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NON-AQUEOUS BIOCATALYSIS BY CATALASE IMMOBILIZED ON TI/SILICATE

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INTRODUCTION

Native catalase is one of the most efficient biocatalysts whose high enzymatic activity at hydrogen peroxide decomposition with no radical formation has potential practical applications in medicine, food-processing industry and biochemical analysis. In this connection, catalase immobilization has been extensively researched and different solid carriers, such as artificial membranes [1,2] alum, gelatine, polyacrylamide and egg shells [3], carbonaceous materials [4] alumina pellets [5], etc. have been tested for that purpose. The biocatalytic activity of catalase in non-aqueous media has been examined for designing biocatalysts applicable in organic synthesis [6] or for creating organic phase enzyme electrodes (OPEE′s) making possible peroxide substrates' determination [7-13]. Besides hydrogen peroxide determination in cosmetic or pharmaceutical products [7,8] the use of non-aqueous media provides the opportunity to determine variety of water-insoluble peroxide compounds: tret-butylhydroperoxide [10]; cumene hydroperoxide [9,13]; benzoyl peroxide and meta-chloro peroxibenzoic acid [14,15].

In this work a new bio-heterogeneous catalyst designed for work in organic medium is reported. The catalyst under study was obtained by means of catalase adsorption immobilization on an inorganic silicate carrier. The catalytic activity of the biocatalyst has been examined at the cleavage of 3-chlorperoxibenzoic acid in acetonitrile.

EXPERIMENTAL

Reagents: Catalase (EC 1.11.1.6) was from Penicillium chrysogenum 245 (Biovet-Peshtera, Bulgaria) with specific activity of the enzyme was 1000 U/mg. Buffer solutions were prepared with $Na_2HPO_4.12H_2O$, KOH, H_3PO_4, and citric acid all of analytical grade.

Acetonitrile for UV spectroscopy (Fluka), was used as reaction medium; 3-chloroperoxibenzoic acid (m-CPBA or 3-CPBA) of analytical grade, was purchased from Fluka and used without further purification.

The immobilization matrix used was a silicate adsorbent, kindly provided by the "Synthesis and reactivity of oxide systems" group - Bulgarian Academy of Sciences. The silicate adsorbent was possesses a specific surface of 1000 $m^2.g^{-1}$; a pore size of ~39Å and will be further denoted as TiS within the text.

Preparation of biocatalysts: Catalase immobilization on TiS and the kinetic studies protocol by UV spectrophotometry were described in details in [14]. The spectrophotometer used was Specord UV VIS (Carl Zeiss, Jena, Germany).

RESULTS AND DISCUSSION

The trend of the kinetic curves of 3-chlorperoxibenzoic acid cleavage in acetonitrile catalyzed by catalase, immobilized on TiS is hyperbolic in character, which is typical for the formal Michaelis – Menten kinetics. The rate of the process, catalyzed by immobilized on TiS catalase depends on the temperature since the 3-chlorperoxibenzoic acid decomposition in acetonitrile takes place more rapidly at 25° than at 10°. The parameters of the enzymatic process catalyzed by CAT/TiS – the apparent Mihaelis' constant K_M^{app} and maximum rate V_{max}^{app} were determined from a Lineweaver – Burk plot: the reciprocal initial rate of the enzyme-catalyzed reaction versus the reciprocal substrate initial concentration. Compared with the values of the apparent Michaelis constant when the process is catalyzed by catalase enzyme immobilized on carbonaceous carriers, reported previously [14], for the process catalyzed by CAT/ TiS K_M^{app} is twice as low (Table 1). The values of V_{max}^{app} determined for CAT immobilized on different matrices correlate to the corresponding values of K_M^{app} (Table. 1). The apparent Michaelis constants could be affected considerably by such factors as substrate distribution between the bulk solution and the matrix for enzyme immobilization [16].

Table 1. *The kinetic parameters of 3-chlorperoxibenzoic acid cleavage in acetonitrile by catalase, immobilized on different matrices; the adsorption capacity and specific surface of the matrices*

Catalytic system	Kinetic parameters			Matrix characteristics	
	K_M^{app}, μM	V_{max}^{app} μmol·min^{-1}·mg^{-1}	$k_{sp} \cdot 10^4$ s^{-1}·mg^{-1}	Adsorption capacity mg·g^{-1}	specific surface m^2·g^{-1}
CAT/TiS	333.3	11.1	7.28	30	1000
CAT/NORIT	714.3	18.1	6.48	60	1500
CAT/PM-100	666.7	15.3	3.89	40	100

It is evident from the data of the first order specific rate constants (Table 1) that the activity of catalase in the process under study is the highest when the enzyme is immobilized on TiS. For a comparison, the effective rate constant was found twice as

low when it is immobilized on the carbonaceous matrix PM-100 and 1.12 times lower – when immobilized on NORIT activated carbon. The values of K_M^{app} and k_{sp} (Table 1) for the catalysts CAT/TiS and CAT/PM-100 shows that a higher k_{sp} corresponds to the lower value of K_M^{app} (with CAT/TiS), and vice versa. This fact can be assigned to the effect of the substrate distribution between the solution and the matrix for enzyme immobilization [16]. At an equilibrium distribution of the substrate between the solution and the enzyme carrier, the apparent Michaelis constant (K_M^{app}) is strongly dependent on the distribution coefficient P $\{P = \frac{[S]^b}{[S]^m}\}$ of the substrate between the matrix for enzyme immobilization $[S]^m$ and the bulk solution$[S]^b$: $K_M^{app} = K_M \times P$, where K_M is the constant of Michaelis for a reaction, catalyzed by a non-immobilized enzyme. (Because of catalase insolubility in acetonitrile, K_M in this solvent could not be determined). The comparison of the apparent Michaelis constant value of the catalytic system studied with those reported previously (Table 1.) suggests that in the first case the distribution coefficient is about two times as small than in the case of adsorbed on carbonaceous matrices enzyme. In other words, the concentration of 3-CPBA on TiS carrier is much bigger than on the carbonaceous ones, which could be assigned to hydrophobic interactions between the silicate and the organic peroxide. Another possible reason for the substrate to be concentrated in the matrix with the adsorbed enzyme, with catalyst CAT/TiS could be the smaller amount of adsorbed on TiS catalase and respectively, bigger surface free for adsorption, in comparison with the carbonaceous matrices, NORIT and PM-100 (Table.1).

The overall rate of the decomposition reaction catalyzed by the immobilized enzyme given by the equation of Michaelis – Menten is inversely proportional to the apparent Michaelis constant, so that a decrease of K_M^{app} leads to the increase of the effective reaction rate, and of the observable specific rate constants, accordingly.

The activation energy, rate –limiting stage and the pre-exponential Arrhenius factor (which takes into account the number of the catalyticaly – active centers on the surface of the heterogeneous catalyst under study) of 3-chlorperoxibenzoic acid decomposition in acetonitrile by catalase, immobilized on TiS were determined using the effect of temperature on the rate of the process. The values of the rate constants at different temperatures were calculated by means of a first order kinetic equation, plotted in coordinates ln C – time (Table 1). The increase of the temperature by 10° leads to the acceleration of the rate of the process 1.2 times when using CAT/TiS: for T = 283 K, $k_{sp} = 7.28 \cdot 10^{-4}$ $s^{-1} \cdot mg^{-1}$; for T = 293 K, $k_{sp} = 9.03 \cdot 10^{-4}$ $s^{-1} \cdot mg^{-1}$.

The activation energy (Table 2) of 3-chlorperoxibenzoic acid decomposition by catalase, immobilized on TiS was calculated according to Arrhenius' equation: $E_a = \frac{RT_1T_2}{T_2 - T_1} ln \frac{k_2}{k_1}$. The activation energy value (E_a = 14.85 kJ·mol^{-1}; Table.2) and the insignificant influence of the temperature on the rate of the process, catalyzed by CAT/TiS prove that with this catalyst, the process takes place in the diffusion area of

the catalysis. The Gibbs energy of activation ΔG* is practically identical with the previously reported values for CAT immobilized on carbonaceous matrices [14], i.e. it does not depend on the nature of the matrix, whereas ΔH* and ΔS* depend on the matrix, used for immobilizing the enzyme. The process catalyzed on CAT/silicate matrix TiS is characterized by considerably lower values of ΔH* and ΔS* in comparison with CAT, immobilized on the carbonaceous materials – activated carbon or carbon black. The activation parameters, presented in Table 2: the entropy of activation (ΔS*), the enthalpy of activation (ΔH*) and the Gibbs energy of activation (ΔG*), were calculated according to the basic equation of the transition state theory. The steric factor P (dimensionless) was calculated by the expression: $P = e^{\Delta S^*/R}$, and the pre-exponential multiplier Z_0 in the equation of Arrhenius – from the expression $k = Z_0 \cdot e^{\frac{-E_a}{RT}}$.

Table 2. *Parameters of the bioheterogeneous systems CAT/TiS, in the breakdown of 3-chlorperoxibenzoic acid in acetonitrile.*

Kinetic parameters	Activation parameters
$k_{sp.} = 7.28 \cdot 10^{-4}$ $s^{-1} \cdot mg^{-1}$	$\Delta G^* = 86.19$ $kJ \cdot mol^{-1}$
$E_a = 14.85$ $kJ \cdot mol^{-1}$	$\Delta H^* = 12.49$ $kJ \cdot mol^{-1}$
$Z_0 = 0.4$ s^{-1}	$\Delta S^* = -260.4$ $J \cdot K^{-1} \cdot mol^{-1}$
	$P = 2.5 \cdot 10^{-14}$

Concluding, all above discussed results contribute to our understanding how the matrix for enzyme immobilization can affect the parameters of a heterogeneous-biocatalytic process.

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