Production of α-amylase by *Bacillus licheniformis* ATCC 6346 in fermenter under controlled conditions

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Application of enzymes in industrial processes requires their production at large scale in bioreactors. Product yield depends on cell density and also on cell specific productivity. Already the production of α-amylase by *Bacillus licheniformis* ATCC 6346 has been optimised in shake flasks and this is scaled up to large laboratory fermenter level. Production of α-amylase by *Bacillus licheniformis* ATCC 6346 was studied in 3L laboratory scale fementer which contained 2L fementation medium. Single colony of *Bacillus licheniformis* ATCC 6346 from nutrient agar slants (grown at 37°C for 24h) was transferred to activation medium incubated at 42°C in a rotary shaker (100rpm) for 12 hours and used as inoculum. The nutrient agar medium contained (gl^{-1}) nutrient agar 25.0 and starch 3.0 and the activation medium contained (gl^{-1}) meat extract, 10.0; peptone, 10.0; NaCl, 5.0 and soluble starch 3.0 at pH 7.0. The fermentation medium was inoculated with 20% (v/v) inoculum and incubated at 42°C and 100rpm. The fermentation medium contained (gl^{-1}) soluble starch, 4.0; $(NH_4)_2SO_4$, 5.0; peptone, 6.0; $FeCl_3$, 0.01; $MgCl_2.6H_2O$, 0.01; $CaCl_2.2H_2O$, 0.01; KH_2PO_4, 4.0 and K_2HPO_4, 7.5. In shake flasks 48.82Uml^{-1} α-amylase activity was obtained at 36 hours, at100rpm and at 42°C but in 3L fermenter no amylase activity was obtained at 36 hours, at 100rpm, 42°C and at 0.6 vvm aertion. When the fermentation was continued the enzyme production was started (1.50 Uml^{-1}) at 48 hours and reached the maximum (13.59 Uml^{-1}) at 104 hours. When the agitation rate of the medium in the fermenter was varied from 100 to 200rpm, the activity of amylase and growth of organism (OD_{600nm}) at 100, 150 and 200 rpm obtained were 5.15, 12.32 & 36.76Uml^{-1} and 2.02, 2.14 & 3.28 respectively at 72 hours, at 0.6 vvm aeration and at 42°C. After the inoculation, the dissolved oxygen percentage immediately reduced to zero and started to increase after 57 hours at 200rpm and this could be the reason for the delayed enzyme production. Therefore the aeration was increased from 0.6 to 1.2 vvm. At 1.2vvm aeration and at 200rpm maximum α-amylase activity (35.05Uml^{-1}) was obtained at 52hours. At 1.2 vvm aeration, the mixing of the medium in the fermenter was varied, the activity of α-amylase and growth of the organism (OD_{600nm}) at 200, 300 and 400 rpm were 4.61, 51.17 & 8.76 Uml^{-1} and 3.17, 3.78 & 4.96 respectively at 28hours and 42°C. When the temperature of the medium in fermenter was varied from 37 to 46°C , the activity of amylase, growth of organism (OD_{600nm}) and dry mass of cells at 37,

42 and 46°C were 50.422, 51.17 & 34.71 Uml^{-1}, 4.17, 3.78 & 3.63 and 2.36, 2.02 & 1.18 $mgml^{-1}$ respectively at 28h, 300rpm and at 1.2vvm aeration. For further scaling-up studies 42°C, 1.2 vvm and 300 rpm were selected. Under these conditions 51.17 Uml^{-1} of α-amylase was produced at 28 hours. In shake flask, maximum α-amylase activity (48 Uml^{-1}) was obtained at 36 hours, at 42°C and 100rpm but in fermenter, maximum α-amylase activity (51.17 Uml^{-1}) was obtained at 28 hours, 42°C, at 1.2 vvm and 300rpm therefore fermentation in 3L fermenter improved the α-amylase production by 1.06 fold and reduced the time by 8 hours.

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