



**Purification of Thermophilic Lipase  
*E.coli* BL21(DE3)-pET30(a)-lipITB1.2 Using Immobilized  
Metals Affinity Chromatography Ni-NTA**

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**Abstract:** Lipase as biocatalyst displays three major types of selectivity (chemo-, regio- and diastereo-, and enantio-selectivity) and useful in many industrial applications. In order to increase the enzyme performance, purification of lipase is the essential thing and need to be investigated. In this research, Immobilized Metals Affinity Chromatography (IMAC) Ni-NTA was applied to purify a local thermophilic lipase lipITB 1.2. The purification of lipase was performed at the optimum condition of reaction (Ni-NTA - enzyme supernatant ratio of 1:1 and continuous stirring at 100 rpm for 1 hour). Elution was carried out at a flow rate of 0.02 m/s. 50 mM phosphate buffer pH 7.5, 300 mM NaCl, and 5mM imidazole solutions were used as eluents. Based on the results, purification of thermophilic lipase lipITB1.2 by IMAC Ni-NTA was successfully performed by increasing 17-folds the specific activity of heated supernatant of crude enzyme.

**Keywords:** thermophilic lipase; enzyme; immobilized metals affinity chromatograph (IMAC); Ni-NTA; specific activity.

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