IMMOBILIZATION OF CRUDE LACCASE ONTO CHITOSAN BEADS TO ENHANCE ITS THERMAL AND pH STABILITY

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Abstract

Laccases are essential enzymes in various industrial applications, playing a vital role in numerous industrial processes. When immobilized, laccases demonstrate enhanced resistance to environmental changes and offer the added benefit of being easily recoverable and recyclable, which makes them superior to their free forms. Immobilization primarily protects the enzymes from harsh conditions, such as high temperatures and extreme pH levels, significantly improving their stability and lifespan. In this study, we explored the impact of immobilization of crude laccase, isolated from Ganoderma sp., on its thermal and pH stability. The crude laccase was immobilized onto chitosan beads that were pre-activated with 0.5% glutaraldehyde. The study focused on determining the effects of crosslinking and immobilization time on the relative activity of the immobilized enzyme. The thermal and pH stability of both free and immobilized laccase were evaluated over an 8-hour incubation period, across a pH range of 4-8 and temperatures between 20°C and 50°C. The optimal crosslinking time was found to be 2h, which resulted in a relative activity of 34.04% for the immobilized enzyme with a corresponding immobilization time of 2 h. Further optimization of the immobilization time yielded the highest relative activity of 51.31% when the crosslinking time was 2h and the immobilization time was extended to 3 h. In contrast, the lowest relative activity was observed when the crosslinking time was 4 h and the immobilization time was 2h. Both free and immobilized laccases exhibited maximum stability at pH 5 and 20°C. However, immobilized laccase showed superior stability compared to the free form. After an 8-hour incubation at 4°C and pH 5, the immobilized laccase retained 68.25% of its initial activity, whereas the free laccase showed 59.64% of the residual activity. For thermal stability, after 8 h at pH 5 and 20°C, the immobilized laccase demonstrated a residual activity of 58.89%, outperforming the free laccase, which retained 53.29% of the activity. These results underscore the importance of optimizing the immobilization process to enhance the stability of laccases, demonstrating that immobilization significantly improves both thermal and pH stability.

Keywords: *crude laccase, white rot fungi, immobilization, chitosan beads*