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EFFECT ON STABILITY OF TRYPSIN ENZYME BY INTERCALATION WITH MONTMORILLONITE

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Trypsin is a proteolytic enzyme found in the digestive system of many vertebrates. This enzyme cleaves peptide bonds specifically at the carboxyl side of the amino acids lysine and arginine. Trypsin is made up of 223 amino acid residues and its molecular weight is 23 000 Da. Trypsin has an optimum operating temperature at about 37 ^{0} C and optimum operating pH of about 8. This enzyme is thermally unstable and undergoes self digestion at high temperatures. To prevent autolysis, trypsin should be stored at -20 to -80 ^{0} C.

Montmorillonite (MMT) is a naturally occurring phyllosilicate mineral. The crystalline structure of montmorillonitis made up of multiple layers and each layer consists of two tetrahedral silica sheets sandwiching an octahedral alumina sheet. MMT has been successfully used as a support as well as a catalyst for the past few decades due to its greater swelling ability, high surface area and cation exchange capacity. Properties of MMT can be tuned with acid treatment, cation exchange, pillaring with robust metal ions, intercalation with polymeric organic molecules etc. Since MMT is an aluminosilicate, it possess acidic sites which can be used as binding sites through the $-NH_2$ groups of enzymes during immobilization. Immobilized enzymes are preferred over free enzymes owing to their multiple and repetitive use. Enzyme immobilization improves the stability of the enzyme by increasing its half life. Immobilization allows the enzyme to work in a large range of environments. It allows the enzyme to remain active at different temperatures and pH than would be predicted for the enzyme if it was not immobilized.

In this study, trypsin was immobilized on montmorillonite clay via adsorption. The immobilized enzyme was characterized by XRD measurements, FTIR measurements and activity for casein hydrolysis. The purpose of immobilization of trypsin on MMT was to enhance the thermal and storage stability of the enzyme.

X-Ray diffraction results demonstrated that the intercalation of the enzyme into the interlayer space of montmorillonite was through the side chains of amino acid residues of trypsin, indicating that trypsin was not intercalated between clay layers but was adsorbed on the surface. Activity for casein hydrolysis results demonstrated that the percent immobilization yield was about 12 % and the activity of the enzyme was lost significantly during the immobilization. Though the percent immobilization yield was low, thermal and storage stability enhancement of the enzyme due to immobilization could be observed. Retention of the activity of immobilized enzyme was higher than that of free enzyme at high temperatures.