviations seems to be reasonably small if the range of measurement errors is considered.

**Table 5** Estimated parameters of the complete model for the enantioselective transesterification of (R, S)-2-methyl-1-pentanol.

| *Parameter* | | |
|---|---|---|
| $V_{max\,S}$ | $mmol \cdot (g \cdot h)^{-1}$ | 73 |
| $V_{max\,R}$ | $mmol \cdot (g \cdot h)^{-1}$ | 28 |
| $K_{mS}$ | $mmol \cdot l^{-1}$ | 240 |
| $K_{mR}$ | $mmol \cdot l^{-1}$ | 560 |
| $K_{mVAc}$ | $mmol \cdot l^{-1}$ | 972 |

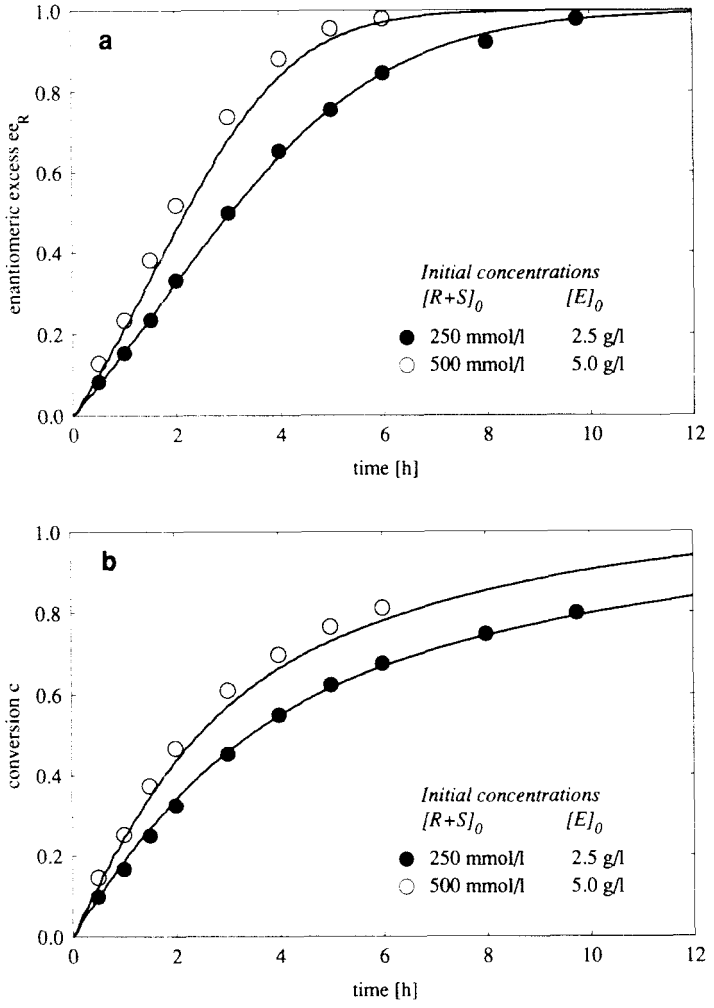


**Figure 6** Dynamic evolution of $ee_R$ and c for varying initial concentrations of (R, S)-2-methyl-1-pentanol and $[VAc]_0 = 2000\ mmol \cdot l^{-1}$; comparison of experimental observations (symbols) with model predictions (lines) a) enantiomeric excess $ee_R$ b) conversion c.

The approach introduced by Chen *et al.* (1982) to quantitatively analyze the enantioselectivity of kinetic resolution experiments mainly rested on the prediction of $ee_R$ as a function of conversion by assuming first order reaction rates with respect to each enantiomeric concentration. To apply their model to the resolution process investigated in this paper, results of $ee_R$ as a function of c for various batch transesterification experiments are summarized in Figure 8 and compared to the relationship given by equation (3) as well as to the proposed complete reaction model. Results demonstrate, that the enantioselectivity is indeed independent of initial concentration values and can be entirely described by a single curve. The apparent enantiomeric ratio $E$ for the resolution of

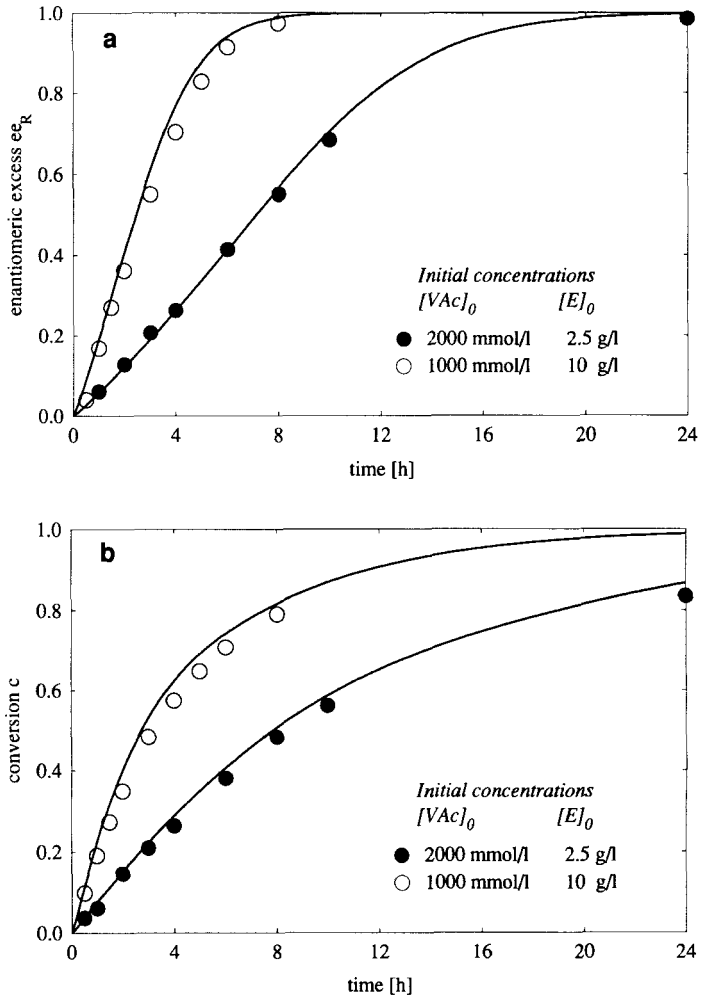


**Figure 7** Dynamic evolution of $ee_R$ and c for varying initial concentrations of vinylacetate and $[R+S]_0 = 1000\,mmol \cdot l^{-1}$; comparison of experimental observations (symbols) with model predictions (lines) a) enantiomeric excess $ee_R$ b) conversion c.

(R, S)-2-methyl-1-pentanol according to the model of Chen *et al.* (1982) was calculated by least squares estimation giving a value of $E = 6.23$, represented by the dashed line in Figure 8. If $E$ is evaluated from the parameter estimates of the complete model listed in Table 5, one obtains a comparable value of $E = 6.08$, indicating that the model is consistent with the theory derived by Chen *et al.* Simulation studies have shown that integration of equations (11), (12) and (13) with varying initial concentrations of R, S and VAc leads to identical ee-c dependencies, represented by the solid line in Figure 8. Therefore, enzyme selectivity is not affected by different concentrations of the nonchiral acyl donor. However, it should be noted again that absolute reaction rates and the entire time

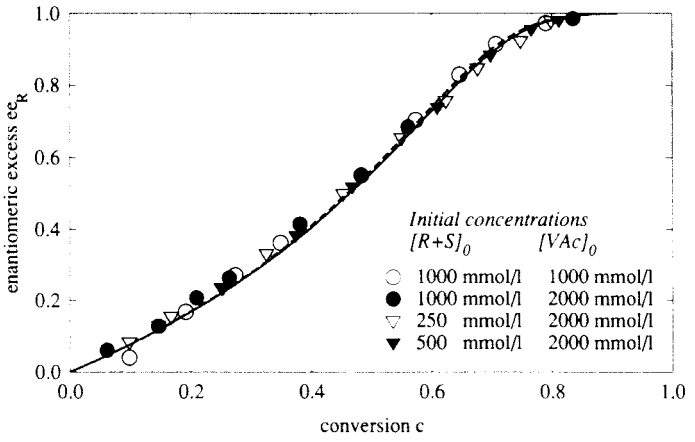


**Figure 8** Enantiomeric excess $ee_R$ as a function of extent of conversion for varying initial substrate concentrations as indicated. Symbols = experimental observations, dashed line = model according to Chen *et al.* (1982), solid line = model presented in this paper.

progress of the resolution process can only be predicted by considering the complete mechanistic model that takes all substrate concentrations into account.

### *Theoretical Analysis of Continuous Optical Resolution*

Investigations of enzyme-catalysed resolution processes have mainly dealt with batch reactor kinetic data. Few results have been published with enantiomeric separations performed in continuously operated systems. However, the detailed kinetic analysis presented in this paper should provide a useful basis for the prediction of enzyme specificity and activity in a continuous reactor. Hence, the proposed rate equations have been used to theoretically analyze the process under study. Most attention has been given to the influence of the residence time distribution on the competition between the enantiomeric compounds, in order to quantitatively compare reactor configurations which are commonly used in nonaqueous enzyme catalysis such as fixed bed or mechanically agitated membrane reactors. To account for this, the kinetic model has been coupled with a simple series of continuously stirred tank reactors (CSTR), the material balances for vessel $i$ being expressed by the following set of differential equations:

$$\frac{d[\mathrm{R}]_i}{dt} = ([\mathrm{R}]_{i-1} - [\mathrm{R}]_i) \cdot \frac{\mathrm{N}}{\tau} - [\mathrm{E}]_0 \cdot V_\mathrm{R} \tag{14}$$

$$\frac{d[\mathrm{S}]_i}{dt} = ([\mathrm{S}]_{i-1} - [\mathrm{S}]_i) \cdot \frac{\mathrm{N}}{\tau} - [\mathrm{E}]_0 \cdot V_\mathrm{S} \tag{15}$$

$$\frac{d[\mathrm{VAc}]_i}{dt} = ([\mathrm{VAc}]_{i-1} - [\mathrm{VAc}]_i) \cdot \frac{\mathrm{N}}{\tau} - [\mathrm{E}]_0 \cdot V_\mathrm{R} - [\mathrm{E}]_0 \cdot V_\mathrm{S} \tag{16}$$

with $\tau$ = overall mean residence time, N = total number of CSTR's in series and $[\mathrm{R}]_{i-1} = [\mathrm{R}]_0$, $[\mathrm{S}]_{i-1} = [\mathrm{S}]_0 = [\mathrm{R}]_0$, $[\mathrm{VAc}]_{i-1} = [\mathrm{VAc}]_0$ giving the input concentrations in the first vessel if $i = 1$.

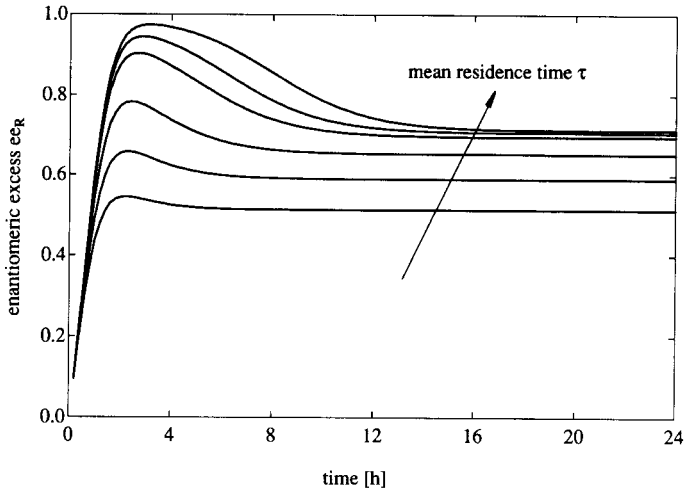


**Figure 9** Simulated enantiomeric excess $ee_R$ as a function of time for varying mean residence times $\tau$ in a single CSTR, $[R+S]_0 = [VAc]_0 = 1000\,mmol \cdot l^{-1}$, enzyme concentration $[E]_0 = 30\,g \cdot l^{-1}$, $2 \leq \tau \leq 100\,h$.

Simulations were performed assuming initial conditions to be equal to the input concentrations $[R]_0$, $[S]_0$ and $[VAc]_0$. From the concentrations in the outlet stream of the final CSTR the enantiomeric excess $ee_R$ and the total substrate conversion c were calculated as previously described.

Results for a single CSTR are shown in Figure 9, where the dynamic progress curve of $ee_R$ is plotted for varying values of the mean residence time $\tau$. When the reaction is started, the $ee_R$-values rapidly increase until a maximum is observed. As the conversion proceeds further, the enantiomeric excess slowly decreases until the final steady state is reached. The maximum $ee_R$ is enhanced by a prolonged duration of the fluid in the reactor represented by increasing values of the mean residence time $\tau$. However, simulations demonstrate that steady state $ee_R$-values beyond an asymptotic number of approximately 0.7 cannot be achieved, independent of the range $\tau$ is increased to. Thus,

$$\lim_{\tau \to \infty} ee_R = 0.7.$$

An explanation might be found in the lower concentrations that occur in an ideally mixed tank, resulting in a partly reduced inhibitory effect of S on the rate of reaction of R. It is evident that stirred tank reactors seem to be unfavourable for the continuous resolution of racemic mixtures when enzymes with modest enantioselectivity ($5 < E < 10$) have to be used. These findings suggest that the residence time distribution predominantly affects the apparent selectivity of the continuous resolution process. The relationship between the degree of backmixing and the maximum $ee_R$ achieved is clearly demonstrated by the theoretical predictions shown in Figure 10. In this plot, the commonly used ee-c curves were obtained from steady state calculations and are drawn for different numbers of CSTR's in series. These are compared to the result previously obtained for the batch resolution process (dashed line). Again, one can see that the maximum

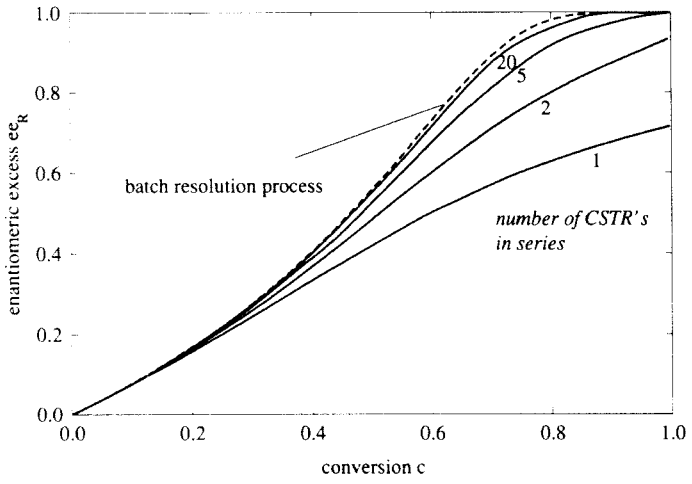


**Figure 10** Simulated enantiomeric excess $ee_R$ as a function of extent of conversion for different numbers of CSTR's in series compared to the results obtained from batch kinetic data.

attainable $ee_R$ for a single ideally mixed reactor is unsatisfactorily low. By increasing the number of theoretical stages from one to twenty it becomes obvious that the kinetically controlled enantioselectivity of the lipase is only seen if deviations from plug-flow are negligible. Hence, a packed bed immobilized enzyme reactor should prove to be most favourable if one wants to design a continuous lipase-catalysed resolution process, even if additional mass transfer problems may occur.

## CONCLUSIONS

The lipase-catalysed optical resolution of racemic 2-methyl-1-pentanol by transesterification using vinylacetate as acylating agent is described in this paper. The enzyme showed good activity in media of extremely low water content and at high substrate concentrations. Optically pure (R)-2-methyl-1-pentanol with $ee_R > 98\%$ excess could be obtained by driving the extent of conversion to approximately 80%, the enzyme exhibiting moderate enantioselectivity. A complete mechanistic model was developed to quantitatively describe the time course and the selectivity of the resolution process, providing velocity equations for the conversion of each enantiomer under competitive conditions. The influence of all substrate concentrations on the individual rates for competing ping-pong bi-bi reactions has to be considered if one aims to design and scale-up the process, even if the enantioselectivity for the reaction under study seems to be independent of initial conditions in the range of experimental observations.

Though the estimated model parameters are subject to some uncertainty because of the limited information obtained from initial rate data, reasonable predictions of dynamic experiments could be achieved. Therefore, the basic model appears to be generally applicable to similar lipase-catalysed resolution

processes. However, it should be emphasized that reliable parameter estimation for competitive reactions should rest on homochiral initial rate data for at least one enantiomer in order to overcome limitations in parameter sensitivity.

Application of the model to the theoretical analysis of a continuous separation process revealed a substantial decrease in selectivity compared to results observed in batch experiments. As a conclusion, plug-flow type reactors have to be considered if the amount of the undesired reaction pathway is significant. An immobilized enzyme fixed bed reactor should permit the continuous resolution of racemic 2-methyl-1-pentanol with reasonable optical and chemical yields. Future research will therefore concentrate on the question of how the enantioselectivity is affected by relevant immobilization techniques.

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