

Immobilization of L-asparaginase towards surface-modified carbon nanotubes

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Abstract

L-asparaginase (LA) is an enzyme that catalyzes L-asparagine hydrolysis into L-aspartic acid and ammonia and is mainly applied in pharmaceutical and food industries. The LA currently commercialized for pharmaceutical purposes is produced from two main bacterial sources: recombinant *Escherichia coli* and *Erwinia chrysanthemi*. However, some disadvantages are associated with its free form, such as the shorter half-life. Immobilization of LA has been proposed as an efficient approach to overcome this limitation. In this work, a straightforward method, including the functionalization of multi-walled carbon nanotubes (MWCNTs) through a hydrothermal oxidation treatment and the immobilization of LA by adsorption over pristine and modified MWCNTs was investigated. Different operation conditions, including pH, contact time, ASNase/MWCNT mass ratio, and the operational stability of the immobilized LA, were evaluated. The characterization of the LA-MWCNT bioconjugate was addressed using different techniques, namely Transmission Electron Microscopy (TEM), Thermogravimetric analysis (TGA), and Raman spectroscopy. Functionalized MWCNTs

showed promising results, with an immobilization yield and a relative recovered activity of commercial LA above 95%, under the optimized adsorption conditions (pH 8, 60 min of contact, and 1.5×10^{-3} g.mL⁻¹ of LA). The LA-MWCNT bioconjugate also showed improved enzyme operational stability (6 consecutive reaction cycles without activity loss), proving its suitability for application in industrial processes.

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