



EXTENDED ABSTRACTS

Optimization of Biodegradation Process for Disperse Textile Dyes Using Brown Rot Fungi

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ABSTRACT

The protease enzyme from *Cucurbita maxima* peel was isolated and purified and its application in removing blood stain was studied. The optimum pH and temperature for this newly isolated protease was found to be 7.0 and 40°C respectively. The relative molecular mass of isolated protease decided by SDS PAGE and it had been found to be 31 KD. This new protease enzyme was applied to blood stained cloth to gauge the stain removing capacity of the enzyme within the presence and absence of detergents. From the results, it's confirmed that the newly isolated protease removes stain completely with detergents and even within the absence of detergents. This study confirms the appliance of *Cucurbita maxima* peel protease in removing blood stains. The thermostable crude proteolytic extract and purified protease produced by *Aspergillus tamarii* URM4634 were investigated at different temperatures. The activity results were used to estimate the energy of activation of the hydrolysis reaction catalyzed by crude extract and purified protease ($E^* = 34.2$ and 16.2 kJ/mol) also because the respective standard enthalpy variations of reversible enzyme unfolding ($\Delta H^{\circ}u = 31.9$ and 13.9 kJ/mol). When temperature was raised from 50 to 80 °C in residual activity tests, the precise rate constant of crude proteolytic extract thermoinactivation increased from 0.0072 to 0.0378 min⁻¹, while that of purified protease from 0.0099 to 0.0235 min⁻¹. These values, like half-life decreases from 96.3 to 18.3 min and from 70.0 to 29.5 min, respectively, enabled us to estimate the energy of activation ($E^*d = 49.7$ and 28.8 kJ/mol), enthalpy ($\Delta H^*d = 47.0$ and 26.1 kJ/mol), entropy ($\Delta S^*d = -141.3$ and -203.1 J/mol K) and Gibbs free energy ($92.6 \leq \Delta G^*d \leq 96.6$ kJ/mol and $91.8 \leq \Delta G^*d \leq 98.0$ kJ/mol) of thermoinactivation. Such values suggest that this protease, which proved to be highly thermostable in both forms, might be profitably exploited in industrial applications. To the simplest of our knowledge, this is often the primary comparative study on thermodynamic parameters of a serine protease produced by *Aspergillus tamarii* URM4634. Proteases which also are called "enzymes of digestion" are documented biocatalysts. They're commercially utilized in various industries like detergents, food, pharma, diagnostic etc. It's reported that 60% of total enzyme market is roofed by the protease and are considered because the most precious commercial enzyme. The source of proteases are enormous and bacterial proteases are more significant as compared to plant and animal proteases due to their rapid climb and may be easily manipulated genetically. However, plant proteases which has unique substrate specificity are free from undesirable side enzyme activities which is absent in microbial or animal systems. This makes the plant based protease resources as valuable source having profound applications in enzyme industry. There are minimal reports about the characterization of plant proteases. Thus, the arduous look for new potential plant proteases continues so as to form them industrially applicable

and price effective. Within the present investigation the plant pomelo L. (Rutaceae family) was selected as there's no report available on the protease enzyme and its characterization. The plant selected is well documented with its medicinal uses: antispasmodic, anti-inflammatory, anti-bleeding, bronchodilator, antidiabetic, anthelmintic, disinfectant, etc. Therefore, the plant looked to be a promising candidate for the protease source which may be exploited for its biotechnological applications. Therefore, the aim of this study is to characterize the protease and partially purify it from the leaves of pomelo L. with a view of that these proteolytic enzymes are often commercialized as alternative source. Alkaline protease from alkaliphilic *Bacillus* sp. NPST-AK15 was immobilized onto functionalized and non-functionalized rattle-type magnetic core@mesoporous shell silica (RT-MCMSS) nanoparticles by physical adsorption and covalent attachment. However, the covalent attachment approach was superior for NPST-AK15 protease immobilization onto the activated RT-MCMSS-NH₂ nanoparticles and was used for further studies. As compared to free protease, the immobilized enzyme exhibited a shift within the optimal temperature and pH from 60 to 65 °C and pH 10.5-11.0, respectively. While free protease was completely inactivated after treatment for 1 h at 60 °C, the immobilized enzyme maintained 66.5% of its initial activity at similar conditions. The immobilized protease showed higher k_{cat} and K_m , than the soluble enzyme by about 1.3-, and 1.2-fold, respectively. Additionally, the results revealed significant improvement of NPST-AK15 protease stability in sort of organic solvents, surfactants, and commercial laundry detergents, upon immobilization onto activated RT-MCMSS-NH₂ nanoparticles. Importantly, the immobilized protease maintained significant catalytic efficiency for ten consecutive reaction cycles, and was separated easily from the reaction mixture using an external magnetic flux. To the simplest of our knowledge this is often the primary report about protease immobilization onto rattle-type magnetic core@mesoporous shell silica nanoparticles that also defied activity-stability tradeoff. The results clearly suggest that the developed immobilized enzyme system may be a promising nanobiocatalyst for various bioprocess applications requiring a protease.

Keywords: Biodegradation; Textile Dyes; Optimization