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Approximation of the thermodynamic parameters for the sunflower seed oil epoxidation reaction with enzyme treated under atmospheric pressure

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Abstract

After determination of optimal parameters for epoxidation reaction at atmospheric pressure we proposed to calculate some thermodynamic parameters of this enzymatic reaction. Activation energy was determined from Arrhenius plots on the basis of initial rates. Activity of Novozym 435 increased between 30 and 45°C, but with further temperature increase thermal deactivation occurred. The thermal deactivation constant was 0.26 after 6 hours of reaction and 0.2 after 24 hours, and the deactivation enthalpy was around 20 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, enzyme deactivation, Arrhenius diagram, thermodynamic properties.

Introduction

Lipases (or triacylglycerol acylhydrolases) belong to the class of hydrolases and usually they do not require the presence of cofactors. In the case of fungal lipases the production is in correlation with the availability of triglycerides. Besides this, free fatty acids, salts or glycerol stimulate lipase production [17]. The lipase concentration depends by optimal substrate composition. So, recently, was studied the effect of inorganic nitrogen and phosphorous sources on hydrolase's production by a *Bacillus subtilis* strain [1].

The determination of the thermodynamic parameters for any enzymatic reaction is in close connection with the enzyme activity. In the case of lipases activity and stability, an optimal domain of temperatures between 35...45°C is considered. Thus, for example, the pancreatic lipase loses its activity for temperatures higher than 40°C, whereas some microbial lipases are much more resistant at high temperatures.

It is considered that lipases released by *Aspergillus niger, Rhizopus javanicus* are stable at 50°C. The lipases of the thermal-tolerant species as *Humicola lanuginosa*, *Psedudomonas* sp. are stable at 60°C and 70°C as well. However, the lipases of *Candida* genus show half-cooling times much smaller with increasing the temperature. For example, the lipase released by *Candida gigantea* shows half-cooling times for 45°C, 50°C and 55°C: 35,7 min., 46,4 min., and, respectively, 22,9 min. The maximum of activity for lipases by *Candida gigantea* is between 30...35°C, whereas the lipases released by *Aspergillus terreus* maintain 100 % activity at 60°C for 24 hours [2,16].

In the case of enzymatic epoxidation reaction catalyzed by the immobilized lipase released by *Candida antarctica,* it is considered that the approximation of the thermodynamic

parameters begins after finalizing the addition of the whole solution amount of H_2O_2 35 %, that is after 6 hours.

The importance of estimation of thermodynamic parameters is due to influence on the position of equilibrium of an enzyme catalyzed reaction and on the yield of product that can be expected. All these factors determine free energy changes of reactions and variations of activation energy.

Materials and methods

Enzymes and chemicals

Commercial lipase – Novozym 435 (EC 3.1.1.3.) in immobilized form on a macro-porous anion exchange resin was kindly donated by Novo Nordisk A/S (Copenhagen, Denmark). The commercial sunflower oil, oleic acid with 99 % purity and starch were used as they were received.

Supercritical carbon dioxide (with 99.995% volume pure) was supplied by Messer MG (Ruše, Slovenia). All the other chemicals were from Merck KDaA (Darmstadt, Germany), Riedel-de Haen (Seelze, Gemany) and Aldrich Chemical Co. (Diesenhofen,Germany).

Results and discussion

The thermodynamic properties of the lipase catalyzed epoxidation reaction were estimate on the basis of the concentrations of the epoxi acids that was achieved (figure 1). The regression coefficients were estimated by analytical (using statistical program data Analysis – table 1) and graphical methods. Activation energy can be properly determined from an Arrhenius plot.

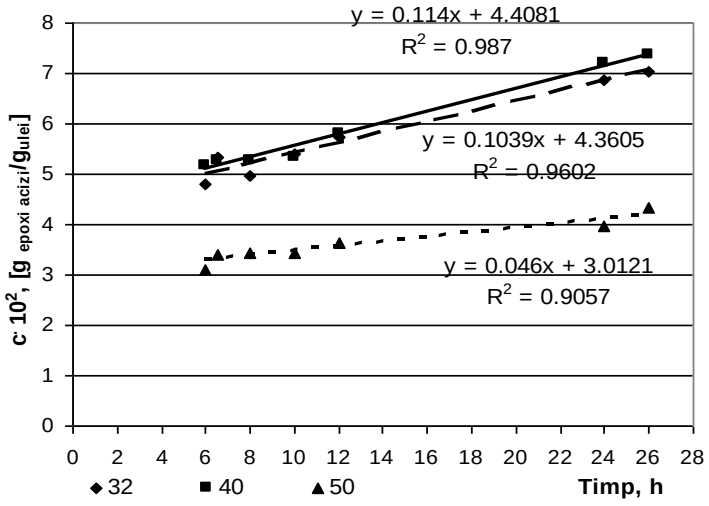

Figure 1. The concentrations of epoxi acids between 6 - 24 hours

Table 1. Estimation of regression coefficients for epoxidation reaction of sunflower oil with enzyme untreated in supercritical conditions

t = 32°C	*Regression*		*t = 40°C*	*Regression*	
R^2 = 0,960248	*Coefficients*	*Error standard*	R^2 = 0,987027	*Coefficients*	*Error standard*
Intercept	4,360505	0,144565	Intercept	4,408089	0,089403
X Variable 1	0,103854	0,00945	X Variable 1	0,113982	0,005844

t = 50°C	*Regression*	
R^2 = 0,905674	*Coefficients*	*Error standard*
Intercept	3,01207	0,101577
X Variable 1	0,046005	0,00664

From figure 1 and table 1 it is to be noted a good correlation between experimental values and lines of regression bigger than 90 % (R^2 = 0.9057 for a temperature of 50°C), the experimental results thus being correlated also with very small values of standard errors.

In this case, the X variable from table 1 represents the concentration of reaction products. At a certain time of developing the enzymatic reaction (e.g. after 6 hours) it can be noticed a decrease by 32 % of the concentration, together with an increase in the temperature from 32°C to 50°C and a bigger decrease, of 39.72 % respectively, in the same time with the temperature variation from 40°C to 50°C. This effect is due to the temperature increase over the optimal value for the lipase action, which is 40°C. It is appreciated that, past this temperature value, the lipase is deactivated, the tendency of decreasing the concentration value being maintained also after 24 hours, a case in which it can be noticed a decrease by 41.3% when the temperature increases from 40°C to 50°C.

With respect to the time evolution of concentrations for each temperature, a value increase was noticed, so that for all temperatures the concentrations value after 24 hours are approximately 30 % higher as compared to those obtained after 6 hours.

The time and temperature influence on the concentration of reaction products for the reaction interval situated in between 6–26 hours was analyzed by using the ANOVA two-way method (table 2).

In this regard two hypotheses are presented: a null hypothesis, H_0, according to which the values are not significantly influenced by the cause factors, and an alternative hypothesis H_1 which presents the significant influence of the values by the tested variables.

Table 2. Bifactorial Anova analysis for the study of time and temperature influence on the concentration of reaction products

ANOVA						
Variation sources	*SS*	*df*	*MS*	*F*	*P Value*	F_{critic}
Time	9,823780952	6	1,637297	15,17499	$5{,}66 \times 10^{-5}$	2,996117
Temperature	22,77526667	2	11,38763	105,5442	$2{,}42 \times 10^{-8}$	3,88529
Error	1,294733333	12	0,107894			
Total	33,89378095	20				

From table 2 it is to be noticed a certain influence of the two cause-factors, time and temperature, on the tested values of concentration. Thus, an alternative hypothesis H_1 is taken into consideration, with a probability of 95 %, as the $F_{calculated} >> F_{critical}$ in the case of both variation sources.

The values obtained for the concentrations of reaction products allow for the calculation of the initial reaction velocities that are represented in semi-logarithmical coordinates according to the inverse of the thermodynamic temperature in Arrhenius diagrams (figure 2 and figure 3).

For the estimation of thermodynamic parameters were used the following relations:

- ✓ In an Arrhenius plot, *log v* is graphed against *1/T* to give a straight line with a slope of $-E_a / R$ (the error is equal with T, that is usually not significant). With the value of the slope can be calculated the activation energy, E_a, J/mol.
- ✓ The slope of the other line obtained at higher temperature was $\frac{\Delta H_d - E_a}{R}$, so can be estimated the enthalpy of deactivation, ΔH_d, J/mol.
- ✓ Thermal deactivation of enzyme may be reversible, irreversible or a combination of the two. The constant of deactivation, K_d was calculated with relation:

$$k_d = \frac{E_a + R \cdot T_{max}}{\Delta H_d - \frac{E_a}{R \cdot T_{max}}} \quad (1)$$

- ✓ The free energy of deactivation, ΔG_d, J/mol was calculated from relation:

$$k_d = e^{-\frac{\Delta G_d}{R \cdot T}} \quad (2)$$

- ✓ The entropy of deactivation, ΔS_d, kJ/(mol·K) may be estimated after noting that, at the temperature T_{max} the *log* v_{max} is maximized.

$$\Delta G_d = \Delta H_d - T \cdot \Delta S_d \quad \rightarrow \quad \Delta S_d = \frac{\Delta H_d - \Delta G_d}{T} \quad (3)$$

Where: T – maximal thermodynamic temperature, K.

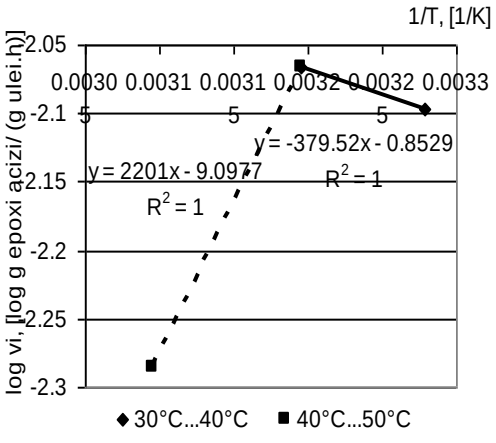

Figura 2. Arrhenius plot for epoxidation reaction with enzyme treated at atmospheric pressure (after 6 hours of reaction)

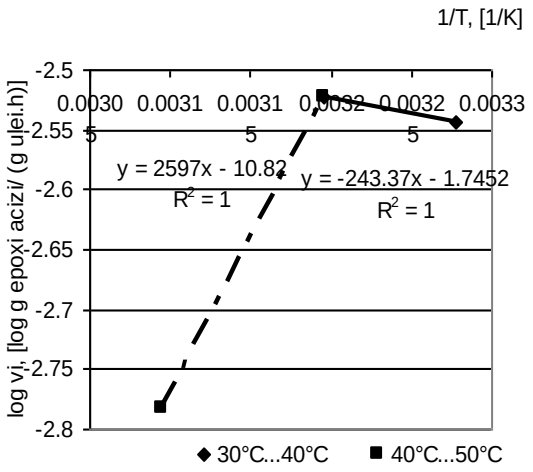

Figura 3. Arrhenius plot of epoxidation reaction with enzyme incubated at atmospheric pressure (after 24 hours of reaction)

Using all the relations presented below we present, in table 3, the thermodynamic parameters values calculated for epoxidation reaction with enzyme non-incubated in supercritical conditions.

Table 3. Thermodynamic Values for Epoxidation of Sunflower Oil with Novozym 435 at Atmospheric Pressure

Thermodynamic parameter	***Symbol and U.M.***	***Values at***	
		6 hours	***24 hours***
Activation energy	E_a, kJ/mol	3,15	2,20
Deactivation enthalpy	ΔH_d, kJ/mol	21,45	23,61
Deactivation constant	K_d	0,26	0,20
Free energy of deactivation	ΔG_d, kJ/mol	3,42	4,18
Entropy of deactivation	ΔS_d, kJ/(mol·K)	0,57	0,6

The results in table 3 allow us to state that: an increase by 18 % of the free energy Gibbs and by 9 % of the deactivation enthalpy is noticed. On the other hand, the values of the activation energy decrease by 30 % after 24 h, while the deactivation constant remains unchanged for values almost constant.

If the calculation of the activation energy is confirmed, it is noticed an increase by 92 %, and 93 % respectively, for the values registered after 48 h and 50 h, as compared to those calculated at 24 h, which suggest that after two days the reaction develops much more slower. In these conditions it is appreciated that after 48 h or 50 h a halt of the reaction can be realized when the maximum of concentration of reaction products is accumulated.

Conclusions

- The deactivation enthalpy is bigger, thus the proportion of active enzyme is sensitive, depending on temperature variation. Because of the fact that the values $\Delta H > 0$, the reactions are endo-thermal, thus proving the increase of the equilibrium constant for high temperatures.
- 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.
- It is not justified the development of enzymatic reaction for sunflower seed oil epoxidation for more than 48 h or 50 h, because it was noticed an increase of the energy activation values for those time sets, as compared to those calculated at 24 h, a phenomenon that indicates that reactions are more slower after 48 h.
- In general, it is believed that at smaller values for K_d the enzyme is much more active. From the analysis of the obtained values, it is noticed that for the reaction with enzyme treated at 20 MPa (at 6 h) the K_d values are smaller by 47 % as compared to the system of un-incubated enzyme in SC CO_2, and by 39 % smaller as compared to the system with incubated enzyme at 10 MPa in supercritical fluid.

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