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| Title: | Purification, Characterization and Immobilization of the Thermophile-Derived Cellulase Rhodothermus Marinus Cel12A onto Amberlite Beads and Mix Bed Resin: Optimization and Characterization |
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| Abstract: | Rhodothermus marinus Cel12A, or RM Cel12A is a highly thermostable, family 12 endoglucanase. RM Cel12A was previously cloned in our lab. The clone was in the pQE 30 expression vector containing a N-terminal 6x histidine tag and it was expressed in XL1 blue. Recombinant Cel12A has been purified to electrophoretic homogeneity with Ni-NTA-based affinity purification with a final specific activity of 69.6 units/mg. SDS-PAGE of the final preparation revealed a single protein band of 28.4 kDa. The enzyme exhibited maximum activity at a temperature of 90 $^{O}$C at pH 7.0. The apparent Michaelis constant [Km(app)] for CMC was 10 μM. Purified Cel12A showed the highest activity upon its primary substrate, carboxymethyl cellulose (CMC), followed by p-nitrophenyl-β-D-cellobioside and avicel. Cellulases are widely used enzymes in the food industry, paper industry, and textile industry etc. Despite these advantages, the use of enzymes in industrial applications has been limited by several factors, mainly the high cost of the enzymes, their instability, and availability in small amounts. Also the enzymes are soluble in aqueous media and it is difficult and expensive to recover them from reactor effluents at the end of the catalytic process. In this respect, immobilization and characterization of this industrially important thermostable cellulase Cel12A was carried out on two different matrices i.e. Amberlite XAD-7 and Mix Bed Resin. Maximum immobilization of 53 % and 70% was obtained with Amberlite and Mix Bed Resin respectively. The immobilized enzyme showed a high operational stability by retaining 70% and 90% of initial activity after 20 uses for Amberlite and Mix Bed Resin respectively. Both the matrices used for enzyme immobilization are non-toxic, cheap, renewable, biodegradable and have importance in food, cosmetics, biomedical, or pharmaceuticals applications. Immobilized cellulase can offer the possibility of a wider and more economical exploitation of biocatalysts in food, paper, textile industries etc. |
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