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Fructose-Aided Cross-Linked Enzyme Aggregates of Laccase: An Insight on Its Chemical and Physical Properties

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Abstract : Laccase, a multicopper oxidase (EC 1.10.3.2) have been at the forefront as a superior industrial biocatalyst. They are versatile in terms of bestowing sustainable and ecological catalytic reactions such as polymerisation, xenobiotic degradation and bioremediation of phenolic and non-phenolic compounds. Regardless of the wide biotechnological applications, the critical limiting factors viz. reusability, retrieval, and storage stability still prevail. This can cause an impediment in their applicability. Crosslinked enzyme aggregates (CLEAs) have emerged as a promising technique that rehabilitates these essential facets, albeit at the expense of their enzymatic activity. The carrier free crosslinking method prevails over the carrier-bound immobilisation in conferring high productivity, low production cost owing to the absence of additional carrier and circumvent any non-catalytic ballast which could dilute the volumetric activity. To the best of our knowledge, the ε-amino group of lysyl residue is speculated as the best choice for forming Schiff's base with glutaraldehyde. Despite being most preferrable, excess glutaraldehyde can bring about disproportionate and undesirable crosslinking within the catalytic site and hence could deliver undesirable catalytic losses. Moreover, the surface distribution of lysine residues in Trametes versicolor laccase is significantly less. Thus, to mitigate the adverse effect of glutaraldehyde in conjunction with scaling down the degradation or catalytic loss of the enzyme, crosslinking with inert substances like gelatine, collagen, Bovine serum albumin (BSA) or excess lysine is practiced. Analogous to these molecules, sugars have been well known as a protein stabiliser. It helps to retain the structural integrity, specifically secondary structure of the protein during aggregation by changing the solvent properties. They are comprehended to avert protein denaturation or enzyme deactivation during precipitation. We prepared crosslinked enzyme aggregates (CLEAs) of laccase from T. versicolor with the aid of sugars. The sugar CLEAs were compared with the classic BSA and glutaraldehyde laccase CLEAs concerning physico-chemical properties. The activity recovery for the fructose CLEAs were found to be ~20% higher than the non-sugar CLEA. Moreover, the □□□□□□/ values of the CLEAs were two and three-fold higher than BSA-CLEA and GACLEA, respectively. The half-life (t1/2) deciphered by sugar-CLEA was higher than the t1/2 of GA-CLEAs and free enzyme, portraying more thermal stability. Besides, it demonstrated extraordinarily high pH stability, which was analogous to BSA-CLEA. The promising attributes of increased storage stability and recyclability (>80%) gives more edge to the sugar-CLEAs over conventional CLEAs of their corresponding free enzyme. Thus, sugar-CLEA prevails in furnishing the rudimentary properties required for a biocatalyst and holds many prospects.

Keywords : cross-linked enzyme aggregates, laccase immobilization, enzyme reusability, enzyme stability
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