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Research Article

Exploiting endo-β-1, 4-xylanase enzyme from fungal origin for green leather processing: A novel and sustainable strategy

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ABSTRACT

The linear polysaccharide β-1, 4-xylan (hemicellulose), one of the major components of plant cell walls, is considered as by product in paper making process. The enzymatic hydrolysis of β-1, 4-xylan by xylanases into xylose can be useful for preparation of different value added product with wide industrial applications. Microorganisms are the rich sources of xylanases, produced by diverse genera and species of bacteria, actinomycetes, and fungi. Several species of Bacillus and filamentous fungi secrete high amounts of extracellular xylanases .However; the principal producer of xylanase is filamentous fungi. Hence, xylanase can be explored in various industrial applications including greener processing of hides and skins. It can be used for the soaking, greener cleaning of hides and skin, bleaching of pigments from skin in leather industries. In this work, a Three Phase Partitioning (TPP) method has been used for isolation of this enzyme from *Agaricus bisporus* which is an edible basidiomycete mushroom. The isolated enzyme extract was assayed to find out the xylanase activity. The enzyme production was optimized under variable experimental conditions such as ammonium sulphate concentration, ratio of culture filtrate to tertiary butanol (v/v) and pH. It was found that all the three experimental variables influence the degree of enzyme production and its activity. Maximum enzyme was obtained in the inter-facial phase at 50 % ammonium sulphate saturation (w/v) when the other conditions were maintained constant, whereas at 1:2 ratio of culture filtrate to *t*-butanol (v/v) and at pH 6 keeping the remaining experimental variables constant individually the same result has been achieved. This work might pave the way towards a greener eco-friendly enzyme based leather processing.

Keywords- Hemicellulose, hydrolysis, xylanase, basidiomycete, inter-facial phase, leather processing.

1. INTRODUCTION

Bio refinery of lignocellulosic biomass is gaining considerable economic importance as it can be platform intermediate chemicals with respect to current ones. Xylose, the 2nd most abundant sugar present in lignocellulosic biomass after glucose, is the hydrolysed product of xylan (Basinskiene L., Garmuviene S, Juodeikiene G., Haltrich D, 2006). Xylanase catalyzes the hydrolysis of glycosidic linkage of linear polysaccharide β-1, 4-xylan, leading to the formation of xylose, xylobiose and others sugars (Aspinall, G.O 1959). Indeed, xylanase is ubiquitous among microorganisms (fungi, bacteria and actinomycetes) belonging to several ecological niches. It plays a major role in micro-organisms thriving on plant sources for the degradation of plant matter into usable nutrients, increasing the sustainability of lignocellulosic biomass. Several efforts are underway to achieve an efficient commercially viable process development for xylose production and purification.

Three-phase partitioning (TPP) is one of the novel bio separation approaches for industrially important enzymes with the enhancement of catalytic power of the enzyme (Agnihotri S, Dutt D, Tyagi C H, Kumar A, Upadhyaya JS, 2010). The method involves the addition of a salt followed by the addition of a water miscible organic solvent to the crude protein extract (Amnison, G. 1992). In TPP, proteins are excluded from two immiscible liquid phases into a middle zone that becomes concentrated by low speed centrifugation into a thin disk (Roy I and et al 2000). Kosmotropy, electrostatic forces, conformation tightening and protein hydration shifts have been suggested as the physico-chemical basis for underlying TPP of protein (Kalyanpur, M 2000).

As a ‘‘proof of the concept’’, TPP approach was adopted for the commercial downstream processing of xylanase using agro-residues in a cost effective manner (Wati RK, Theooakron T, Benjakul S, Rawdkuen S 2009). *Agaricus bisporus* is an edible basidiomycete mushroom native to grassland in Europe and North America. It represents a very attractive lignocellulosic feedstock in bio refinery schemes. It is shown that xylanase preparation, subjected to TPP, resulted in recovery of xylanase activity as well as significant purification of the enzyme. Various parameters affect the efficient partitioning of xylanase. Therefore, concentration of ammonium sulphate added, pH of the culture medium and ratio of volume of *t*-butanol to the culture filtrate were varied to optimize in order to get highest purity fold and yield in partitioning (Ohkoshi A, Kudo T, Mase T, Horikoshi K, 1985).

2. Materials and methods

2.1 Microorganism and Inoculum Preparation.

The fungus *Agaricus bisporus* was used for the submerged fermentation of xylanase production system. The *A.bisporus* was maintained on potato dextrose agar slants at 4 °C. The active *A.bisporus* was inoculated in a complex medium containing 10%potato extract, 1% glucose, 0.15 % KH_2PO_4 and 2% malt extract. The mycelial culture of *A. bisporus* was grown at 25 °C for 7 days (Fig. 1).

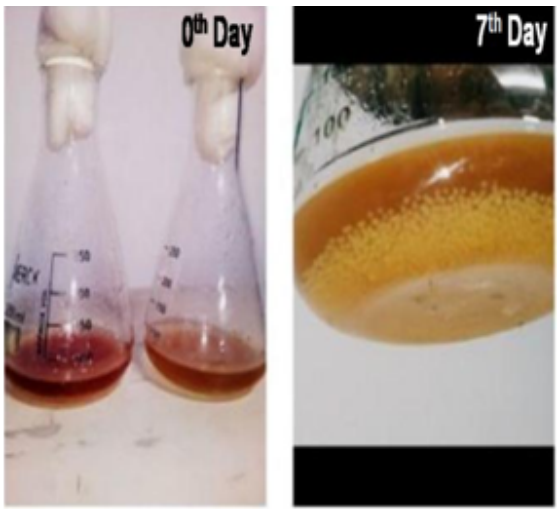

Fig. 1. Pure culture preparation of *A. bisporus* in synthetic media

2.2. Medium Preparation for enzyme production

Xylanase production medium containing 2 % tamarind kernel powder, 0.2% yeast extract, 0.15 % KH_2PO_4, 0.037% $CaCl_2$,H_2O, 0.05 % $ZnSO_4$, $7H_2O$,0.05 % $MgSO_4$, $7H_2O$, 0.05% H_3BO_3, 0.05% $MnCl_2$, $5H_2O$, 0.0036%$NaMoO_4$, H_2O, 0.0036$FeSO_4$, $7H_2O$and 10 % (v/v) spore suspension. TKP (Tamarind Kernel Powder) was used as carbon source fermentation was carried out at about 30°C for a week in shake flask culture and culture filtrate was used for the enzyme protein partitioning system.

2.3. Three-phase partitioning of xylanase

Three phase partitioning uses *t*-butanol and ammonium sulphate to precipitate xylanase from aqueous solution (Pike R N, Dennison C, 1987). The crude culture filtrate was mixed with ammonium sulphate at 25 °C and then vortexed gently to dissolve the salt. The mixture was vortexed gently for 1 min and then allowed to stand for 1 h at room temperature. Afterwards, the mixture was centrifuged at 5000 rpm for 10 min again at room temperature and the three phases were observed. Tertiary butanol is normally completely miscible with water, but upon the addition of enough salt, such as ammonium sulphate, the solution separates into two phases, a lower aqueous phase and an upper organic *t*-butanol phase. Presence of protein in the original aqueous phase leads to the formation of an intermediate phase containing xylanase–*t*-butanol co-precipitates between the lower aqueous and upper *t*-butanol phase (Fig 2).

They float above denser aqueous salts because bound *t*-butanol increases the buoyancy. Concentration of ammonium sulphate added, pH of the culture medium and ratio of volume of *t*-butanol to the culture filtrate were optimised as the floating is the function of these process parameters (Sharma Shweta, Gupta M N, 2000)

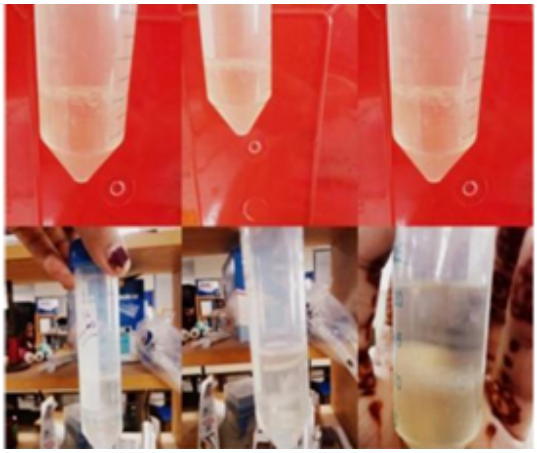

Fig. 2. Three phase partitioning of enzyme in lab scale

2.4. Xylanase activity assay

For xylanase activity determination xylan was used as a substrate. 0.2 gm of xylan was dissolved in 10 ml of distilled water. Four test tubes were marked as blank, control, $test_1$ and $test_2$. In the blank test tube, 2ml of distilled water and 3ml of DNSA was added and mixed together and kept aside. The remaining three marked test tubes contain the assay samples. The assay samples contain 3ml of DNSA, 200 µl of acetate buffer (pH 5.0), 1.4 ml of the substrate and 200 µl of an appropriate enzyme solution volume. The reaction mixtures were incubated at 40 °C for 10 min, and reducing sugars liberated were determined by measuring O.D.

3. RESULT AND DISCUSSION

In order to optimize best three-phase partitioning system for purification of endo-xylanase from culture filtrate, effect of various process parameters such as percent saturation of ammonium sulphate, crude extract to *t*-butanol ratio and pH of the culture medium were analyzed.

3.1. Effect of ammonium sulphate concentration

In order to determine the effect of ammonium sulphate concentration on partitioning, the assay

was carried out over the concentration range of 30-70 %.The relative activities (%) were expressed as the ratio of the xylanase activity obtained at a certain temperature to the maximum activity obtained at the given temperature range. Fig 3 shows the partitioning of endo-xylanase into the interfacial phase with different percent saturation of ammonium sulphate with *t*-butanol equal in volume to the starting crude enzyme solution. The results demonstrate that optimized ammonium sulphate concentration for the enzyme extraction in TPP was found to be 50%.

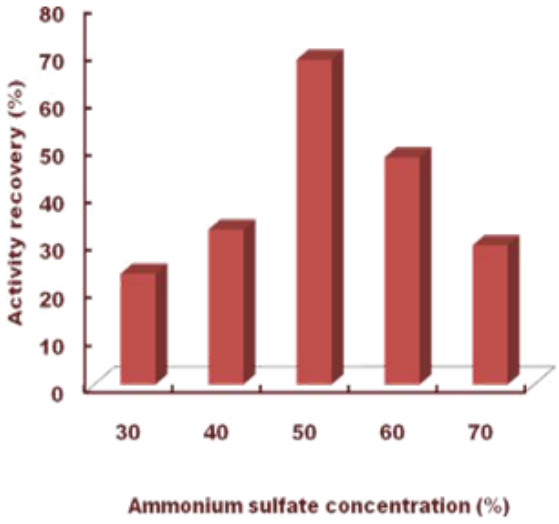

Fig. 3. Effect of varying concentration of ammonium sulphate on the enzyme activity recovery of endo-xylanase in three-phase partitioning system.

3.2. Effect of culture filtrate to *t*-butanol concentration

Various amounts of *t*-butanol was added to crude extract and saturated with 50% ammonium sulphate in the volumetric ratio viz. 1:0.5, 1:1, 1:1.5 and 1:2 (shown in Fig. 4) shows the optimization of crude extract to *t*-butanol ratio for the recovery of enzymes.

The best result was obtained at 1:2 ratio of ammonium sulphate and *t*-butanol interfacial phase.

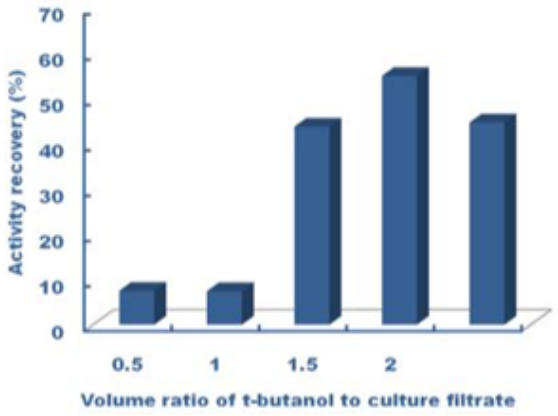

Fig. 4. Effect of varying ratio of culture filtrates to *t*-butanol on the enzyme activity recovery of endo-xylanase in three-phase partitioning system

3.3. Effect of pH on the activity and stability of partitioned xylanase

The effect of pH on the activity of xylanase was investigated by incubating samples with acetate buffer of different pH, ranging from 2.6 to 7.0 at 37 °C. The crude culture filtrate of Xylanase was brought to 50% ammonium sulphate saturation. The pH was adjusted to different pH value in the desired range, followed by addition of *t*-butanol in the ratio of 1:1 (v/v). After incubation, the residual activity (%) with respect to control was assayed under standard activity assay conditions. Fig. 5 shows the effect of pH on the enzyme recovery. It has been found that maximum enzyme was recovered at pH6.

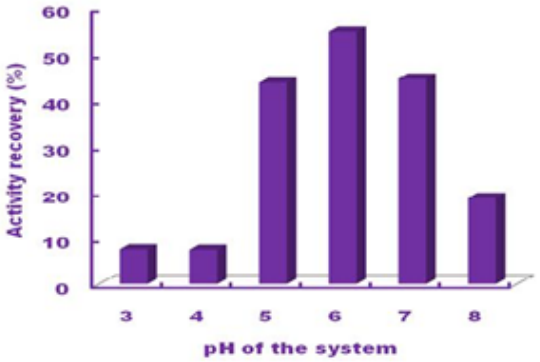

Fig. 5. Effect of pH of the system on the enzyme activity recovery of endo-xylanase in three-phase partitioning system.

4. CONCLUSION

Three – phase partitioning has so far been shown to be useful for downstream operation in protein recovery. It is a simple, quick and economical technique and scaling up is convenient. I can conclude from the present set of researches that with necessary optimization, the combination of 50 %(w/v) ammonium sulphate saturation with 1:2 ratios of xylanase to *t*-butanol (v/v) at ph 6.0 was optimal for attaining the best recovery of xylanase of *A.bisporus*. As TPP is a scalable process, the process described here has the potential to be carried out at the production level. The data given in the experiments also shows the efficiency of TPP as an initial step for bio separation of xylanase. Applications of xylanase avoid the use of chemicals that are expensive and cause pollution. Thus xylanase produced by this technique can be used for eco friendly and greener leather production.

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