



## ABSTRACT

The work describes the purification and immobilization of alkaline protease produced from *Aspergillus oryzae*. Enzyme was partially purified with 70% ammonium sulphate precipitation where the enzyme showed 1.5 fold purification with 24.89 U/mg specific activity. Suitability of 15 different carriers for immobilization was investigated using different methods like adsorption, entrapment and covalent bonding. Their protease activities and immobilization yields were measured and cellulose, charcoal, artemether nanoparticles, cotton gauze bandage and loofa showed highest activities 8.05 U/g, 12.24 U/g, 7.47 U/g, 8.88 U/g and 9.7 U/g of carrier, respectively and highest immobilization efficiencies of 41.83%, 63.6%, 38.28%, 46.71% and 50.41%, respectively. Enzyme immobilized on sponge, polyurethane and cellulose showed greater storage stability more than five weeks as compared to the free enzyme. The thermal and pH stability of the enzyme was significantly improved by the immobilization process. Temperature and pH maxima of the enzyme showed no changes before and after immobilization and remained stable at 50 °C and 8.0, respectively but in some cases, the optimum temperature and pH of enzyme changed to 60 °C and 9.0, respectively. The reusability study showed that immobilized enzyme can be used upto three cycles. The effect of pH on immobilization efficiency was studied and maximum immobilization efficiency was achieved at pH 8.0. The effect of different concentrations of enzyme on immobilization efficiency showed that increasing concentrations of enzyme decreased the immobilization efficiency. From all the immobilization methods used, covalent bonding exhibited lowest leakage rate of protease from support. The immobilized enzyme had higher  $K_m$  and lower  $V_{max}$  than the free enzyme. The immobilized enzyme efficiently carried out dehairing of goat skin and synthesis of dipeptide.

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