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LACCASES IMMOBILIZED ON MESOPOROUS SILICA PARTICLES AND THEIR APPLICATION IN A CONTINUOUS STIRRED REACTOR FOR THE ELIMINATION OF ENDOCRINE DISRUPTING CHEMICALS

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Abstract

Immobilization of enzymes increases stability of enzymes and their re-use in multiple cycles. Mesoporous silicates (MPs) is an established support for enzyme immobilization with respect to the requirements for enzyme carriers such as high surface area, chemical and thermal stability, uniform pore distribution, high adsorption capacity, ordered porous network, mechanical strength and toxicological safety. Organic micropollutants present in wastewater at very low concentrations such as the plasticizer bisphenol A, the detergent ingredient nonyphenol, and the antibacterial triclosan, are receiving increasing attention because of their adverse effects on human health and on the environment. To date there is no effective and sustainable physico-chemical remediation strategy available. Laccases from white rot fungi were found to be particularly active in the removal of these micropollutants. Biological treatment of wastewater can therefore be enhanced by the use of immobilized laccases. To identify and optimize the factors of immobilization with impact on biocatalyst activity, recovery and thermal stability, a central composite precision design was used. Three factors were chosen for the optimization of the immobilized biocatalyst production using response surface methodology: laccase concentration, glutaraldehyde concentration and bovine serum albumin concentration. Crude laccase preparation from *Coriolopsis gallica HAI 1184* was immobilized in a two-step reaction. Laccase was first adsorbed to the MPs, followed by a cross-linking step using glutaraldehyde. After successive washings, no activity was found in the supernatant. Based on multi-criteria optimization for biocatalyst production (initial laccase activity of 1500 Ug^{-1} and glutaraldehyde concentration of 225 mM), a maximum biocatalyst activity of 383 Ug^{-1} (determined in 100 mM sodium tartrate buffer (pH 4.5) with 2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulfonate as substrate) was obtained. The immobilized biocatalyst was found to be more stable at higher temperature and pH conditions compared to free enzyme. The immobilized biocatalyst was applied for the elimination of 50 µM bisphenol A in a continuous membrane reactor with a working volume of 0.05 L. More than 90 % BPA was successfully removed for more than 30 h of continuous treatment by the biocatalyst with a half-life of 52 h and a low rate of inactivation (0.013 h^{-1}). This clearly shows the potential of these biocatalysts in the treatment of micropollutants in wastewaters.