Highly Efficient and Stable Novel NanoBiohybrid Catalyst to Avert 3,4-Dihydroxybenzoic Acid Pollutant in Water

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The present study reported for the first time covalent immobilization of protocatechuic 3,4-dioxygenase (3,4-POD) onto functionalized multi-walled carbon nanotubes (F-MWCNT) for degrading the toxic 3,4-dihydroxybenzoic acid (3,4-DHBA) pollutant in water. The F-MWCNTs had a maximum 3,4-POD loading of 1060 µg/mg. Immobilized 3,4-POD had 44% of relative structural changes to its free configurations. Nevertheless, >90% of relative activity and about 50% of catalytic efficiency were retained to the free enzyme. Immobilized 3,4-POD demonstrated higher alkaline stability and thermostability than the free 3,4-POD. The free and immobilized 3,4-POD lost 82% and 66% of relative activities, respectively after 180 min of incubations at 90°C. Excellent shelf-life was observed for the immobilized 3,4-POD with residual activity of 56% compared with 41% and 39% of the free 3,4-POD at 4°C and 25°C over 30 days storage. Immobilized 3,4-POD showed >60% of catalytic activity retention even after ten-cycle uses, defraying the expenses of free 3,4-POD productions for long term uses. Finally, the immobilized 3,4-POD removed 71% of 3,4-DHBA from water in <4 h, paving its future application for water purification with reduced costs and time.

Industrial food processing waste water effluents account for the 3,4-dihydroxybenzoic acid (3,4-DHBA) concentrations in the environment¹. This dwindling of the finite fresh water resources, seriously affects the terrestrial, aquatic, and aerial flora and fauna. The 3,4-DHBA has shown contradictory biological effects on the animal and human tissues. Some authors hypothesize the 3,4-DHBA can inhibit chemical-actuated-carcinogenesis of various mouse tissues such as liver, kidney, skin and so on²; whereas others have proved that the compound has decreased the level of glutathione – a major cellular antioxidant. It induces oxidative stress; and causes hepatotoxicity, nephrotoxicity, tumor productions, and inflammations³,⁴,⁵,⁶. Babich et al.⁷ found that the 3,4-DHBA with a concentration from 5 to 25 mM could be significantly toxic for normal human cells and nontoxic to malignant cells. Therefore, effective and inexpensive regulatory tool should be developed to remove the 3,4-DHBA from water.

Several studies employing Fenton¹, adsorption⁶, O₃/UV or H₂O₂/UV⁷ and microbial degradation⁸ have been adopted to remove the 3,4-DHBA from water but these methods are less selective, ineffective for dilute solution, time consuming, energy intensive, and generate toxic byproducts⁹. In contrast, the judicious choices of using enzyme for water purification are due to its high selectivity and sensitivity, fast reaction kinetics, fewer byproducts formation, minimal energy consumption and finally benign for the environment as compared with the physical and chemical methods¹⁰. However, the free enzyme is not stable under mechanical and chemical stresses and difficult to separate from the substrates in a reaction vessel. In order to overcome these hurdles, immobilization of enzymes onto a physical support is a must¹¹ for water purification.

The protocatechuic 3,4-dioxygenase (3,4-POD; EC: 1.13.1.3, MW: 700 kDa) is an intradiol cleaving enzyme commonly found in Pseudomonas putida¹² which consists of α- and β-subunits (αβ)n, where n = (2–12)¹³. It has a non-heme Fe (III) at the active site that participates in the direct degradation of 3,4-DHBA¹⁴ to 3-carboxy-cis, cis-muconic acid (CMA) without any byproducts formations¹⁵. The MA has not shown toxicity effects on normal cells¹⁶, and is an industrially valuable compound for adipic acid production (2.3 million metric tons/year

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