analysis indicated that the linkage between the enzyme and the affinity matrix was specific and substantially stable. The half life of the immobilized carbamolyase CBL303 could reach 210 h at 45 °C whereas its free form had that of 17 h. In particular, the immobilized enzyme could be recycled for 16 times to achieve 100% conversion of D-HPG. Along with our previous illustration of D-hydantoinase immobilization (Chern and Chao, 2005), the success achieved by immobilization of the evolved carbamoylase in this work apparently offers a promising way for the efficient production of D-HPG from D,L-HPH.

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Chitosan—Unique matrix for protease immobilisation

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Immobilized proteases can be used in two reversed processes: hydrolysis of peptides or proteins in water (food industry and proteomics research) and peptide bond formation in nonaqueous media (synthesis of pharmaceuticals, substrates, inhibitors, etc. of peptide nature). The main goal of this work was to create immobilized biocatalyst capable to work both in aqueous and low water media with high efficiency. Enzyme (subtilisin, chymotrypsin and papain)/chitosan biocomposite films were prepared as described in Bacheva et al. (2008). Amidase activity of subtilisin was tested using Glp-Ala-Ala-Leu-*p*NA (Bacheva et al., 2008), of chymotrypsin—by Glp-Phe-*p*NA and of papain—by Glp-Phe-Ala-*p*NA (Semashko et al., 2008). Protease activity was checked against azocasein and esterase activity—using *p*-nitrophenylacetate. Peptide synthesis by subtilisin/chitosan and by papain/chitosan was carried out as described in (Bacheva et al., 2008). The distinctive feature of these biocomposites was the preparation technique providing for high loading and uniform enzyme distribution. Comparing chemical modification and physical adsorption methods it was found that biocomposites treated with glutaraldehyde (optimum 1–2%, v/v) were more effective than untreated, especially in aqueous media. Higher glutaraldehyde concentration leads to lower activity of biocatalyst. Raising of starting enzyme concentration (2–150 mg/mL) lead to increase of activity and degree of loading, but after 75 mg/mL it reached a plateau (80 mg protein/g biocatalyst) and further concentration increase had no effect. The kinetics parameters, temperature and pH-dependence of subtilisin/chitosan hydrolytic activity were studied. All the biocomposites have high storage and operational stability in aqueous and nonaqueous media. The samples possessed high synthetic activity and were capable to catalyze peptide bond formation in DMFA/CH_3CN (6/4) mixture in reaction Z-Ala-Ala-Leu-OCH_3 + Phe-*p*NA → Z-Ala-Ala-Leu-Phe-*p*NA for subtilisin, Z-Ala-Ala-OCH_3 + Leu-*p*NA → Z-Ala-Ala-Leu-*p*NA for papain with the product yield 60–100% after 24 h of reaction. Thus biocatalytic films prepared in this work are characterized by preparative simplicity, high activity and stability in different media. This work was supported by RFBR 06-03-33056a.

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Application of stereoselective biocatalysts for the enantiomeric resolution of beta-hydroxynitriles

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A nitrile functional group is a precursor of a number of valuable organic intermediates. Besides adding an additional carbon atom to a molecule, a nitrile group can also be considered as a protected form of the corresponding amides and acids. Chemical hydrolysis of nitriles to amides and acids requires harsh reaction conditions (strong acids and high temperature), and is therefore non-stereoselective. With the advance of biotechnology, biocatalytic methods of hydrolyzing nitriles using nitrilases and nitrile hydratase is supplementing classical chemical methods. However, there are some stumbling blocks in the wide application of these enzymes, including limited commercial availability, thermal instability, limited range of substrates and poor stereoselectivity. Hence, new sources of efficient nitrile biocatalysts are of considerable interest.

Asymmetric hydrolysis of 3-hydroxy-3-arylpropanenitriles and 3-hydroxy-4-aryloxybutanenitriles were studied using a *Rhodococcus* sp. Enantio-pure beta-hydroxy amides and acids were obtained in excellent enantiomeric excess with moderate to high yields when the 3-hydroxy-4-aryloxybutanenitriles were employed as substrates. Conversely, a high enantiomeric excess was not achieved during the hydrolysis of the structurally related 3-hydroxy-3-arylpropanenitriles even though the biocatalyst provided the corresponding amides and acids at high yields.

Keywords: Biocatalysts; Beta-hydroxynitriles; Nitrilase

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