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# The kinetic behavior of catalase immobilized on Amberlite IRA-410

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Beef liver catalase was immobilized on the strongly basic ion-exange resin Amberlite IRA-410, and used to study the kinetics of the reaction of hydrogen peroxide decomposition. The process of the immobilization was also followed. Rate constants for the enzymatic reaction were determined for the initial period and for the reaction as a whole. Reuse of the immobilized enzyme was also examined. Rate constants at four temperatures in the range 20—40 °C were determined and the energy and entropy of activation were calculated from rate data.

Enzymes are proteins which catalyse chemical reactions in living organisms.[1] Recent investigations show that enzymes can catalyse numerous reaction which do not occur in nature and consequently can be used, with advantage, in synthetic organic chemistry,[2–4] and also in many technological applications. However, high cost makes repeated or continuous use desirable in most cases. Use of enzyme in processing has also been limited by the difficulty and expense of their isolation, their instability, and by the fact that in freely soluble form can usually be used only once.[5,6] Successful immobilization can solve these problems. By definition, immobilized enzymes are enzymes which are physically confined or localized in certain defined region of space with retention of their catalytic activities, and which can be used repeatedly and continuously.[7] Major advances in immobilized enzyme technology occured in the mid to late 1960s.[8]

The advantage of immobilization include the possibilitiy of reuse, enhanced stability, rapid separation of catalysts from the reaction mixture, no product contamination by the enzyme, continuous processes based on bound enzyme are readily

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automated, may exibit selectively-altered chemical or physical properties, rapid termination of reactions, controlled product formation, possible greater efficiency in consecutive multiple-step reactions.[9] Immobilized enzymes can serve as good model systems to study the functioning of enzymes and to obtain information about the effects of different microenvironments on the properties of enzymes.[10] The actual and potential uses of immobilized enzymes are numerous.[7]

Immobilized enzymes, can be prepared using several techniques,[5,6,9] such as physical adsorbtion to a solid phase, entrapment within a gel matrix, containment behind a semipermeable membrane (microcapsules, hollow fibers, etc.), covalent attachment to an inert support, incorporation directly into a polymer, intermolecular crosslinking of enzyme molecules and the use of "live" or "dead" cells.

Beef liver catalase is a heme-containing enzyme of molecular weight 250.000 (Summer, Grallen, 1938) made up of four subunits arranged with a point group symmetry of 222 (Kiselev *et al.*, 1968; De Rosier, 1971; Vainshtein, 1973).[11]

The basic reaction in which catalase acts is the degradation of hydrogen peroxide produced in living cells. The reaction is as follows[1]

$$2H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2$$

Hydrogen peroxide is a particularly suitable germicide for the food industry; it may be used to destroy harmful organisms and then may be rapidly and completely decomposed by the enzyme catalase. Hydrogen peroxide-catalase applications are possible in cheese production, the desugaring of egg whites, the removal of oxygen from food products, and the enzymatic production of gluconic acid.[12]

Investigations concerning immobilized catalase kinetics have been reported in literature. The kinetics of the hydrogen peroxide decomposition reaction by immobilized catalase has been studied in order to determine the efficiency and stability of immobilized enzyme[12,13] and the effects of diffusion.[14]

Considering the advantages of immobilized enzymes, it is possible that the application of immobilized catalase in the processing industry will become more common. Therefore, we thought it important to investigate the kinetic behaviour of catalase immobilised on a suitable ion exchange resin, and possibly get an insight into the mechanism of its catalytic action.

## EXPERIMENTAL

Beef liver catalase from Boenringer-Mannhaim GmbH was immobilized on resin Amberlite IRA-410 ($d$=1 mm) using the procedure given in Scheme 1 as suggested by Đ. Vasić-Rački.[15] Immobilization was investigated at 21 °C in a batch reactor, $V$=45 cm$^3$ at $n$=200 rpm. Degradation of the hydrogen peroxide was investigated in the temperature range 20—35 °C in a batch reactor ($V$=160 cm$^3$ $n$=125 rpm) and for the initial period of reaction in the temperature range 25—35 °C.

Amberlite IRA-410, $d=1$ mm
kept in distilled water
↓ rinsing
Activation in a column
with 0.1 M NaOH
↓ rinsing
Keeping in bidistilled water, 24 hours

1% Solution of catalase
in bidistilled water
(25 cm³)
↓
20 cm³ → Immobilization, $t=21$ °C
2 hours, $n=200$ rpm
↓
Glutaraldehyde, 1% solution,
$t=21$ °C, 30 min
↓
Rinsing with bidistilled
water, $3\times10$ cm³
↓
Reaction with $H_2O_2$, batch reaction, 10 cm³
of resin, $c_{H_2O_2}$=const., $n=125$ rpm, $t$=const

Scheme 1. Schematic presentation of catalase immobilization on basic ion-exchanger Amberlite IRA-410

The kinetics of both the immobilization and of the reaction was followed by volumetric titration of the samples with potassium permanganate in the presence of sulphuric acid (5 cm³ sample, 5 cm³ 0.1 M $H_2SO_4$, 0.1 M $KMnO_4$). For the calculation of the rate constants integral method was used.

## RESULTS

The data from three experiments for the immobilization of catalase on the ion-exchange resin in a two hour period are given in Fig. 1. In that period over 90% of the enzyme was taken from the solution. This was determined from the

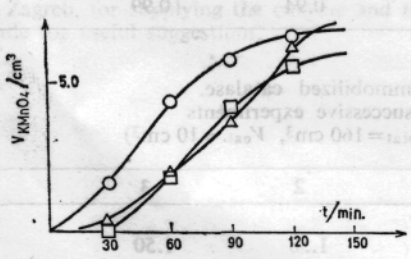


Fig. 1. The rates of catalase immobilization on ion-exchange resin Amberlite IRA-410 in three experiments

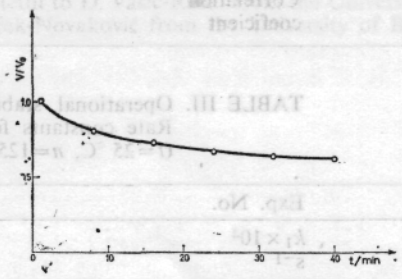


Fig. 2. The rate of the reaction of hydrogen peroxide degradation by catalase immboilized on Amberlite IRA-410 at 30 °C.

difference in the enzyme content in the control sample and the sample after immobilization, and calculated from the hydrogen peroxide decomposition in both samples. The concentration of immobilized catalase amounts to approximately 0.25 mg/cm³.

In general, catalase as any other enzyme, shows characteristic kinetic behaviour which is presented in Fig. 2, for the reaction at 30 °C as an example. Rate constants for the first order reaction of immobilized catalase in the degradation of hydrogen peroxide at four temperatures are given in Table I.

TABLE I. Rate constants for the hydrogen peroxide decomposition reaction. Temperature dependence ($n$=125 rpm, $V_{total}$=160 cm³, $V_{cat.}$=10 cm³)

| $t$ °C | 20 | 25 | 30 | 35 |
|---|---|---|---|---|
| $k_1 \times 10^4$ s$^{-1}$ | 1.02 | 1.46 | 1.83 | 2.33 |
| Correlation coeficient | 0.96 | 0.96 | 0.98 | 0.99 |

Although the reaction in question is considered to conform to the first order rate law as a whole,[12–14] certain irregularities in the initial period prompted us to investigate that region separately, and the corresponding rate data are listed in Table II. The results of the examination of operational stability, given in Table II, indicate satisfactory behaviour of the immobilized catalase in three successive experiments with same sample of immobilized enzyme. The apparent increase of the reaction rate is probably due to the experimental error (*ca.* 7.5%). Titration with $KMnO_4$ is not an ideal method for the study of hydrogen peroxide decomposition reaction.[16]

TABLE II. Rate constants for the initial period of the hydrogen peroxide decomposition reaction. Temperature dependence ($n$=125 rpm, $V_{total}$=160 cm³, $V_{cat.}$=10 cm³)

| $t$ °C | 25 | 30 | 35 |
|---|---|---|---|
| $k_1 \times 10^4$ s$^{-4}$ | 8.10 | 7.66 | 9.16 |
| Correlation coeficient | 0.97 | 0.94 | 0.99 |

TABLE III. Operational stability of immobilized catalase. Rate constants for three successive experiments ($t$=25 °C, $n$=125 rpm, $V_{total}$=160 cm³, $V_{cat.}$=10 cm³)

| Exp. No. | 1 | 2 | 3 |
|---|---|---|---|
| $k_1 \times 10^4$ s$^{-1}$ | 1.46 | 1.58 | 1.50 |
| Correlation coeficient | 0.96 | 0.95 | 0.96 |

## DISCUSSION

The process of catalase immobilization on strongly basic ion-exchange resin Amberlite IRA-410, using the absorption method appears to be satisfactory and rather fast. Over 90% ot the enzyme was immobilized within two hours in three experiments and the data fit the curves in Fig. 1. fairly well.

Immobilized catalase prepared in the present work decomposes hydrogen peroxide very fast in the temperature range 20—30 °C; about 80% reacted in 50 minutes. The high values for the correlation coefficients given in the Tables show that the assumption of the first order of the reaction as a whole was correct. The same applies to the intial period but the constants are much higher.

Energy of activation $E_A$ and the corresponding entropy of activation $\Delta S^{\neq}$ are calculated from the relevant formulae,[17] for the reaction as a whole and for the initial period. The results are as follows:

*For the whole reaction* $E_A = 42.74$ kJ mol$^{-1}$ $\Delta S^{\neq} = -149.31$ JK$^{-1}$ mol$^{-1}$

*For the initial period* $E_A = 9.15$ kJ mol$^{-1}$ $\Delta S^{\neq} = -247.61$ JK$^{-1}$ mol$^{-1}$

The much lower energy of activation in the initial period which is very important for enzyme action, indicates that the reaction in this period goes very smoothly. The high negative value of the entropy difference indicates that the orientation of the reacting species on the active catalyst cites is very important. This is also important for the reaction as a whole, which is normal for enzymatically catalyzed reactions. It is well known, that enzyme catalysed reactions frequently change both the mechanism and the order of the reaction in its course. However cosidering that there is no change in the order of reaction, we believe that the apparantly lower orientation requirements and higher energy of activation for the reaction as a whole are due to diffusional limitations. We could not be certain about it, as we did not examine the effect of mixing on the reaction rate.

Reuse of the immobilized enzyme showed very good operational stability in the reaction in which not only hydrogen peroxide is decomposed but also a certain degree of catalase deactivation could be expected. This is of importance for eventual industrial application where low operation cost is demanded. Further investigations of the system are necessary for reactor design and eventual pilot scale operation.

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ИЗВОД

## КИНЕТИЧКО ПОНАШАЊЕ КАТАЛИЗЕ ИМОБИЛИСАНЕ НА АМБЕРЛИТУ IRA-410

ДУШАН МИЈИН, ВЕСНА НИКОЛИЋ, МИЛИЦА МИШИЋ-ВУКОВИЋ и ДРАГОЉУБ В. ВУКОВИЋ

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Каталаза из говеђе јетре је имобилисана на јако базној јоноизмењивачкој смоли Амберлит IRA-410 и коришћена за испитивање кинетике реакције разлагања водоник-пероксида, у којој учествује као катализатор. Такође је праћен и процес имобилизације. Константне брзине реакције првог реда су одређиване за почетни период реакције и за целокупни ток. Испитивано је и узастопно коришћење истог узорка имобилисане каталазе. Одређене су константе брзине реакције за почетни период и укупну реакцију у температурном интервалу 20—40 °C и израчунате вредности за енергију и ентропију активације.

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