DOI: 10.19184/ICL.v1i1.3

# Determination Kinetic Parameters of Endo-β-1,4-D-Xylanase from Abdomenal Termites with Xylan Oat and Birchwood

Anak Agung Istri Ratnadewi,*[a] Linda Faiqotul Himmah, [a] Tri Mulyono, [a] Wuryanti Handayani, [a] NG. Krishnabudi,[b] and Sudarko[a]

Abstract: Parameters kinetic ($K_M$, $V_{Max}$, and $k_{Cat}$) endo-β-1,4-D-xylanase under optimum conditions with oat spelt xylan and birchwood substrate have been investigated in this study. Hydrolysis of endo-β-1,4-D-xylanase using variation of substrate concentration (b/v) ranging from 0.2 to 1.2%. Variation of incubation time is up to 20 hours with 4 hours interval at the optimum temperature of the enzyme, 40°C. The results obtained from this study were the KM of endo-β-1,4-D-xylanase for oat spelt xylan and birchwood were 4.10 mg/ml and 0.681 mg/ml, respectively. $V_{Max}$ values, and $k_{Cat}$ for oat spelt xylan substrate of 0.28 mg/ml.jam and 1.7 x $10^{-3}$ $s^{-1}$. While $V_{Max}$, and $k_{Cat}$ for birchwood substrate that is 0.117 mg/U/jam and 7 x $10^{-4}$ $s^{-1}$. From the results of this study we found that endo-β-1,4-D-xylanase can hydrolaze substrates which have differences solubility.

Keywords: endo -β-1,4-D-xylanase, kinetic parameters, oat spelt xylan, birchwood.

## INTRODUCTION

The wall of plant cell are composed of hemicellulose, cellulose, and lignin. Hemicellulose is the second largest constituent in plant cell walls. The main component of hemicellulose is xylan [1]. Xylan is heteropolysaccharide with xylopiranosil residue as the main chain compiler. Subtituents of xylan can be α-arabinofuranosil, o-acetil, and 4-O-methyl-D-glucuronic acid [2]. The β-1,4 bond between D-xylopiranosil is the main characteristic of xylan. The abundance and type of bonding of the subtituents can differ depending on the source [3].

Hemicellulase is a group of enzymes that can hydrolyze hemicellulose. These enzymes have various applications of biotechnology, structure and function [3]. One of them is endo-1,4-β-D-xylanase (EC 3.2.1.8). Endo-1,4-β-D-xylanase can break the glycosidic β-1,4 bond from within the main xylan chain. This enzyme is generally produced by microorganisms. The most studied type is fungi and bacteria [2]. The enzyme used in this study was isolated from *Bacillus* sp. which comes from the termite abdominal system. Enzyme purification is carried out partially under optimal conditions [4].

Each enzyme has diverse characteristics, structure, activity, hydrolysis results, efficiency, and the level of hydrolysis of xylan [5]. One of the observed enzyme characteristics is the determination of the value of the kinetic parameters ($K_M$, $k_{Cat}$, and $V_{Max}$). Kinetic parameters are measured by observing the rate of formation of products with low enzyme concentrations. The theory for this analysis is based on Michaelis-Menten [6]. The parameter values obtained will indicate the characteristics of the enzyme. Kinetic parameter measurements were carried out on two different substrates namely oat spelt xylan and birchwood. In this study we report the results of research on the kinetic parameters ($K_M$, $V_{Max}$, and $k_{Cat}$) endo-1,4-β-D-xylanase from termite abdomen using the steady state approach.

## METHODS

### Production and partial purification of endo-β-1,4-D-xylanase

Production of Endo-β-1,4-D-xylanase, bacteria cultured in LB (Luria Bertani) medium were incubated at 37 °C. Crude enzymes are collected by centrifugation (8,000 rpm for 20 minutes at 4 °C). The crude enzyme is isolated by ammonium sulfate precipitation (40-50% saturation) and centrifugation (8,000 rpm for 20 minutes at 4 °C). After that, the precipitate is dissolved in phosphate buffer (pH 5.0). The final step of purification is dialysis using a membrane by Elkay for 24 hours. Citrate phosphate solution changes at 2, 4, 6, and 12 hours.

### Determination of Enzyme Activity and Protein Levels

The enzymatic activity of xylanase was measured by spectrophotometry (Hitachi U-2001). The xylanase activity was calculated by measuring the amount of reducing sugar released from 0.8% (w/v) of the oat spelt xylan (Sigma, USA). The enzyme was incubated with 0.8% (w/v) xylan solution in a citrate phosphate buffer, pH 5.0 at 40 °C for 60 minutes. DNS reagents (750 µl) were added to 250 µl of mixed enzymes and oat spelt xylan. The mixture is boiled for 15 minutes and cooled for 20 minutes. Reducing sugar is determined by measuring absorbance at 550 nm. One unit of xylanase activity is defined as 1 umol reducing the sugar produced, by hydrolyzing the xylan substrate, per minute at 40 °C.

The total amount of protein was determined according to the Coomassie blue method described by Bradford using bovin serum albumin as a standard.

### Determination of Kinetic Parameters

The influence of oat spelt xylan and birchwood (Sigma, USA) silane concentrations, ranging from 0.2 to 1.2% (w/v), on the xylanase activity of both fungal strains was evaluated under optimal testing conditions. Kinetic parameters, $K_M$, $V_{Max}$ and $k_{Cat}$ are estimated by linear regression of the plot according to Lineweaver and Burk.

[a] AAI. Ratnadewi, LF. Himmah, T. Mulyono, W. Handayani, Sudarko
Department of Chemistry, Faculty of Mathematics and Natural Sciences, University of Jember
*e-mail: istri_dewi.fmipa@unej.ac.id
[b] NG. Krishnabudi
Department of Economics, Faculty of Economics and Business, University of Jember

## RESULT AND DISCUSSION

### Purification of Endo-β-1,4-D-xylanase

The activity of crude extract, fractionation results, and endo-β-1,4-D-xylanase dialysate can be seen in Table 1. This enzyme was purified to a partial stage. Under optimum conditions the largest endo-β-1,4-D-xylanase activity was obtained by dialysate as a result of the dialysis process which was 0.093 U/ml and then fractionated 40 - 50% by 0.057 U/ml, and crude extract by 0.046 U/ml.

The smallest protein content was obtained by dialysis results of 0.046 mg/ml while the highest protein content was indicated by crude extracts which was 0.103 mg/ml. Specific activity indicates the amount of enzyme activity per amount of protein contained in the enzyme mixture tested. The final specific activity of endo-β-1,4-D-xylanase is 2.04 U/mg with a purification of 4.62 times that of crude extracts, and a final yield of 21%. The results of this dialysis process are used to determine the kinetic parameters at a later stage.

Table 1. Purification of endo-$\beta$-1,4-D-xylanase

| Pemurnian | Volume (ml) | Activity (U/ml) | Protein (mg/ml) | Yield (%) | Specific activity (U/mg) | Purification fold |
|---|---|---|---|---|---|---|
| Crude | 48 | 0,046 | 0.103 | 100 | 0.442 | 1 |
| fractionation results | 24 | 0,057 | 0.073 | 35 | 0.780 | 1.77 |
| dialysat | 23 | 0,093 | 0.046 | 21 | 2.04 | 4.62 |

### Kinetics Parameter

The initial velocity is determined at each concentration with a variation of 0.2-1.2 %. Initial velocity data against substrate concentration were plotted using the Lineweaver-Burk curve (Figure 1). This is done for each substrate both oat spelt xylan and birchwood.

The kinetic parameters, $K_M$ and $V_{max}$ for endo-β-1,4-D-xylanase are 4.10 mg/ml and $3.1x10^{-2}$ µmol/ml/min for oat spelt xylan. As for the birchwood substrate, the $K_M$ and $V_{Max}$ values were 0.681 mg/ml and 1.3x10-2 µmol/ml/min (Table 2). $K_M$ and $V_{Max}$ from xylanases isolated from *Paenibacillus* sp. NF1 is oat spelt xylan with KM value of 5.64 mg/ml and 6.32 mg/ml for birchwood [7]. While the $V_{Max}$ value for oat spelt xylan which is greater than birchwood is 3364.57 µmol/min.mg protein while the $V_{Max}$ for birchwood is 3126.44 µmol/min.mg protein. $K_M$ and $V_{Max}$ data from endo-β-1,4-D-xylanase isolated from Trichoderma reesei on oat spelt xylan and birchwood, 1.8 mg/ml and 2.1 mg/ml, respectively. The other kinetic data obtained were $k_{Cat}$ values of 168.7 ± 11.6 $s^{-1}$ for oat spelt xylan and 205.7 ± 19.5 $^{s-1}$ for birchwood [8].

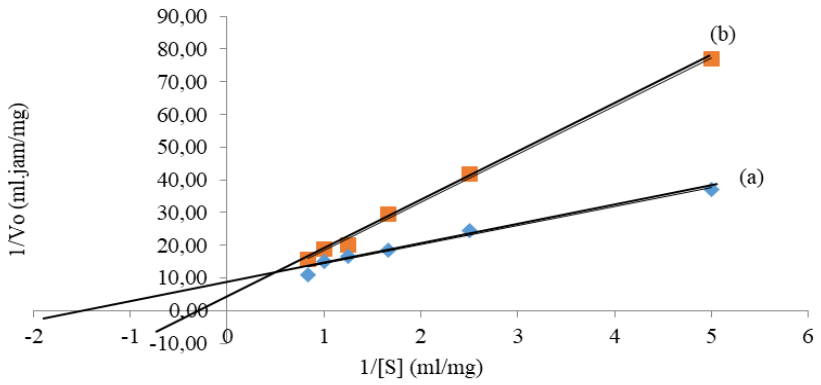


Figure 1. Figure 1/V relationship with 1/[S] based on the Lineweaver-Burk equation of substrate (a) oat spelt xylan (b) birchwood

Table 2. Kinetic parameters of endo-β-1,4-D-xylanase

| Substrate | $K_M$ (mg/ml) | $V_{Max}$ (µmol/ml/menit) | $k_{Cat}$ ($s^{-1}$) | $k_{Cat}/K_M$ (ml/s/mg) |
|---|---|---|---|---|
| Oat spelt xilan | 4.10 | $3.1x10^{-2}$ | $1.7 \times 10^{-3}$ | 0.925 |
| *birchwood* | 0.681 | $1.3x10^{-2}$ | $7 \times 10^{-4}$ | 1.72 |

The next kinetic parameter values that can be seen in Table 2 are $k_{Cat}$. The $k_{Cat}$ values for oat spelt xylan and birchwood substrates were $1.7 \times 10^{-3}$ $s^{-1}$ and $7 \times 10^{-4}$ $s^{-1}$, respectively. The $k_{Cat}$ /$K_M$ value is 0.925 ml/s /mg for oat spelt xylan and 1.72 ml/s/mg for birchwood.

The results showed that with the same time and substrate concentration, endo-β-1,4-D-xylanase can produce more xylooligosaccharides from birchwood than oat spelt xylan. This difference can be seen in the measured reducing sugar yield. This can be caused by differences in the composition of the two substrates. Birchwood contains more than 90% water soluble and consists of a high percentage of neutral sugar (87.7% mainly xylose residues, small amounts of glucose, arabinose, and galactose) and 10.2% hexuronic acid. While the components of oat spelt xylan have a high percentage of xylose (84%) and some arabinose, glucose, and galactose. Also water solubility varies greatly depending on temperature and centrifugation. As a result endo-β-1,4-D-xylanase works faster in birchwood. This is also

supported by kinetic parameter values, $K_M$ measured for birchwood.

The value of $K_M$ is a desociation constant where this constant is inversely proportional to the enzyme affinity. The small $K_M$ value indicates the smaller the tendency of the substrate and the enzyme to dissociate, the enzyme affinity towards the large substrate. This results in a very complex [ES] complex, so the reaction equilibrium shifts toward the product. If the $K_M$ value is large, then the equilibrium will shift towards the enzyme and substrate [9]. Based on the comparison of $K_M$ values, endo-β-1,4-D-xylanase has a greater affinity on the birchwood substrate.

Where $k_2$ is $k_{Cat}$, the catalytic constant that determines the rate of product formation. This value also affects the value of $V_{Max}$ [10]. A low $K_M$ value can be obtained from a high value and a low $k_2$ value or high and a high $k_2$ value. In experimental data it was found that the value of $k_{Cat}$ obtained by birchwood was smaller than oat spelt xylan, this resulted in the $V_{Max}$ value obtained was also low.

The catalytic efficiency ($K_{Cat}/K_M$) value in Table 2 where the value for birchwood substrate is greater than oat spelt xylan which is 1.72 ml/s/mg. According to Eisenthal et al. that this specific constant value can be used to compare enzymes with two different substrates. If the $K_M$ value is used as an indicator of specificity, then to determine the better substrate can be seen from the comparison of [S] and $K_M$ values. The increasing value of [S] /$K_M$ becomes a good parameter to describe a better substrate for an enzyme. This is what underlies $K_{Cat}/K_M$ as a specific constant [8]. The increasing value of specific constants will produce a reaction speed to form a better product [10]. The specific constant values obtained from this study indicate that the birchwood substrate is a better substrate for endo-β-1,4-D-xylanase.

## CONCLUSION

Endo-β-1,4-D-Xylanase from abdomenal termites has different kinetic parameters in hydrolyzing oat and birchwood xylan substrates. The results of the kinetic parameters showed that endo-β-1,4-D-Xylanase from abdominal termites was more effective on birchwood substrates with a $k_{cat}/K_m$ value greater than that of oat spelt xylan.

## REFERENCES

[1] B. D. Amel, B. Nawel, B. Khelifa, G. Mohammed, J. Manon, K. G. Salima, N. Farida, H. Hocine, O. Bernard, C. J. Luc, and F. M. Laure, "Characterization of a Purified *Thermostable Xylanase* from Caldicopro *Bacteralgeriensis* sp. nov.strain TH7C1T", *Carbohydrate Research*, vol. 419, pp. 60-68, 2015.

[2] S. Subramaniyan, and P. Prema, "Biotechnology of Microbial Xylanases: Enzymology, Molecular Biology and Application", *Critical Reviews in Biotechnoloy*, vol. 22, pp. 1, pp. 33-46, 2002.

[3] D. Shallom, and Y. Shoham, "Microbial Hemicellulases", Current Opinion in Microbiology, 2003, pp. 216-228.

[4] A. A. Kurniawan, "Optimasi Kondisi Hidrolisis Xilan Oat dengan Endo β-1,4-D-Xilanase asal Mikroorganisme dalam Sistem Abdomenal Rayap untuk Produksi xilooligosakarida", Skripsi, Jember: Universitas Jember, 2015.

[5] Knob, Adriana, and C. C. Eleonora, "Purification and Characterization of Two Extracellular Xylanases from Penicillium Sclerotiorum : A Novel Acidophilic Xylanase", *Biochemistry and Biotechnology*, vol. 162, no. 2, pp. 429-43, September 2010.

[6] D. L. Nelson, and M. M. Cox, "Lehninger Principal Biochemistry Fourth Edition", New York: W.H. Freeman and Company, 2010.

[7] H. Zheng, M. Sun, L. Meng, H. Pei, X. Zhang, Y. Zheng, W. Zeng, J. Zhang, J. Hu, F. Lu, and J. Sun, "Purification and Characterization of a *Thermostable Xylanase* from *Paenibacillus* sp. NF1 and its Application in Xylooligosaccharides Production", *J. Microbiol Biotechnol*, vol. 24, no. 4, pp. 489-496, 2014.

[8] J. He, B. Yu, K. Zhang, X. Ding, and D. Chen, "Expression of endo-1, 4-beta-xylanase from *Trichodermareeseiin Pichia pastoris* and functional characterization of the produced Enzyme", *BMC Biotechnology*, vol. 9, pp. 56-66, 2009.

[9] Saropah, A. Dyah A. Jannah, and A. Maunatin. "Kinetika Reaksi Enzimatis Ekstrak Kasar Enzim Selulase Bakteri Selulolitik Hasil Isolasi dari Bekatul", *Alchemy*, vol. 2, no. 1, pp. 35-45, 2012.

[10] N. Atkins, and J. Paula, "Physical Chemistry Ninth Edition", New York: W. H. Freeman and Company. 2010.

[11] Eisenthal, Robert, M. J. Danson, and D. W. Hough, "Catalytic Efficiency and KCat/KM : A Useful Comparator", *TRENDS in Biotechnology*, vol. 25, no. 6, pp. 3-5, 2007.