



AQUEOUS TWO PHASE PURIFICATION OF XYLANASE OBTAINED FROM A BACTERIAL ISOLATE

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ABSTRACT

The aim of this work was to extract and to purify xylanase produced by the bacillus strain from the crude fermentation broth using aqueous two phase system (ATPS). Xylanase is an important industrial enzyme that finds use in several industries like paper and pulp, textiles, food and feed and production of bioethanol from agri-residues. There is a huge demand for a cost effective xylanase in the industry. The xylanase was extracted by partitioning in ATPS composed of phosphate and polyethylene glycol (PEG). The technique resulted in 1.35 fold purification. The specific activity of the xylanase obtained was 6.27mu/mg

KEYWORDS: Xylanase, Aqueous two phase systems, ATPS, Lignocellulose degradation, Enzyme purification



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INTRODUCTION

Hemicellulose is the second most abundant plant components of plants and the waste plant residues contain up to 40% hemicellulose formed by pentose sugars.¹ Recently enormous interest has been generated in process that converts the plant waste into biofuels. The enzymatic hydrolysis of abundantly available xylan leads to formation of fermentable sugars *i.e* xylose which can be fermented by several species of microorganisms into many useful products.^{2,3,4,5,6} Xylanases are elaborated ubiquitously in many types of microorganisms such as bacteria, fungi, protozoans and some yeast. Recently there is an increasing demand for cost effective microbial xylanolytic enzymes which benefits the industrial applications. Aqueous two phase systems (ATPS) can be considered as an integrated technique where extraction, concentration and primary purification are in a single unit operation. This effectively diminishes production enzyme cost resulting in increased economy of enzyme application. Liquid – Liquid solvent extraction process is a well-established industrial unit operation. The existing processes can easily be adapted for aqueous two phase systems. ATPS are characterized by their versatility, easy scale up parameters, process integration capability and relative low cost. This technique is commonly regarded as a primary recovery stage mainly due to its low selectivity. However, the use of strategies involving the modification of ATPS with affinity ligands have resulted in significant increases in recovery yields and purification folds of biological products. In the present paper we have attempted the purification of xylanase from culture broth of bacterial isolates and the efficiency and specific activity of the enzyme produced was determined.

MATERIALS AND METHODS

Routine culture maintenance

The bacterial isolate was maintained in liquid medium as well as solid medium in medium containing 0.5% (w/v) xylan adjusted to pH 8.0. The isolate was cultured at 32°C and preserved at 4°C for short term.

Screening and isolation of thermophilic xylanase producing bacteria

The thermophilic bacteria used in this study were isolated from Shiv kund (hot water geyser), Sohna, Haryana, India. The water temperature of this hot spring varies between 46°C to 51.7 °C. The muddy water samples of the hot springs were brought to the laboratory at 4°C. The samples were serially diluted in sterile distilled water and spread on to xylan agar plates with following composition in g/l: wheat bran, 5.0; yeast extract, 3.0; peptone, 5.0; NaCl, 5.0 and agar, 20.0, pH-9.0. These plates were incubated at 45-50 °C for 3 days. The xylanase producing capability of the cultures was identified by flooding the plates with 0.1% aqueous congo red. for 15 min followed by repeated washing with 1 M NaCl.⁷ The bacterial colonies that exhibit xylanase activity can be visualized have clear zones around the colonies. Pure cultures for these colonies were established and the bacterial isolate A was

selected on the basis of high xylanase production capacity.

Production of xylanase

The bacterial culture was activated and pre-cultured for 48h in a basal salt (BSS) medium supplemented with 0.5 % (w/v) xylan. Erlenmeyer flasks (500ml) having 100 ml of basal salt medium were inoculated (5% v/v) with the activated cultures. The Erlenmeyer flasks were incubated at 32°C in an incubator shaker at 100 rpm. Samples were collected regularly at 24h interval and further analysed for xylanase.

Xylanase assay

Xylanase hydrolyzes the polymer xylan into the xylose monomers. In this assay 1% (w/v) birchwood xylan solution was prepared by dissolving 1gm of xylan in 100ml of sodium citrate buffer. Xylanase activity was measured according to Bailey *et al.*⁸ Buffered solution of 1% (w/v) birchwood xylan (900 µL) was added to enzyme solution (100µL) in a test tube. The enzyme-substrate mixture was allowed to react at a temperature of 50°C for 5min in water bath. The reaction was stopped by adding 1.5mL DNS reagent and this mixture was boiled in a boiling water bath for 5 minutes for development of colour.⁹ The absorbance was measured at 540 nm. One unit of xylanase activity was defined as the amount of enzyme that liberates 1micromole of reducing sugars equivalent to xylose per minute under the assay conditions described. Solubilised xylan was prepared by stirring birchwood xylan with 1M NaOH for six hours at room temperature followed by centrifugation and freeze drying the supernatant after neutralising the alkali with 1M HCl.

Protein estimation

Total soluble protein was measured according to Lowry *et al.*¹⁰ Protein concentration was determined using bovine serum albumin (BSA) as a standard.

Preparation of ATPS

Polyethylene glycol of molecular weight PEG 6000 was used for the preparation of ATPS. 10 -gram phase systems were prepared by adding appropriate amount of 22% PEG, 10% K₂HPO₄ and 12% NaCl in crude enzyme solution. If required, pH of the crude enzyme was adjusted to 8.5 by addition of alkali. The mixtures were vortexed for 5 min and the phases were allowed to separate in graduated cylinders for 12 h. Then the top phase was carefully removed with a pipette, leaving a small amount at the interface, and the bottom phase was then sampled through the interface. Samples of each phase were analysed for enzyme activities and protein.

RESULTS AND DISCUSSION

The bacteria possessing xylanolytic potential were isolated from the soil sample collected from Dumdama lake, Sohna using enrichment culture technique. A high yielding isolate of mesophilic gram positive bacteria (Isolate A) was purified and used in the present study. Isolate A was inoculated in shake flasks (250 ml) having 50 ml of basal medium where oats spelt xylan was the only carbon source. The medium consisted of (g/L):

xylan, 5.0; peptone, 5.0; yeast extract, 5.0; K_2HPO_4 , 1.0 and $MgSO_4 \cdot 7H_2O$, 0.2; (pH 9.0). Sample of the culture broth was withdrawn every 24 h and cell growth as well as enzyme concentration was determined. All of the

studies were carried out in triplicates and mean of three readings were plotted with the standard deviation as given in Figure 1.

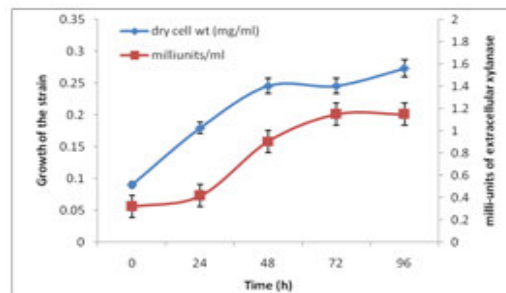


Figure 1
Time-course of cell growth and xylanase production by Isolate A

It is evident from the Figure 1 that the Isolate is well adjusted to the culture medium and the lag phase is imperceptible in the growth curve. The log phase of cell growth is observed to last for 48 h after inoculation. Subsequently, the cells enter stationary phase where no cell growth occurs. Enzyme production commences only after the completion of 24 hours of culture and continues well into the stationary phase of growth. After 72 h no further increase in cell mass or enzyme concentration can be achieved. The batch culture was terminated after 96 h. The cell culture broth so obtained was centrifuged to separate the cell debris and the cell culture broth. The

cell culture broth so obtained was subjected to purification using aqueous two phase system (ATPS). The xylanase was extracted by partitioning in ATPS composed of phosphate and polyethylene glycol (PEG) as given in the previous section. Once the top and bottom phases separate the top phase was carefully removed using a pipette. Samples of each phase were analysed for enzyme activities and protein. Table 1 shows the enzyme activity, protein concentration and specific activity obtained before and after the aqueous two phase extraction.

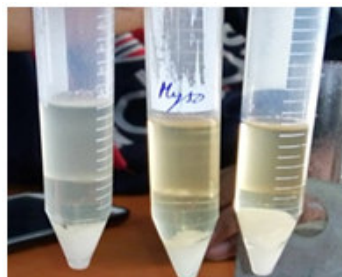


Figure 2
Aqueous two phase systems separation of xylanase

Table 1
Summary of aqueous two phase extraction of xylanase from Isolate A

	mu/ml	Protein mg	Specific activity mu/mg	% recovery	Fold Purification
Crude	1.852	0.3993	4.6381	100	1
Top- Phase	1.8173	0.29	6.2666	98.1264	1.3511
Bottom Phase	0.0062	0.01	0.0617	01.8736	

The xylanase was found to get concentrated in the top phase as has been reported by other researchers too.¹¹ A negligible loss of enzyme activity occurred in ATPS purification step as shown by the recovery of 98% in the top phase. Moreover the specific activity of xylanase increased 1.35 fold. It can therefore be concluded that ATPS systems can be used conveniently as the first step of recovery and concentration of xylanase from the bacteria Isolate A.

FUTURE SCOPE

Further optimization of production would lead to increase in activity. The parameters for ATPS production such as salt and PEG concentration *etc.* can be optimized to increase the purification fold.

CONFLICT OF INTEREST

Conflict of interest declared none.

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