

Investigate of Process Parameters on Xylanase Enzyme Activity in *Melanocarpus Albomyces* Batch Culture

Vipan Kumar Sohpal¹, Apurba Dey² Amarpal Singh³

Abstract— The thermal and operational inactivation of a commercial Xylanase from *Melanocarpus Albomyces* was studied in several buffered solutions usually employed to study the activity of this enzyme. Some previous works have been done to understand the effect of the of the buffers sample on the enzyme activity. Afterwards, data obtained at temperatures from 40 to 80 °C at pH 7.0 were used to find activity, half life and de-activation constant. Both half time ($t_{1/2}$) and deactivation constant have (K_d) significant effect of temperature and value of (K_d) increased by 2-3 times for each step of 10°C. The parameters of V_{max} and K_m obtained using Lineweaver-Burk plot method were 1.935 μ Mol /L min and 0.131 mol /L correspondingly. Enzyme activity was investigated with different buffers over wide range of pH (5.0-10.0) at room and optimum kinetic temperature indicates that pH 6.0 - 7.0 found highly suitable. The experimental results revealed good thermal stability, with greater stability at higher pH value for Xylanase in hemi- cellulosic reaction.

Index Terms—Xylanase, *Melanocarpus Albomyces*, Temperature, pH, Activity and Lineweaver-Burk plot

I. INTRODUCTION

The discovery of a variety of thermo stable enzymes has led to expanded growth in the industrial enzyme market. Interest in thermo stable enzymes has grown mainly due to fact that most of the existing industrial processes are run on high temperature. The use of xylanase to break down hemi-cellulosic material has been extensively proved in paper industrial process. Xylanase is composed of endo-xylanase and exo- xylanase are major components of enzymatic consortium act in nature by depolymerizing xylan molecules into monomeric pentose units that are used by bacterial and fungal populations as primary carbon source. Lignocelluloses materials are widespread in nature and xylan is a polysaccharide found in the hemicelluloses fraction of lignocelluloses. Xylan is a potential significant resource for renewable biomass, which can be utilized as a substrate for

the preparation of many products such as fuels, solvents and pharmaceuticals. Corn, wheat stem, barley shells and similar material contain xylan (Gomes et al [12], Gawande and Kamat [11], Beg et al [3] and Subramaniyan and Prema [24]). On the other hand, xylanases (E.C. 3.2.1.8) are needed for making use of hemicelluloses. For most bioconversion processes, xylan must first be converted to xylose or xylo-oligosaccharides. This can be done either by acid hydrolysis or by the use of xylanolytic enzymes (Chivero et al [7]). Xylanase enzyme deconstructs plant structural material by breaking down hemi-cellulose, a major component of the plant cell wall. Xylanase are produced from many different fungi and bacteria. Xylanase enzymes are used commercially in the pulp and paper, food, beverage, textile and animal feed industries (Tan et al [25], Wong et al [26] and Li et al [18]). Most commercial xylanases are produced by *Trichoderma*, *Bacillus*, *Aspergillus*, *Penicillium*, *Aureobasidium*, and *Talaromyces* sp. (Li et al [18]).

In this study, some physiological conditions affecting the activity of xylanase enzyme produced from *Melanocarpus Albomyces* IIS 68, namely pH, temperature and substrate concentration were determined. In addition, the effect of temperature on deactivation and Michaelis-Menten constants were determined and studied in liquid cultivation media

II. MATERIAL AND METHOD

In the study, *Melanocarpus Albomyces*, which was isolated and classified from the micro-flora of India by Institute of Microbial Technology, was used for production of the xylanase enzyme. Stock cultures were maintained on potato dextrose agar at 4°C. Liquid medium described by Kim et al. [16] was used with some modification for growth and enzyme production. It contained ($g\ l^{-1}$); 0.5 Proteous-peptone, 0.3 urea, 0.2 KH_2PO_4 , 0.3 $CaCl_2$, 0.2 Tween-80, 1.4 $(NH_4)_2SO_4$, 0.3 $MgSO_4 \cdot 7H_2O$ and 1 % xylan (sigma) as carbon source. In 250 ml flasks, 100 ml of medium was sterilized in autoclave at 121°C, 1.5 atm for 15 min. After media preparation 1ml spore suspensions containing $15 \cdot 10^6$ spores were inoculated into the growth media. The liquid medium was incubated at 30°C for 13 days and agitated at 150 rpm. Finally the enzyme activity was measured by modifying the method described by Khanna and Gauri [15], using a 0.1 M sodium-acetate buffer with pH 5. The liquid culture filtrate was centrifuged at 7,200 rpm for 15 min. One ml of 1 % xylan solution and 0.5 ml enzyme sample were

Manuscript received 13 March, 2010 This work was supported in part by the Department of Chemical Engineering & Bio Technology.

1. Vipan Kumar Sohpal ,Senior Lecturer, Department of Chemical Engineering & Bio-Technology ,BCET, Gurdaspur- 143521, Punjab, India (corresponding author to phone:91-98882-20918; Fax:91-1874-221463; e-mail: vipan752002@ indiatimes.com)

2. Apurba Dey Dr., Professor, Department of Biotechnology, NIT, Durgapur, West Bengal, India (e-mail:apurbadey2003@yahoo.co.in).

3. Amarpal Singh,Dr., Assistant Professor, Department of Electronics, BCET, Gurdaspur- 143521, Punjab, India (e-mail: s_amarpal @yahoo.com).

added to the reaction tubes and incubated for 30 min at 37°C and then centrifuged at 7,200 rpm for 10 min. The amount of reducing sugar in the reaction tubes was measured using the Dinitrosalicylic Acid Method (DNS) described by Miller [21]. The absorbance was read at 540 nm using U.V. Visible spectrophotometer. The amount of reducing sugar was calculated from the standard curve based on the equivalent glucose. One unit xylanase activity was described as the amount of enzyme producing 10 µg of reducing sugar in 1 ml medium in 1 minute under standard test conditions. The amount of protein was determined by Lowry Method (Lowry et al [19]).

III. NOMENCLATURE & CALCULATION

[S]=Substrate Concentration (mol/L)
V=Velocity of Xylanase (µ Mol /L min)
V_{max}= Maximum Velocity (µ Mol /L min)
K_d=Deactivation constant (1/min)
K_M=Michaelis constant (mol /L)
t_{1/2}= Half Life (min)
T temperature (°C)

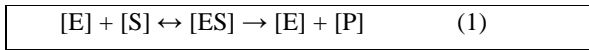
$$\text{Enzyme Activity (IU/ml)} = \frac{(\text{mg xylose in assay tube}) * (1000 \mu \text{ mole /m mole}) * \text{Dilution Factor}}{(150 \text{ mg /m mole}) * (5 \text{ min}) * (0.2 \text{ ml})}$$

$$K_d = A e^{-E_d/RT}$$

$$t_{1/2} = 0.693/K_d$$

IV. MATHEMATICAL MODELING

The mechanism of enzyme substrate interaction is generally expressed by the as follows



where S, P, E and ES are substrate, product, enzyme and enzyme-substrate complex concentrations respectively. k₁ and k₂ and k₃ represent, catalytic rate constant respectively. The dependence of the reaction rate on the substrate concentration can be derived as:

$$V_0 = V_{max} [S] / K_m + [S] \quad (2)$$

where V_{max} is the maximum reaction rate and K_m is Michaelis- Menten constant. Lineweaver Burk is double reciprocal of Michaelis- Menten equation (2), which can be represented by following relation

$$1/V_0 = 1/V_{max} + K_m/V_{max} [S] \quad (3)$$

Results

With respect to the temperature effect, as occurs with all chemical reactions, the reaction velocity catalyzed by enzyme increases exponentially with temperature increase over a determined range in which enzyme is stable. Since enzymes

are fragile protein structures projected to operate at physiological temperatures, when used at temperature above 40°C, they loose their activity due to the denaturing of various enzyme molecules present as the temperature or the reaction time increase. Previous experiment has revealed that rate of reaction and activity of enzyme increase with temperature of reactions. Experiments were performed over the temperature range of 10 to 70°C to examine the effect of temperature on the activity PGA enzyme. From figure 3, enzyme activity or rate of reaction was found to continuously increase with increase in temperature, with a maximum activity 256.74 (µ/g) at 68°C. The optimized value was calculated from the curve was equal to 250.11(µ/g) at 65°C.

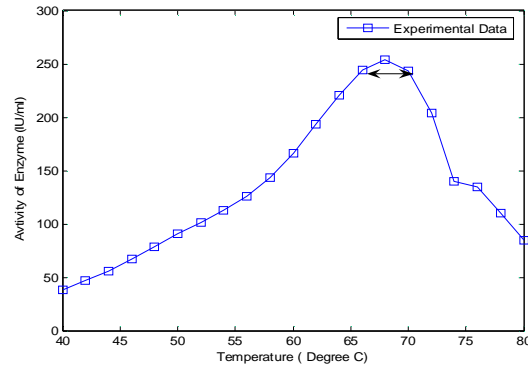


Figure 1 Temperature effect on activity of Xylanase enzyme

The majority of the enzymes present a characteristic pH value where its activity is maxima. Above and below this pH, the activity reduces. The enzyme's three-dimensional structure, responsible for its catalytic activity, is stabilized by a hydrogen bond, hydrophobic interaction and a di-sulfate bond. Altering the hydrogen concentration modifies the equilibrium of these forces, irreversibly deactivating the enzyme. Other factors where enzymatic activity is related to pH depend on the enzyme's acid and alkaline behavior and the substrate itself

The activity of enzyme is very high at pH below than 6, but activity steadily down with pH. On other hand beyond the pH value 7, the activity reduces drastically at room temperature. Using optimized temperature as shown in Figure 1. The maximum activity observed at pH 6 using phosphate citrate. The corresponding value of activity observed 225.23 (IU/ml) is almost similar tris-HCL as shown in Figure 2. On other hand Sodium carbonate and bicarbonate shows least enzyme activity.

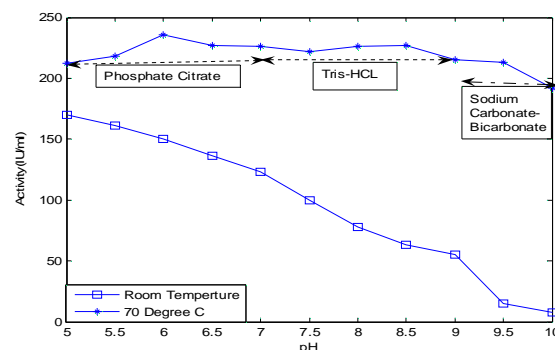


Figure 2 Comparison of the pH effect on activity of Xylanase enzyme at room and optimized temperature

The Michaelis-Menten constants were determined by measuring the activity of the xylanase with varying amounts of substrate. The experiments were conducted at pH 7, 300 rpm, and 68°C for xylanase. The kinetic data found in above conditions is placed in Table 1

Table 1 Kinetic data obtained at different substrate concentration

Concentration of Substrate (Mol/L) [S]	1/[S] L/mol	Velocity [V] μ Mol /L min	1/[V] L.min/mol* 10^{-3}
1/60	60	216	4.6
1/30	30	424	2.3
1/20	20	519	1.9
1/15	15	620	1.6
1/12	12	729	1.3
1/10	10	830	1.2
1/8.5	8.5	859	1.1
1/7.5	7.5	971	1.0

The parameters of V_{max} and k_m obtained using the Lineweaver-Burk plot method were 1.935 μ Mol /L min and 0.131 mol /L respectively. According to the calculated and linear approximation values of k_m and V_{max} , are almost same as shown in Figure 3.

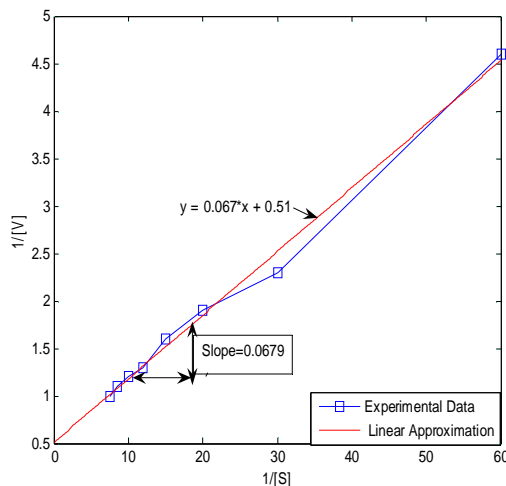


Figure 3 Comparison of Experimental & Linear approximation for Lineweaver Burk Plot

The half life of xylanase enzyme reduces by step with increases of temperature. The half life reduced to $\frac{1}{2}$ with rise of temperature up to 10°C temperature. Moreover thermal deactivation constant increased with rise of temperatures. K_d

values increase drastically but in systematic fashion as indicated in Table 2.

Table 2 Half life and Deactivation Constant variation with thermal effect

Temperature (°C)	50	55	60	65	70
Half Life (min)	.0031	.00305	.003	.00295	.00291
De-activation Constant (K_d)	0.0041	0.0078	0.0568	0.068	0.082

V. DISCUSSION

In order to maximize enzyme production and decrease production costs, substrates such as wheat bran are used to produce xylanase enzyme from different fungi. In previous studies, solid fermentation was used especially with *Trichoderma sp.* mainly due to economic reasons (Atev et al [1] & Deschamps and Huett [9]). Our experimental results revealed that xylanase activity was higher in liquid production medium. However, it should be noted that production time for the liquid medium was 13 days. Therefore, liquid production media have their own advantages and disadvantages.

Our studies revealed that the xylanase activity was maximum around pH 6. In studies carried out with *Trichoderma sp.*, it was also concluded that the most suitable pH value for xylanase activity was 5 (Dekker Final [8], Gomes et al [12] & Royer and Nakkas [22]). The optimum pH was also around 5 in similar experiments on fungal xylanases (Milagres et al [22], Gandhi et al [10] and Sigoillot et al [23]). In some other studies, in order to determine xylanase activity generally pH was kept around 5 (Bailey et al [2]). But optimum pH value was found out to be 3 in a study carried out with *T. reesei Rut C-30* (Couchon and LeDuyt [4]). Results of similar experiments in literature reveal that the optimal pH for fungal xylanases is, in general, slightly higher than the other sources.

Our results on optimum xylan concentration were similar with the results of the previous studies (Atev et al [1], Leathers et al [17], Deschamps and Huet [9] Keskar et al [14] Royer and Nakas [22] and Gomes et al [12]). Optimum temperature for xylanase activity was found out to be 68°C. Similarly, in other studies carried out with for fungal xylanases the optimum temperature was 65°C (Chandra and Chandra [5] and Gandhi et al [10]). *Trichoderma sp.* it was concluded that the optimum temperature varied between 45 and 60°C (Dekker [8] and Gomes et al [12]). Besides, similar to our findings, experimental results of *Melanocarpus Albomyces* of halftime deactivation constant, V_{max} & K_m are similar to other fungal strains.

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