CHAPTER 3

RESULTS AND DISCUSSIONS

3.1 Estimation of the Coefficients in Mathematical Model and Model Setup.

Central Composite Design was used to predict the possible condition for xylanase production from *Streptomyces* sp. Ab106.3. Eleven conditions were established using computer simulation as shown in Table 2.2. *Streptomyces* sp. Ab106.3 was cultured in all 11 conditions to obtain xylanases in 5 days in shaked flasks. It was found that conditions No.8, 9,10 and 11 showed the high level of xylanase activities as shown in Figure. 3.1. The amounts of enzyme activity were 11.96, 12.47, 13.4 and 13.9 IU, respectively. No.9, 10, 11 were the same condition. It suggested the data accuracy of the flask experiments. It seemed that this microorganism produced xylanases at relatively high temperature and neutral or alkaline conditions.

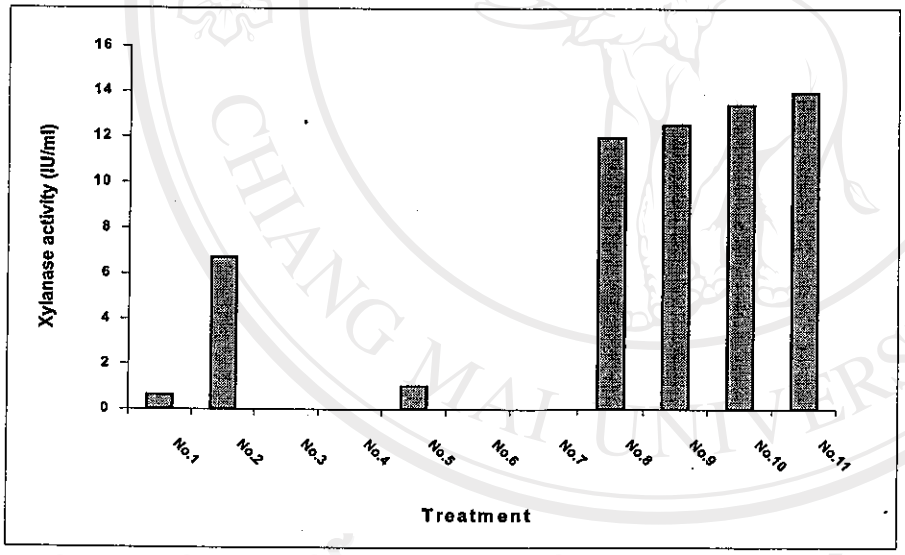


Figure.3.1 Xylanase Productions From Conditions Simulated by Central Composite Design.

3.2 Model Analysis.

Quadratic model analysis by "SX. Version 7 " program (Analytical Software) was employed to find out the quadratic mathematical model and could be written as follows:

$$Y = 13.26 - 1.09X_1 + 2.82X_2 - 6.72X_1^2 - 4.04X_2^2 - 1.52X_1X_2$$

Where ,Y is the predicted xylanase yield, X_1 and X_2 were coded variables of temperature and pH, respectively. The summary of variance analysis for the model was shown in Tables 3.1

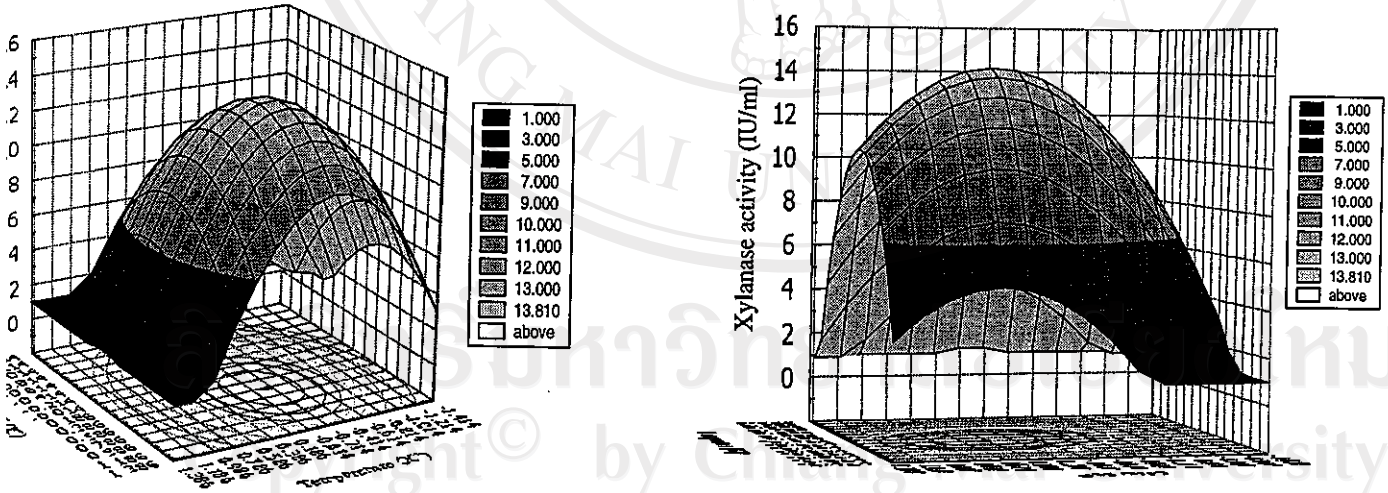
Table 3.1. Least Squares Linear Regression Analysis for Xylanase Production (quadratic model).

Variable	**Parameter estimate**	**Degree of freedom**	**Computation *t***	***P* > *t***
Intercept	13.2658	1	10.80	0.0001
X_1	-1.09870	1	-1.46	0.2040
X_2	2.82863	1	3.76	0.0132
X_1^2	-6.71524	1	-7.50	0.0007
X_2^2	-4.04640	1	-4.52	0.0063
$X_1.X_2$	-1.52400	1	-1.43	0.2114
Source	Sum of squares	Degree of freedom	Mean squares	*F*-value ($p < 0.05$)
Model error	363.964	5	72.7928	16.08
Residual error	22.6314	5	4.52628	($p = 0.0042$)
Total	386.596	10		
Coefficient of correlation (R^2) = 0.9415				

The computed *F* value (16.08) in Table 3.1 was equal to the *F* value (16.08) in the statistic table, indicated that the model was significant . The probability *P* value (0.0042) was also relatively low indicating the significance of the model. The value R^2 = 0.9415 indicated a high correlation between the experimental data and predicted values. The coefficient of variations (0.9415) indicated the degree of precision. Table 3.1, also showed the student *t* distributions (10.80, -1.46, 3.76, -7.50, -4.52 and -1.43) and corresponding values of the parameter estimation (13.2658, -1.09870, 2.82863, -6.71524, -4.04640 and -1.52400). The *P* values were used to check the significance of each coefficient. The low value of *P*, (*P* <0.05) indicated the more significant correlation of coefficient.

3.3 Optimization of pH and Temperature for Xylanase Production.

To investigate the effects of pH and temperature on xylanase production, The three dimension contour plot (Statistica version 5 , SIEGE Production Inc. USA) was employed. The maximum xylanase activity of 14 IU could be estimated at 50 °C , pH 7.2 (Figure 3.2). It was no doubt that *Streptomyces* sp. Ab106.3 could produce xylanases at 50 °C.



Figuer.3.2 3-D Graphics for Quadratic Response Surface Optimization for Xylanase Production.

Response surface methodology for quadratic model was used to optimize the condition. There were reports on cellulase-free xylanase productions mainly form *Bacillus* sp., *Cellulomonas* sp. and *Streptomyces* sp. (Beg *et al* ,2000 , Antanopoulos *et al* , 2000 and Maheswari and Chandra , 2000). In addition, most of *Streptomyces,* which produced cellulase-free xylanases, were mesophillic microorganisms. *Streptomyces* sp. QG113 (Beg *et al* ,2000) produced 7.5 IU of xylanases on wheat bran medium at 37^{o}C,pH 8.0 after 5 days of cultivation.; *Streptomyces albus* (Antanopoulos *et al* , 2000) produced 12 IU of xylanases in xylan medium at 30^{o}C,pH 7.5 after 5 days of cultivation ; *Streptomyces cuspidosporous* (Hoq *et al* , 1994) produced 18 IU of xylanases in xylan medium at 37^{o}C,pH 7.5 after 5 days of cultivation. So, *Sterptomyces* Ab 106.3 was not only thermotolelant strain but also comparable to those microorganisms in terms of xylanase production.

3.4 Test for the Accuracy of the Model.

To test for the accuracy of the model, *Streptomyces* sp. Ab106.3 was cultured at the optimized condition (50^{o}C at constant pH 7.2) on rotary shaker. The enzyme production was shown in Figure.3.3. They was 14.1IU/ml . This fitted very well with the quadratic response surface optimization (Figure 3.2).

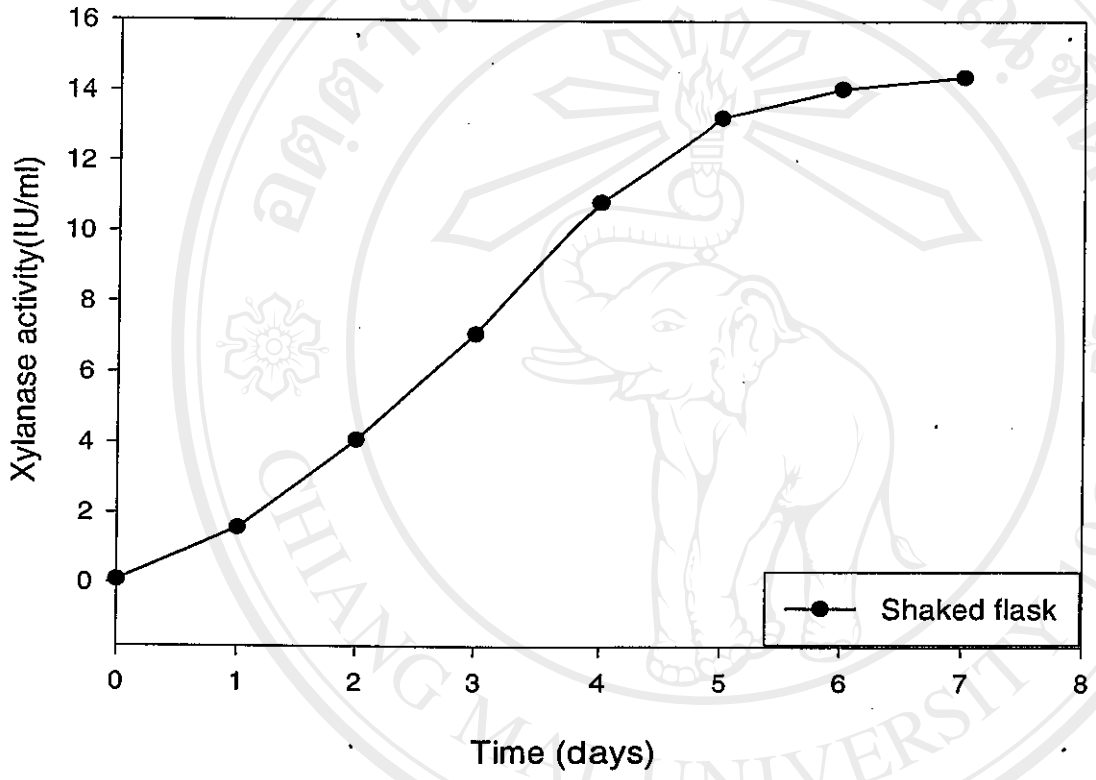


Figure. 3.3 Xylanase Production at Optimized Condition, 50 ^{O}C , pH 7.2.

3.5 Some Properties of Xylanases

3.5.1 Optimum Temperature and pH of Crude Xylanases

The temperature used in the pulp bleaching industry is 60°-90°C and under the alkaline condition of mainly pH 9-10 (Antanopoulos *et al* , 2000, Beg *et al* ,2000 and Balakrishman *et al* , 1992). Therefore, the enzyme characteristics were investigated. Figure 3.4 showed the xylanase activities at various temperatures and pH values. The result revealed that the optimum pH and temperature of xylanases were 6.0 and 60° - 65 °C, respectively . There were quite closed to the xylanases from *Streptomyces albus* ATCC3005 (50 °C and pH 6.5)(Antanopoulos *et al* , 2000) and *Streptomyces roseisclerestcus* (60 °C and pH7)(Grabski and Jeffries , 1991). It also showed that more than 80 per cent of xylanase activity still remained at 60 °C and pH 9. This could be the advantage of this enzyme for industrial applications.

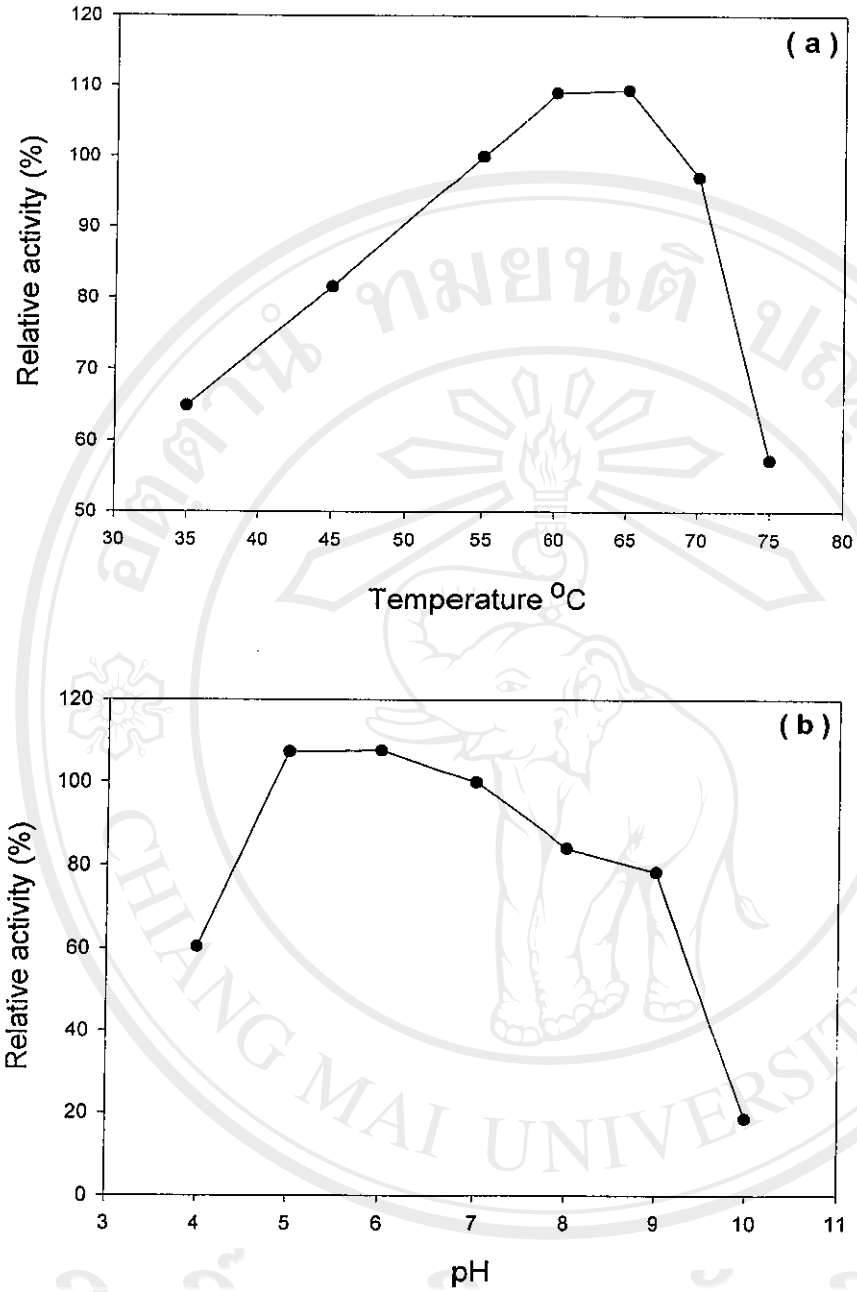


Figure. 3.4 Optimum Temperature and pH of Crude Xylanases Obtained from *Streptomyces* sp. Ab106.3

(a) Temperature profile at pH 7

(b) pH profile at 60 °C

3.5.2 Thermal Stabilities of Crude Xylanases.

Thermal stabilities of enzyme obtained from *Streptomyce* sp. Ab106.3 were investigated at 55°, 65° and 75°C. Figure 3.5b showed that the xylanases had the half life of 3 h at 65°C, pH 9.0. It was longer than those found in other reports, as summarized in Table 3.2. Relatively high stability at high alkaline condition (pH 9.0) has not yet been reported elsewhere. Even at 55°C, the relative enzyme activity was 50 per cent after 24 h of incubation (Figure 3.5a). This characteristic is significant for the practical use as crude enzyme . At higher temperature, 75 °C, the xylanases rapidly lost their activities in the range of pH 6-9 for a short period of time (Figure3.5c). At neutral pH, the enzyme was more stable than that at pH 9.0 at any temperature ranges. The enzyme was likely alkaline tolerant and thermotolerant.

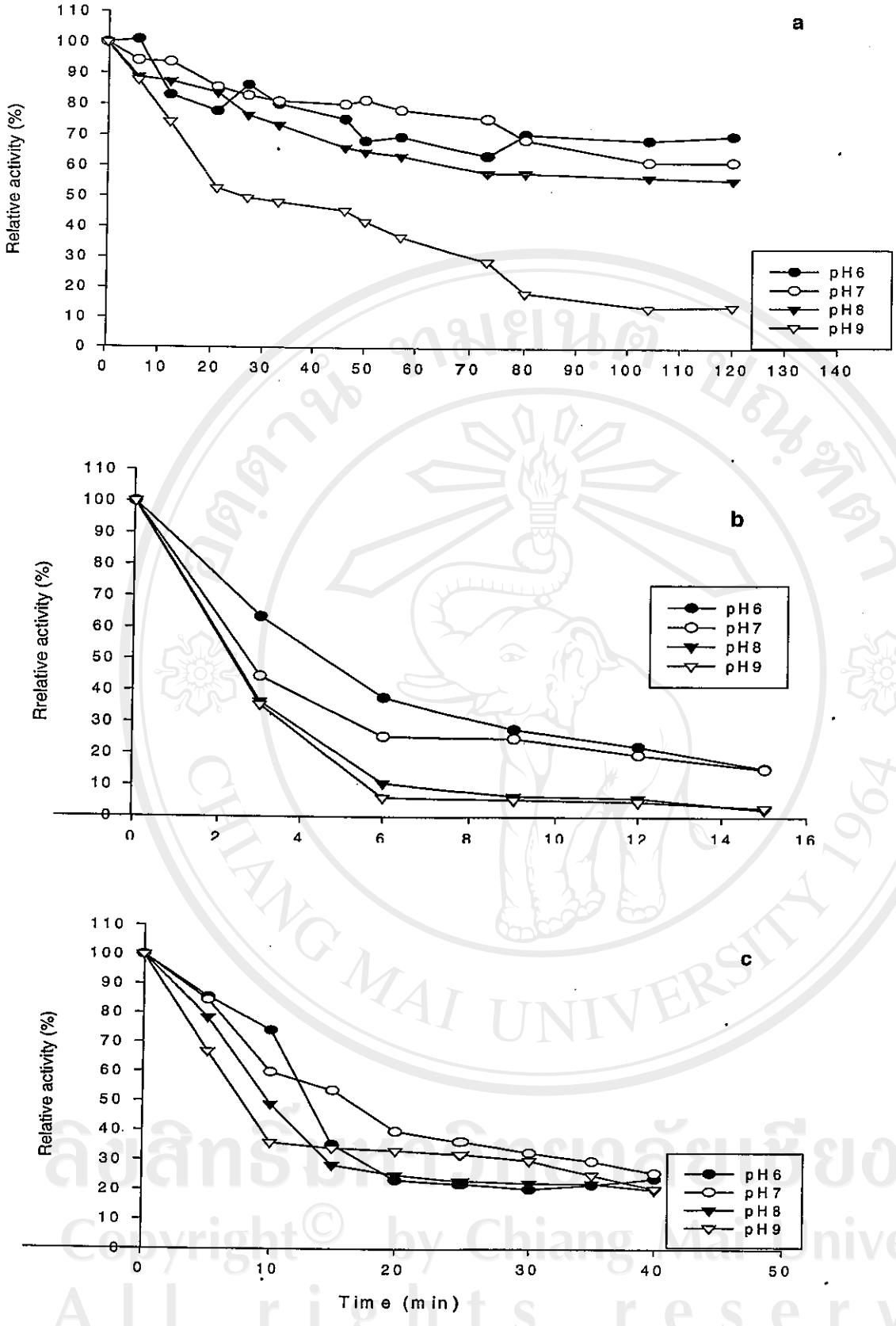


Figure. 3.5 Stability Profiles of Xylanases under Various Temperature and pH Values.
(a) ;55^{O}C , (b) ; 65^{O}C and (c) ; 75^{O}C.

TABLE 3.2. Comparison of Cellulase-Free Xylanase Production and Properties of *Streptomyces* sp.Ab106.3 to other Mesophilic and Thermophilic Actinomycetes.

Microorganism	Substrate	Growth Condition	Xylanase (IU)	Opt. Temp (°C)	Opt. pH	Half life: t_d (h)	References
Thermoactinomyces thalophilus	1% xylan +peptone	50°C, pH8.5,4 d	18	65	8.5	2.0 (65°C, pH8.5)	Kohli *et al* , 2001
Streptomyces sp. QG113	wheat bran	37°C, pH8.0, 5 d	7.5	50	8.5	1.0 (50°C, pH9.0)	Beg *et al* , 2000
Streptomyces albus ATCC 3005	1%xylan +yeast extract.	30°C, pH7.5, 5 d	12	60	6.5	0.5 (55°C, pH6.5)	Antanopoulos *et al* , 2001
Streptomyces cuspidosporous	1% xylan	37°C, pH7.5, 5 d	22	65	5.5	5.0 (55°C, pH5.5)	Maheswari and Chandra , 2000
Streptomyces roseisclereoticum	1% xylan.	37°C, pH7.5, 5 d	16	60	7.0	NR	Grabski and Jeffries , 1991
Streptomyces sp.	1 %xylan 1% straw	NR NR	5 14	50 50	8.0 8.0	NR NR	Lumba and Penninnckx , 1992
Streptomyces sp. Ab106.3	**1% xylan**	**50°C, pH7.2, 5 d**	**8**	**65**	**6.0**	**NR**	**Present work**
	1%bagasse	**50°C, pH7.2, 7 d**	**14**	**65**	**6.0**	**3.0 (65°C, pH9.0)**	**Present work**

NR = not recorded.

3.5.3 Effect of Bleaching Reagent on Xylanase Stability

Hydrogen peroxide and sodium hypochlorite were employed as bleaching reagents. The effect of hydrogen peroxide concentrations on xylanase stability was shown in Figure 3.6. At 0.1-0.5 per cent (w/v) of hydrogen peroxide, 45 ^{O} and 55^{O}C, for 180 min, it was found that the relative enzyme activities were more than 50 per cent (Figure 3.6a and 3.6b). At 65^{O}C, it retained 50 per cent of the activity at hydrogen peroxide concentration of 0.1 per cent w/v (Figure 3.6c). This characteristic was considered for the practical use of crude enzyme in cooperation of hydrogen peroxide in pulp bleaching process. For the effect of sodium hypochlorite concentrations on xylanase stability, it was shown in Figure 3.7. It were revealed that, the xylanases ost activities in the presence of sodium hypochlorite. This could be the increase of pH by sodium ion (pH11), resulting in more than 90 per cent decrease of the xylanase activity.

In the pulp and paper industrial processes, the combination of hydrogen peroxide bleaching condition is as follow; 0.4 to 1.5 per cent w/v, pH 9.2 to 10.8, at 60^{O} to 85 ^{O}C, for 1 to 3h, and 10 to 20 per cent pulp consistency. For sodium hypochlorite bleaching of pulp, the condition; was 5-7 per cent chlorine as hypochlorite, pH 8.5 to 11.0, at 40^{O} to 50^{O} C, for 5 to 8 h, and 3 to 6 per cent pulp consistency (Kenneth , 1970). It was revealed that, the use of enzyme cooperated with bleaching reagents such as hydrogen peroxide and sodium hypochlorite in pulp bleaching process was not suitable. It was suggested elsewhere that, the use of hydrogen peroxide in enzyme pretreated pulps, the condition was 1.5 per cent H_2O_2; 0.7 per cent NaOH; 0.5 per cent $MgSO_4$; 70 ^{O}C for 3 h and 12 per cent pulp concentration (Christov and Prior 1997).

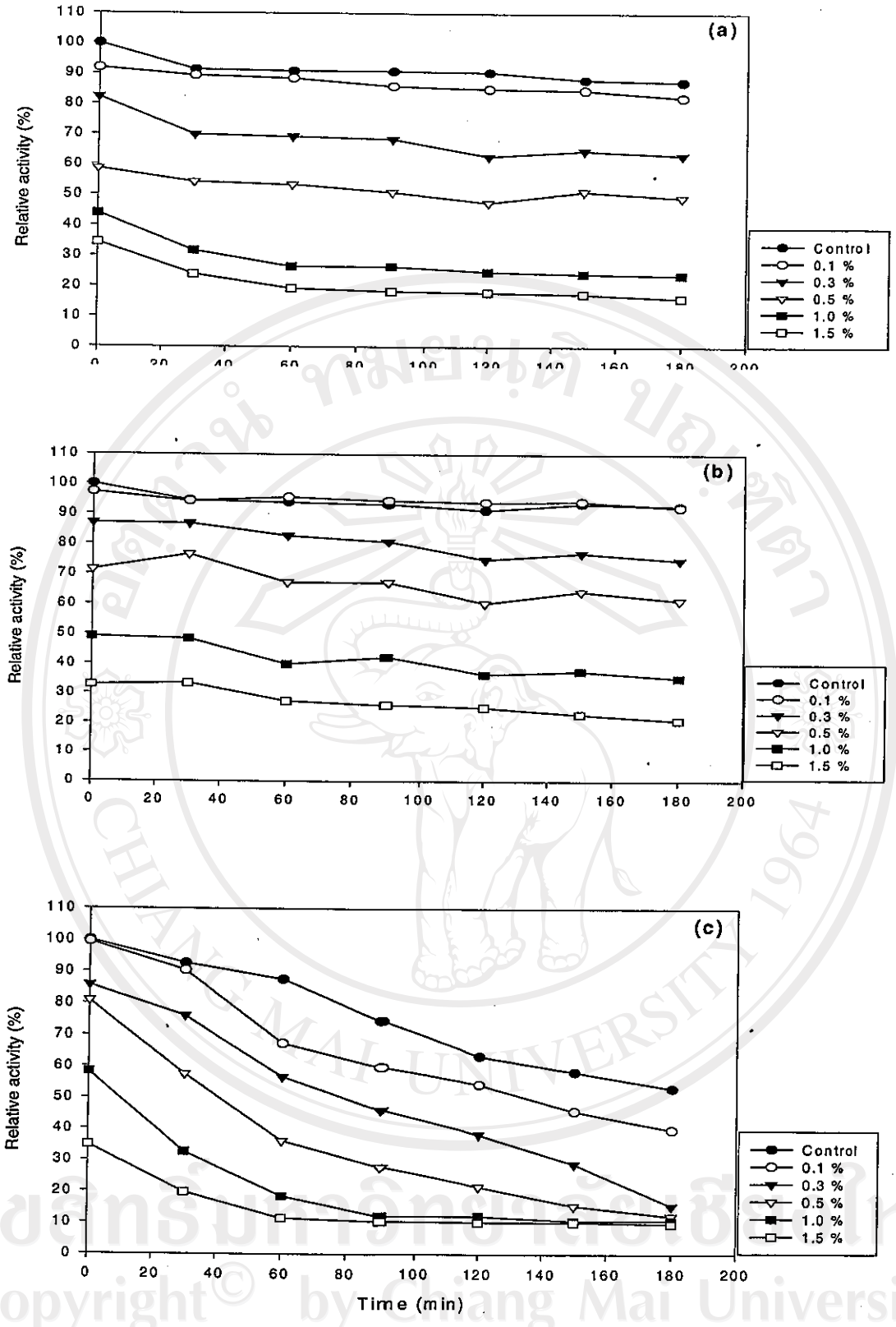


Figure. 3.6 Stability Profiles of Xylanases Under Various Temperature and Hydrogen Peroxide Concentrations. (a) ; 45^{o}C , (b) ; 55^{o}C and (c) ; 65^{o}C.

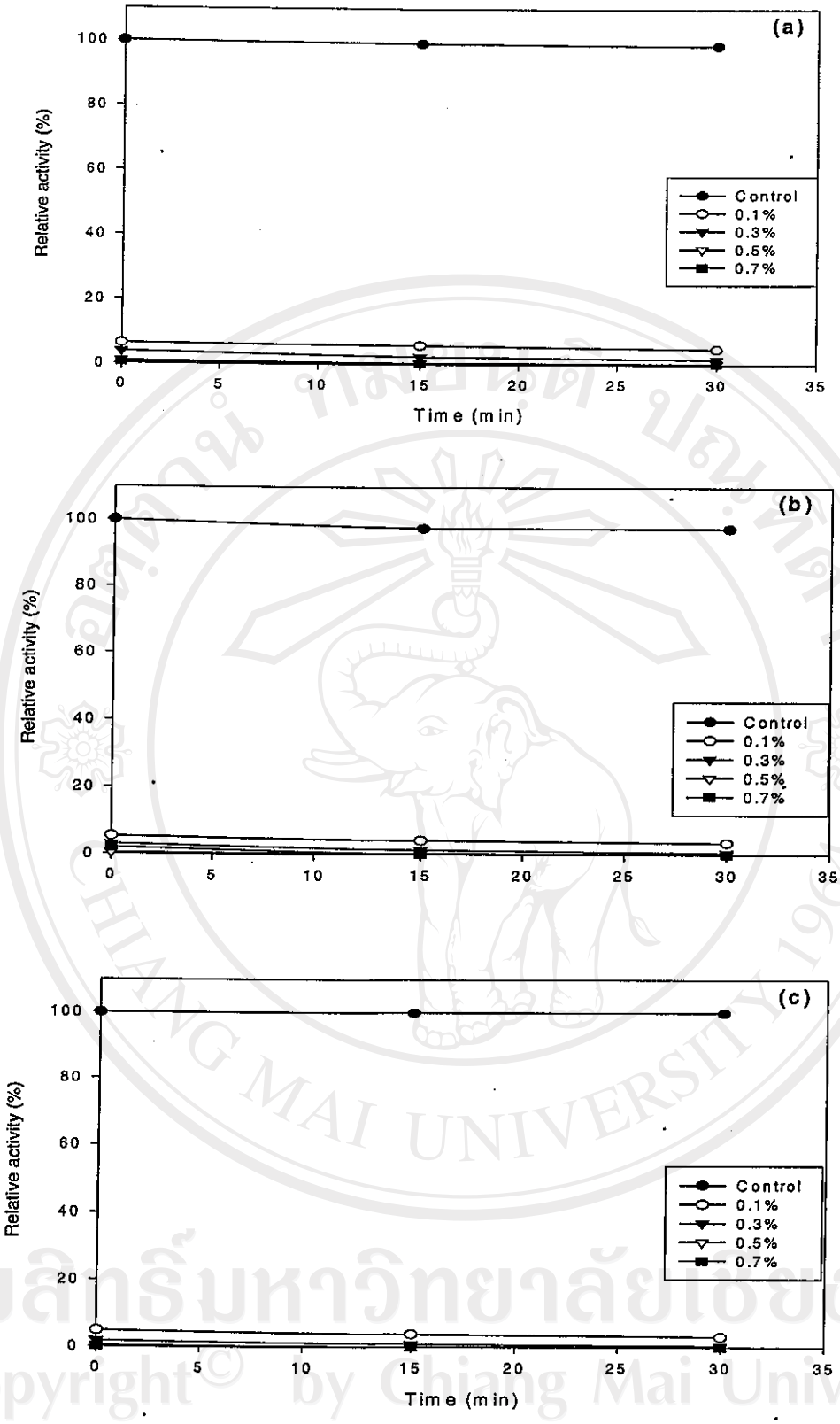


Figure. 3.7 Stability Profiles of Xylanases Under Various Temperature and Sodium Hypochlorite Concentrations. (a) ; 45^{O}C , (b) ; 55^{O}C and (c) ; 65^{O}C.

3.6 Enzyme Kinetics

The results obtained form enzymatic hydrolysis of oat spelt xylan , concentrations of oat spelt xylan 1 – 20 g/l with constant concentration of enzyme (5 IU/ml) , at 65 ^{o}C was shown in Figure 3.8. The amount of xylose liberated was proportional to the initial substrate concentrations.

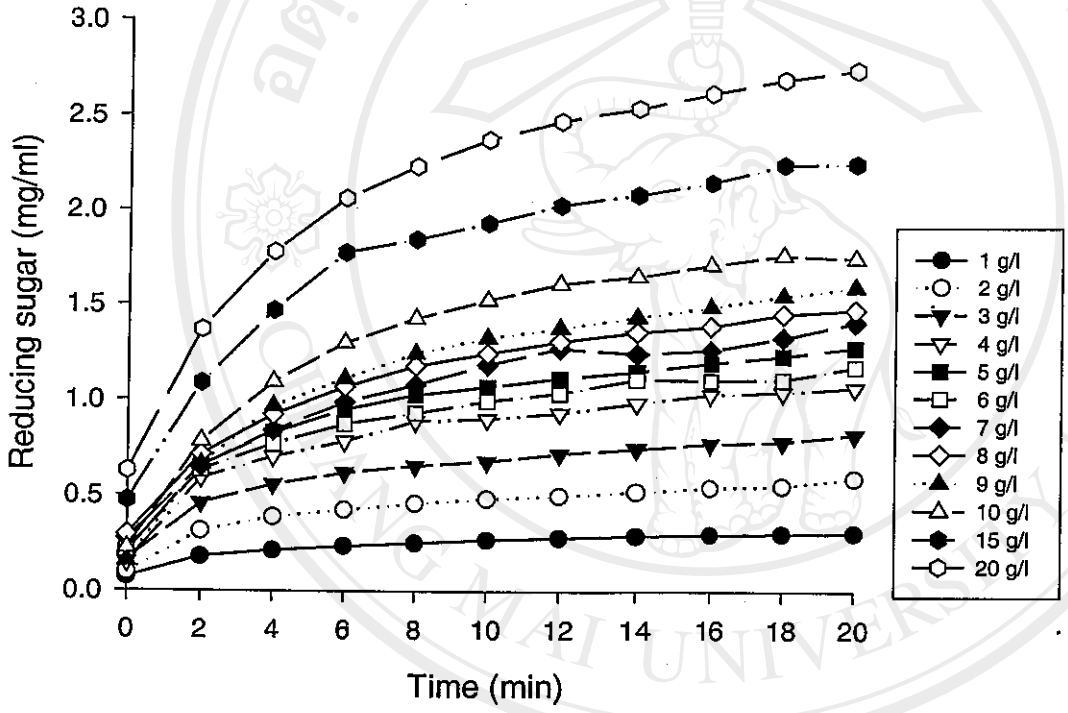


Figure. 3.8 Enzymatic Hydrolysis of Oat Spelt Xylan (1 – 20 g/l) with Constant Concentration of Xylanases (5 IU/ml), at 65 ^{o}C.

The kinetics were illustrated by using Linewever – Burk plot shown in Figure. 3.9 . The Michaelis constant, K_M , and maximum reaction rate, V_{max},of crude enzyme were 18.66 mg/ml and 12.91 μmol/mg protein-min, respectively. The high value of K_M showed the high affinity of the enzyme to convert xylan to xylose compared to the other reports, as summarized in Table 3.3.

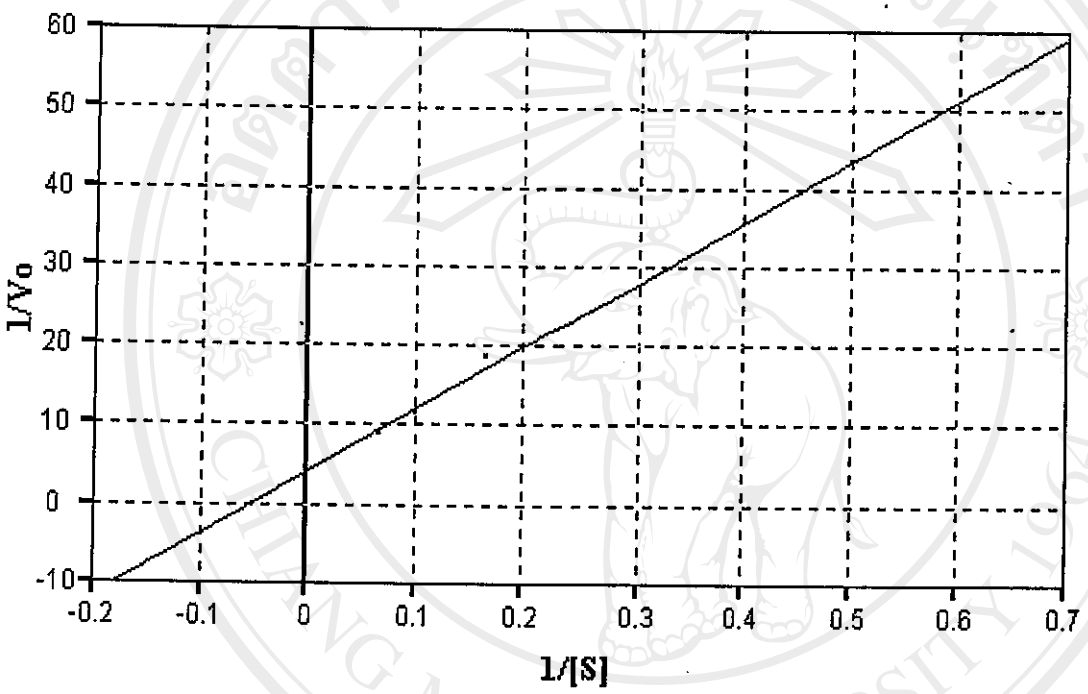


FIgure. 3.9 Lineweaver –Burk Plot of the Enzymatic Hydrolysis of 1 – 20 g/l Oat Spelt Xylan with Constant Concentration of Xylanase (5 IU/ml) at 65 ^{o}C.

Table 3.3 Comparison of Xylanase Kinetic Constants of *Streptomyces* sp.Ab106.3 to other Actinomycete Xylanases.

Microorganisms	K_M (mg/ml)	V_{max} (μ mol /mg protein- min)
Streptomyces sp. EC10	3	NR*
Streptomyces sp. B-12-2	0.8-5.8	162-470
Streptomyces sp. T7	10	7610
Streptomyces chattanoogensis CECT 3336	4,0.3	78.2,19.1
Streptomyces sp. QG-11-3	1.2	158.85
Thermomonospora curvata	1.4-2.5	NR*
****Streptomyces* sp. Ab 106.3**	**18.66**	**12.91**

*NR = not recorded

**Present work

Source: Beg *et al* , 2001.

The inhibition effect of hydrogen peroxide on the xylanases was determined by using the soluble fraction of oat spelt xylan as substrate. When the enzyme was incubated in the presence of a various concentrations of hydrogen peroxide, the xylanase activities were dramatically reduced when the hydrogen peroxide concentration increased. The reduction rates of the enzyme were shown in Figure 3.10. Increasing of hydrogen peroxide concentrations led to further reduction of the xylanase activity. Consequencing of this, the hydrogen peroxide concentrations of 0.1 and 0.2 M were employed for the estimation of inhibition constants (Ki). The enzyme was incubated with oat spelt xylan (2 – 15 g/l) together with and without the inhibitor (H_2O_2). From each inhibitor concentration, the apparent K_M and V_{max} were determined by double reciprocal plot. These data indicated that the inhibition effect of hydrogen peroxide on xylanases was mixed-inhibition. It showed the increase of slope ($\alpha Km/V_{max}$) and intercept (α'/V_{max}), (where α=1+ [I]/Ki , α'= 1+ [I]/Ki'), when the hydrogen peroxide concentrations increased (Figure 3.11) .

For mixed-inhibition, the inhibition constants (Ki , Ki') were estimated by computer program (Enzyme Kinetic Pro. Version1.1). Kinetic constants of mixed-inhibition compared to non-inhibition were shown in Table 3.4.

Table 3.4 Comparison of Xylanase Kinetic Constants of *Streptomyces* sp.Ab106.3 for Mixed-Inhibition to Non-inhibition.

Type of inhibitions	V_{max} (μ mol /mg protein- min)	K_M (mg/ml)	Ki	Ki'
Non-inhibition	13.91	18.66	-	-
Mixed-inhibition	13.05	18.74	0.0704	0.038

It was reported that the xylanase from *Penicillium funiculosum* was strongly inhibited by three wheat xylanase inhibitors (XIP-I, TAXI I and TAXI II). Without inhibitor the kinetic constants Km and V_{max} were 0.47 per cent w/v and 2,540 μmol xylose / mg protein-min, at pH 5.5 and 30 ^{o}C, respectively, when birchwood xylan was used as substrate. When three xylanase inhibtiors were present, kinetic analysis showed that the inhibition was competitive for all three enzyme/inhibitor complexes, and the inhibition constants, Ki, were 3.4, 16 and 17 nM, respectively.(Caroline *et al* , 2002).

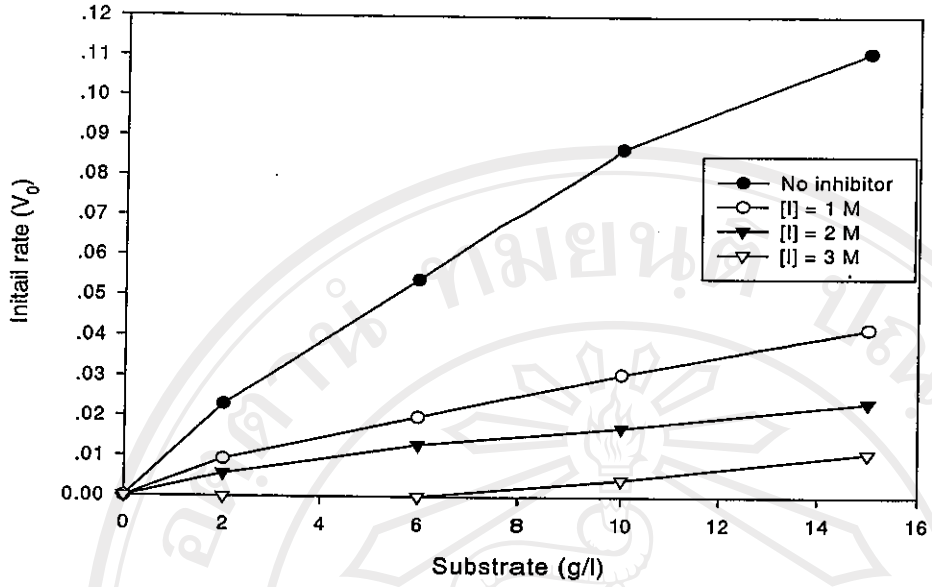


Figure 3.10 Effect of Hydrogen Peroxide on Initial Rates of Xylanase Activity.

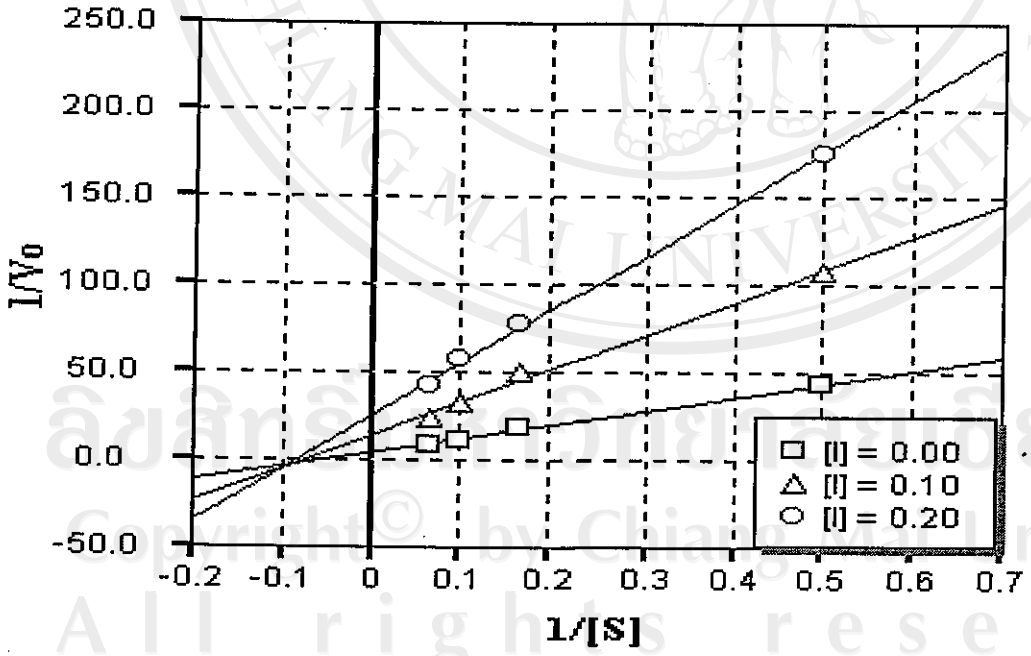


Fig 3.11 Inhibition Kinetic analysis of Xylanase by Hydrogen Peroxide Using Soluble Oat Spelt Xylan as Substrate.

3.7 Prebleaching and Bleaching of Kraft Pulp

3.7.1 Biobleaching Pulp with Xylanases

Kraft pulp (5 g) was incubated with 5,10 and 15 IU/g of crude xylanases obtained from *Streptomyces* sp. AB 106.3, at 55 °C pH 7 for 3,24,36 and 48 h. Release of reducing sugar was shown in Figure 3.12. The amounts of reducing sugar increased with time in the filtrates after enzyme treatment and flatted out after 24h of incubation. About 0.1 mg/ml of reducing sugar was detected in the control. It was revealed that, when the xylanase doses increased from 5 to 15 IU /g of pulp, there was no significant difference in the liberation of reducing sugar. The highest amount of reducing sugar of 0.60 mg/ml was detected when 15 IU /g of enzyme dose was used after 24 h of incubation at 55°C. In addition, the amounts of reducing sugar (0.55 and 0.58 mg/ml) were obtained when 5 and 10 IU/g pulp were employed, respectively.

It was found that releasing of lignin derived compounds (LDCs) at 280nm and chromophore at 465 nm increased with incubation time. The release of LDCs (280nm) was gradually increased with time of incubation (Figure. 3.13a) and chromophore (465nm) was flatted out after 24 h of incubation time,(Figure 3.13b). The correlation among the release of LDCs (280nm), chromophore (465nm) and reducing sugars suggested the dissociation of lignin-carbohydrate complex from the kraft pulp fibers. Kulkarni and Rao (1996) reported that the OD of 0.1900 at 465 nm could be achieved when 10 IU of the xylanase obtained from *Bacillus* sp was used per gram of washed and oven-dried bagasse pulp at 50 °C for 4 h. The release of reducing sugars and the release of lignin and phenolic compounds were interrelated phenomena. When the pulp was pretreated with xylanase, the xylose and other reducing sugars were released from the hemicellulose layer that ultimately resulted in an increase in the free sugar content in the pulp sample. Xylan is actually a part of hemicellulose and is sandwiched between lignin and cellulose layers. When xylan is degraded by the xylanases, in addition to xylose, it also results in the release of lignin and phenolic compounds from the pulp fibers those ultimately cause the enhancement in absorbance of pulp samples compared to the control.

Brightness of xylanase treated pulp comparatively increased by 2 per cent compared to control after 24 h of incubation as shown in Figure 3.14. The brightness of control was approximately of 65 per cent. The brightness of treated kraft pulp with 5 – 10 IU/g dried kraft pulp varied from 65.86, 65.95 and 66.21 to 66.67, 66.71 and 66.95 per cent after incubation for 3 and 24 h, respectively. From the result, it suggested that higher enzyme dose or longer incubation time (more than 24 h) did not significantly enhance the biobleaching benefit. It was reported elsewhere that cellulase-free xylanase obtained from *Streptomyces* sp. QG-11-3 was used in biobleaching of eucalyptus kraft pulp. The xylanase dose of 3.5 U/g moisture free pulp exhibited the optimum bleach boosting of eucalyptus kraft pulp at pH 8.5, 50°C after 2 h of treatment (Beg *et al* , 2001).

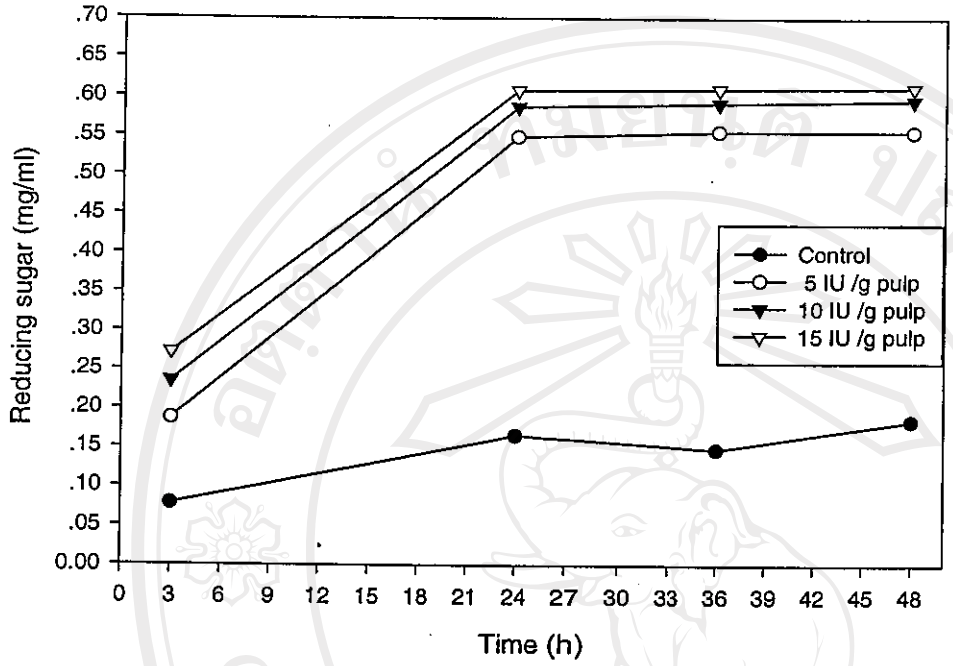


Figure 3.12 Release of Reducing Sugar from Enzymatic Pretreatment of Kraft Pulp with Different Xylanase Doses at 55°C, pH 7.0.

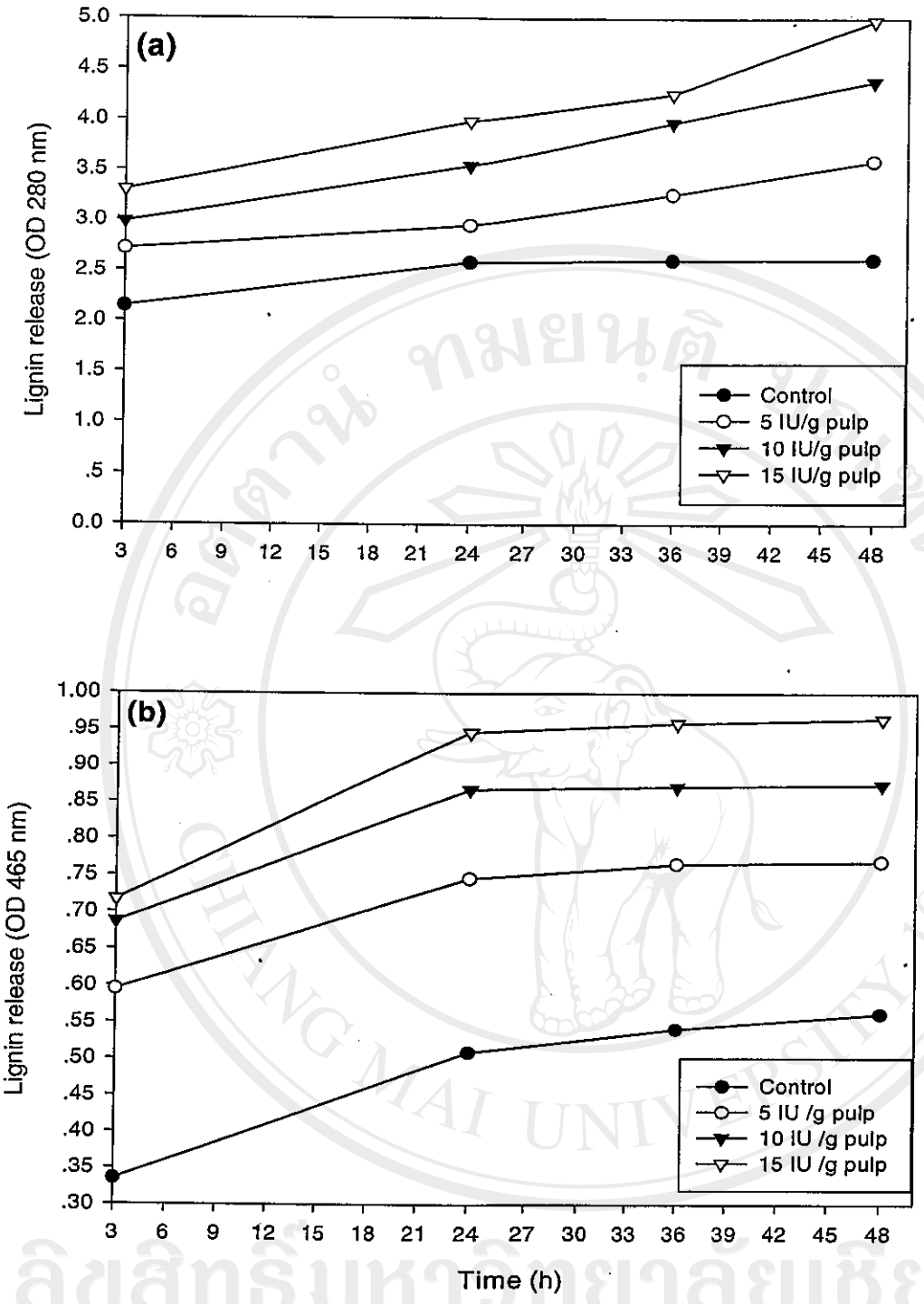


Figure 3.13 Release of Lignin from Enzymatic Pretreatment of Kraft Pulp with Different Xylanase Doses at 55°C, pH 7.0.

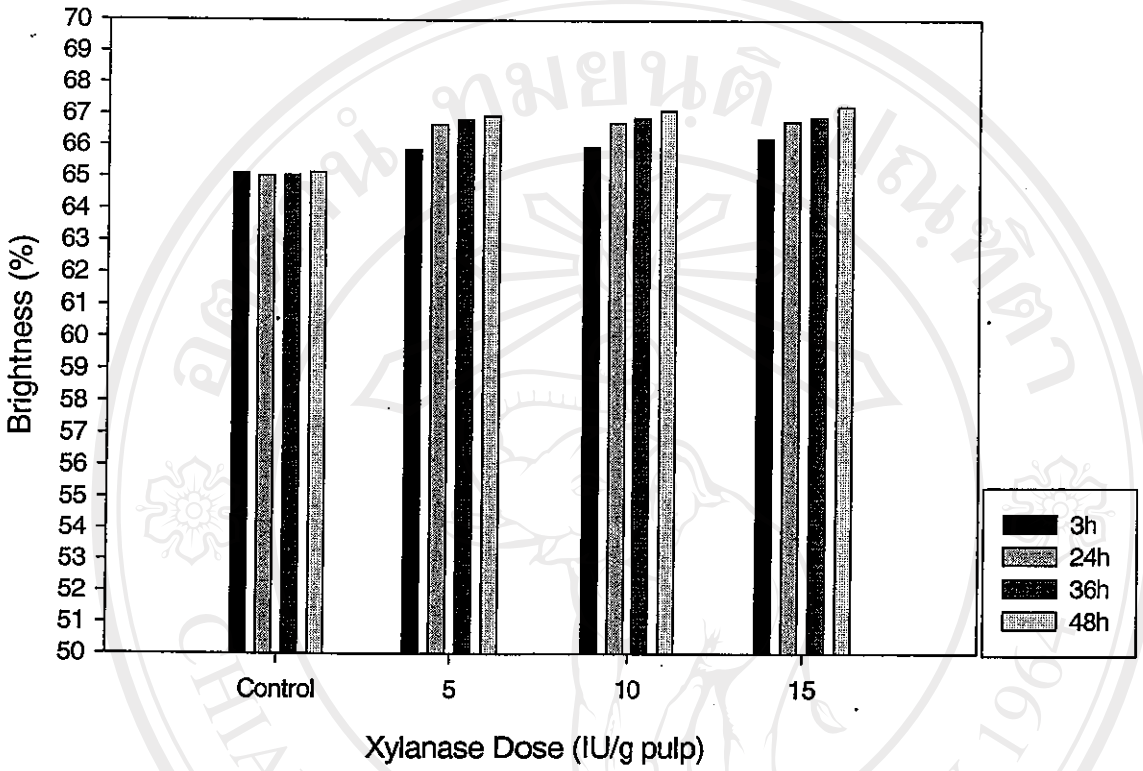


Figure 3.14 Effect of Various Xylanase Doses on the Kraft Pulp Brightness at Different Incubation Times.

3.7.2 Hydrogen Peroxide Bleaching of Enzyme Pretreated Pulp

The biobleaching ability of xylanases form *Streptomyces* sp.Ab106.3 was tested in hydrogen peroxide bleaching pulp. In each, enzymes pretreated dose performed in a similar way as judged by the pulp properties.

The brightness of xylanase pretreated pulp bleached by hydrogen peroxide comparatively increased by 2 per cent compared to control after 24 h of incubation as shown in Figure 3.15. The brightness of the control was approximately of 77 per cent. The brightness of hydrogen peroxide bleaching pretreated kraft pulp with 5 – 10 IU/g dried kraft pulp varied from 78.34, 78.41 and 78.63 to 79.01, 79.02 and 79.10 per cent after incubation for 3 to 24 h. It was found that, the results were correlated with the brightness of pulp in xylanase treatment(Figure 3.14), indicated that peroxide bleaching affected the brightness gain that could be obtained from xylanase pretreatment. On the other hand, it was reported that there was no clear improvement in brightness after peroxide bleaching of sulfite spruce pulps pretreated with purified xylanases and mannanases obtained from *Trichoderma reesei* (Christov and Prior 1997). Hence, these results may lead to the following suggestions: firstly, the biobleaching effect may be strongly dependent on the properties of the particular microbial xylanase used; secondly, since, xylan is structured and localized in a different manner in softwood pulps compared to hardwood pulps, its response to enzyme degradation would differ as well.

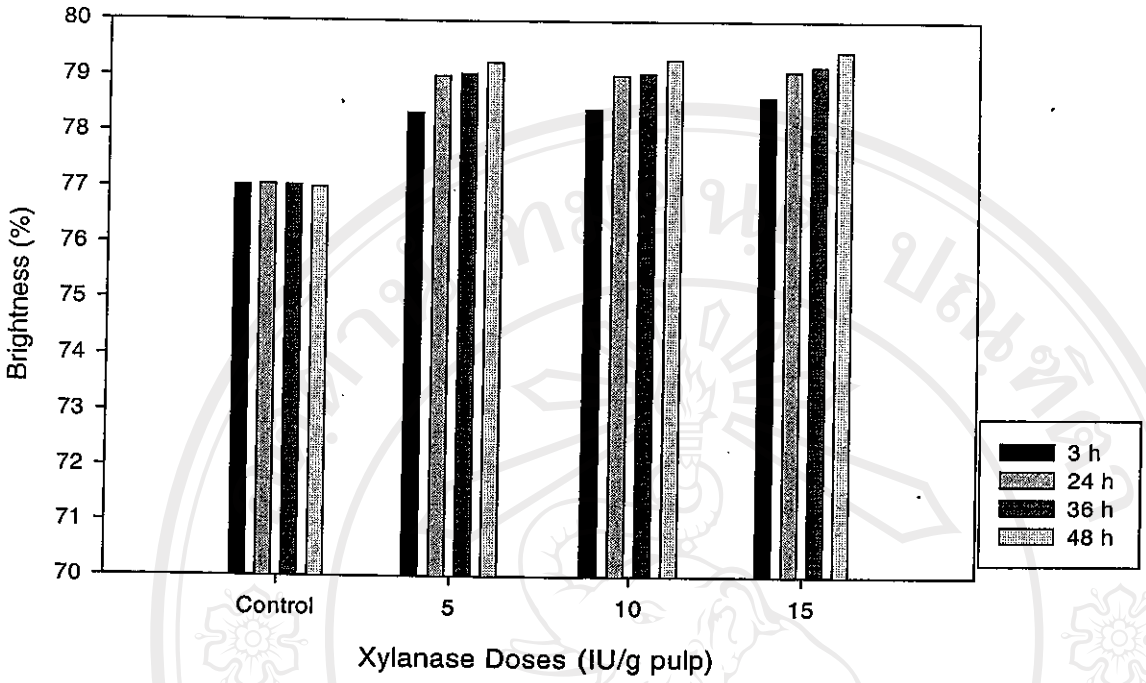


Figure 3.15 Effect of Hydrogen Peroxide Bleaching on Brightness of the Pretreated Kraft Pulp with Various Xylanase Doses.

3.7.3 Enzyme Bleaching of Hydrogen Peroxide Pretreated Pulp

Kraft pulp (5 g) was pretreated by hydrogen peroxide. The enzyme concentrations of 5,10 and 15 IU/g of xylanases were used at 55 °C, pH 7 for 3,24,36 and 48 h. Releasing of reducing sugar was shown in Figure 3.16. The amounts of reducing sugar increased with time in the filtrates after enzyme treatment and flated out after 36 h of incubation. About 0.01 mg/ml of reducing sugar was detected in the control. It was revealed that, when pretreated pulp with hydrogen peroxide was further treated with the enzyme, comparatively lower reducing sugar was released. The highest amount of reducing sugar of 0.33 mg/ml was detected when 15 IU /g of enzyme dose was used after 48 h of incubation, at 55°C. In addition, reducing sugar of 0.27 and 0.31 mg/ml were obtained when 5 and 10 IU of

enzyme doses /g were employed, respectively. The alkaline condition in hydrogen peroxide pretreatment may be the cause of the comparatively lower of reducing sugar.

It was found that, the XH treated pulp (xylanase pretreated pulp was further treated with hydrogen peroxide) showed higher reducing sugar concentrations about 3.4, 2.6 and 2.4 fold than HX treated pulp (hydrogen peroxide pretreated pulp was further treated with xylanases, when 5, 10 and 15 IU/g pulp were employed at 55°C, respectively (Figure 3.12 and 3.16). The release of LDCs (280nm) and chromophore (465nm) of XH treatment were higher than that of HX treatment about 2.3 and 4.3 fold at similar condition of enzyme treatment (Figure 3.13 and 3.17).

In addition, the brightness gain of the XH treatment (Figure 3.15) was a little more (around 1.5 per cent) than that of the HX treatment (Figure 3.18), when 5, 10 and 15 IU of enzyme /g pulp were employed at 55°C. It was reported elsewhere that to achieve the brightness gain, it was depended on the position of enzyme stage in the bleaching sequence (Wong *et al*, 1996). On the other hand, It was suggested that the final pulp brightness was not greatly dependent on the location of the xylanase stage in an oxygen-peroxide-ozone bleaching sequence (Ledoux *et al*, 1993). Although, the importance of direct brightening might be limited to peroxide bleaching.

These results indicated that enzymatic prebleaching could have facilitated increasing brightness in fibers and might be led to reduction of bleaching chemical used in pulp bleaching process. It was reported elsewhere that using xylanases from *Staphylococcus* sp. SG-13, pretreatment of pulp and its subsequent treated with 8 per cent hypochlorite, reduced the kappa number by 30 per cent, enhanced the brightness of 11per cent (Gupta *et al*, 2000). Commercial xylanases, such as, Novozyme 473, VAI xylanase and Cartazyme HS-10 resulted in a 31per cent reduction in chlorine consumption, a 30 per cent reduction in total organic chlorine content in the extraction stage effluent with an increase in the brightness, tensile strength and burst factor by 3, 26, and 32 per cent, respectively(Bajpai *et al*, 1994). The pretreatment of bagasse pulp with xylanases from thermophilic *Bacillus* sp. NCIM 59 resulted in the reduction of kappa number by 21 per cent and increase in the brightness by 2.5 per cent, whereas viscosity of enzyme treated

samples were unaltered (Kulkarni and Rao 1996).The application of xylanases form *streptomyces* as prebleaching agent in pulp bleaching for pulp improvement has been reported by using xylanases from *Streptomyces. lividans* (Ragauskas *et al*, 1994) and *Streptomyces* sp. TUB B-12-2 (Elegir *et al*, 1994).

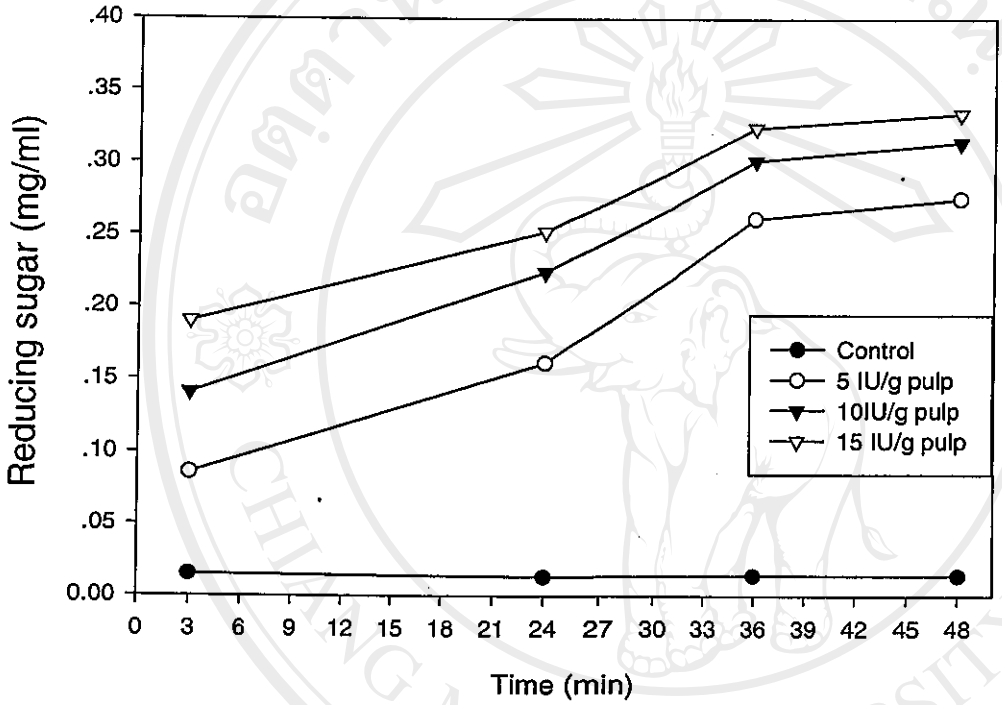


Figure 3.16 Release of Reducing Sugar from Enzymatic Treatment of Kraft Pulp with Different Xylanase Doses After Hydrogen Peroxide Bleaching.

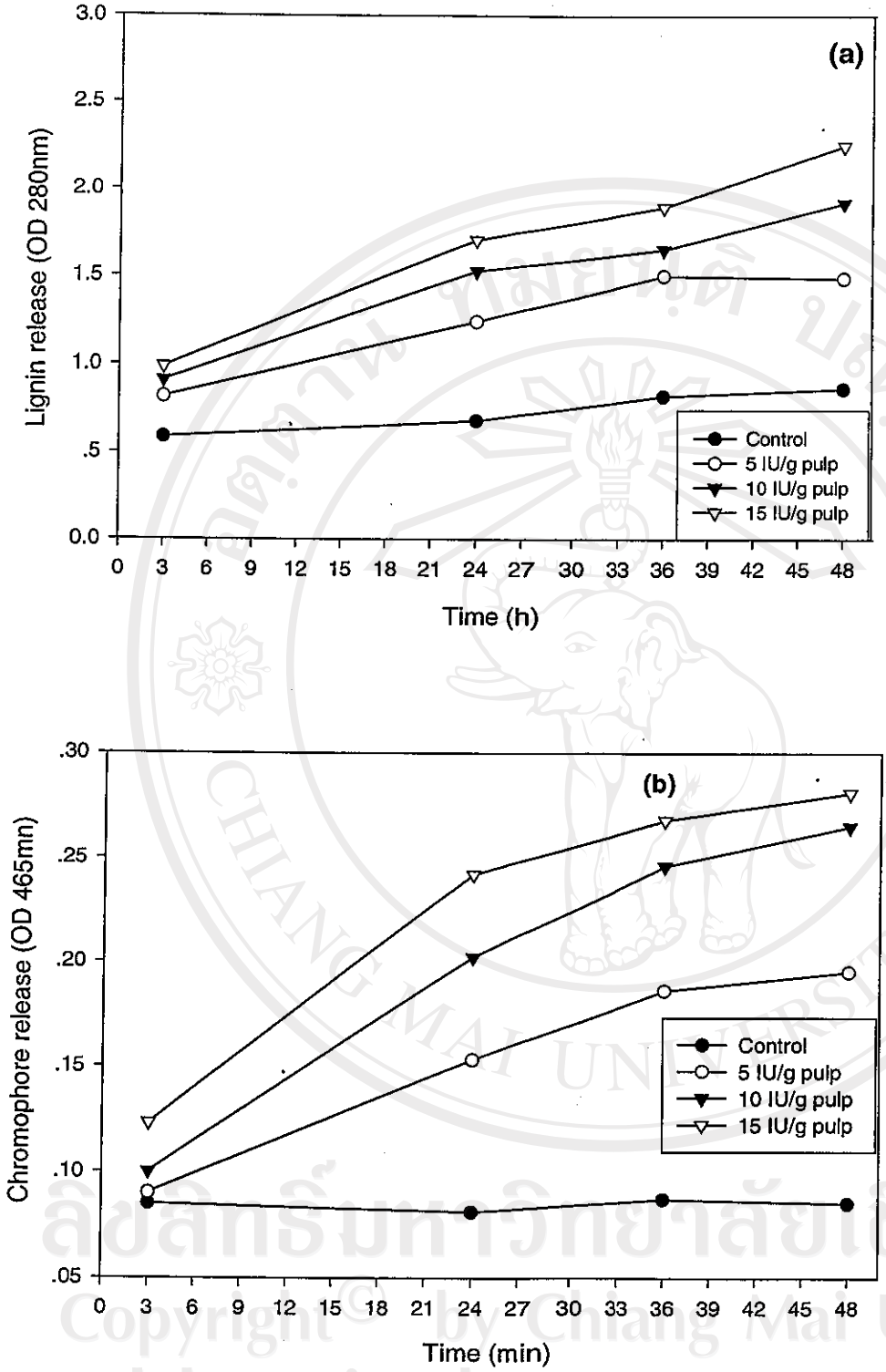


Figure 3.17 Release of Lignin from Enzymatic Treatment of Kraft Pulp with Different Xylanase Doses After Hydrogen Peroxide Bleaching.

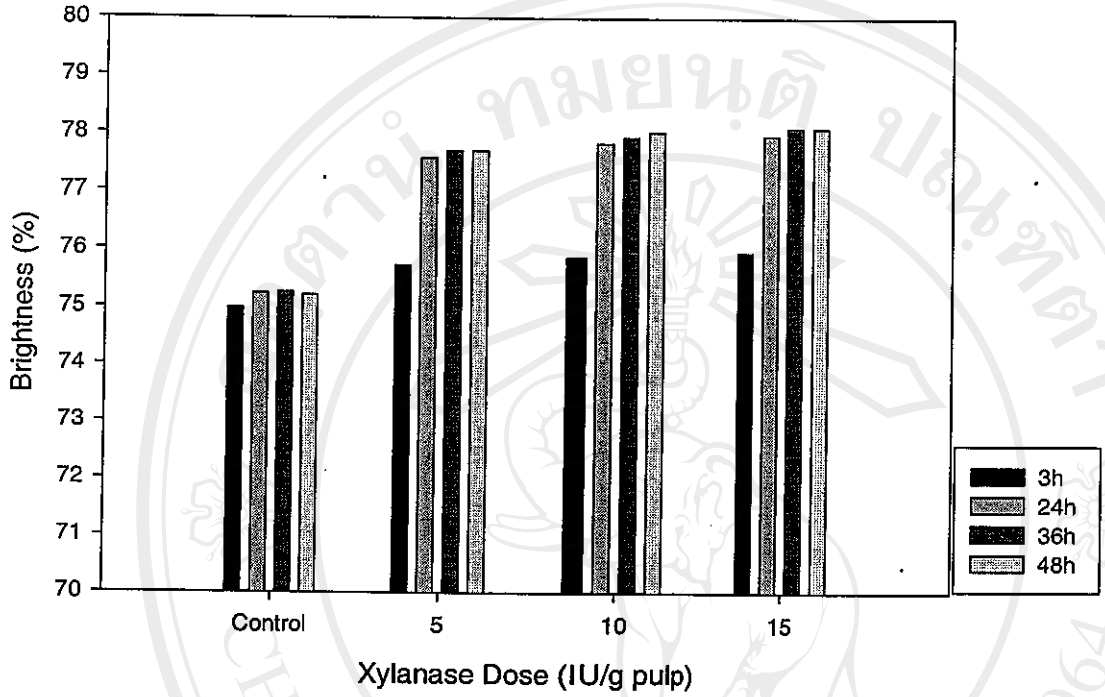


Figure 3.18 Effect of Xylanase Doses on Brightness of the Pretreated Kraft Pulp with Hydrogen Peroxide.

3.8 Conclusions

1. It was found that the CCD, regression analysis and response surface methodology were effective in finding the optimum point of 2 factors, temperature and pH, for xylanase production which was 50 °C, pH 7.2.
2. Xylanases of 14 IU/ml could be obtained from shaked flask. Furthermore, *Streptomyces* sp. Ab106.3 could grow very easily in low cost and simplified substrate such as cane bagasse.
3. For enzyme characterization point of view, it was likely alkaline tolerant and thermotolerant, as summarized in Table 3.5

Table 3.5 Summary of Some Properties of Xylanases Obtained form *Streptomyces* sp. Ab106.3

Xylanase Activity (IU)	Opt. Temp. (°C)	Opt. pH	Half life: t_d(h)											
			55 °C				65 °C				75 °C			
			pH6	pH7	pH8	pH9	pH6	pH7	pH8	pH9	pH6	pH7	pH8	pH9
14	60-65	6.0	> 120 h	> 120 h	> 120 h	30 h	5 h	3 h	3 h	3 h	15 min	18 min	10 min	10 min

4. Enzyme kinetic constants of xylanases from *Streptomyces* sp. Ab106.3 showed high values of K_M indicated that this enzyme has high affinity to convert xylan to xylose, as summarized in Table 3.6

Table 3.6 Summary of Kinetic Constants of Xylanases Obtained form *Streptomyces* sp. Ab106.3

Type of inhibition	V_{max} (μ mol /mg protein- min)	K_M (mg/ml)	Ki	K'i
Non-inhibition	13.91	18.66	-	-
Mixed-inhibition (0.1-0.2M Hydrogen Peroxide)	13.05	18.74	0.0704	0.038

5. The bioblaching of pulp revealed that the XH treatment could be suitable for kraft pulp bleaching step, because reducing sugar, lignin release and also brightness of pulp were higher than those of the HX treatment.

3.9 Suggestions

The scale up process by using fermentor should be investigated. In addition, agitation and aeration rates should be considered.