ESTIMATION OF THE KINETIC PARAMETERS FOR H2O2 ENZYMATIC DECOMPOSITION AND FOR CATALASE DEACTIVATION

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Abstract - Catalase is a potentially useful biocatalyst in various industrial bioprocesses (textile industry, food processing, and pulp and paper) that require removal of hydrogen peroxide. This process can be achieved in such reactors even under isothermal conditions. However, it is usually connected with a long duration of the process or with spending a considerable amount of biocatalyst for a unit mass of the transformed substrate, which in turn leads to an increase in operating costs. They can be limited by applying the optimal temperature control, which requires the values of the thermodynamic parameters - the activation energy for reaction and the activation energy for deactivation must be known. This work reports these parameters for hydrogen peroxide decomposition and Aspergillus niger catalase deactivation at temperatures ranging from 35°C to 50°C.

Keywords: Aspergillus niger catalase; Deactivation; The activation energy for deactivation.

INTRODUCTION

Catalase (EC 1.11.1.6) decomposes hydrogen peroxide to water and oxygen

\[ 2\text{H}_2\text{O}_2 \xrightarrow{\text{catalase}} \text{O}_2 + \text{H}_2\text{O} \]  

(1)

The catalytic reaction takes place in two steps. The first hydrogen peroxide molecule oxidizes the heme to an oxyferryl species in which one oxidation equivalent is removed from the iron and one from the porphyrin ring to generate a porphyrin cation radical (reaction 2a). The second hydrogen peroxide is then used as a reagent with compound 1 - \( \text{Enz}(\text{Por}^\text{III} - \text{Fe}^\text{IV}) = 0 \) to regenerate the resting state enzyme, water, and oxygen - reaction 2b (Switala and Loewen, 2002).

\[ \text{Enz}(\text{Por}^\text{III} - \text{Fe}^\text{IV}) + \text{H}_2\text{O}_2 \rightarrow \text{H}_2\text{O} + \text{Enz}(\text{Por}^\text{II} - \text{Fe}^\text{III}) = 0 \]  

(2a)

\[ \text{Enz}(\text{Por}^\text{II} - \text{Fe}^\text{III}) = 0 + \text{H}_2\text{O}_2 \rightarrow \text{H}_2\text{O} + \text{O}_2 + \text{Enz}(\text{Por} - \text{Fe}^\text{II}) \]  

(2b)

Industrial applications for catalase include removal of hydrogen peroxide after cold sterilization steps in food processing (Farkye, 2004; Lee, 2004; Tarhan, 1995). Catalase has also been used in the manufacturing of semi-conductors (Akyilmaz and Kozgus, 2009; Liu et al. 2016) and in the textile industry (Arabaci and Usluoglu, 2013; Costa et al. 2002). Moreover, catalase is indispensable for carrying out biotransformation processes with the use of oxidases to cause decomposition of hydrogen.
peroxide being formed in the reaction (Ene and Maria, 2012; Maria et al. 2012).

Deactivation of catalase by the substrate is a significant limitation for any broader use of catalase. Previous attempts to stabilize *Aspergillus niger* catalase by immobilization have not been successful enough as yet (Grigoras, 2017; Hooda, 2014; Milek et al. 2011; Yoshimoto et al. 2005).

In the present study the deactivation of catalase by hydrogen peroxide was examined. It is not possible to analyze catalase deactivation by hydrogen peroxide decomposition in a batch reactor without knowing the enzymatic reaction kinetics. Catalase from *Aspergillus niger* appears to show the dependence of reaction rate $v_R$ on concentration $C_S$ observed for the Michaelis-Menten equation:

$$v_R = \frac{v_{max} C_S}{K_m + C_S}$$  \hspace{1cm} (3)

where: $V_{max} = k_R C_E$, is the maximum reaction rate, $K_m$ is the apparent Michaelis-Menten constant. $K_m$ values, as determined for catalase from *Aspergillus niger*, are in the range 0.322-0.465 mol/L (Lardinois et al. 1996; Switala and Loewen, 2002). Hence, for typical applications of catalase in decomposition of residual hydrogen peroxide, for which the concentration is lower than 0.02 mol/L (Arvin and Pedersen, 2015; Herdt, 2012; Ghadermarzi and Moosavi-Movahedi, 1996) Eqn (3) is simplified and assumes the following form:

$$v_R = k_R C_E C_S$$  \hspace{1cm} (4)

where $k_R$ is the reaction rate constant, L/(mol·h).

When conducting the study of decomposition kinetics of hydrogen peroxide by catalase we need to take into account the phenomenon of deactivation. Georg (1947) was the first to propose the following experimental kinetic equation which describes the catalase deactivation rate $v_D$:

$$v_D = C_E \left( \frac{a C_S}{b + C_S} + c C_S \right)$$  \hspace{1cm} (5)

where $a$, $b$, and $c$ are experimental constants.

The above equation for hydrogen peroxide concentrations $C_S < b$ is simplified to obtain a first order reaction with respect to the substrate and the enzyme concentrations:

$$v_D = k_D C_E C_S$$  \hspace{1cm} (6)

where $k_D$ is the deactivation rate constant, L/(mol·h).

The value of the $b$ constant, as established by Georg (1947) for catalase from erythrocytes, was 0.15 mol/L, which means that, for substrate concentrations below 0.015 mol/L Eqn (5) can be well approximated Eqn (6). This kinetic equation of deactivation was used a number of times in studies on immobilized catalase of either animal or microbiological origin (DeLuca et al., 1995; Herdt, 2012; Milek and Wójcik, 2011; Tarhan, 1995; Tse and Gough, 1987, Vasudevan and Weiland, 1990).

Vasudevan and Weiland (1990) studied deactivation of catalase from beef liver and from *Aspergillus niger* by hydrogen peroxide. Experiments were conducted in a continuous stirred tank reactor (CSTR) at a temperature of 25°C and initial concentration of hydrogen peroxide in the range from 0.05 to 1 mol/L.

DeLuca et al. (1995) studied deactivation of native catalase from beef liver and from *Aspergillus niger*. They used Eqn (6) for analysing hydrogen peroxide decomposition at a temperature of 25°C and at hydrogen peroxide initial concentration 0.02 mol/L. The reaction rate constant for deactivation of catalase from *Aspergillus niger* $k_D$ equals 0.00851 L/(mol·s), 17 times lower than that for beef catalase.

Lardinois et al. (1996) also confirmed that the deactivation of catalase from *Aspergillus niger* proceeded according to Eqn (6). Agreement of experimental data with this kinetic equation was obtained for a very wide range of hydrogen peroxide concentrations (0.01- 2 mol/L) but at the temperature of 25°C.

The effect of the deactivation of *Aspergillus niger* catalase by hydrogen peroxide in a wider range of temperatures has not been shown in the literature yet. The present studies were conducted with the concentration of hydrogen peroxide lower than 0.015 mol/L at temperatures ranging from 35°C to 50°C. The obtained parameters for deactivation of *Aspergillus niger* catalase by hydrogen peroxide can be used in modeling and optimization of batch bioreactors (Grubecki, 2016; Vasić-Rački et al., 2011).

**MATERIALS AND METHODS**

**Reagents**

Catalase (E.C. 1.11.1.6) from *Aspergillus niger* was purchased from Sigma-Aldrich (No. catalog C3515). Perhydrol (30% hydrogen peroxide) was procured from POCH, Poland. All other chemicals used were of analytical quality.
Reaction study procedure

Assay of the rate reaction for the decomposition of hydrogen peroxide was measured at the temperatures 20°C, 25°C, 30°C, 35°C, 40°C, 45°C and 50°C. In the spectrophotometer tank (isothermal batch reactor) was placed 2 mL of hydrogen peroxide of concentration 0.015 mol/L, pH 6.9, and catalase added (30 U/mL). The mixture was gently homogenized and incubated at the reaction temperature for 1 min. Catalase activity was monitored by observing the rate of decomposition of hydrogen peroxide using a spectrophotometer UV - VIS JASCO V -530. Absorbance of H₂O₂ was measured at a wavelength of 240 nm (Bayramoglu et al., 2016). From the change of the concentration of hydrogen peroxide in the time from 0 s to 15 s, which has a linear relationship, the values of reaction rate constant were determined.

Deactivation study procedure

Assay of the rate of deactivation catalase by hydrogen peroxide was measured at the temperatures 35°C, 40°C, 45°C and 50°C, respectively. The reaction was carried out in Erlenmeyer flasks (250 mL) in a water bath shaker (250 rpm). Reaction volume was 200 ml hydrogen peroxide at an initial concentration of 0.015 mol/L and pH 6.9 and different amounts of catalase were used (2.5 U/mL or 5.0 U/mL).

All the deactivation experiments were pre-incubated (1.5 h) in the water bath shaker to set the reaction conditions. Samples of the reaction solution were taken out at intervals of 20 minutes and the conversion of hydrogen peroxide by catalase was measured spectrophotometrically. Deactivation rate constants kᵦ were determined on the basis of the conversion of hydrogen peroxide at an initial concentration of 0.015 mol/L and pH 6.9 at temperatures of 35°C, 40°C, 45°C and 50°C.

Kinetic model

Based on an analysis that was conducted in the theoretical part it was assumed that a kinetic equation for the reaction (Eqn 4) and a kinetic equation for deactivation (Eqn 6) may be applied for the description of hydrogen peroxide decomposition at an initial concentration of 0.015 mol/L. The mass balance for the substrate and active catalase in an isothermal batch reactor leads to a system of two ordinary differential equations:

\[
\frac{dC_S}{dt} = - kSC_SE_S \tag{7}
\]

Initial conditions were defined as follows: \( C_S (t = 0) = C_{S0} \) and \( C_E (t = 0) = C_{E0} \). When the dimensionless activity of catalase is \( a = C_E / C_{E0} \), then Eqs (7) and (8) become:

\[
\frac{dC_S}{dt} = - k_a aC_S \tag{9}
\]

\[
\frac{da}{dt} = - k_0 aC_S \tag{10}
\]

where: \( k_a = k_E k_R \) initial conditions \( C_S (t = 0) = C_{S0} \) and \( a (t = 0) = 1 \).

Further, it is convenient to introduce the fractional conversion \( X = (C_{S0} - C_S)/C_{S0} \) and the system of eqs. (9)-(10) can be written:

\[
\frac{dX}{dt} = k_a a(1 - X) \tag{11}
\]

\[
\frac{da}{dt} = - k_0 aC_S(1 - X) \tag{12}
\]

with initial conditions: \( X(t = 0) = 0 \) and \( a (t = 0) = 1 \).

Dividing eq. (11) by (12) and using \( a(t=0)=1 \) and \( X(t=0)=0 \) for a reaction that proceeds in a batch reactor, it defines the relationship between activity and conversion

\[
a(X) = 1 - \frac{k_a}{k_0} C_SX \tag{13}
\]

where \( k_a = k_E k_R \).

A change in the conversion with time, \( t \), is described by the following relationships:

\[
X = 1 - \frac{1 - \exp[(k_a - k_0 \cdot C_S)t]}{C_S k_0 - \exp[(k_a - k_0 \cdot C_S)t]} \quad \text{for} \quad k_0 C_S \neq k_a \tag{14a}
\]

\[
X = 1 - \frac{1}{(k_a \cdot t + 1)} \quad \text{for} \quad k_0 C_S = k_a \tag{14b}
\]

Preliminary analysis of the experimental data showed that the reaction rate constant \( k_a \) and deactivation rate constant \( k_a \) in Equations (14a) and (14b) are strongly correlated and the reaction rate constant \( k_a \) changes much less with changes in temperature than do the rate constants for typical enzymatic reactions. Therefore, independent spectrophotometric measurements of the rates of hydrogen peroxide decomposition for reaction times below 1 minute were made, using many times as high catalase concentrations. Such conditions enable enzyme deactivation by the substrate to be practically eliminated. The reaction rate constant \( k_a \) at the initial
concentration of hydrogen peroxide of 0.015 mol/L and in the temperature range from 20°C to 50°C and changed every 5°C, was thus established.

For known values of $k_R^*$, based on Equation (14a) the values of $k_D$ were found using nonlinear regression with the Levenberg-Marquardt procedure (Freitas et al. 2012; Frutiger et al. 2016). It is a standard technique used to solve nonlinear equation by the least squares method and is the most popular alternative to the Gauss-Newton method of finding the minimum of the sum of the squares. If experimental values are expected to vary along the curve, then the points should be weighted differentially. The most often used weighting method is called "weighting by $1/y^2$" and in this case it is described as follows:

$$\text{SEE}(k_d) = \sum_{i=1}^{n} \left( \frac{1}{(X_{exp})_i} - (X_{cal}(k_D,t))_i \right)^2$$  \hspace{1cm} (15)$$

where $(X_{exp})_i$ is the conversion of hydrogen peroxide by Aspergillus niger catalase determined experimentally; $X_{cal}(k_D,t)$ is the conversion of hydrogen peroxide by Aspergillus niger catalase calculated by Eqn (14a).

Eqn (15) allows one to find the objective function with a given set of parameters. The obtained values of the parameters were calculated using nonlinear regression with SigmaPlot 12.3.

RESULTS AND DISCUSSION

The present study shows the effect of temperature on the process of hydrogen peroxide decomposition by catalase from Aspergillus niger to complete limited information on the subject in the literature. Tse and Gough (1987) and also DeLuca et al. (1995) analyzed hydrogen peroxide decomposition by Aspergillus niger catalase at the temperatures of 25ºC and 37ºC, respectively. However, the researchers did not report data for higher temperatures of the process, which are commonly used in industry.

On the basis of this study, the reaction rate constants $k_R$ were determined for temperatures 20ºC, 25ºC, 30ºC, 35ºC, 40ºC, 45ºC and 50ºC. The rate of decomposition of 0.015 mol/L hydrogen peroxide was measured during 15 seconds. The dependence between the $k_R$ constant and temperature was in agreement with the Arrhenius equation and this dependence is presented in Figure 1.

The value obtained for the activation energy for the reaction, $E_a$, equals 12.9±0.7 kJ/mol. This value is similar to the activation energy for decomposition of hydrogen peroxide by catalase, found earlier for catalase Terminox Ultra (Milek and Wójcik, 2011). The activation energy was a little lower than that reported in the literature (19.7 kJ/mol) by Altamore (1974). Hooda (2014) reported that the value of the activation energy for decomposition of hydrogen peroxide equals 23.5 kJ/mol. However, the value of $E_a$ was also evaluated from an Arrhenius plot by plotting the inverse of temperature vs. the log of enzyme activity. The temperature of the reaction mixture was varied from 15°C to 75°C and, above the temperature of 35°C, the deactivation of catalase influenced the value of the activation energy $E_a$.

On the basis of the deactivation study, the deactivation rate constants $k_D$ were determined for the entire studied range of temperature (35-50ºC), using nonlinear regression with the Levenberg-Marquardt optimization procedure. The obtained $k_D$ values have been collected in Table 1. Comparison between calculated conversion and experimental conversion for all temperatures in the range from 35°C to 50°C was made and the standard errors of estimate SSE were lower than 0.07.

![Figure 1. Arrhenius plot for decomposition 0.015 mol/L hydrogen peroxide by Aspergillus niger catalase (30 U/mL).](image-url)
The increase in temperature caused an increase in the value of the rate constant for deactivation \( k_D \). This value almost doubled at 40°C compared to the value obtained for the temperature 35°C. For the decomposition of hydrogen peroxide at 45°C, the value of \( k_D \) was four times higher than the value \( k_D \) obtained for the temperature 40°C.

The Pearson correlation coefficient \( r \) for the obtained parameters was higher than 0.994 and the determination coefficient (correlation coefficient squared, \( r^2 \)) was higher than 0.988. Figure 3 shows a comparison between experimental conversions and those found with the use of the deactivation constants given in Table 1. All the data relate to the same quantity of catalase (2.5 U/mL), which enables their direct comparison.

The first order model is able to fit adequately all the kinetic data at all the temperatures. The enzyme is totally deactivated at the end of the reaction at 50°C. Figure 4 shows the comparison of the experimental conversion data with the calculated conversion for temperatures ranging from 35°C to 50°C by catalase from *Aspergillus niger*.

Data in Table 1 were used for making the Arrhenius plot (Figure 5).

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**Table 1.** Deactivation rate constants for catalase from *Aspergillus niger* for an initial concentration of 0.015 mol/L hydrogen peroxide.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>35°C</th>
<th>40°C</th>
<th>45°C</th>
<th>50°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>( k_D ) (L/(mol·h))</td>
<td>30.5±2.98</td>
<td>58.6±5.71</td>
<td>235.9±6.23</td>
<td>469.5±4.99</td>
</tr>
<tr>
<td>SEE</td>
<td>0.066</td>
<td>0.022</td>
<td>0.018</td>
<td>0.006</td>
</tr>
<tr>
<td>( r )</td>
<td>0.997</td>
<td>0.997</td>
<td>0.994</td>
<td>0.998</td>
</tr>
<tr>
<td>( r^2 )</td>
<td>0.992</td>
<td>0.993</td>
<td>0.988</td>
<td>0.996</td>
</tr>
</tbody>
</table>
Deactivation rate constants varied depending on temperature in accordance with the Arrhenius equation. The activation energy for deactivation of *Aspergillus niger* catalase was calculated to be 158.7±1.7 kJ/mol. The correlation between the data obtained from the mathematical model and experimental data was high (correlation coefficients higher than 0.988). The value of the activation energy for deactivation of catalase *Aspergillus niger* was 3.4 times higher than the value of the activation energy for deactivation of catalase Terminox Ultra (Miłek and Wójcik, 2011).

The established values of rate constants for reaction and rate constants for deactivation $k_D$ based on equation (13) allow definition of the change of catalase activity during the decomposition of hydrogen peroxide with the initial concentration of 0.015 mol/L. Figure 6 shows the change of enzyme activity with time calculated at 40°C, using two quantities of the enzyme.

From the data shown in Figure 6, it was observed that the activity of catalase (2.5 U/ml) during hydrogen peroxide decomposition at time 1.6 h and at the temperature 40°C decreased to about 50% of the initial activity of catalase.

Figure 7 shows the changes in the activity of the enzyme, which depends on temperature ranging from 35°C to 50°C using 2.5 U/mL catalase. Figure 7 shows that, during decomposition of hydrogen peroxide at 45°C, the catalase activity falls to 20% of the initial activity after 0.58 h. On the other hand, during decomposition of hydrogen peroxide at 50°C, the catalase activity falls to 20% of the initial activity after 0.26 h.

Thus, due to the significant deactivation of catalase at temperatures above 40°C, it is recommended to use larger quantities of enzyme during the course of the reaction of hydrogen peroxide decomposition by catalase.

Table 2 shows values of the deactivations constant $k_D$ for catalase of different origins at hydrogen peroxide concentrations lower than 0.02 mol/L, which are presented in the literature.

The effect of temperature on the deactivation of catalase from *Aspergillus niger* by hydrogen peroxide so far has not been the object of systematic research. In the literature only the values of $k_D$ for *Aspergillus niger* catalase for the temperatures 25°C and 37°C are presented: 30.6 L/(mol·h) and 54.0 L/(mol·h), respectively (DeLuca *et al.* 1995; Tse and Gough, 1987).
Estimation of the kinetic parameters for H$_2$O$_2$ enzymatic decomposition and for catalase deactivation

The kinetic deactivation parameters obtained for the *Aspergillus niger* catalase allow the calculation with the Arrhenius equation of the deactivation rate constants $k_D$ for specific temperatures. The calculated value of $k_D$ of 3.54 L/(mol·h) for *Aspergillus niger* catalase at the temperature of 25ºC is lower than that determined by DeLuca *et al.* (1995). The calculated value of $k_D$ of 42.7 L/(mol·h) for *Aspergillus niger* catalase at 37ºC is lower by about 21% than that determined by Tse and Gough (1987). However, it appears that there is a divergence in the results when enzymes of different origins are used. Comparing the calculated values of $k_D$ for *Aspergillus niger* catalases with $k_D$ values for bovine liver catalase (DeLuca *et al.*, 1995), it can be concluded that microbial catalases are more stable than those obtained from animal tissues.

However, the inactivation constant is reported to be strongly influenced by the other enzymes and reactions producing hydrogen peroxide, e.g., an oxidation reaction catalyzed by an oxidase (Ene and Maria, 2012; Maria *et al.* 2012). So it is necessary in the future to study and estimate kinetic parameters for deactivation of catalase in bi-enzymatic reactions.

The parameters obtained for deactivation of *Aspergillus niger* catalase by hydrogen peroxide can be used in modeling and optimization of batch bioreactors (Grubecki, 2016; Vasić-Rački *et al.*, 2011).

**CONCLUSIONS**

Hydrogen peroxide decomposition in the concentration range from 0.001 mol/L to 0.015 mol/L by catalase from *Aspergillus niger* is associated with a noticeable deactivation of the enzyme by the substrate at temperatures ranging from 35ºC to 50ºC. Good agreement between experimental data and the model simulations was obtained. The deactivation rate is described by a first-order kinetic equation in relation to the enzyme and the substrate concentrations. The parameters, namely, the deactivation rate constants in this mathematical model, were estimated by the Levenberg-Marquardt method. Dependence of the reaction rate constant for deactivation on temperature is in agreement with the Arrhenius equation. The activation energy obtained for decomposition of hydrogen peroxide equals 12.9±0.7 kJ/mol. The activation energy for deactivation of *Aspergillus niger* catalase was 158.7±1.7 kJ/mol.

In summary, the results obtained could be very useful in order to improve the application of *Aspergillus niger* catalase in industrial processes. The kinetic deactivation parameters that were determined and the appropriate mathematical model could be used to significantly optimize hydrogen peroxide decomposition by using *Aspergillus niger* catalase. In the future, it is necessary to determine the kinetic parameters for the reaction of in situ production H$_2$O$_2$ by another enzyme.

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**NOMENCLATURE**

| $a$ | dimensionless activity of catalyst |
| $C_E$ | concentration of enzyme (mol/L) |
| $C_S$ | concentration of hydrogen peroxide (mol/L) |
| $E_D$ | activation energy for deactivation (kJ/mol) |
| $E_R$ | activation energy for reaction (kJ/mol) |
| $k_D$ | deactivation rate constant (L/(mol·h)) |
| $k_{D0}$ | pre-exponential deactivation rate constant (L/(mol·h)) |
| $k_R$ | reaction rate constant (L/(mol·h)) |
| $k_R^*$ | reaction rate constant (1/h) |
| SEE | standard error of estimate |
| $T$ | temperature (ºC) |
| $t$ | time (h) |
| $X$ | fractional conversion |

**REFERENCES**


Estimation of the kinetic parameters for H$_2$O$_2$ enzymatic decomposition and for catalase deactivation


