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Production & Partial Purification of Xylanase Enzyme from Soil Isolate

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Abstract

Enzymes can carry out myriads of biochemical reactions under ambient conditions. Because of their eco-friendly nature, they are the best alternative to polluting chemical reactions in many industrial applications. Interest in xylanases has increased over the last few years mainly due to the applications of these enzymes in paper industries for pulp treatment as well as for improving the effectiveness of conventional bleaching chemicals [8, 10]. Present work was conducted to isolate xylanase producing soil bacteria. The birchwood xylan, along with local agricultural sources like wheat bran, rice straw, rice husk, sugar cane bagasse, etc. were tested as raw materials. Amongst all the raw materials used, wheat bran showed maximum xylanase activity 149.2±0.2 IU/mL, at optimum temperature 37^{o}C and pH 8.0. While 2 % of wheat bran along with yeast extract gave highest production in present studies. The purified enzyme showed the specific activity of 20.7 IU/mg protein as compared to crude enzyme 17.2 IU/mg protein in case of wheat bran.

Keywords: Xylanase, Wheat bran, Birchwood xylan, Yeast extract, Beef extract, Bleaching.

1. Introduction

Xylan is one of the major components of hemicelluloses found in plant cell wall while hemicelluloses is the second most abundant polysaccharide next to cellulose [2, 7, and 30]. Xylan is a heteropolysaccharide having O-acetyl, arabinosyl and 4-O-methyl-D-glucuronic acid substituent & have a backbone consisting of β-1, 4 linked D-xylosyl residues [24]. Complete xylan degradation requires the combined action of different xylanolytic enzymes, such as endo-1, 4-β-xylanases, β-xylosidase, α-arabino furanosidase, and esterase [28, 29]. Endo-1, 4-β-xylanases (1, 4-β-D-xylan xylanohydrolase, E.C.3.2.1.8) depolymerise xylan by the random hydrolysis of xylan back bone and 1, 4- β-D-xylosidase (1, 4-β-D-xylan xylohydrolase E.C.3.2.l.37) split off small oligosaccharides [15]. This can be used by microbial systems as a primary source of sugar [13]. The products of xylan hydrolysis are xylose, xylobiose, xylotriose, xylotetrose and xylo-oligosaccharides. These compounds have possible applications as food additives in poultry, in wheat flour for improving dough

handling and the quality of baked products and for extraction of coffee and plant oils. [23]. A large number of bacteria and fungi are known to produce xylanases [1, 21, and 25]. Xylanases derived from microorganisms have immense potential applications in various industries including food, feed, textile and paper processing industry [31]. The use of xylanase in the paper and pulp industry is related to reduction of environmental pollution caused by the use of large amounts of chlorine and chlorinated compounds during pulp bleaching [6].

Xylanase production can be carried out using agricultural waste materials; those are used as substrates which provide carbon and mineral nutrients to the organisms under the controlled conditions [2, 27]. As the price of the substrate plays a crucial role in overall processing cost, cheap substrates such as agroresidues are nowadays becoming choice of many large scale enzyme productions [26]. The production process of any enzyme is affected by nutritional and physiological factors such as carbon source, nitrogen source, additives, inoculum size, pH of the media, incubation temperature, agitation rate, and others. Higher production of industrial enzymes by optimizing these parameters is of prime importance, because an improper optimization of these factors leads to a lower production of the enzyme [14].

The recent studies are focusing on the optimization of various parameters such as carbon & nitrogen sources, temperature, & pH. As the purity of enzymes plays major role in their applications, partial purification of enzyme was also performed.

2. Materials & Methods

2.1. Microorganism & Maintenance

Y2 isolate, obtained from soil, was used in this study. Stock cultures were maintained on xylan agar medium [19] at 4°C.

2.2 Inoculum preparation

The erlenmeyer flask (250 mL) containing 50 mL of nutrient broth was inoculated with the Y2 isolates and incubated at 37^0C for 24 h under shaking conditions.

2.3 Procurement & Pre-treatment of substrates

Indigenous carbon sources like wheat bran & rice straw were obtained from local farmhouses of Surat, Gujarat, while rice husk & sugar cane bagasse were obtained from local flour-mill & sugar factory of Surat, Gujarat. Selected agricultural residues were washed 2-3 times with distilled water followed by drying, mashing and then treated with 1.0% (W/V) NaOH for 2 h. Finally, treated residues were washed with distilled water for several times, and allowed to air dry [12]. Commercially available birchwood xylan (HiMedia) was also used as a carbon source for comparative studies.

2.4 Production of Xylanase Enzyme by Submerged Fermentation

Xylanase production by submerged fermentation was carried out using medium containing Bergey's mineral salts like 0.3% $NaNO_3$, 0.05% K_2HPO_4, 0.02% $MgSO_4.7H_2O$, 0.002% $MnSO_4.H_2O$, 0.002% $FeSO_4.7H_2O$, 0.002% $CaCl_2.2H_2O$, 0.1% yeast extract and 0.5% of various raw materials along with commercially available birchwood xylan; pH of the medium was set to 8.0. The flasks were inoculated with 1.5% (w/v) of 24 h old inoculum and incubated at 37^0C on an orbital shaker at 120 rpm. The resulting bacterial growth was

separated from the culture medium by centrifugation at 10,000 g at 4°C and supernatant was used as crude enzyme.

1.5. Enzyme assay

Xylanase activity was determined using birch wood xylan as substrate [5]. One mL of the reaction mixture containing 0.5 mL of appropriately diluted enzyme and 0.5 mL of 1% birch wood xylan (in Phosphate buffer, pH 7.0), was incubated for 10 min at 60^{o}C. The reducing sugars released were determined by the dinitrosalicylic acid method [18]. Xylose was used as a standard.

1.6. Protein determination

Protein concentration was estimated by the Folin-Lowry method [17]. Bovine serum albumin (sigma) was used as a standard to calculate the concentration of proteins.

1.7. Optimization of production parameters

2.7.1. Effect of carbon sources on xylanase production

Enzyme production was carried out using various carbon sources viz. wheat bran, rice straw, rice husk, sugarcane bagasse and birch-wood xylan, used at 0.5% (w/v). All flasks having 50 mL of xylanase production medium were kept on shaker at 37^{0}C, at 120 rpm.

2.7.2. Effect of nitrogen source on xylanase production

Enzyme production was monitored using various inorganic nitrogen sources viz. KNO_3, NH_4Cl and $(NH_4)_2SO_4$ and organic sources viz. peptone, yeast extract, beef extract, at 0.5% (w/v) and in combination also. Wheat bran (0.5% w/v) was used sole carbon source in the production medium. A control devoid of nitrogen source was also kept.

2.7.3. Effect of substrate concentration on xylanase production

Enzyme production was carried out by taking different concentration of wheat bran viz. 0.5 %, 1.0 %, 1.5 %, 2.0 % & 2.5 % in the flask containing 100 mL production medium & inoculated with 1.5% of bacterial isolate, incubated at 37^{0}C & 120 rpm.

2.7.4. Effect of temperature on xylanase production

Xylanase production was studied by inoculating each flask containing 100 mL of production medium, with 1.5 % of 24 h old inoculum and incubated at different temperatures viz. 30^{0}C, 37^{0}C, and 45^{0}C at 120 rpm. The enzyme was assayed in the culture filtrate.

2.7.5. Effect of pH on xylanase production

Xylanase production was studied at pH values ranging from 6.0-10.0. Erlenmeyer flasks containing production medium of different pH viz. 6.0, 7.0, 8.0, 9.0 & 10.0 was inoculated with 1.5 % of 24 h old inoculum and incubated at 37^{0}C in a rotary shaker incubator at 120 rpm.

1.8. Purification of Xylanase

1.8.1. Ammonium sulphate precipitation

The crude enzyme was purified from the culture supernatant by precipitating the proteins using ammonium sulphate precipitation procedure. Initially the needed concentration of

$(NH_4)_2SO_4$ was obtained by performing precipitation at various ammonium sulphate concentrations i.e. 30, 40, 50, 60, 70 and 80% by carried out this procedure at 4°C for few hours with continuous stirring. After complete dissolution, supernatant was kept at 4°C overnight, for allowing proteins to precipitate. The precipitates were then collected and analysed for xylanase activity [16].

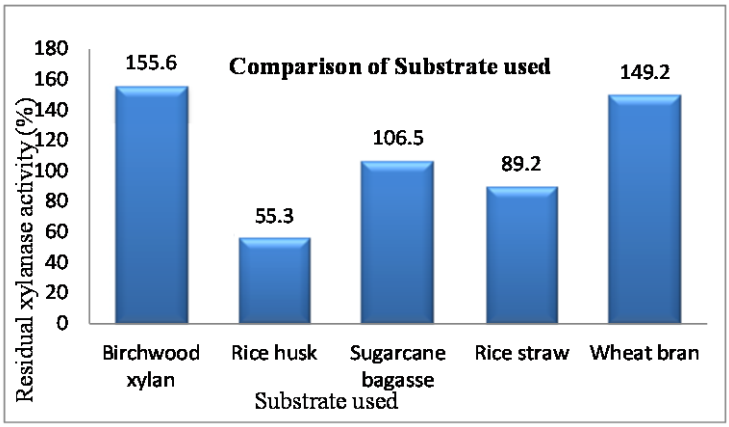
1.8.2. Desalting by dialysis

After precipitation, the ammonium sulphate present in the enzyme solution was removed by subjecting the solution to dialysis in a phosphate buffer (pH 7.0) for 24 h at 4°C [9]. The concentrated protein sample was then carefully withdrawn and stored at 4°C. Enzyme activity was estimated using standard protocol [5, 18] to calculate the fold of purification.

2. Results & Discussion

3.1. Optimization of production parameters

3.1.1 Effect of carbon sources on xylanase production



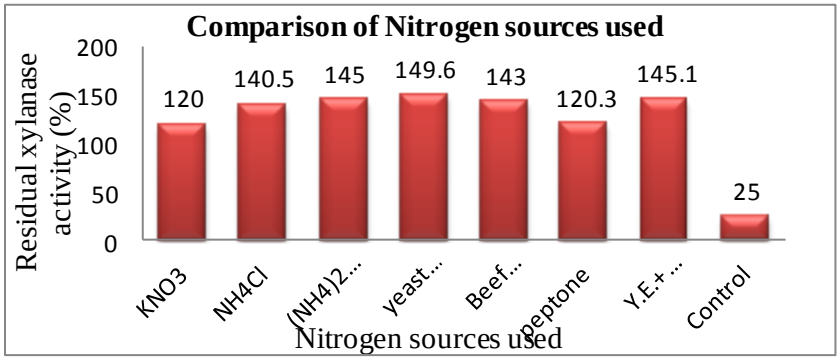
Graph 1: Comparison of carbon sources used for xylanase production using Y2 isolate.

The use of purified xylan as a substrate is uneconomical for large scale production of xylanase. So effect of some industrial and agricultural residue as carbon source was determined [3, 4]. From results, it is observed that xylan (155.6 IU/mL) & wheat bran (149.2 IU/mL) showed the highest production of xylanase, followed by sugarcane bagasse, rice straw & rice husk. As the use of wheat bran showed highest production, it can be considered that agricultural sources are economically viable & are able to give higher yield of extracellular xylanase.

3.1.2. Effect of nitrogen source on xylanase production

Nitrogen sources are also having major role in microbial growth and metabolism. Xylanase production was estimated in the presence of several organic and inorganic nitrogen sources using 0.5% wheat bran as a substrate. In the case of wheat bran as a carbon source, organic nitrogen sources such as yeast extract, peptone and beef extract resulted in higher enzyme titre compared to inorganic compounds such as KNO_3 and NH_4Cl. The effect of different combinations of nitrogen sources was also analysed on enzyme production. It was observed that a combination of yeast extract, and $NaNO_3$ also resulted in efficient enzyme production. Yeast extract has shown the highest xylanase production (149.6 IU/mL) using wheat bran

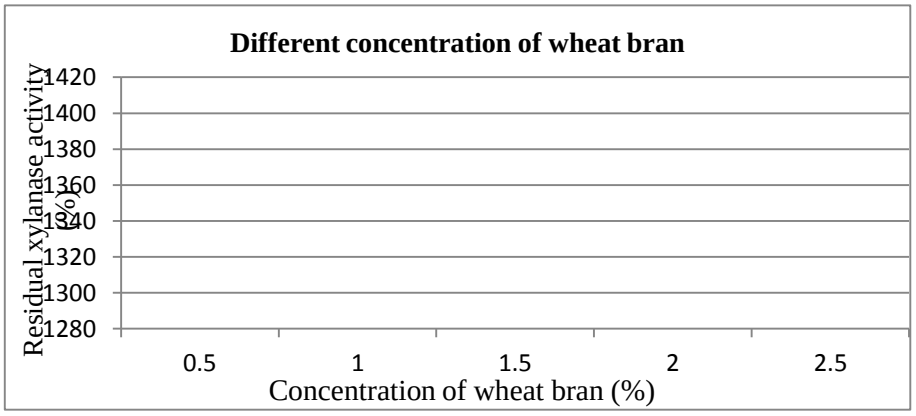
containing medium. So, the large scale enzyme production can be carried out using yeast extract as only nitrogen source.



Graph 2: Comparison of nitrogen sources using wheat bran as sole carbon source.

3.1.3. Effect of substrate concentration on xylanase production

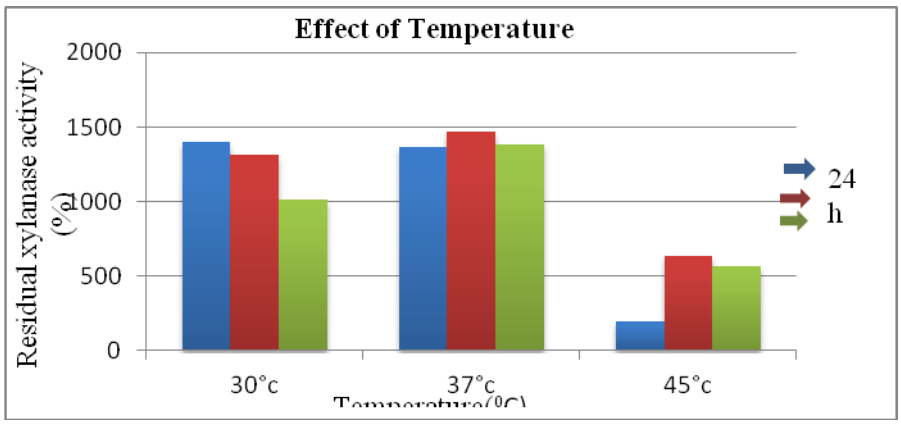
Xylanase production was found to vary with change in the concentration of substrate. Enzyme production was carried out in the presence of 0.5–2.5 % wheat bran. The highest activity was found to be at 2.0 % wheat bran, and decline in xylanase production was found by increasing the concentration of wheat bran beyond 2.0 %. This could be due to formation of a thick suspension and improper mixing of the substrates in shake flasks. So, excess concentration of substrate results in decline in enzyme activity.



Graph 3: Effect of wheat bran concentration (%) on xylanase production in submerged fermentation.

3.1.4. Effect of temperature on xylanase production

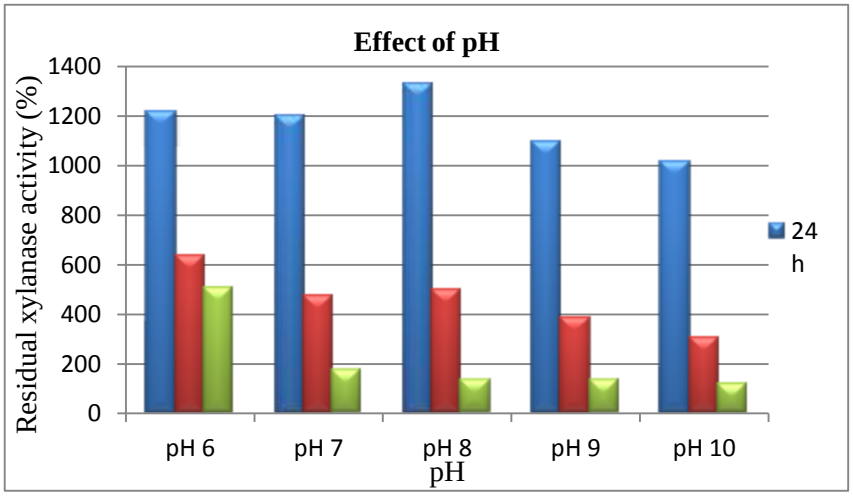
Generally, microbes are known to produce high enzyme titre at their optimum growth temperature. So, xylanase production was studied using wheat bran at different temperature viz. 30^0C, 37^0C & 45^0C. The optimum temperature for xylanase production was found to be 37^0C.The highest enzyme activity at 37^0C was 146.8 IU/mL. While at 45^0C, the production was 63.8 IU/mL using wheat bran, which was comparatively very less.



Graph 4: Effect of different temperature on xylanase production using wheat bran as carbon source.

3.1.5. Effect of pH on xylanase production

The initial pH of the media strongly influences many enzymatic systems and transport of several species of enzymes across the cell membrane [20]. Xylanase production was studied using wheat bran at different pH viz. 6, 7, 8, 9 & 10. Maximum production of xylanase was obtained at pH 8 (132.6 IU/mL). Which confirms that optimum pH for the xylanase production using Y2 isolate is 8.



Graph 5: Effect of pH on xylanase production using wheat bran.

3.2. Partial purification of xylanase

Table 1: Purification steps of xylanase enzyme produced using wheat bran.

Step	Total protein (mg)	Total activity (IU)	Specific activity (IU/mg)	Yield %	Purification fold
Crude extract	76.1	1312	17.2	100.00	1.0
$(NH_4)_2SO_4$ precipitation & After Dialysis	32	665	20.7	50.68	1.20

Xylanase produced using wheat bran as substrate by Y2 isolate was further purified by the ammonium sulphate fractionation followed by dialysis. Initially, the crude filtrate containing xylanase was subjected to different ammonium sulphate concentration (40-80%) for precipitation. It was observed that highest enzyme activity was found at 80% concentration of $(NH_4)_2SO_4$. And the precipitated enzyme was further dialyzed against the same buffer, showing purification fold of 1.20 with specific activity of 20.73 IU/mg and 50.68% of enzyme yield.

4. Conclusion

The paper demand increases every day as a result of developed population and industrialization. Water and energy utilization and in particularly waste generation are becoming more important concern ever worldwide. A major goal is to decrease damage to environment by waste minimization, reuse and recycle. So, by the use of xylanase enzyme in pulp & paper industries rather than chemical treatment the degree of waste generation can be minimized. Recent studies focuses on the large scale xylanase production using agro residues, which can be easily procured at lower cost.

Amongst all the raw materials used, wheat bran showed maximum xylanase activity 149.2±0.2 IU/mL, at optimum temperature 37^{o}C and pH 8.0. In present studies 2 % of wheat bran along with yeast extract gave highest production. The purified enzyme showed the specific activity of 20.7 IU/mg protein as compared to crude enzyme 17.2 IU/mg protein in case of wheat bran.

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