Politechnika Śląska

# ROZPRAWA DOKTORSKA

„Monolity krzemionkowe jako nośniki enzymów wykorzystywanych w wybranych procesach biotransformacji”

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ABSTRACT OF PhD DISSERTATION

# Silica monoliths as enzyme carriers in selected biotransformation processes

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The main goal of the PhD thesis was the application of modified silica monoliths, with hierarchical pore structure as enzyme carriers, and evaluation of their utility in selected biotransformation processes, including multi-enzymatic/cascade processes.

As the part of the dissertation, research on the development and application of the following immobilized biocatalysts: covalently/noncovalently immobilized lipase CalB from *Candida antarctica*; covalently immobilized UDP-glucose pyrophosphorylase isolated from *Thermocrispum agreste,* (*Ta*GalU); covalently immobilized/co-immobilized enzymes, constituents of enzymatic cascade for the L-erythrulose synthesis (namely D-amino acid oxidase isolated from *Rhodotorula gracilis* ($DAAO_{Rg}$), commercially available catalase, and transketolase isolated from *Geobacillus stearothermophilus* ($TK_{gst}$)) was conducted. The activity and stability of the obtained biocatalysts were determined in selected reactions: hydrolysis of *p*-nitrophenyl acetate and esterification of levulinic acid (lipase CalB), synthesis of UDP-glucose (*Ta*GalU) and trehalose (*Ta*GalU in cascade), as well as in the synthesis of L-erythrulose (enzyme cascade: $DAAO_{Rg}$, catalase and $TK_{gst}$).

The research has shown, that conditions of the process in which enzyme is used, should be taken into account while selecting the immobilization method. Both immobilization methods (covalent and adsorption) enabled to obtain highly active lipase biocatalysts, but their stability was different in aqueous and non-aqueous medium. Covalently immobilized lipase showed high stability in hydrolysis as well as esterification processes, while lipase immobilized by adsorption, was stable only in the esterification process. It was also shown, that covalent immobilization of UDP-glucose pyrophosphorylase allowed to obtain biocatalyst with broader range of the pH and temperature optimum as well as improved thermal stability (compared to the native form). In addition, the enzyme immobilized inside the silica microreactor, showed high process stability and could be used as a part of enzymatic cascade. This was demonstrated in the synthesis of trehalose, where the cascade consisted of *Ta*GalU and trehalose transferase was used. Another example of a cascade process was the enzymatic synthesis of L-erythrulose from D-serine. It was proven that covalent immobilization of D-amino acid oxidase, catalase and transketolase contributed to the significant improvement of their thermal stability (compared to the native form). The best yield of L-erythrulose

was obtained in the cascade, where $DAAO_{Rg}$ and catalase were co-immobilized and $TK_{gst}$ was immobilized separately.

In summary silica monoliths are very good carriers for enzymes used both in batch and in continuous processes. The obtained results, especially involving cascade processes, can be a starting point for application of the presented enzyme systems in the production of other valuable compounds.