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Biotransformation of phenolic compounds by use of tyrosinase from *Agaricus bisporus* in native and immobilized form

SUMMARY

Tyrosinase is an enzyme that has unique dual catalytic activity. This enzyme is capable to hydroxylate monophenols to diphenols and oxidize diphenols to quinones. In addition, tyrosianse has a wide substrate specificity, which makes it very interesting from the industrial point of view. However, numerous studies on the catalytic mechanism of tyrosinase show that a significant difficulty associated with the use of tyrosinase may be the suicide inactivation phenomenon, occurring during the reaction with diphenol compounds. Despite this fact, numerous publications describing the potential of tyrosinase application do not consider the suicide inactivation, which from a practical point of view seems to be a key element of tyrosine usage.

The main goal of the dissertation was to examine the applicability of tyrosinase from *Agaricus bisporus* for production of L-DOPA, 3-hydroxytyrosol and cinnabarinic acid, including studies on the impact of suicide inactivation on processes and the possibilities of reducing this phenomenon.

In the first part of the research, on the basis of mechanical stability tests of Grancel carriers and attempts to immobilize tyrosinase in the CLEA form, it was found that the separation of crosslinked aggregates is difficult due to their non-uniform size, therefore for the next part of the research tyrosinase was covalently bonded into the Granocel carrier. After fulfilling certain conditions (volume of the preparation, bed stabilization), this carrier can be used to conduct processes in the stirred tank reactor and the packed bed column. In addition, the advantages of using this carrier is the absence of sorption of monophenol substrates and hydroxylation products and the lack of internal diffusion resistance, which is connected to the binding of the enzyme into the carrier surface.

Processes of L-DOPA and 3-hydroxytyrosol production were carried out in a similar manner. In both cases, the need of reaction mixtures aeration was demonstrated. Reactions were conducted using a native and immobilized enzyme, in systems containing (i) 1mM L-tyrosine or 2,5 mM tyrosol and a double excess of AH_2 in phosphate buffer; (ii) 1mM L-tyrosine or 2,5 mM tyrosol and double excess of AH_2 and 6,7 mM HA in a borate buffer at pH

9,0, 8,0 or 7,0 and, in the case of 3-HTyr production, the system (i) was also used, containing in addition 6,7 mM of HA (iii). In the first system maximum of 34% L-DOPA and 50% 3-HTyr was obtained. Double volume of immobilized preparation and the additional supplementation of AH_2 allowed to increase the degree of conversion to 69%. Introducing to the system with the native enzyme 6,7 mM HA which converts the *met*-tyrosinase to *oxy*-Tyr allowed to obtain 98,5% of 3-Htyr. While conducting processes, the phenomenon of suicide inactivation caused by diphenol compounds was observed, what caused that higher conversion degree was impossible to obtain due to low operational stability of immobilized tyrosinase. Introduction of borate ions to the system (ii) allowed to limit suicide inactivation by complexing diphenol products, thanks to which at pH 7,0 a 95% conversion was obtained with native enzyme and 70% with enzyme immobilized in the case of L-DOPA and almost 100% in 3-HTyr production processes. Despite the high efficiency of reactions achieved in processes with immobilized enzyme, it was found that too low operational stability excludes the use of this preparation. Optimal reaction systems for production of these two diphenols are systems with native enzyme and, respectively, with 1 mM L-tyrosine, 2 mM AH_2, 6,7 mM HA in borate buffer pH 7,0 or 2,5 mM tyrosol, 5 mM AH_2, 6,7 mM HA in 0,5 M phosphate buffer pH 7,0.

In tyrosinase reaction with 3-HAA, cinnabarinic acid has been shown to be the main product. Based on substrate and product stability at a given pH, sorption phenomena and tyrosinase stability in dimerization processes, it was found that the optimal pH of reaction is pH 6,0. Tests shown that there was no need of additional aeration of reaction mixture, so processes were carried out in stirred tank reactors, a microreactor and a packed bed column. These studies proved that tyrosinase has very high stability in the presence of the reaction mixture components, and hence, 3-HAA can be included in compounds that induce suicide inactivation to a very small extent. The maximum conversion obtained in the stirred tank reactor was 80%, and its increase was impossible due to the reaction by-products. Since the phenomenon of suicide inactivation in this reaction is negligible, to carry out the 3-HAA dimerization processes both native and immobilized enzymes can be successfully used.