SOUTHERN BRAZILIAN JOURNAL OF CHEMISTRY 2021 VIRTUAL CONFERENCE

ACTIVITY-BASED KINETIC MODELLING OF LIPASE CATALYSED SYNTHESIS OF PEROCTANOIC ACID

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Received 10 December 2021; received in revised form 10 January 2021; accepted 28 January 2022

ABSTRACT

Carboxylic peroxy acids are organic oxidants of relevance in the cosmetic, food, and agrochemical industries. However, they are traditionally used as intermediaries in a process known as Prileschajew epoxidation and synthesized using sulfuric acid as a catalyst, promoting undesirable reactions on the final epoxides. Therefore, the study of selective catalysts such as enzymes is a topic of interest. In this work, peroctanoic acid synthesis was carried out using n-hexane as the solvent and an immobilized *Candida Antarctica* Lipase B commercial preparation as the catalyst. On the other hand, the oxidant, hydrogen peroxide was supplied as an aqueous solution, comprising the disperse phase of the reacting system. The reaction progression was quantified by iodometric and cerimetric titration of the peroctanoic acid concentration in the hexane phase. Four different initial amounts of octanoic acid were tested (0.74, 1.57, 10, and 20 millimoles). Substrate inhibition by octanoic acid was observed having at 1.57 millimoles the maximum initial reaction rate. The experimental data were fitted to a ping-pong bi-bi enzymatic kinetic model to estimate the initial reaction rate. Since this system constituted a liquid-liquid (organic-aqueous) two-phase system, the model was evaluated employing the thermodynamic activities of all species involved, assuming phase equilibria with time. The activities of all species were estimated using UNIFAC. As a result, the model was able to reproduce the trend of the initial rate with the change of the initial amount of octanoic acid.

Keywords: Kinetic modeling, *Candida Antarctica* Lipase B, UNIFAC, Heterogeneous catalysis.

1. INTRODUCTION

Carboxylic peroxy acids are organic oxidants of relevance in the cosmetic, food, and agrochemical industries. On the other hand, they are used as intermediates in the Baeyer-Villiger reaction and the Prileschajew epoxidation. The peroxy acids synthesis consists of the reversible reaction of a carboxylic acid and hydrogen peroxide to give a peroxy acid and water as in Equation 1:

In the Prileschajew epoxidation, the formed peroxy acid reacts with an unsaturated organic substrate to produce an epoxide. Traditionally, sulfuric acid was the catalyst used in the peroxy

acid synthesis despite being abrasive, difficult to handle at industrial scale, and unselective, thus promoting oxirane ring-opening of final epoxides. Therefore, the study of alternative environmentally friendly catalysts as lipases is a topic of interest. Particularly, the immobilized lipases, as heterogeneous catalysts, have the advantage that they are not required to be separated from the final reaction products, allowing them to be reused in multiple batches. The widely widespread technical *Candida Antarctica* Lipase B (CALB) was proved to be active in this reaction, known as a promiscuous lipase activity (Ortiz, 2019). This reaction was initially proposed by Björkling (Björkling, 1990). The mechanism proposed for peroxidation consists of a strict order entrance and outlet of substrates and products known as ping-pong bi-bi. First, the carboxylic acid must enter the CALB's active site pocket and be esterified with

the alcohol group of Ser105, liberating a water molecule in the process. Then, hydrogen peroxide can perhydrolyze the Ser105-Carboxylic acid ester leaving a peroxy acid as a product (Warwel, 2000). This system is conformed by two liquid phases: a continuous organic phase, containing the substrates of epoxidation, and a disperse phase of aqueous nature, which contains hydrogen peroxide. Some authors postulated that this reaction occurs in the organic phase by H_2O_2 molecules transferred from the aqueous droplets. (Orellana-Coca, 2005). However, reaction at the organic-aqueous double layer interface was also postulated (Schmid, 1998; Verger, 1997; Silvestrini, 2020).

2. MATERIALS AND METHODS

2.1. Materials

Candida Antarctica Lipase B IMMCALB-T1-350 from ChiralVision (Den Hoorn, The Netherlands) (particle size: 0.35 mm, activity: 11000 PLU/g) was used as the catalyst. The solvent utilized was n-hexane from Cicarelli (Santa Fe, Argentina). Hydrogen peroxide 30% w/w analytical grade solution (Taurus, Argentina) and octanoic acid p.a (Tetrahedron, India) were used as reagents. Potassium iodide, ammonium cerium (IV) nitrate, sodium thiosulfate, sulfuric acid 95-98%, and potassium hydroxide of analytical grade were used for the analytical procedure.

2.2. Methods

The experimental setup consisted of a 4.6 cm of i.d glass reactor, not baffled, with a maximum capacity of 100 ml. The reactor was submerged in a thermostatic bath to maintain the temperature at a constant value of $30 \pm 1^\circ\text{C}$. The system was mechanically agitated at 600 rpm with a helix-type agitator with 2.5 cm of diameter.

Peroctanoic acid synthesis runs were carried with 1.799 g of hydrogen peroxide aqueous solution 25% w/w (0.01323 moles of hydrogen peroxide). The amounts of octanoic acid fed to the reactor were 0.1073, 0.2267, 1.4421, and 2.8842 g (0.74, 1.57, 10, and 20 millimoles, respectively). The amount of n-hexane loaded on each essay was 52.245, 52.113, 50.768, and 49.173 ml, respectively, to produce a total reaction volume of 54 ml. For all experimental runs, 0.5 g of catalyst were used.

The sampling methodology consisted of stopping the mechanic agitation for 30 seconds to allow the complete separation of the liquid phases. Then, 0.5 ml of the upper phase (organic) were

sampled. The amount of peroctanoic acid produced was quantified by iodometric and cerimetric titration (Bhattacharya, 2011).

The Nelder-Mead algorithm of the function `fminsearch` in the software Matlab 8.4.0 was used to fit the kinetic parameters to the experimental data. First, the initial reaction rate was calculated from an order 2 polynomial fit of the initial condition of the system and the first 2 measurements of the reaction progress. The polynomial was derived concerning time and evaluated in time zero.

3. RESULTS AND DISCUSSION

3.1. Synthesis runs

Figure 1 shows the production of peroctanoic acid with time for each run.

Figure 1. mmoles of peroctanoic per gram of catalyst produced for each octanoic acid initial load. ●: 0.74 mmol; ○: 1.57 mmol; ▼: 10 mmol; △: 20 mmol.

The increase of the initial load of octanoic acid from 10 mmoles produced a decrease in the initial reaction rate. Therefore, the system exhibited an inhibitory effect by the octanoic acid. The initial reaction rate at 20 mmoles loading was the lowest, with a value of $0.0244 \text{ mmol g}^{-1} \text{ min}^{-1}$. At the 120 min of the reaction, the value of the reaction rate raised to $0.033 \text{ mmol g}^{-1} \text{ min}^{-1}$ despite the consumption of the substrate, showing that the octanoic acid initial amount is a critical parameter to calibrate. In a dynamic system where the peroxy carboxylic acid is used as an intermediary for an epoxidation series reaction, its consumption will turn the peroxy acid synthesis back to an initial rate condition. Therefore, the maximization of the initial rate for peracidation rather than the chemical equilibrium will probably increase the productivity

of the epoxidation.

3.2. Kinetic-thermodynamic model

A Michaelis-Menten ping-pong bibi mechanism with an octanoic acid reversible inhibition term was proposed to fit the initial rate of reaction (Eq. 2).

$$V_0 = \frac{k_r a_A a_B}{K_{M,B} a_A \left(1 + \frac{a_A}{K_{i,A}}\right) + K_{M,A} a_B + a_A a_B} \quad (\text{Eq. 2})$$

Where a_A is the initial activity of octanoic acid, a_B is the initial activity of hydrogen peroxide, $K_{M,B}$ is the Michaelis-Menten constant of hydrogen peroxide, $K_{M,A}$ is the Michaelis-Menten constant of octanoic acid, $K_{i,A}$ is the inhibitory constant of octanoic acid and k_r is the main kinetic constant.

The kinetic law is evaluated in the initial thermodynamic activities of the reaction substrates. This methodology is known as TABEK (thermodynamic activity-based enzyme kinetic), which was used to fit kinetic data of lipase-catalyzed esterifications (Castillo, 2016; Sandoval, 2002). In order to use this procedure is required to assume that interfacial diffusional resistances do not affect the reaction rate and that all species in both liquid phases are in thermodynamic equilibrium. However, considering this is not necessary to assume in which liquid phase or interface the reaction is taking place because the activity has the same value in both liquid phases.

Table 1 shows the value of the initial thermodynamics activities of the substrates for each run.

Table 1. Initial activities of the reaction substrates on each essay - UNIFAC 30 °C

Oc. Acid initial load (mmol)	a_{acid}	$a_{\text{H}_2\text{O}_2}$	a_{water}
0.74	0.0073	0.1325	0.8438
1.57	0.0152	0.1326	0.8438
10	0.0847	0.1326	0.8435
20	0.1471	0.1329	0.8430

The thermodynamic model UNIFAC was used for the phase stability analysis and the calculation of the initial activities (Fredenslund, 1975). In Table 2 are summarized the activity coefficients of the reagents on each octanoic acid loading condition. These UNIFAC predictions indicate the deviation of the ideal behavior. As the activity coefficient becomes higher than 1, more non-ideal is the system.

Since hydrogen peroxide is added as an aqueous solution to the system, water activity is

different from zero at the initial time. However, since no peroctanoic acid is present, the backward reaction can be neglected at time zero. As shown, the initial activities of water and hydrogen peroxide were maintained practically constant, and the octanoic acid activity increases according to the increased feeding from run to run. As the initial activity of water is constant, any effect that it may have on the initial reaction rate can be neglected in the kinetic model (Sandoval, 2001).

Table 2. Initial activity coefficients on each phase of the reagents – UNIFAC 30 °C

Oc. Acid initial load (mmol)	Aqueous phase		Organic phase	
	$\gamma_{\text{Oc. Acid}}$	$\gamma_{\text{H}_2\text{O}_2}$	$\gamma_{\text{Oc. Acid}}$	$\gamma_{\text{H}_2\text{O}_2}$
0.74	270.6	0.891	3.791	8240
1.57	270.2	0.891	3.740	7667
10	266.3	0.890	3.280	4007
20	262.5	0.890	2.848	2139

Figure 2 shows the initial experimental velocities and the model estimation as a function of the initial activity of octanoic acid. These experiments were utilized to obtain the kinetic parameters (Table 3).

Figure 2. Experimental and model estimation of initial reaction rate vs. octanoic acid activity.

Table 3. Kinetic parameters

k_r (mmol g ⁻¹ min ⁻¹)	$K_{M,A}$	$K_{M,B}$	$K_{i,A}$
0.9561	0.1324	0.2466	0.0067

The inhibitory effect of butyric acid was reported previously in the literature (Dutta Banik, 2016). However, it is an uncommon behavior and apparently occurs around certain concentrations

of carboxylic acids. Therefore, the inhibition mechanism of carboxylic acids is not specified. Considering the reaction mechanism proposed, where the octanoic acid is the first substrate that interacts with the active site, the inhibitory effect should not be due to octanoic acid interfering with the normal functionality of the active site amino acids. It is assumed that the inhibitory mechanism could be that octanoic acid interacts with another structure of the enzyme, minimizing its catalytic activity. The other possibility is that the large amounts of octanoic acid are competing to enter the active site leading to a steric problem. This behavior indicates the existence of an optimum initial octanoic acid activity for each initial hydrogen peroxide activity, and it can be evaluated with equation 3.

$$a_A = \sqrt{\frac{K_{iA}K_{mA}a_B}{K_{mB}}} \quad (Eq. 3)$$

This equation is obtained deriving the initial velocity concerning the octanoic acid activity, with constant hydrogen peroxide activity and equating to zero.

For the particular initial hydrogen peroxide activity used in this work, the optimal initial activity of the octanoic acid, obtained from equation 2, is 0.02184, which corresponds with an initial load of 2,2024 mmole (0.3176 g).

4. CONCLUSIONS

The TABEK methodology results in a satisfactory tool to fit the experimental data of the initial peroctanoic synthesis rate considering its inhibitory effect in the reaction. The mechanism of the inhibitory effect of the carboxylic acid is still undetermined and needs more deep investigation in further works.

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