Kinetic analysis of enzymatic esterification of fatty acids and ethanol

V. U. Miguel[(*)], G. C. Trubiano, G. Pérez, D. O. Borio and A. F. Errazu
Planta Piloto de Ingeniería Química (UNS-CONICET)
Camino La Carrindanga km 7. (8000) Bahía Blanca, Argentina

Abstract

The esterification of oleic acid and ethanol with immobilised lipase in a solvent-free media is presented in this work. The reaction has been carried out at a laboratory scale in a stirred tank reactor operating batchwise. The evolution of oleic acid conversion to ester was determined by using two analysis techniques: measurement of the acid index and gas chromatography. The influence of the operating conditions on the equilibrium conversion and conversion-time profiles was analysed. The amount of enzyme, temperature, initial alcohol/oleic acid molar ratio and initial water content were considered. A kinetic model has been developed for the enzymatic esterification of oleic acid and ethanol catalysed by *Candida Antartica* immobilised lipase. Some kinetic models were considered and a second order reaction kinetic was selected. The parameter adjustment was carried out by using a computer program based on Marquardt algorithms. The results from the simulated experiments by using the proposed model showed a satisfactory agreement with the experimental data.

1. INTRODUCTION

Most esters are easily produced by acid or base catalysed condensation of an alcohol and an acid at elevated temperatures. In many cases these temperatures cause a degradation of the reactants and/or the product itself. Resulting consequences are not only a loss of substrate but also a laborious processing to obtain the desired ester in reasonable purity. Modern enzymology has achieved improvements in the development and application of lipases as catalysts. New immobilisation techniques make possible to use enzymes in industrial processes in a similar way to the classical catalysis for heterogeneous reactions [1] [2].

Lipases belong to the enzymatic class of hydrolases and catalyse both the hydrolysis and synthesis of esters at the interface between water and insoluble substrate. This interface, consisting of water and oil, is essential for the activity of lipase. Some of the advantages of lipases are the specificity for different types of substrate, high yields and the possibility of working under mild operating conditions. In the case of the immobilised lipase, the interface is obtained by immobilisation of the necessary water together with the lipase. The main advantages of immobilised lipases are the easy recovery and reusability of enzymes and low contamination levels for both the final product and the environment [3] [4].

In the present work, the enzymatic esterification was investigated in order to analyse the influence of the operating conditions on the reaction yield and develop a kinetic model to describe the reaction rate for different operating conditions.

(*) E-mail: vmiguel@plapiqui.edu.ar

2. MATERIALS AND METHODS

2.1. Reagents

Enzyme: The catalyst used was Novozym 435. It is a *Candida Antartica* lipase, immobilised on a macroporous acrylic resin. The product consists of bead-shaped particles with the diameter in the range of 0.3-0.9 mm. The product was delivered with the water content of 1-2% w/w. It was kindly donated from Novo Nordisk AS (Copenhagen, Denmark).

Lipase Activity: The ester synthesis activity of Novozym 435 is expressed in Propyl Laurate Units (PLU/g). The activity of Novozym 435 used for our synthesis was 7000 PLU/g.

Chemicals: Alcohol was purchased from Dorwil (Ethanol 96% v/v and Ethanol 99% v/v) Fatty acids (mainly oleic acid) were kindly donated by Materia Hnos (Mar del Plata, Argentina). Standards and other chemicals were provided by Sigma.

2.2. Procedure

Experimental equipment: The experimental apparatus consisted of a well mixed glass reactor operating batchwise (BSTR), provided with a heat transfer jacket and with a blade type mixer (Fig. 1). Reactants were introduced in the reactor where they were heated up to the desired temperature. When the reaction temperature was reached, the catalyst was added. Stirring speed was set at 300 rpm. Samples of 0.1 cm^3 were taken at regular time intervals for analysis. Once the reaction was finished, the catalyst was separated by filtration, washed with a solvent and dried. In this way the lipase was recovered for a next operation.

Analytical Methods: The reaction progress was followed by the determination of the acid index, and the product distribution was analysed by means of capillary gas chromatography.

The product composition was analysed following the AOCS official method Cd 11b-91 based on capillary gas chromatography. 10mg of reaction sample were dissolved in pyridine (containing 0.1mg/ml of n-tetradecane as internal standard), before being silylated with BSFTA/TMCS, allowing the resulting mixture to react at 70°C for 20 minutes. Finally, about 1 μl was injected on a

Figure 1: Experimental set up: 1, jacket; 2, reactor; 3, pressure sensor; 4, temperature sensor; 5, stirrer; 6, sampling; 7 enzyme loading

Varian 3700 chromatograph equipped with a split/splitless capillary injector, a flame ionisation detector and a SPB1 (30m x 0.32mm x 0.25μm) Supelco column. Helium was used as carrier gas, the oven temperature was programmed from 80°C to 290°C with a constant ramp of 10°C/min. The response time and response factors were calculated by comparison with Sigma reference standards.
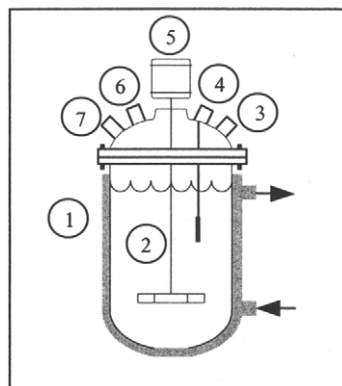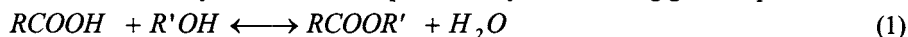
3. KINETIC MODEL DEVELOPMENT

The esterification of fatty acids could be represented by the following global equation:

$$RCOOH + R'OH \longleftrightarrow RCOOR' + H_2O \tag{1}$$

Different kinetic models have been proposed by others authors [5]. A simplified model based on a reversible model with second-order equations, both the forward and the reversal reaction, was considered as a first approach. The reaction rate equation was expressed as:

$$r = -\frac{d[Ac]}{dt} = Et\ k_1\left([Ac][Al] - \frac{1}{K_{eq}}[Es][W]\right) \tag{2}$$

An Arrhenius-type equation was proposed for the rate constants with activation energy and the pre-exponential for each reaction rate constant.

$$k_1 = k_{1\infty}\exp\left(-E_1/RT\right) \tag{3}$$

$$K_{eq} = K_{eq\infty}\exp\left(-\Delta H/RT\right) \tag{4}$$

On the other hand, an ordered reaction mechanism (bi-bi model) was also suggested. The reaction sequence is expressed by the following reaction scheme:

$$Ac + E \xleftrightarrow{k1,k-1} AcE \tag{5}$$

$$AcE + Al \xleftrightarrow{k2,k-2} AcEAl \tag{6}$$

$$AcEAl \xleftrightarrow{k3,k-3} EsE + W \tag{7}$$

$$EsE \xleftrightarrow{k4,k-4} E + Es \tag{8}$$

This model was considered taking into account different controlling steps and keeping the remaining steps at equilibrium. The derived kinetic equations are shown in Table 1.

Table 1
Kinetic Models

Model	Controlling step	Resulting kinetic equation	Parameters
2nd order reversible	----	$r = Et\ k_1\left([Ac][Al] - \dfrac{1}{K_{eq}}[Es][W]\right)$	k_1 K_{eq}
Ordered mechanism	Eq. (5)	$r = \dfrac{Et\left(\overrightarrow{k}[Ac][Al] - \overleftarrow{k}[W][Es]\right)}{[Al]K_2K_3K_4 + [W][Es] + [W][Es][Al]K_2 + [Es][Al]K_2K_3}$	$\overrightarrow{k} = k_1K_4K_2K$ $\overleftarrow{k} = k_{-1}$
Ordered mechanism	Eq. (6)	$r = \dfrac{Et\left(\overrightarrow{k}[Ac][Al] - \overleftarrow{k}[W][Es]\right)}{1 + [Es][W]K_3K_4 + [Es]K_4 + [Ac]K_1}$	$\overrightarrow{k} = k_2K_1$ $\overleftarrow{k} = k_{-2}K_3K_4$
Ordered mechanism	Eq. (7)	$r = \dfrac{Et\left(\overrightarrow{k}[Ac][Al] - \overleftarrow{k}[W][Es]\right)}{K_4 + K_1K_4[Ac] + [Ac][Al]K_2K_1K_4 + [Es]}$	$\overrightarrow{k} = k_3K_1K_2K_4$ $\overleftarrow{k} = k_{-3}$
Ordered mechanism	Eq. (8)	$r = \dfrac{Et\left(\overrightarrow{k}[Ac][Al] - \overleftarrow{k}[W][Es]\right)}{[W] + K_1K_3K_2[Ac][Al] + [Ac][Al][W]K_2K_1 + K_1[Ac][W]}$	$\overrightarrow{k} = k_4K_1K_2K_3$ $\overleftarrow{k} = k_{-4}$

4. RESULTS AND DISCUSSION

A set of experiments was designed and carried out in order to examine the desired range of process variables. In the following figures the influences of operating variables are shown. These figures contain both the experimental data (as marked points) and the simulation results obtained from the second order reversible model (as continuous lines).
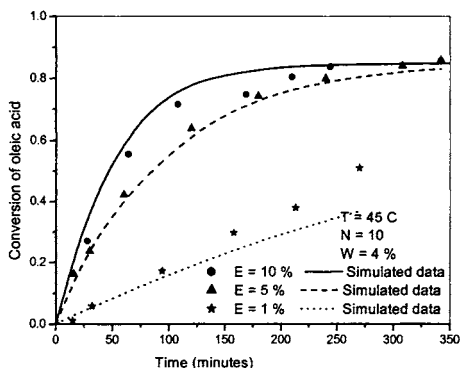
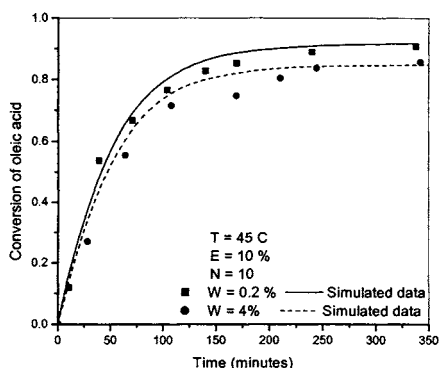

Figure 2: Influence of enzyme content

Figure 3 : Influence of initial water content

4.1. Influence of the enzyme content
The influence of weight of catalyst (E) on the reaction progress can be seen in figure 2. As the value of E increases, the reaction rate rises at the same proportion. It is also demonstrated that the initial reaction rate presents a lineal relationship with the enzyme content. The conversion value reached at equilibrium is not shown for all the experiences but it was not affected by the enzyme weight.

4.2. Influence of the initial water content
Water is a product of esterification reaction and a reactant for the hydrolysis (reverse reaction). Therefore, the initial water content (W) affects the equilibrium. For this enzymatic reaction, the equilibrium conversion increases as the initial water concentration decreases (figure 3). The water content affects the reaction in two ways: the reaction rate is decreased along the course of reaction and the final conversion is lower because water concentration shifts the equilibrium towards the reactants. This effect has also been reported by other authors [6].

4.3. Influence of temperature
Esterification of oleic acid with ethanol has been carried out at three different temperatures (T) as it is shown in figure 4. As expected, a temperature rise increases the reaction rate. The simulated results from reversible kinetic model were according to this behaviour and from the adjusted parameters, an endothermic reaction was validated.

4.4. Influence of initial molar ratio of alcohol/fatty acid
The initial molar ratio of alcohol/fatty acid (N) has an important effect on the equilibrium conversion, which increases with alcohol excess (figure 5). As expected, the increment in the

amount of one of the reactants shifts the chemical equilibrium towards the product side. On other hand, alcohol excess has an unfavourable effect on the reaction rate. The reversible kinetic model offered a good fit all experiences at different N, although for N=1 (stoichiometric value) higher differences between calculated and experimental values were found.
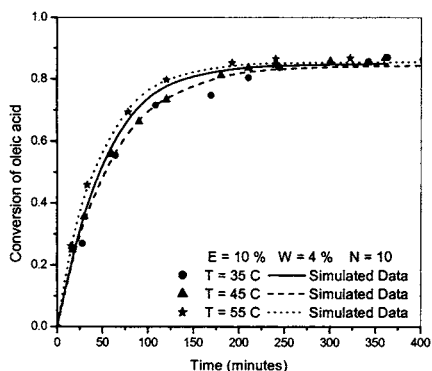
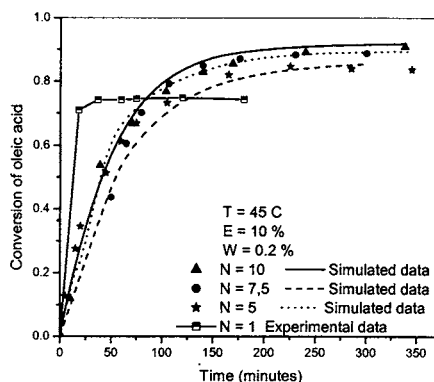Figure 4: Influence of temperature on the conversion

Figure 5: Influence of initial molar ratio on the conversion

4.5. Model parameters adjustment

Kinetic model parameters were adjusted to find the best values fitting experimental data with simulation results. A multiparametric, non-linear regression based on Marquardt algorithm was employed. The agreement of adjustment for each model can be compared in Table 2 by means of the variance values:

Table 2
Kinetic parameters

Models (controlling step)	Variance
2nd order model (--)	1.18×10^{-02}
Ordered model (5)	4.93×10^{-03}
Ordered model (6)	1.40×10^{-01}
Ordered model (7)	1.35×10^{-01}
Ordered model (8)	1.20×10^{-01}

The lowest value of variance from ordered model (5) indicates the best fit. However, the second order model was selected because the variance is similar and the equation representing the model is simpler. The resulting parameters for this model are shown in Table 3.

The positive value of ΔH obtained from the adjustment is in concordance with an endothermic reversible reaction. According to the variance values, the second order model was selected to represent the reaction rate as shown in the previous Figs. 2-5.

Table 3
Kinetic parameters for the second order model

Kinetic parameter	Fitted value
$k_{1\infty}$	5.39×10^{-02}
E_1	3447
$K_{eq\infty}$	4.69
ΔH	918

5. CONCLUSION

The solvent-free esterification of oleic acid and ethanol was carried out using a commercial enzyme as catalyst. The enzymatic esterification seems to be an interesting way to obtain fatty acid ester due to several advantages such as the mild operating conditions, the product quality and energetic saving, among others.

The experimental results allowed knowing the effect of reaction variables on the reaction rate and the ester yield. The esterification rate can be described adequately by the selected model and the simulated results showed a good prediction of the reaction rate for the different operating conditions. Therefore, this model and its parameters can be considered valid either for reactor design or for simulation purposes.

NOMENCLATURE

Ac: Oleic acid
Al: Ethanol
E: immobilised lipase
Es: Ester
W : water
ΔH = heat of reaction [cal/mol]
[i] = concentration of component i [mol/l]
E_1 = activation energy of k_1 [cal/mol]
Et = enzyme/substrate ratio [%w enzyme/acid]

k_{-1} = rate constant for equation i (reverse)
$k_{1\infty}$ = pre-exponential factor of k_1
K_{eq} = equilibrium constant
k_i = rate constant for equation i (forward)
K_i = desorption/absorption constant for equation i
N = initial molar ratio (alcohol/fatty acids)
r = reaction rate [mol/(l gcat min)]
R = gas constant [cal/mol k]
T = temperature [K]

REFERENCES

1. E. Lie, G. Molin, Journal of Chemical Technology and Biotechnology 50 (1991) 549-553
2. L. Mojovick, S. Siler-Marinkovic, G. Kukic, G. Vunjak-Novakovic. Enzymes in Microbial Technology 15 (5) (1993) 438-443
3. L.H. Posorske, Journal of the American Oil Chemists Society 61 (11) (1984) 38-42
4. G. Langrand, C. Triantaphylides, J. Baratti, Biotechnology Letters 10(8) (1988) 549-554
5. T. García, M. Martinez, D. García and J. Aracil , Reactions kinetics and the Development of Catalytic Processes, G.F. Froment and K. C. Waugh (eds.) (1999)
6. M. Leitgeb, Z. Knez, JAOCS Vol.67 (1990) n11