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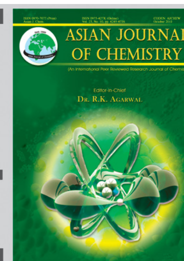

Xylanase Activity and Kinetics Comparison During Pulping of *Gossypium arboreum* and *Gossypium barbadense*

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In this paper, xylanase enzyme was employed for pulping of two cotton species *Gossypium arboreum* and *Gossypium barbadense*. Initially ground cotton was used to investigate the enzymatic hydrolytic effect. Xylanase activity was optimized at various substrate concentrations, different pH, temperature and for the presence of various metal ions. Xylanase gave best activity with 1 g substrate, pH 8 at 50 ºC. Xylanase activity for *Gossypium arboreum* was 1.132 µmol/min/mL at pH 5 and 20 ºC. Xylanase activity for *Gossypium barbadense* was determined at pH 6-9. K_m was found to be 722 mol and V_{max} was 109 µmol/min. Xylanase shows good potential to replace the toxic chemicals of paper and pulp industry.

Key Words: Enzymes, *Gossypium arboreum*, *Gossypium barbadense*, Optimization, K_m and V_{max}.

INTRODUCTION

Today enzymes are helping industry to make the products used by society in a way that is less harmful to the environment. Xylanase is a class of enzymes which degrade the linear polysaccharide β-1,4-xylan into xylose, thus breaking down hemicelluloses major component of the cell wall of plants. Xylanases are also being used in bread making. Enzymatic hydrolysis of non-starch polysaccharides leads to the improvement of properties of dough and crumb firmness[1].

Xylose, (wood sugar), is an aldopentose a monosaccharide containing five carbon atoms and including an aldehyde functional group. It has chemical formula $C_5H_{10}O_5$. It is found in the embryos of most edible plants. Xylan is widely distributed in plant cell walls and forms a main part of the hemicellulose fraction[2].

An attractive application of this hydrolysis process is the removal of xylan from wood pulp for manufacturing of dissolved pulp. Recently, the interest in cellulase-free xylanases has focused on pulping and bleaching processes, in which chlorine and hypochlorine for biobleaching can be reduced. Naturally occurring microbial strains capable of secreting xylanases free of cellulase activity would be attractive for such applications. The most important enzyme needed for enhancing the bleaching of pulp is endo-B-xylanase. Xylanases enhance the cleaving of reprecipatated xylan formed on the outer surfaces of the cellulose fibers after pulping[3].

Xylan is the most abundant noncellulosic polysaccharide present in both hardwoods and annual plants and accounts for 20-35 % of the total dry weight in tropical plant biomass. In temperate softwoods, xylans are less abundant and may comprise about 8 % of the total dry weight. Xylan is found mainly in the secondary cell wall and is considered to be forming an interphase between lignin and other polysaccharides. Many structural aspects of xylans are not clear because of the difficulties associated with the isolation of xylans from natural raw materials without significant alteration or loss of the original structure and association with other components[4].

Viikari *et al.*[5] showed the important properties of the xylanases for use in the pulp and paper industry, the effect of pulping operations on the hemicellulose component of the plant biomass subjected to kraft pulping. Grabski and Jeffries[6] emphasized the diversity observed in pulp operations and therefore there exists a vast potential for variability in the choice of enzymatic treatments as well as in the choice of various microbial enzymes[7].

Suurnakki[8] studied that enzyme-aided, totally chlorine-free bleaching of different industrial softwood kraft pulps was studied using purified Trichoderma reesei xylanase and mannanase. Rifaat *et al.*[9] studied that potential applications of microbial xylanases in the pulp and paper industry was gradually increasing and several were approaching to commercial use. This industry needed a xylanase, which was free of cellulase.

Twenty isolated Streptomycetes strains from Egyptian soils, which produce cellulose free-xylanase and were easily grown using a low-cost agriculture waste substrate, were investigated.

In the present study activity of xylanase for pulping the stems of *Gossypium arboretum* (Tula or wild cotton) and *Gossypium barbadense* (pima cotton) was investigated. These cotton species are extensively cultivated in Pakistan and their stems are being destroyed or burnt. Estimated cotton production of Pakistan was over 2.387 million hectares in Punjab province which produced 7.702 million bales while in Sindh it was cultivated over 0.561 hectares and its yield was 2.243 million bales[10].

EXPERIMENTAL

Lignocellulosic substrate was cotton stalk (Bt-121) grinded into small particles to increase surface area for the enzyme activity. The cotton stalk was pretreated with 2 % NaOH and Na_3PO_4 in the ratio of 1:20 (w/v) for 2 h at room temperature. The treated sample was thoroughly washed with water in order to remove alkali and acid to neutralize and then dried at 50 ºC in an oven to obtain a constant weight.

Assay the xylanase activity at pH 6 with respect to change in concentration of substrate and time of action: Different substrate concentrations were taken into separate conical flasks. Assigned the number of each sample named as 1, 2, 3 and 4. A sample number 1 contain 1 g of substrate, sample number 2 contains 2 g of substrate, sample number 3 contains 3 g of substrate and sample number 4 contains 4 g of substrate.

After addition of 2 mL of xylanase enzyme in each sample and add 25 mL of buffer (pH 6) was also added in reaction mixture. Then the samples were placed in incubator at 50 ºC for 3, 6 and 9 h.

After giving the above mentioned time to enzyme, the amount of reducing sugar was determined by UV VIS spectrophotometer (Lebomed UVD 3200) using 3,5-dinitro-slicylic acid method. Kinetics were determined for xylanase activity at *Gossypium arboretum* (Tula or wild cotton) and *Gossypium barbadense* (pima cotton), stems in the ground form.

Xylanase activity was assayed using 1 % birchwood xylan as the substrate. Xylan was dissolved in 50 mM glycine NaOH buffer (pH 9.0). The reaction mixture, containing 10 µL of an appropriate dilution of the enzyme and 250 µL of the substrate, was incubated for 10 min in an incubator maintained at 10 ºC. The amount of reducing sugars liberated was determined by using 3,5-dinitrosalicylic acid method (DNS method). The absorbance of the reference samples (substrate solution incubated without enzyme and diluted enzyme solution in buffer) was deduced from the values of the test samples. One unit of xylanase activity was expressed as µmol of reducing sugars (xylose equivalent) released in 1 min under the above conditions. Cellulase activity was assayed as above, using low-viscosity carboxymethylcellulose (1 %) in place of xylan.

RESULTS AND DISCUSSION

Xylanase activity during pulping of *Gossypium arboreum*: Xylanase gave following activity against *Gossypium arboreum* as substrate. The temperature was set at 20 ºC constant and pH range was used from 4.1-7.0. In this experiment enzyme activity µmol/min/mL, total protein mg/mL and specific enzyme activity µ mol/min/mg protein was found on different pH (Table-1). Optimum activity of enzyme was found to be 1.132 µmol/mL at pH 5. Temperature was optimized in the initial studies of enzyme catalysis and 20 ºC was found to be best temperature for enzyme activity.

TABLE-1
ENZYME ACTIVITY OF *Gossypium arboreum*

pH	Enzyme activity (µmol min/mL)	Total protein (mg/mL)	Specific enzyme activity (µmol min/mg protein
4.1	0.24	0.28	0.86
4.4	0.536	0.316	1.69
4.7	1.007	0.48	2.1
5.0	1.132	0.525	2.16
5.3	0.998	0.516	1.93
5.6	0.872	0.49	1.78
5.9	0.281	0.261	1.08
6.2	0.009	0.11	0.82
6.5	0.001	0.08	0.3
7.0	0	0.061	0

*Temperature for all measurements was maintained at 20 ºC and all values are average of triplicate.

Kinetics studies of xylanase were carried out at pH 5, temperature 20 ºC, substrate concentration 200 µmol. Xylanase activity of *Gossypium arboreum* at optimal conditions was 1.132. Table-1 also represents the activity relationship with pH of the medium. Specific activity was found to be 2.16 (µmol min/mg protein).

Effect of incubation time interval on the xylanase activity: The aim of this experiment is to determine the effect of duration of temperature on the xylanase activity. This experiment investigated at different incubation time interval. It was found considerable effect of interval of time of temperature on the xylanase activity. The concentration of substrate, enzyme and pH were remained constant in this experiment. Enzyme activity at pH = 8, temperature of incubator = 50 ºC (Fig. 1).

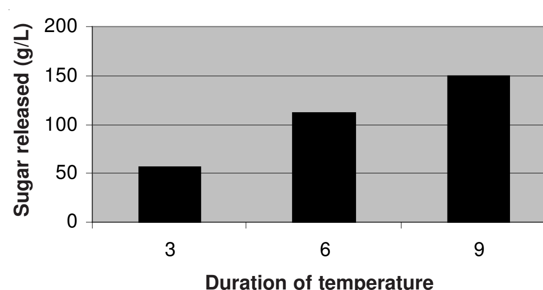

Fig. 1. Effect of incubation time on xylanase activity

Kinetics of xylanase for *Gossypium arboreum* is given in Table-2. V_{max} and K_m has been calculated using lineweaver and Burk model for enzyme calculations.

Xylanase activity during pulping of *Gossypium barbadense*: The experiment investigated the effect of enzyme (xylanase) activity on Pakistan second most common cotton species *Gossypium barbadense* as substrate. The temperature was set at 20 ºC constant and pH range was found at 4.1-7.0. In this experiment enzyme activity u mole/min/mL, total protein mg/mL and specific enzyme activity µmol/min/mg protein was found on different pH. Maximum activity of xylanase was found to be 1.282 µmol/cm^3 of enzyme added at pH 5.6 and temperature for this activity was at 20 ºC for the substrate Table-3 showed enzyme activity and specific enzyme activity

TABLE-2
KINETIC STUDIES OF XYLANASE FOR *Gossypium arboreum* AT OPTIMUM CONDITIONS

Time	Substrate (µmol)	OD	Product formed (µmol)	Substrate µmol remaining	1/[S]	V_0	$1/V_0$
1	200	0.456	27.5625	172.4375	0.0057992	27.5625	0.03628118
2	200	0.897	55.1250	144.8750	0.0069025	27.5625	0.03628118
3	200	1.123	69.2500	130.7500	0.0076482	23.0833	0.04332130
4	200	1.225	75.6250	124.3750	0.0080402	18.9063	0.05289256
5	200	1.259	77.7500	122.2500	0.0081799	15.5500	0.06430868
6	200	1.458	90.1875	109.8125	0.0091064	15.0313	0.06652807
7	200	1.900	117.8125	82.1875	0.0121673	16.8304	0.05941645

Kinetics calculations by using lineweaver burk equation of double reciprocals. Slope = K_m/V_{max} = 4.3372. Y intercept = $1/V_{max}$ = 0.0155, K_m = 279.819 mol and V_{max} = 64.516 µmol/min.

TABLE-4
KINETIC STUDIES OF XYLANASE FOR *Gossypium barbadense* AT OPTIMUM CONDITIONS

Time	Substrate (µmol)	OD	Product formed (µmol)	Substrate µmol remaining	1/[S]	V_0	$1/V_0$
1	200	0.564	34.3125	165.6875	0.00603546	34.3125	0.0291439
2	200	0.689	42.125	157.875	0.00633413	21.0625	0.04747774
3	200	0.895	55	145	0.00689655	18.3333	0.05454545
4	200	1.123	69.25	130.75	0.00764818	17.3125	0.05776173
5	200	1.425	88.125	111.875	0.00893855	17.625	0.05673759
6	200	1.544	95.5625	104.4375	0.0095751	15.9271	0.06278613
7	200	1.1002	67.825	132.175	0.00756573	9.68929	0.10320678

Kinetics calculations by using lineweaver burk equation of double reciprocals. Slope of the straight line was (K_m/V_{max}) = 6.572, Y intercept = $1/V_{max}$ = 0.0091, K_m = slope/Y intercept = 722.197 mol, V_{max} = K_m/slope = 109.890 µmol/min.

at pH 4.1-7.0. Temperature was optimized in the initial studies of enzyme catalysis and 20 ºC was found to be best temperature for enzyme activity. Kinetics studies of xylanase were carried out at pH 5, temperature 20 °C, substrate concentration was 200 µmol (Table-3). Xylanase activity of *Gossypium barbadense* at optimal conditions was 1.282 (Table-4) Fig. 1 represents the activity relationship with pH of the medium. Specific activity was found to be 2.27 (µmol min/mg protein).

TABLE-3
ENZYME ACTIVITY OF *Gossypium arboreum*

pH	Enzyme activity (µmol HQ/min/mL)	Total protien (mg/mL)	Specific enzyme activity (µmol HQ/min/mg protein
4.1	0.233	0.27	0.86
4.4	0.281	0.322	0.87
4.7	0.525	0.491	1.06
5.0	0.893	0.538	1.66
5.3	1.026	0.549	1.87
5.6	1.282	0.565	2.27
5.9	0.38	0.412	0.92
6.2	0.072	0.126	0.57
6.5	0.011	0.093	0.12
7.0	ND	0.066	ND

Xylanase is the name given to a class of enzymes which degrade the linear polysaccharide β-1,4-xylan into xylose, thus breaking down hemicellulose, which is a major component of the cell wall of plants.

The relative xylanase activity was determined at various pH conditions. The pH range used varied from 6-9. Three different buffers (0.05 M) were used. Phosphate buffer was used for pH 6-9. To test the pH stability, the purified enzymes was diluted using respective buffers having pH ranging from 6-9 as described above and were incubated for 3-9 h at 50 ºC temperature. The residual enzyme activity was estimated at 3 h intervals during the 3-9 h period of incubation.

It was found that Xylanase activity was maximum at pH 8. The amount of sugar released was equal to 32.00g/L, at this pH by taking the substrate concentration 1.00 g and the interval of incubation was about 3 h. It was found that enzyme activity at pH 7 was very close to pH 8. It was found after performing the numbers of experiment that the optimum pH range of the xylanase was 7-8. Result of relative enzyme activity at different substrate concentration with respect to different pH has been plotted in graph. Released sugar is decreased with the increase of pH.

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