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Entitled

*ENZYMATIC PRODUCTION OF CYCLODEXTRIN USING CYCLODEXTRIN GLYCOSYLTRANSFERASE
IMMOBILIZED IN METAL ORGANIC FRAMEWORKS (MOFS)*

By

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Abstract

Cyclodextrins (CDs) and their derivatives have attracted significant attention in the pharmaceutical, food, and textile industries, which has led to an increased demand for their production. CDs are typically produced by the action of cyclodextrin glycosyltransferase (CGTase) on starch. Owing to the relatively high cost of enzymes, the economic feasibility of the entire process strongly depends on the effective retention and recycling of CGTase in the reaction system, while maintaining the enzyme's activity and stability. Previous supports used for this purpose have numerous drawbacks, including enzyme leaching, activity loss and significant mass transfer limitations. The aim of this dissertation was to improve performance of immobilized CGTase by using metal-organic frameworks (MOFs), possessing better properties than conventional supports, as immobilization support. CGTase was immobilized on different synthesized MOFs, namely MIL-101, Cu-BTC, using either surface, covalent attachment or entrapment and compared to conventional support, namely Zeolite Y as well as Graphene nano-particles (GNP). The use of a calcium-based two-dimensional MOF, namely Ca-TMA, and modified Cu-BTC using N,N-dimethylcyclohexylamine to produce hierarchical H-Cu-BTC were also tested for enhanced enzyme capacity and reduced diffusional limitations of the large starch molecules. The adsorption capacity, the effect of immobilization on the secondary structures of CGTase and on the characterization of the support as well as the kinetic parameters of the free CGTase were assessed. The adsorption isotherms of CGTase on the tested MOFs were best represented by the Langmuir isotherm, with maximum adsorption capacities reaching 21, 30.6, 37.5, 40 mg/g over Ca-TMA, Cu-BTC, microporous MIL-101 and GNP, respectively. The adsorption capacity was improved to 49.5 mg/g over H-Cu-BTC. These capacities were significantly higher than that observed using conventional Zeolite-Y, which did not exceed 6.1 mg/g, as well as other supports reported in previous literature. Characterization of the empty supports using combination of X-ray diffraction (XRD), scanning electron microscopy (SEM), Thermal Gravimetric analysis (TGA) revealed that the structures of the MOFs remained intact post-CGTase immobilization. The immobilized CGTase on the different MOFs were tested for CDs production from starch, and the relative activity, reusability and mass-transfer limitations were investigated. The specific activity of the free CGTase used was 167 U/mg, which dropped upon immobilization to 28, 38, 65.2 and 98.5 U/mg protein on GNP, Ca-TMA, Cu-BTC, and H-CU-BTC respectively. Reusability studies revealed that based on α -CD, MIL-101 showed 29% residual enzyme activity, which improved with covalent attachment via glutaraldehyde to 40%, Ca-TMA gave 33%, and GNP showed 74% relative activity after eight reaction cycles. Entrapment of CGTase within H-Cu-BTC led to residual CGTase activity of 87% after ten reaction cycles, compared to 70% over microporous Cu-BTC and presence of macropores and mesopores enhanced substrate mass transfer from 0.68 min^{-1} over microporous MOFs to 0.89 min^{-1} on macroporous H-Cu-BTC, thus, improved cyclodextrin production. This dissertation provides information on the effect of MOFs properties on immobilized CGTase performance, which can be used in developing robust CGTase-based biocatalysts for industrial application.