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Pinewood Nanobiochar: a Unique Carrier for the Immobilization of Crude Laccase by Covalent Bonding

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Abstract

Nanotechnology-inspired biocatalytic systems attracted attention for many applications since nanosized supports for enzyme immobilization can improve efficiency-determining factors e.g. enhancing the surface area and loading capacity and reducing the mass transfer resistance. Among the nanomaterials, nanobiochar has unique features as a support for enzyme immobilization i.e. high surface to volume ratio, porous structure, and presence of functional groups on its surface. However, the performance of the immobilization is highly dependent on the immobilization conditions and the properties of the enzyme and the support material. In this research, crude laccase was covalently immobilized onto functionalized nanobiochar using a two-step method of diimide-activated amidation. The effect of different parameters were investigated. The optimal conditions were found to be 14 mg/mL of laccase concentration, 5 mg/mL of nanobiochar, 8.2 mM of cross-linker and 3 h of contact time. For investigating the pH, thermal, storage, and operational stability, the sample obtained from the optimized conditions was used. The results showed the higher stability of immobilized laccase against temperature and pH variation compared to free laccase. In addition, immobilized laccase maintained its catalytic performance up to seven cycles of utilization and showed more than 50% of initial activity after two months of room temperature storage.

Keywords: Nanobiochar, Laccase, Immobilization