

ABSTRACT

The present study is concerned with the optimization of cultural conditions for alpha amylase production by *Bacillus amyloliquefaciens* NRRL-14394 in 7.5 L stirred fermentor having 5.0 L working volume. Among the four different fermentation media analyzed, M4 was declared best for optimum production of the enzyme. Inoculum size which best suited the process was 8 % v/v while production of the enzyme following growth of the organism was found optimum when temperature was 37C and pH 7.0. The agitation and aeration which best supported the growth of the organism was 400 rpm and 1.0 vvm respectively. In order to combat problem of foam formation, suitable antifoam is require which not only break the foam but also should have no or very low effects on the production capacity of the organism. So here in this study among the four different antifoams applied, synthetic antifoam DA (W)-12, gave better results even at a very low concentration. The production of the enzyme reached optimum (82.54 U/ml/min) at 56 h after inoculation. Further increase in incubation time caused degradation of the enzyme.

After optimization of the cultural conditions, the alpha amylase was subjected to purification by precipitation first with 80% ammonium sulphate. In order to completely purify alpha amylase, the precipitated proteins were subjected to FPLC (ion-exchange chromatography). The specific activity of the enzyme increased almost 2000 times while the percent yield after purification was about 40.4 %. Purification fold calculated was 5.24 time. Molecular weight of the enzyme was estimated to be in between 52-55 KDa by using SDS-PAGE. The desizing of the already sized cotton cloth by alpha amylase was also carried out. The maximum (100%) desizing was obtained after one hour incubation when the desizing took place at pH 6.5 and at 80⁰C.