

KINETICS OF ENANTIOMERICALLY ENRICHED SYNTHESIS OF SOLKETAL ESTERS USING NATIVE AND SBA-15 SUPPORTED *P. FLUORESCENS* LIPASE

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Dedicated to Prof. Leon Gradoń on the occasion of his 70th birthday

The studies showed that alkaline lipase from *Pseudomonas fluorescens* enables an irreversible transesterification of vinyl esters to give enantiomeric excess (eeR) of about 80% using vinyl butyrate as acyl donor and diisopropyl ether as a solvent, at partially optimized conditions. For the native lipase the process was adequately described by a five-parameter Ping-Pong Bi Bi model for both enantiomers plus expression accounting for the formation of enzyme-acyl donor complex, but for the same lipase supported on mesoporous materials of SBA-15-Oc type, R-product inhibition also had to be taken into account. The use of hydrophobic support increased by more than two-fold the rate of the S-solketal conversion but even more that of R-solketal. Thus the immobilization of lipase had very positive effect on the process kinetics but decreased its enantioselectivity.

Keywords: bioenantioselectivity, solketal, ping-pong kinetics, transesterification

1. INTRODUCTION

Racemic solketal (isopropylidene glycerol, IPG) is an inexpensive reagent accessible from glycerine and acetone, also as a by-product in biofuels manufacturing, whereas enantiomerically pure (R)- and (S)-solketal esters are in high demand, despite high price due to their wide application as precursors of important biologically active compounds such as glycerylphospholipids, β -blockers, e.g. (S)-propranolol, prostaglandin or leucotrienes (Boncel et al., 2013; Hanson, 1991; Hof and Kellog, 1996; Zniszczoł, et al., 2016).

Thus a rational solution would be a resolution of racemic mixtures of IPG using enantioselective catalysts, e.g. enzymes (Majewska et al., 2006). In this light a development of a method affording high enantiomeric purity as well as formulation of a mathematical model of the reaction kinetics emerges as an important goal. The model itself may also foster development of more effective catalysts, for this and related reactions.

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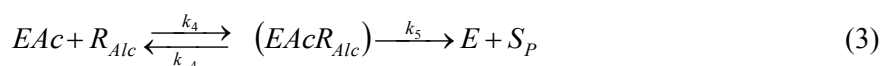
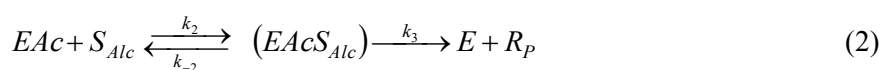
The performed studies (Zniszczoł, 2015) revealed that among three possible synthesis pathways for enantiomerically enriched IPG esters: i) enantioselective esterification involving carboxylic acids, ii) enantioselective transesterification of vinyl esters, and iii) enantioselective hydrolysis of solketal esters, only the second one offered promising results. These studies also showed that alkaline lipase from *Pseudomonas fluorescens* enables an irreversible transesterification of vinyl esters (acyl donors) to give enantiomeric excess (ee_R) of about 80% using vinyl butyrate as acyl donor and diisopropyl ether as a solvent. Experiments with the immobilized enzyme were also performed to demonstrate that the use of carriers with hydrophobic surface: multiwall carbon nanotubes - MWCNT or mesoporous silica of SBA-15 type, the surface of which was modified with n-octyl groups (SBA-15-Oc), significantly increased biocatalysts activity, although internal diffusion limitation of activity was also observed (Boncel et al., 2013; Zniszczoł et al., 2016).

While representative experimental results and details of biocatalysts preparation have already been published (Boncel et al., 2013; Zniszczoł et al., 2016), the kinetics of IPG enrichment has not been reported, and a model capable of describing it can be of major importance for both process engineers and for the development of even more effective biocatalysts. Therefore, the kinetics of racemic IPG resolution was studied in more detail, not only using the native enzyme but also that attached to SBA-15 silicates owing to their widespread application as enzyme carriers.

2. RESULTS AND DISCUSSION

2.1. Base kinetic model

Scores of studies demonstrated that the Ping-Pong Bi Bi family of mechanisms describe lipase-mediated transesterifications and that inhibitory effects usually need to be taken into account (Barros et al., 2010; Pavia et al., 2000; Pilarek and Szewczyk, 2007). Yet, in the reaction system under study the situation is more complex since *P. fluorescence* lipase is not fully (100%) enantioselective to racemic IPG, and in addition to conversion preference for *S* enantiomer, also *R* compound is increasingly involved. Therefore, the conversion of both solketal enantiomers had to be considered, and clearly, the formation of enzyme-acyl complex also had to be taken into account. In effect the base model of the process was described by the scheme:



where *DAc* is acyl donor, *S_{Alc}* and *R_{Alc}* designate alcohols of *S* and *R* configuration, *S_P* and *R_P* are reaction products of *S* and *R* configuration, respectively and *P_U* is product of a side reaction. However, as vinyl alcohol produced in the first reaction is unstable and quickly tautomerizes to vinyl acetate, thus this reaction may be considered irreversible and hence k_{-1} can be assumed to be equal zero, which simplifies the model. Taking this into account and assuming that the reactions have simple order kinetics and also no loss of species to ambient temperature (e.g. by evaporation), the mass balances for each of the species (intermediates included) and the batch process can be written as:

$$\frac{d[E]}{dt} = -k_1[E][DAc] + k_3[EAcS_{Alc}] + k_5[EAcR_{Alc}] \quad (4)$$

$$\frac{d[DAc]}{dt} = -k_1[E][DAc] \quad (5)$$

$$\frac{d[EAc]}{dt} = k_1[E][DAc] - k_2[EAc][S_{Alc}] + k_{-2}[EAcS_{Alc}] - k_4[EAc][R_{Alc}] + k_{-4}[EAcR_{Alc}] \quad (6)$$

$$\frac{d[P_U]}{dt} = k_1[E][DAc] \quad (7)$$

$$\frac{d[S_{Alc}]}{dt} = -k_2[EAc][S_{Alc}] + k_{-2}[EAcS_{Alc}] \quad (8)$$

$$\frac{d[R_{Alc}]}{dt} = -k_4[EAc][R_{Alc}] + k_{-4}[EAcR_{Alc}] \quad (9)$$

$$\frac{d[EAcS_{Alc}]}{dt} = k_2[EAc][S_{Alc}] - k_{-2}[EAcS_{Alc}] - k_3[EAcS_{Alc}] \quad (10)$$

$$\frac{d[EAcR_{Alc}]}{dt} = k_4[EAc][R_{Alc}] - k_{-4}[EAcR_{Alc}] - k_5[EAcR_{Alc}] \quad (11)$$

$$\frac{d[R_p]}{dt} = k_3[EAcS_{Alc}] \quad (12)$$

$$\frac{d[S_p]}{dt} = k_5[EAcR_{Alc}] \quad (13)$$

With all reservations in mind, yet aiming at practical reasons to obtain global expressions for reaction rates, it was additionally assumed that the system attains a quasi steady state, which stipulates that derivatives of all the species are equal to zero.

Bearing in mind the mass balance for the enzyme

$$E_t = E + EAc + EAcS_{Alc} + EAcR_{Alc} \quad (14)$$

and performing a series of transformations, we finally obtained:

$$r_{(R_p)} = \frac{k_1 k_3 [E_t] [DAc] [S_{Alc}]}{K_{M_2} \left\{ [S_{Alc}] \frac{k_3}{K_{M_2}} + [R_{Alc}] \frac{k_5}{K_{M_4}} + k_1 [DAc] \left(1 + \frac{[S_{Alc}]}{K_{M_2}} + \frac{[R_{Alc}]}{K_{M_4}} \right) \right\}} \quad (15)$$

$$r_{(S_p)} = \frac{k_1 k_5 [E_t] [DAc] [R_{Alc}]}{K_{M_4} \left\{ [S_{Alc}] \frac{k_3}{K_{M_2}} + [R_{Alc}] \frac{k_5}{K_{M_4}} + k_1 [DAc] \left(1 + \frac{[S_{Alc}]}{K_{M_2}} + \frac{[R_{Alc}]}{K_{M_4}} \right) \right\}} \quad (16)$$

$$[EAcS_{Alc}] = \frac{[EAc][S_{Alc}]}{K_{M_2}} \quad (17)$$

$$K_{M_2} = \frac{k_{-2} + k_3}{k_2} \quad (18)$$

$$[EAcR_{Alc}] = \frac{[EAc][R_{Alc}]}{K_{M_4}} \quad (19)$$

$$K_{M_4} = \frac{k_{-4} + k_5}{k_4} \quad (20)$$

$$[EAc] = \frac{k_1 [E] [DAc]}{B} \quad (21)$$

$$B = [S_{Alc}] \frac{k_3}{K_{M_2}} + [R_{Alc}] \frac{k_5}{K_{M_4}} \quad (22)$$

$$E = \frac{E_t B}{B + k_1 [DAC] \left(1 + \frac{[S_{Alc}]}{K_{M_2}} + \frac{[R_{Alc}]}{K_{M_4}}\right)} \quad (23)$$

Determination of the model parameters, given in Table 1, performed by fitting them to the experimental data showed that it is capable to represent ($R^2=0.992$) the results obtained for the native enzyme in a broad range of solketal concentrations, Figs. 1 and 2.

Table 1. Kinetic parameters of the base kinetic model evaluated for the native *P. fluorescence* lipase

k_1	$5.49 \cdot 10^{-4}$	$\text{ml} \cdot \mu\text{mol}^{-1} \cdot \text{s}^{-1}$
k_3	5.00	s^{-1}
k_5	$4.92 \cdot 10^{-2}$	s^{-1}
K_{M_2}	$3.43 \cdot 10^{-4}$	$\mu\text{mol} \cdot \text{ml}^{-1}$
K_{M_4}	$2.88 \cdot 10^{-5}$	$\mu\text{mol} \cdot \text{ml}^{-1}$

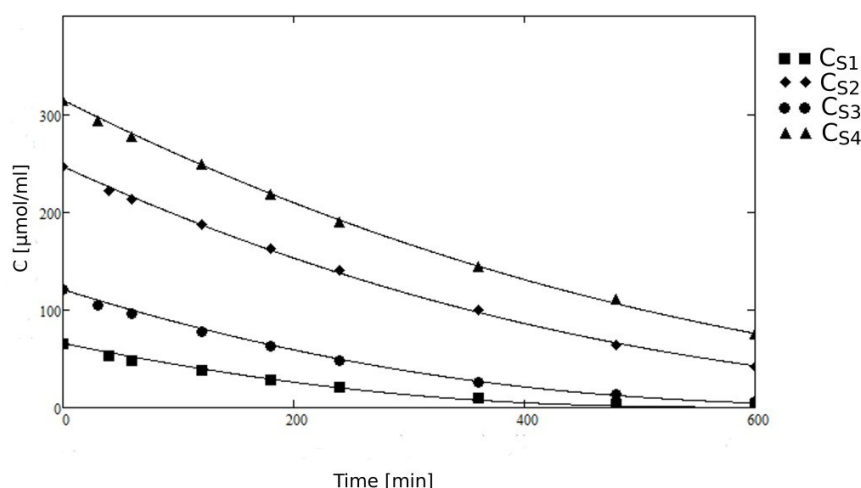


Fig. 1. Progress of S-solketal conversion in time catalyzed by the native enzyme. Solid lines represent the base kinetic model.

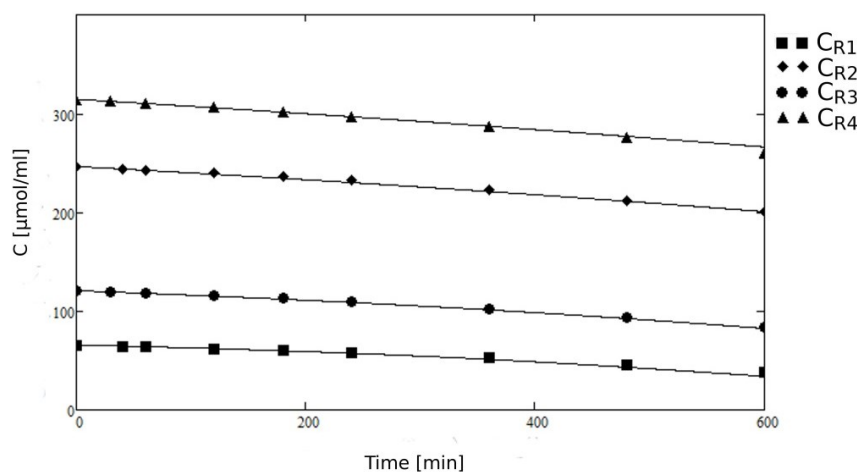


Fig. 2. Progress of R-solketal conversion in time catalyzed by the native enzyme. Solid lines represent the base kinetic model

However, for SBA-15-Oc-supported lipase applied as a slurry catalyst, more notable discrepancies were observed for large solketal concentrations (Majewska et al., 2006) (results not shown here). The use of the same model (pseudo-homogeneity assumption) could be rationalized by the very small sizes (20-40 μm dia.) of the catalysts which made a fine slurry system.

2.2. Extended kinetic model

A number of different modifications of the base kinetic model, which accounted for different inhibitory effects or even adsorption on the carrier, have been tested in order to improve its accuracy for the SBA-15 biocatalysts, but they gave only a moderate improvement. Finally, we tested the effect of the enzyme-acyl complex inhibition by R-product, governed by the equation:



Taking it into account and adopting similar assumptions as before, after numerous transformations the expressions for global reaction rates were determined:

$$r_{(R_p)} = \frac{k_1 k_3 [E_t][DAc][S_{Alc}]}{K_{M_2} \left\{ [S_{Alc}] \frac{k_3}{K_{M_2}} + [R_{Alc}] \frac{k_5}{K_{M_4}} + k_1 [DAc] \left(1 + \frac{[S_{Alc}]}{K_{M_2}} + \frac{[R_{Alc}]}{K_{M_4}} + \frac{R_p}{K_{6R}} \right) \right\}} \quad (25)$$

$$r_{(S_p)} = \frac{k_1 k_5 [E_t][DAc][R_{Alc}]}{K_{M_4} \left\{ [S_{Alc}] \frac{k_3}{K_{M_2}} + [R_{Alc}] \frac{k_5}{K_{M_4}} + k_1 [DAc] \left(1 + \frac{[S_{Alc}]}{K_{M_2}} + \frac{[R_{Alc}]}{K_{M_4}} + \frac{R_p}{K_{6R}} \right) \right\}} \quad (26)$$

For the native lipase they appeared to reproduce the experimental data more precisely ($R^2 = 0.996$, data not displayed here) than the base model, but more importantly the extended model proved capable of portraying quite well ($R^2 = 0.984$) also the data from the SBA-15-Oc-supported lipase (Figs. 3 and 4) using the parameters given in Table 2.

Table 2. Kinetic parameters of the extended kinetic model evaluated for the native and immobilized *P. fluorescence* lipase

	Native enzyme	Enzyme immobilized on SBA-15-Oc	
k_1	$5.56 \cdot 10^{-4}$	$11.8 \cdot 10^{-4}$	$\text{ml} \cdot \mu\text{mol}^{-1} \cdot \text{s}^{-1}$
k_3	1.91	3.65	s^{-1}
k_5	$6.16 \cdot 10^{-2}$	$92.6 \cdot 10^{-2}$	s^{-1}
K_{M_2}	$2.15 \cdot 10^{-5}$	$19.7 \cdot 10^{-5}$	$\mu\text{mol} \cdot \text{ml}^{-1}$
K_{M_4}	$2.53 \cdot 10^{-5}$	$23.9 \cdot 10^{-5}$	$\mu\text{mol} \cdot \text{ml}^{-1}$
K_{6R}	$6.81 \cdot 10^{-6}$	$386 \cdot 10^{-6}$	$\mu\text{mol} \cdot \text{ml}^{-1}$

From the biocatalyst engineering standpoint of interest may be a significant difference in the values of K_{6R} for the native lipase (Table 2) and that attached on to SBA-15-Oc (Table 2). It can be attributed to a large adsorption potential of the applied support, i.e. highly porous silica of SBA-15 type. But even more important are very significant differences in the values of the corresponding kinetic parameters (effective values for heterogeneous catalysts), very much in favor of the SBA-15-based biocatalysts. They clearly demonstrate that specific activity of the lipase attached to SBA-15silicates, the surface of which was modified with octyl groups to impart hydrophobic properties, was over two times more active than the native lipase, which may be of practical significance. Yet, this activation occurred at the expense of increase in R-solketal conversion, and hence enantioselectivity. The former trend does not

come as a surprise and has been reported before by many authors, c.f. critical overviews more recently published (Adlercreutz, 2013; Hrydziuszko et al., 2014). The decrease in enantioselectivity with increase in conversion is also typically observed, and it poses a limit on the useful, economically justified values of conversion coefficients.

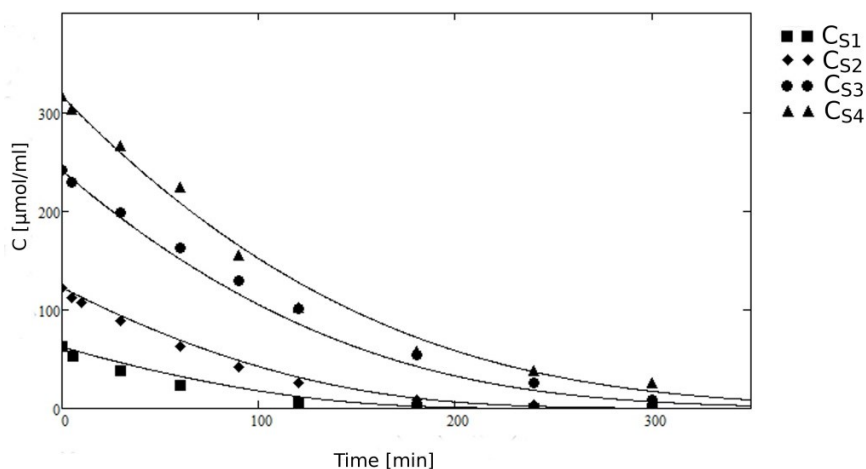


Fig. 3. Progress of S-solketal conversion in time catalyzed by SBA-15-Oc immobilized lipase. Solid lines represent predictions from the extended kinetic model.

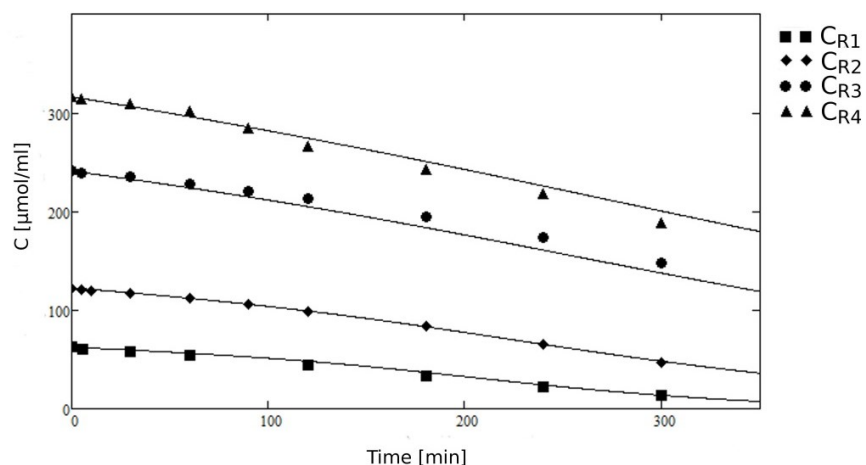


Fig. 4. Progress of R-solketal conversion in time catalyzed by SBA-15-Oc immobilized lipase. Solid lines represent predictions from the extended kinetic model.

3. CONCLUDING REMARKS

The performed studies showed that *P. fluorescence* lipase is an effective catalyst in partial separation of racemic solketal mixtures and that the process can be adequately described by the Ping-Pong Bi Bi model plus an expression accounting for the formation of an enzyme-acyl donor complex. Studies of the biocatalysts made of the same lipase but supported on siliceous mesoporous powders of SBA-15-Oc type, carried out in a batch slurry system, showed that they can be over two times more active than the native enzyme in S-solketal conversion but even more for R-solketal, and the process kinetics can be modeled using the same set of equations, but R-product inhibition needs to be taken into account.

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SYMBOLS

ee_R	enantiomeric excess,
D_{Ac}	acyl donor,
S_{Alc}, R_{Alc}	alcohols of S and R configuration,
S_P, R_P	reaction products of S and R configuration,
P_U	product of a side reaction,
$k_1, k_{-1}, etc.$	reaction rate constant
K_M	Michaelis-Menten constant

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