Production, Purification and Characterization of Xylanase from *Bacillus subtilis* and its applications

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SUMMARY

Thewide scale industrial applications of xylanase require cost effective production of the enzyme which make the process economical. This can partly be achieved by using cheaply available lignocellulosic biomasses. Many of the xylanases are produced by alkalophilic organisms such as *Bacillus* sp. Hence in the present study *Bacillus subtilis* was taken as the study organism. The findings of the study are summarized below:

Bacterial isolates selected after screening of xylanolytic activity by Congo red staining were subjected to liquid state fermentation in xylan production medium. The organism used in this investigation were chosen on the basis of their ability to hydrolyze the commercial xylan.

Bacillus subtilis showed the highest xylanase production. Several natural lignocellulosic biomasses were tested as substrates for xylanase production. Among forty five substrates tested, rice bran was found to be the most suitable substrate for enzyme production.

The substrate concentration is one of the critical factors that influence the fermentation process. The maximum enzyme production occurred at 1% concentration.

Optimal parameters influencing the growth and production of enzyme were investigated. The pH and temperature optima for xylanase production was pH 7 and temperature 37°C respectively for the best enzyme production

Incubation period of 48hrsand agitation rate at200rpm was proved to be the best for maximum production.

Effect of different nutritional parameters such as carbon, nitrogen, bivalent ions and metal ions on the xylanase production was determined.In *Bacillus subtilis* carbon source maltose, nitrogen source peptone, bivalent ions calcium chloride, metal ions Magnesium was found enhanced the maximum enzyme production.

The crude culture filtrate of the bacteria was purified to homogeneity by Ammonium sulphate precipitation followed by Sephadox G 200 column chromatography. The molecular weight of the purified enzyme was showed a single band on SDS-PAGE with the molecular weight of 29 kDa.

The purified enzyme was characterized for optimum pH, temperature stability. Effect of metal ions and inhibitors on enzyme production activity was studied.

In the present study, the waste paper pulp was treated with crude enzyme to study the paper quality. After treatment there was a reduction in kappa number.

The possible usage of xylanase of *Bacillus subtilis* as a bread quality improver was tested in whole wheat bread. The purified enzyme was used as an additive during mixing of wheat flour. The effect of xylanase addition on the fermentation stage and the bread quality was analysed which showed a increase in loaf volume.

Enzyme supplementation to the detergents for the cleaning efficiency of stains was studied. The wash performances of the xylanase were assessed by their ability to remove stains on white cotton cloth. The cloth stained with extracts of mango was found to gain 99% of brightness in crude xylanase when compared to other treatments.

In the present study, the biowaste including kitchen wastes in combination with dry leaves and cow dung was subjected to composting using crude xylanase from *Bacillus subtilis* and with Effective Microrganisms(EM). It was noticed that crude enzyme with the combination of EM increased the level of Nitrogen and Phosphorous.

Biosoftening of coir pith was studied using FTIR analysis. It was observed that the presence of lignin amount was decreased in the enzyme treated sample.