Kinetic Studies of xylanase Produced by Bacillus pasteuri

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The aim of this study is to optimise the conditions for the production of xylanase produced by *Bacillus pasteuri* and to optimise the compositions of the fermentation medium. Single colony of *Bacillus pasteuri* was obtained by cultivating the organism in xylan-agar medium containing (gl^{-1}) nutrient agar 25.0; and xylan, 20.0. Single colonies were selected, activated in xylan-nutrient broth medium (containing (gl^{-1}) xylan, 20.0; and nutrient broth, 25.0) at pH 7.0 and 42°C for 16h and used as inoculum. The inoculum was transferred into the fermentation medium containing (gl^{-1}) xylan, 20.0; peptone, 2.0; yeast extract, 2.5; $CaCl_2.2H_2O$, 0.005; $MgCl_2 6H_2O$, 0.005; FeCl3, 0.005; K_2HPO_4, 2.5; KH_2PO_4, 1.0; NaCl, 0.1 and $(NH_4)_2SO_4$, 2.0. The fermentation was carried out at 42°C and pH 7.0, while shaking at 100 rpm. To improve the production of xylanase, the components of the fermentation medium were changed. The amount of xylan in the fermentation medium was increased from 2 to 80 gl^{-1} and at 48h of fermentation, enzyme activity was measured at pH 7 and 60°C using 10gl^{-1} xylan. The enzyme activity increased from 0.6 to 12.3Uml^{-1} ($U=mgmin^{-1}$) when the xylan concentration was increased from 2 to 80gl^{-1}. The rate of increase was high up to 20gl^{-1}(9.0Uml^{-1}). Therefore for further studies, 20gl^{-1} xylan was used. The amount of $(NH_4)_2SO_4$ in the medium was increased from 0 to 6gl^{-1} while other components of the medium were kept constant and the enzyme produced was monitored at 24, 48, 72, 96 and 116h. The highest enzyme (45.69Uml^{-1}) was produced at 48 hours of inoculation when 2gl^{-1} $(NH_4)_2SO_4$ was added to the medium, while 46.0Uml^{-1} enzyme activity was obtained at 72h when 6gl^{-1} $(NH_4)_2SO_4$ was added to the medium. Therefore 2gl^{-1} was selected for further studies. *B. pasteuri* was cultured at different temperatures of 27, 37, 42, and 50°C xylanase activities were 12.2, 44.7, 48.0 and 3.6Uml^{-1} respectively. Therefore the optimum cultivation temperature for the maximum xylanase production was 42°C. Kinetic properties of xylanase obtained from *Bacillus pasteuri* were determined. Xylan (20gl^{-1}) was allowed to react with xylanase for 4h, the amount of reducing sugar produced was monitored and the reaction time for incubation was fixed as 04min. The pH for the enzyme assay was changed from 4.0 to 9.15 and the optimum pH for the enzyme activity was 6.9 in 0.01M sodium phosphate buffer at 60°C. When the temperature for the assay was changed from 30 to 75°C, the enzyme showed the highest activity at 60°C (49.9Uml^{-1}). The substrate concentration used for the enzyme assay was varied from 10 to 60gl^{-1} and the Michaelis constant was 90gl^{-1}. Further studies are underway to improve the organism and to determine the enzyme stability.