World Academy of Science, Engineering and Technology
International Journal of Biotechnology and Bioengineering
Vol:9, No:07, 2015

ENDO-β-1,4-Xylanase from Thermophilic Geobacillus stearothermophilus: Immobilization Using Matrix Entrapment Technique to Increase the Stability and Recycling Efficiency

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Abstract : Introduction: Xylan is a heteropolysaccharide composed of xylose monomers linked together through 1,4 linkages within a complex xylan network. Owing to wide applications of xylan hydrolytic products (xylose, xylobiose and xylooligosaccharide) the researchers are focusing towards the development of various strategies for efficient xylan degradation. One of the most important strategies focused is the use of heat tolerant biocatalysts which acts as strong and specific cleaving agents. Therefore, the exploration of microbial pool from extremely diversified ecosystem is considerably vital. Microbial populations from extreme habitats are keenly explored for the isolation of thermophilic entities. These thermozymes usually demonstrate fast hydrolytic rate, can produce high yields of product and are less prone to microbial contamination. Another possibility of degrading xylan continuously is the use of immobilization technique. The current work is an effort to merge both the positive aspects of thermozyme and immobilization technique. Methodology: Geobacillus stearothermophilus was isolated from soil sample collected near the blast furnace site. This thermophile is capable of producing thermostable endo-β-1,4-xylanase which cleaves xylan effectively. In the current study, this thermozyme was immobilized within a synthetic and a non-synthetic matrice for continuous production of metabolites using entrapment technique. The kinetic parameters of the free and immobilized enzyme were studied. For this purpose calcium alginate and polyacrylamide beads were prepared. Results: For the synthesis of immobilized beads, sodium alginate (40.0 gL-1) and calcium chloride (0.4 M) was used amalgamated. The temperature (50°C) and pH (7.0) optima of immobilized enzyme remained same for xylan hydrolysis however, the enzyme-substrate catalytic reaction time raised from 5.0 to 30.0 minutes as compared to free counterpart. Diffusion limit of high molecular weight xylan (corncob) caused a decline in Vmax of immobilized enzyme from 4773 to 203.7 U min-1 whereas, Km value increased from 0.5074 to 0.5722 mg ml-1 with reference to free enzyme. Immobilized endo-β-1,4-xylanase showed its stability at high temperatures as compared to free enzyme. It retained 18% and 9% residual activity at 70°C and 80°C, respectively whereas; free enzyme completely lost its activity at both temperatures. The Immobilized thermozyme displayed sufficient recycling efficiency and can be reused up to five reaction cycles, indicating that this enzyme can be a plausible candidate in paper processing industry. Conclusion: This thermozyme showed better immobilization yield and operational stability with the purpose of hydrolyzing the high molecular weight xylan. However, the enzyme immobilization properties can be improved further by immobilizing it on different supports for industrial purpose.

Keywords : immobilization, reusability, thermozymes, xylanase
Conference Title : ICBB 2015 : International Conference on Biotechnology and Bioengineering
Conference Location : Stockholm, Sweden
Conference Dates : July 13-14, 2015