Thermodynamic parameters for the enzymatic epoxidation of sunflower oil at atmospheric pressure with enzyme incubated at 20 MPa in SC CO_2

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Abstract

The stability and activity of lipase from Candida antarctica in supercritical carbon dioxide (SC CO_2) at 200 bar and different temperatures was studied. After incubation in SC CO_2 the enzyme was used as biocatalyst for epoxidation reaction of sunflower oil. On the basis of the results, was calculated some thermodynamic parameters of epoxidation reaction catalyzed by this lipase. Activation energy was determined from an Arrhenius plot.

The constant of deactivation was 0.14 after 6 hours of reaction and 0.202 after 24 hours, and the deactivation enthalpy was around 24,5 kJ/mol.

The Arrhenius equation gives the quantitative basis of the relationship between the activation energy and the reaction rate at which a reaction proceeds.

Keywords: lipase, enzymatic epoxidation, stability, high pressure batch stirred tank reactor, Arrhenius diagram, thermodynamic properties.

Introduction

The use of enzyme in supercritical fluids has been proposed as a means of improving the activity of enzymes in anhydrous environments [8]. The advantages of supercritical fluids as solvents include environmental benefits, health and safety benefits, process and chemical benefits [7].

The enzymatic reactions take place under mild reaction conditions, high catalytic efficiencies and can be obtained pure products with lower energy input.

The stability of enzymes under high pressure is interesting by theoretically and practically point of view. Deactivation of the enzymes and as consequent loss in their activity is determined by the changes in spatial structure of the proteins. Using enzymes in high pressure batch reactors may cause changes in biocatalyst activity due to the pressurization / depressurization steps.

On the basis of the results involving the enzymatic epoxidation of sunflower oil at atmospheric pressure and 40°C, we try to determine some thermodynamic parameters (activation energy, Gibbs energy, enthalpy and entropy of deactivation, deactivation constant) of the reaction that was performed with enzyme incubated in a HP BSTR in SC CO_2 at 20 MPa.

Activation energy can be determined from an Arrhenius plot and the others properties were calculated with specific relations [1].

Materials and methods

Enzymes and chemicals

Lipase (EC 3.1.1.3.) immobilized on a macro-porous anion exchange resin was a gift from Novo Nordisk A/S (Copenhagen, Denmark). Sunflower oil and starch were used as commercial products without any additional changes. Supercritical carbon dioxide (with 99.995 % volume pure) was supplied by Messer MG (Ruše, Slovenia). Oleic acid with 99 % purity and all the other chemicals were from Merck KDaA (Darmstadt, Germany), Riedel-de Haen (Seelze, Gemany) and Aldrich Chemical Co. (Diesenhofen,Germany).

Determination of lipase thermal stability in SC CO_2

Lipase from *Candida antarctica B* was incubated in SC CO_2 at 20 MPa, in the high-pressure batch stirred-tank reactor at different temperatures for 24 hours (Figure 1) [10, 2].

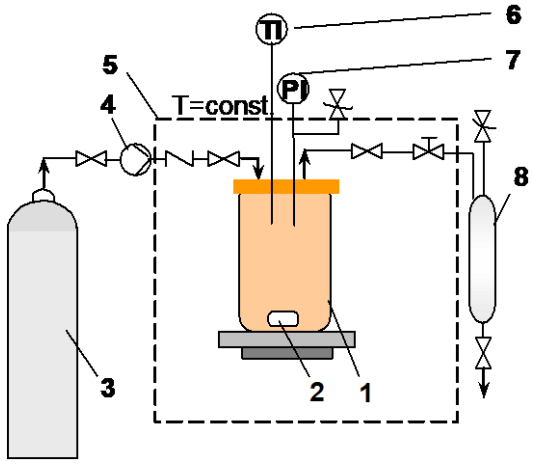

Figure 1. Design of High Pressure Batch Stirred Tank Reactor (HP BSTR)

1 – Bioreactor;
2 – Magnetic stirrer;
3 – Feed of SC CO_2;
4 – High pressure pomp;
5 – T = constant;
6 – Temperature indicator;
7 – Pressure indicator;
8 – Separator.

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Results and discussion

Stability of lipase from Novozym®435 at 20 MPa in SC CO_2

After slow depressurization, the pre-incubated enzyme was used as biocatalyst for epoxidation reaction of sunflower oil, at atmospheric pressure. Optimal reaction parameters was established in a previously paper (lipase amount 0.8 g, temperature 40°C, volume of H_2O_2 2,592 mL that was added slowly, 400 rpm) [3,13,14].

Thermodynamic parameters

As the temperatures increases, the atoms of enzyme molecule have more energies and more tendency to move. To keep the globular protein structure together, the atoms need sufficient energy to overcome the weak interactions [1]. In this experimental work the equilibrium between inactive and active forms of enzyme is at 40°C. At temperatures above 40°C, the deactivation enthalpy, ΔH_d, is higher than the energy of activation. Thermal deactivation of enzymes can be reversible, irreversible or a combination of the two. A simple model of reversible thermal deactivation often suffices to represent thermal activity data for enzyme over large range of temperatures. If we assume that to the equilibrium the enzyme exists in active and inactive form, we can calculated K_d.

In the figure 2 are showed the concentrations of epoxi-acids obtained for epoxidation reaction with enzyme incubated at 20 MPa in SC CO_2 and different temperatures. The

concentrations between 32°C and 40°C are almost closed, but the values at 50°C are with 47.3 %, respectively 48.3 % much lower.

Evolution of concentrations between 6 and 24 hours is: at 32°C the values for concentrations are increasing with 23 % at each 12 h of reactions; at 40°C the increasing is with 24 % and at 50°C with 26.5 %. These values are motivated by the optimal temperature of enzyme action which was demonstrated to be at 40°C. Anyway, the values at 50°C are with 40 % much lower than at 32°C and with 42.3 % than at 40°C.

The regression coefficients and the regression lines are presented in figure 1 and were demonstrated with statistical program Data Analysis (table 1).

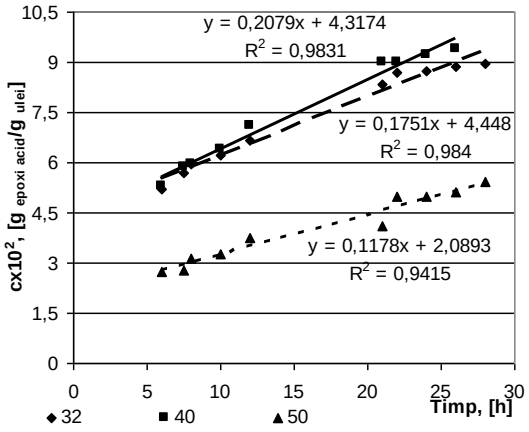

Figure 2. Concentrations of epoxi-acids for epoxidation reactions in time (enzyme incubated at 20 MPa)

Table 1. Regression coefficients for epoxidation of sunflower oil with enzyme incubated in SC CO_2 at 20 MPa

t = 32°C	*Regression*		*t = 40°C*	*Regression*	
R^2 = 0,984656	*Coefficients*	*Error standard*	R^2 = 0,983056	*Coefficients*	*Error standard*
Intercept	4,336252	0,144565	Intercept	4,317422	0,174603
X Variable 1	0,185434	0,00945	X Variable 1	0,207936	0,010318

t = 50°C	*Regression*	
R^2 = 0,941508	*Coefficients*	*Error standard*
Intercept	2,095214	0,186902
X Variable 1	0,117239	0,011045

All the values obtained by analytical or graphical methods for R^2 are the same and are higher (R^2 = 0.94) and, in mean time, the errors standards are lower. So, we can concluded that between experimental and regression values was good correlations.

For estimation of the thermodynamic parameters we considered all the values for initial rates which were plotted for two times of reaction (after 6 hours and 24 hours) in Arrhenius plots (figure 2 and figure 3).

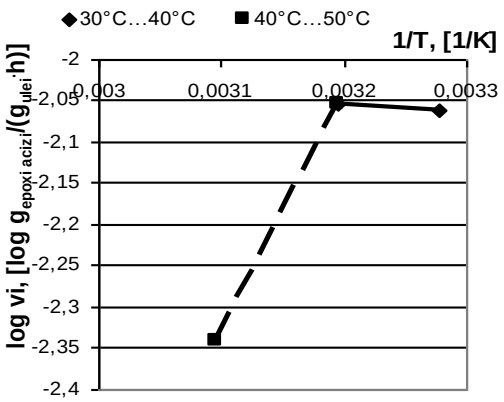

Figure 2. Arrhenius plot for epoxidation of sunflower oil with enzyme treated in SC CO_2 at 20 MPa *(after 6 h of reaction)*

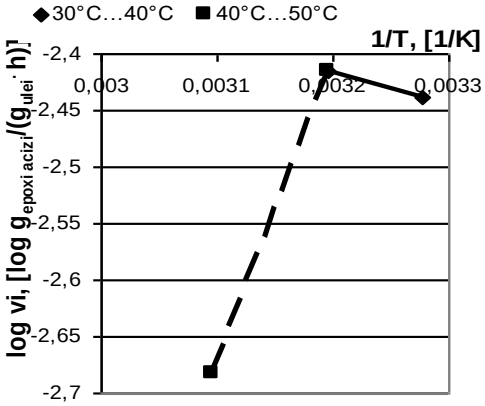

Figure 3. Arrhenius plot for epoxidation of sunflower oil with enzyme incubated in SC CO_2 at 20 MPa *(after 24 h of reaction)*

All the thermodynamic parameters were calculated because we can consider that a simple model of reversible thermal deactivation often suffices to represent thermal activity relationships for enzymes over a wide range of temperatures [1]. We assumed that the lipase existed in inactive and active forms in equilibrium, with equilibrium constant K_d. Considering the slope of the Arrhenius diagrams at higher and lower temperatures was estimated all thermodynamic parameters (table 2).

Generally, for the reactions with enzyme incubated at higher pressures the energy of activation is much lower, so the influence of temperature is much lower. But, at maximum pressure 300 bar the effect is different.

Table 2. Thermodynamic values for epoxidation of sunflower oil with Novozym 435 treated in SC CO_2 at 20 MPa

Thermodynamic parameter	***Symbols and U.M.***	***Values at***	
		6 hours	***24 hours***
Activation energy	E_a, kJ/mol	12,9	2,364
Deactivation enthalpy	ΔH_d, kJ/mol	24,57	24,64
Deactivation constant	K_d	0,14	0,202
Free energy of deactivation	ΔG_d, kJ/mol	5,17	4,17
Entropy of deactivation	ΔS_d, kJ/(mol·K)	0,062	0,065

The activation energy after 24 h of reaction is 2, 36 kJ/mol, which is lower with 82 % than the same reaction, but with enzyme incubated at 100 bar. In the mean time, the deactivation constant is much higher with 31 %, the effect which determined decreasing of free energy of deactivation with 10 %.

If all thermodynamic parameters are calculated after 24 h of reaction, the energy of activation is increasing with 23 % between 24 h and 48 h and with 93 % after 50 h. So, we can conclude that all enzymatic reactions with higher activation energy are much slower.

Conclusions

- In literature it is shown that, for the synthesis reaction of the oleil oleat esther at pressure values higher than 300 bar, it is noticed a decrease of the deactivation enthalpy values of up to 21.11 kJ/mol [10].

- In the case of the enzymatic system under analysis in the present paper, it is noticed a decrease of the ΔH_d values of up to 24 kJ/mol at 200 bar. Thus, the deactivation enthalpy of the system containing untreated enzyme is smaller by 62 % as compared to the system that contains enzyme treated in SC CO_2 at 10 MPa, and by only 13 % smaller than the system that contains enzyme treated at 20 MPa. This indicates the influence of temperature and pressure on the activity of the enzyme treated in SC CO_2.
- As compared to the reaction developed with unincubated enzyme, the E_a values increase by 69 % for the reaction developed with enzyme incubated at 10 MPa, and by 75.5 % for the reaction developed at 20 MPa. Increases in pressure by 100 bars determine an increase of the activation energy by 21 %.
- This can be seen from the Arrhenius diagrams, in which in the interval 30°C to 40°C is noticed activation of the enzyme, but for temperatures bigger than 45°C the lipase deactivation occurs.
- Enzyme deactivation is also justified by the bigger ΔH_d values, as compared to those for E_a, values that determine a decrease of activity, with an increase in temperature of more than 40°C.
- The positive value of the free deactivation energy for the enzyme treated in SC CO_2 indicates a favorable reaction.

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