

PRELIMINARY STUDIES ON α -AMYLASE PRODUCTION
IN SOLID STATE FERMENTATION

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The inoculum preparation of *Bacillus licheniformis* 6346 showed a sigmoid curve with mid log phase at 20h. At this hour the optical density was 0.35 (620nm). This stage of culture (20%, v/v inoculum) was inoculated into fresh nutrient broth. Incubated at room temperature (30°C) for 8h and used as the inoculum. Fermentation on rice bran medium having an initial reducing and total sugar contents of 70g l^{-1} and 210g l^{-1} respectively was compared with rice husk medium which contained no reducing sugar but had starch equivalent to 150g l^{-1} total sugar while other nutrients in the bran medium and husk medium, and the fermentation conditions being the same, the production of enzyme in rich bran and rice husk media were 29 U/gDBM (Dry Bacterial Bran) and 250 U/gDBM respectively. This result indicates that the presence of reducing sugar repressed the α -amylase production. The nutrient requirements of *Bacillus licheniformis* in solid state fermentation on rice husk medium was investigated keeping nitrogen, soluble starch and lipids (coconut oil:gingili oil = 1:1) at constant level but with combination of different nitrogen sources [$(\text{NH}_4)_2\text{HPO}_4$; $(\text{NH}_4)_2\text{SO}_4$ and soya flour] and carbon sources [wheat flour and unpolished red raw rice flour]. Maximum α -amylase activity was observed with the combination of $(\text{NH}_4)_2\text{HPO}_4$ and rice flour (433 U/gDBM). To find the significance of lipid sources, the oils were not added to medium containing $(\text{NH}_4)_2\text{HPO}_4$ and wheat flour. The absence of oils decreased the enzyme production by 16%.

The extraction of enzyme from the rice husk medium was optimized under different conditions. It was observed that the mode of agitation and the contact time did not improve the recovery of the enzyme. The enzyme was extracted best when the DBM to buffer ratio was 1:10. The thermostability of the α -amylase was studied in presence and absence of calcium ion. Presence of calcium ion doubled its stability at 85°C. In the absence of calcium ion at 85°C the activity decreased by 50% in 1h and at 95°C the activity decreased by 87.2% in 20 minutes.