Immobilization of xylanase produced by a new isolate *Bacillus pumilus* MTCC 8964

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INTRODUCTION

There is a high interest in enzymatic hydrolysis of xylan (major component of hemicellulose of plant cell walls) due to its applications in feedstock, fuel, chemical production, paper manufacturing, clarification of fruit juices, wine and recovery of fermentable sugars from hemicelluloses (Coughlan et al, 1993; Lama et al., 2004). β-1,4 xylanases (1,4 β -xylan-xylanohydrolase, E.C. 3.2.1.8) catalyze the hydrolysis of xylan to xylo-oligosaccharides and xylose. A variety of microorganisms, including bacteria, yeasts and filamentous fungi, have been reported to produce xylanases (Reis et al., 2003). Although a large number of bacterial xylanase have been isolated, purified and characterized, yet a very few of them have come up for their use in industrial processes due to high cost, restricted availability, difficulty in recovery from the reaction mixture and above all, the fragile nature of enzyme. Advances in enzyme technology have helped in solving some of these problems by immobilizing the biocatalyst to some extent, with choice of batch or continuous processes, rapid termination of reactions, controlled product formation with ease in removal of the biocatalyst from the reaction medium without any contamination. The immobilized enzymes are more stable at higher temperatures and are active over a wide pH range (Kennedy and White, 1985). In the present study an extra-cellular alkaline xylanase produced by a new isolate *Bacillus pumilus* MTCC8964 was selected for immobilization and characterization by adsorption on different supports.

MATERIAL AND METHODS

Microorganism and inoculum preparation

Oat spelt xylan, 3-amino propyl-triethoxysilane and polyethylenimine were from Sigma Aldrich, USA. The glass beads were from Biomatrix Technologies, India and Fibre glass discs were obtained from Fibre Glass India. All other chemicals were of analytical grade. *Bacillus pumilus* MTCC 8964 used in the present study was isolated from cow feed sample and maintained on Potato Dextrose Agar containing oat-spelt xylan (0.5% w/v) at 40 ^{o}C for 24 h.

Enzyme production and assay

For enzyme production one percent of 24h old seed culture (OD_{660}= 0.5) was inoculated in production medium containing potato 5.0%, dextrose 2.0%, yeast extract 0.5%, oat spelt xylan 0.5% (pH 6.0) and incubated at 40 ^{o}C for 48 h at 150 rpm. The fermented broth was centrifuged at 5000 rpm for 15 min at 4 ^{o}C and the supernatant obtained was used as a crude enzyme. Xylanase activity was determined by measuring the reducing sugar by the dinitrosalicylic acid (DNS) method (Miller, 1959) using D-xylose as the standard. The enzyme assay was carried out at 40 ^{o}C using 0.5% (w/v) oat spelt xylan (50 mM Acetate buffer, pH 6.0) as a substrate. One unit of xylanase activity was expressed as 1µg of reducing sugar (xylose equivalent) released/ml/min under assay conditions.

Activation of supports and characterization of xylanase after immobilization

The activation of glass fibre discs (1x1cm each) and glass beads (4-5 mm) was carried out using Bisswanger (2004) method. Immobilization of xylanase on glass fiber discs was carried out by the modified method of Taylor *et al*. (1977). Immobilization of xylanase on glass beads was carried out as

described by D'Souza *et al*. (1986). For Immobilization five ml of enzyme (100 UmL^{-1}) was added to these supports and the activity of the immobilized enzyme was determined by using 0.1gmL^{-1} of immobilized support under assay condition. The activity yield and other characteristics of immobilized enzyme were compared with that of free enzyme and % yield of immobilized enzyme was calculated using the formula:

% Enzyme yield = Activity in immobilized support/ Activity in free enzyme X 100

The Enzyme activity of free and immobilized xylanase was measured at different pH (5.0-11.0) and temperature (30-60 ^{o}C) under experimental conditions. Thermostability profile of xylanase in free and after immobilization on two supports respectively was studied up to 10 h after incubation at different temperatures (30 ^{o}C-60 ^{o}C) by withdrawing and assaying samples at an intervals of 2h each.

Reusability of immobilized enzyme

The alkaline xylanase immobilized on two supports was tested for its reusability using 0.1g (100 UmL^{-1}) of immobilized support repeatedly up to eight times and percent relative activity determined.

RESULTS AND DISCUSSION

The thermostable and alkaline xylanse of a new isolate of *Bacillus pumilus* MTCC8964 produced at 40^{o}C showed optimal activity at pH 10.0 and 40 ^{o}C respectively. This enzyme was immobilized successfully on to two supports viz., glass fibre discs and glass beads (Fig.1). All the experiments were carried out in triplicate and analyzed. The immobilization was preceded by silanization to introduce reactive groups onto inert glass surface to increase the surface area for immobilization (Bisswanger, 2004). Immobilization of enzyme with polyethylenimine is one of the very quick and cheap procedures and reported to improve the catalytic and stability characteristics of the biocatalyst (D'Souza *et al*., 1986).

After immobilization the enzyme activity yield was found to be 68.26 and 73.33% respectively for glass fibre discs and glass beads. The loss of enzyme activity after immobilization is normal phenomenon (Rosevear, 1988). At least 49% of the activity was retained in controlled pore glass immobilized with *Thermus Rt* 41A enzyme (Wilson *et al*., 1994). Both enzyme immobilized supports showed optimum catalytic activity at pH 10.0 and retained 70% and 63% activity at pH 9.0 respectively with glass fibre discs and glass beads (Fig. 2.) The free enzyme also showed the optimal activity at pH 10.0 as reported earlier (Kumar *et al*., 2009). The extra cellular xylanase produced by *Bacillus pumilus* MTCC8964 had optimum activity at 40^{o}C. The maximum activity of xylanase immobilized on glass fibre discs and glass beads was also observed at 40 ^{o}C (100%) and at 50 ^{o}C it was 80% and 84% respectively in glass fibre discs and glass beads (Fig. 3). Taylor *et al*. (1977) recorded high activity with papain immobilized on silanated controlled pore glass, alumina and titania.

The immobilized xylanase was stable up to 4 h at all temperatures on glass fibre discs and glass beads and stability up to 6 h was observed at 30-50^{0}C with both supports (Fig. 4). The stability of immobilized enzymes at higher temperatures is one of the important improvements that have been achieved with immobilized enzymes on different supports (Hayashi and Ikada, 1990; Manolov *et al*., 1995). The enhancement in activity and stability of immobilized enzymes is important for their industrial applications (Rosevear, 1988). The immobilized xylanase was quite stable and could be reused 3-4 times without any considerable loss in enzyme activity in both supports (Fig. 5). The slight loss in activity after four times was observed in both supports. This may be due to abrasion of supports during repeated use (Rosevear, 1988).The immobilized xylanase (100 UmL^{-1}) was evaluated for xylan hydrolysis from natural apple juice. It took 24h to reduce the hazyness with immobilized supports at ambient room temperature (Fig. 6). Xylanase was already reported to show 27% decrease in insoluble materials during juice clarification (Olfa *et al.*, 2007).

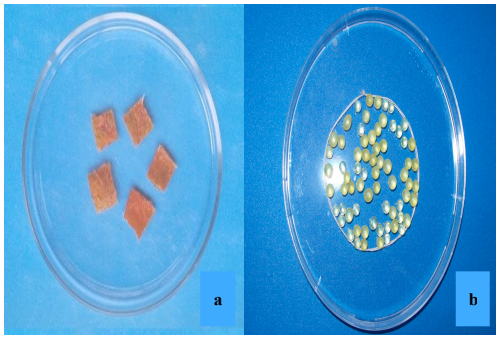

Figure 1. Xylanase of *Bacillus pumilus* MTCC8964 (100 UmL^{-1}) immobilized onto a) glass fibre discs (1x1 cm) and, b) Glass beads (4-5 mm size)

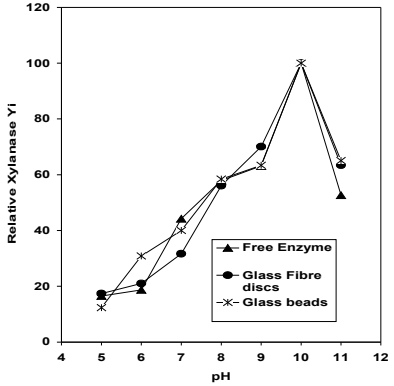

Figure 2. Effect of pH on activity of immobilized xylanase. Optimum value corresponds to relative yield (100%) of immobilized enzyme respectively on glass fibre discs (68.26 UmL^{-1}) and glass beads (73.33 Uml^{-1}).

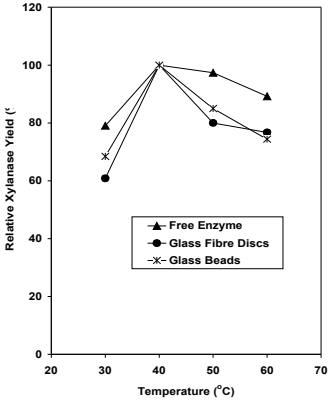

Figure 3. Effect of temperature on immobilized xylanase. Optimum value corresponds to relative yield (100%) of immobilized enzyme respectively on glass fibre discs (68.26 UmL^{-1}) and glass beads (73.33 Uml^{-1}).

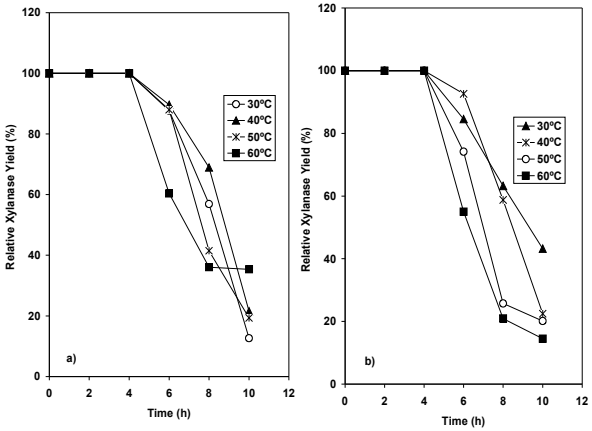

Figure 4. Thermostability profile of immobilized xylanase a) Glass fibre discs and, b) Glass beads.

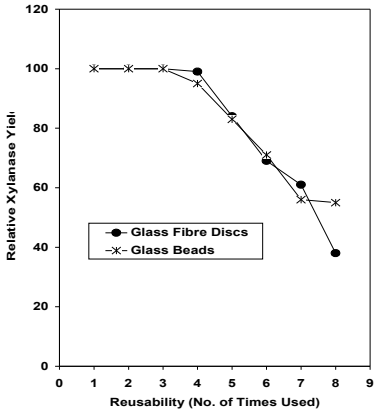

Figure 5. Reusability of immobilized enzyme

Figure 6. Treatment of fresh apple juice with enzyme immobilized glass beads (100UmL^{-1}). a) apple juice before treatment b) after incubation with glass beads for 24 h at 30^{0}C.

CONCLUSION

The present work describes the immobilization of xylanase of a new strain of *B. pumilus* SILB-X MTCC8964. The immobilized xylanase owing to its alkaline, cellulose free nature and activity at high temperature seem to be of considerable use in paper industry and fruit juice clarification. Detailed characterization of the enzyme is in process.

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