CHAPTER 3

RESULTS AND DISCUSSION

3.1 Screening of carbon sources for xylanase production

In this experiment, 4 carbon sources at the concentration of 1 % (w/v) induced xylanase and CMCase productions. Xylanase activity was higher than that of CMCase activity about 30, 20, 8 and 1 folds in baggase, corncob, Avicel and CMC medium, respectively. Xylanase and CMCase productions in various carbon sources were shown in figure 3.1 and 3.2. Baggase seemed to be the best carbon source for xylanase production in this experiment. It was found to be suitable for the production of xylanases by using *Aspergillus awamori* (Sidenberg *et al.*,1997), *Penicillium purpurogenum* and alkaliphilic thermophilic *Bacillus* sp. NCIM 59 (Kulkarni *et al.*, 1999), *Streptomyces albogriseolus, Streptomyces nitrosporous, Micromonospora melanospora* (Van Zyl, 1985), and *Streptomyces* HM-15 (Patel and Ray, 1994).

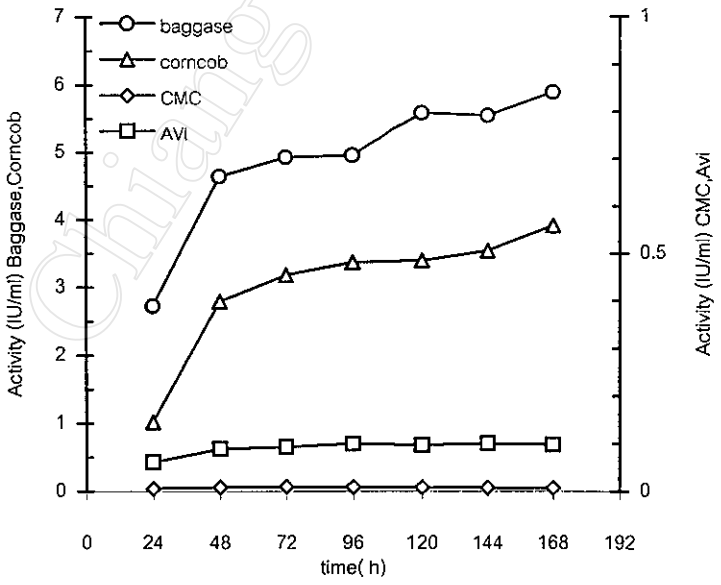

Figure 3.1 Xylanase production in various carbon sources

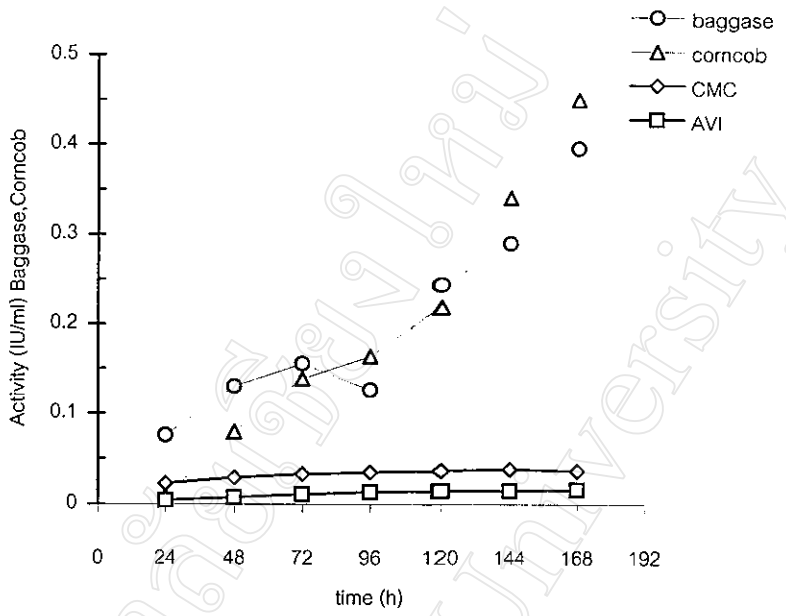

Figure 3.2 CMCase production in various carbon sources

From figure 3.2 CMCase production in baggase and corncob media were higher than those of Avicel and CMC media. CMCase production in corncob medium was slightly higher than that in baggase medium, but the pattern of production was quite the same. CMCase obtained from CMC medium was slightly higher than that in Avicel medium. From the results, cellulosic substrates such as Avicel and CMC could induce xylanase production by *Streptomyces* Ab106.3. It was reported else where that they also induced xylanase production by *Clostridium sterecoraium, Thermonospora curvata* and *Neurospora crassa* (Kulkarni *et. al.*, 1999). Cellulose was the inducer of xylanase in few cases, but it was not clear whether the inducing effect caused by cellulose or the contaminating xylan fraction (Kulkarni *et al.*, 1995). The characteristics of enzyme induction and production of *Streptomyces* Ab106.3 are in agreement with the general concept that fungal and bacterial xylanases are generally associated with cellulases. The synergistic productions of xylanases and cellulases by *Streptomyces* Ab106.3 were observed when cellulose and hemicellulose were used in combination as the carbon source.

To confirm the result, xylanase and CMCase productions in baggase and corncob media were again carried out for three replicates in shaked flask culture. respectively. Xylanase production in baggase medium was approximately of 2.3 times higher than that of corncob medium. A maximum level of xylanase activity of 8.0 (U/ml) was obtained after 144 hr and 3.5 (U/ml) after 96 h of cultivation in baggase and corncob media, respectively.

It is reported that xylanase production by actinomycetes was growth associated product formation. Generally, actinomycetes produce the highest level of xylanase activity within 72-96 h of cultivation in submerge culture (Tuncer *et al.,* 1999). The optimum cultivation time of *Clostridium absonum* CFR-702 was also 72-96 h (Kleupfel *et al.,* 1986, Rani and Nand, 2000). After achieving highest level, enzyme amount was constant for short period, for 24 h or a little bit longer, depended on species of microorganisms, and then decreased concomitantly with fermentation time.

Supplementation of Tween 80 into culture medium was reported to enhance xylanase production (Kuhad *et al.,* 1998, Liu *et al.,* 1998), suggested that Tween 80 might increase oxygen transfer to the culture medium. The amount of xylanase obtained from culture medium without Tween 80 supplementation reduced about 1.6 folds.

3.2 Xylanase production in 5 L fermentor

The productions of xylanase and CMCase were carried out in 5-L fermentor, with working volume of 3.5 l. The fermentation parameters agitation rate, aeration rate, pH, and temperature were 200 rpm, 1 vvm, 7.0, and 55 ^{o}C. The result was shown in figure 3.3. Under the defined fermentation parameters, the highest xylanase activity of 15.9 (U/ml) was obtained after 120 h of fermentation. The amount of both enzymes rapidly increased from 24 h to 48 h. After 48 h of fermentation, the amount of xylanase increased gradually to the highest activity at 144 h. Then, it gradually decreased along with fermentation time.

It was reported elsewhere that xylanase production in the fermentor where fermentation parameters: aeration, agitation, and temperature, were controlled could result in an increase rate of xylanase production (Pham *et al.,* 1998, Palma *et al.,* 1996, Singh *et al.,* 2000b). Increase of oxygen supply resulted in increase of

xylanase production, but excess oxygen supply sometimes reduced xylanase production suggested that oxidation of xylanase was occurred in this condition (Palma *et al.,* 1996).

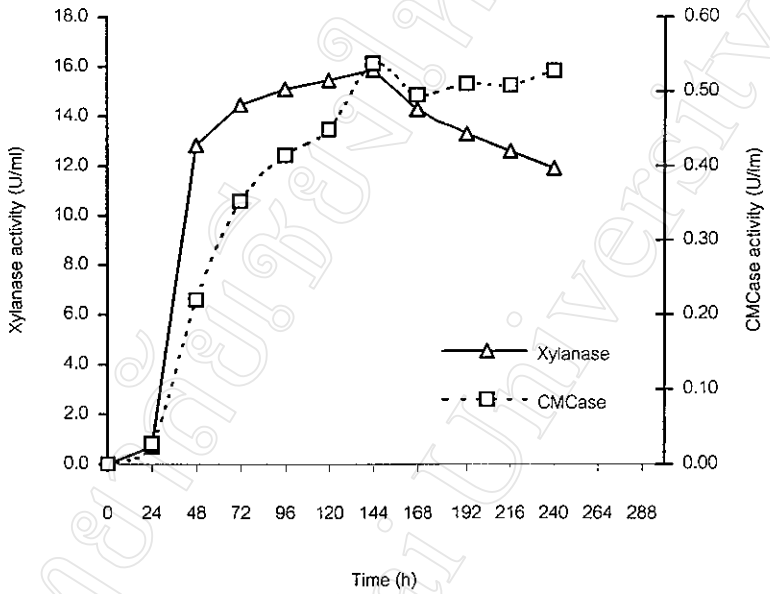

Figure 3.3 Xylanase and CMCase production in 5-L Fermentor with 1% (w/v) Baggase, 55 ^{o}C

CMCase followed the same xylanase production pattern, but it flatted out after 144 h of fermentation. It was reported elsewhere that the xylanase production of *Streptomyces* sp. S38 reached 31±2 (U/ml) after 100 h of fermentation and no cellulase activity was detected (Georis *et al.* 2000). The optimum production for xylanase production of *Streptomyces* sp.1 was 120 h. (Kansoh *et al.,* 2001). The amount of xylanases produced by *Streptomyces lividans* 1326 reached the highest activity of 50 (U/ml) in 72 h. of fermentation, but the amount of cellulase reached the highest activity of 1 (U/ml) in 48 h of fermentation, and then gradually decreased along with fermentation time (Kluepfel *et al.,* 1996). Cellulase produced by *Streptomyces albogriseolus* reached the highest activity of about 0.43 (U/ml) at 96 h of fermentation, and flatted out to 168 h (Van Zyl, 1985).

It was revealed that xylanase production in the fermentor was more effective than that of shaked flask culture, xylanase of 15.9 (U/ml) could be obtained within

48-72 h of fermentation. This may be the increase of oxygen transfer by aeration and agitation, resulting in the increase of mixing rate, oxygen transfer rate, contact surface between microorganisms and hemicellulose substrate. Increasing of agitation and aeration rates resulted in higher xylanase and CMCase productions. However, xylanase production by fungi decreased when agitation rate increased,, suggested that shear force generated by agitation caused hyphae disruption (Siedenberg *et al.*, 1997)

3.3 Partial purification and some properties of xylanases obtained from *Streptomyces* Ab106.3

Purification techniques used in this study were ammonium sulfate precipitation of protein, Ion-exchange chromatography, and Gel-filtration chromatography, respectively. Some properties of enzyme such as, optimum temperature and pH for enzyme activities, temperature and pH stability profiles of crude xylanases and partial purified xylanases were also determined.

3.3.1 Ammonium sulfate precipitation

Ammoniumsulfate of 20-90% (w/v) saturation was used to study protein precipitation from culture supernatant. After centrifugation at 10,000 X g, most of protein precipitant did not sediment, but it floated on the surface of solution. After determination of culture medium composition, it was found that Tween 80 caused the float of protein precipitant. Complete precipitation of protein was archived in the medium without Tween 80 supplementation. Figure 3.4 showed that at 40% saturation of ammonium sulfate there was no xylanase activity in the supernatant. At 60% saturation of ammonium sulfate, most of xylanase activity was found in the protein precipitant. To make sure to obtained all enzyme in the protein precipitant, 80% saturation of ammonium sulfate was used to precipitate xylanases from culture supernatant.

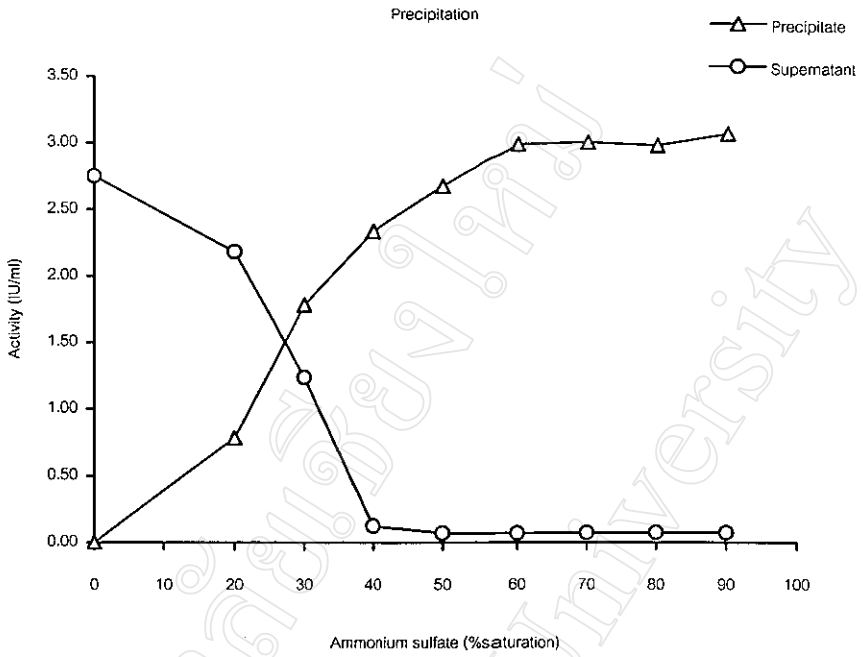

Figure 3.4 Ammonium sulfate precipitation of crude enzyme

3.3.2 DEAE-cellulose column chromatography

It was observed that at pH 8.0 most of xylanases in culture supernatant were bound to DEAE-cellulose. The result of DEAE-cellulose column chromatography was shown in figure 3.5. There were two major active peaks. Xylanases produced by *Streptomyces* Ab1O6.3 might possible be divided into 2 groups. The first group did not bind to DEAE-cellulose at pH 8.0, but another did. Usually, actinomycetes produced more than two forms of xylanases, which were different in sizes and characteristics. *Streptomyces* S38 produced 3 xylanases (Georis *et al.,* 2000). *S. halstedii* JM8 produced 2 xylanases, Xyl1S and Xyl1L, which Xyl1S was a truncated form of Xyl1L (Ruiz-Arribas *et al.,* 1997). *Streptomyces* B-12-2 produced Xyl1a and Xylb, which were different in sizes (Elegir *et al.,* 1994). *S. flavogriseus* also produced 2 xylanases (MacKenzie *et al.,* 1987).

The unbound and bound active fractions were pooled and named Fraction A and Fraction B, respectively.

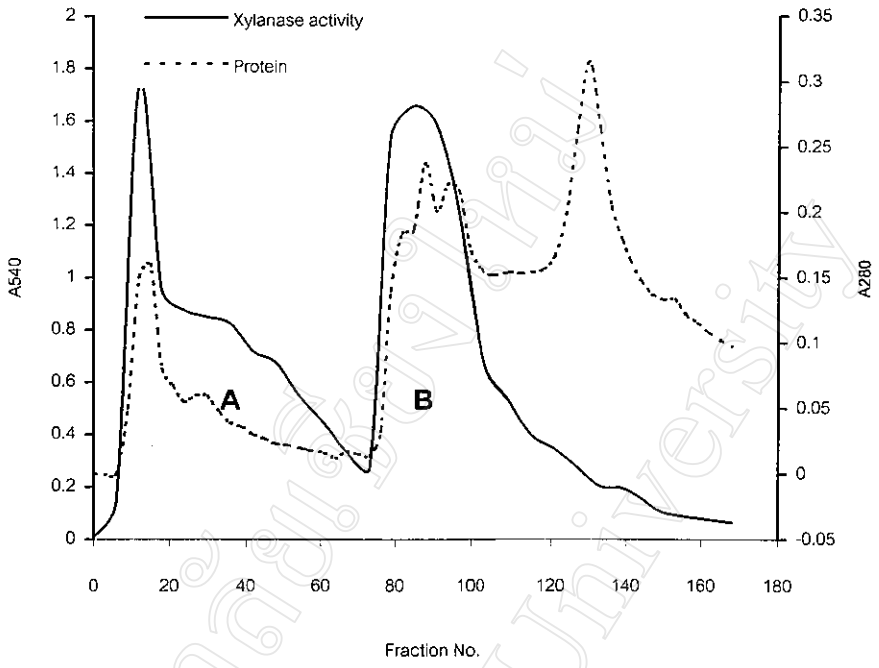

Figure 3.5 Chromatogram of DEAE-cellulose column chromatography

3.3.3 Gel-filtration column chromatography

Fraction A and B obtained from DEAE-cellulose column chromatography were consequently concentrated, by lyophilization. The chromatogram of Fraction A and Fraction B were shown in figure 3.6 and 3.7, respectively. Both fraction A and Fraction B had 3 active xylanase peaks each called Fraction A1, A2, A3, B1, B2, and B3, respectively. So, after gel-filtration chromatography, xylanases obtained from *Streptomyces* AB 106 might have 5-6 forms of xylanase. It was reported else where that five different xylanases were purified from *Aspergillus niger* 11, at least 3 forms of xylanases were purified from *Clostridium stercorarium, Streptomyces* sp. Strain 3137, *Streptomyces exfoliates* MC_1, *Trichoderma harzianum* E85, *Trichoderma reesei* QM9414, *Aeromonas* sp. Strain 212, *Penicillium janthinellum,* and *Talaromyces byssochlamydoides* YH-50 (Ruiz-Arribas *et al.,*1997, Wong *et al.,* 1988).

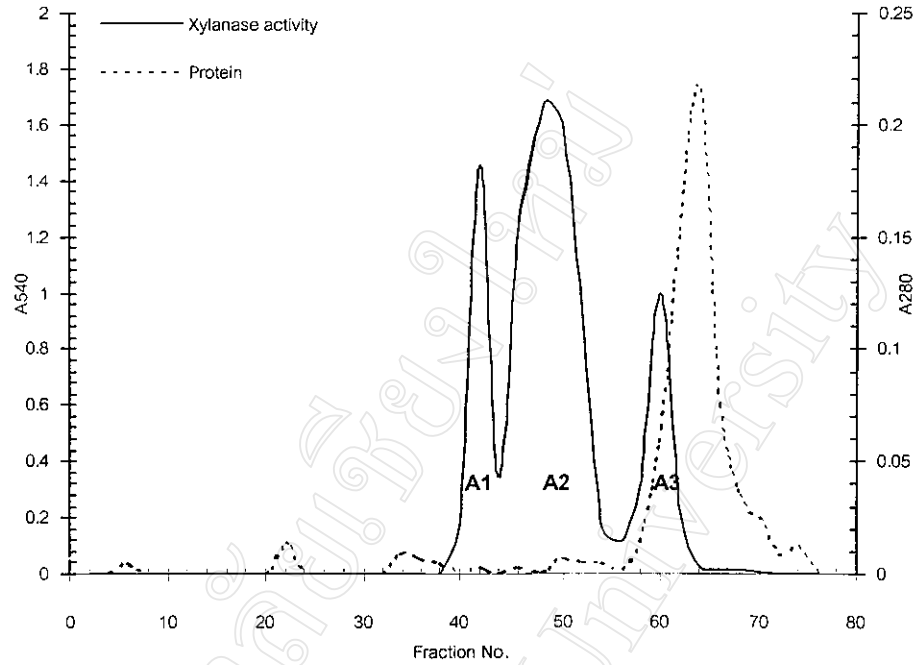

Figure 3.6 Chromatogram of Sephadex G-100 column chromatography of Fraction A.

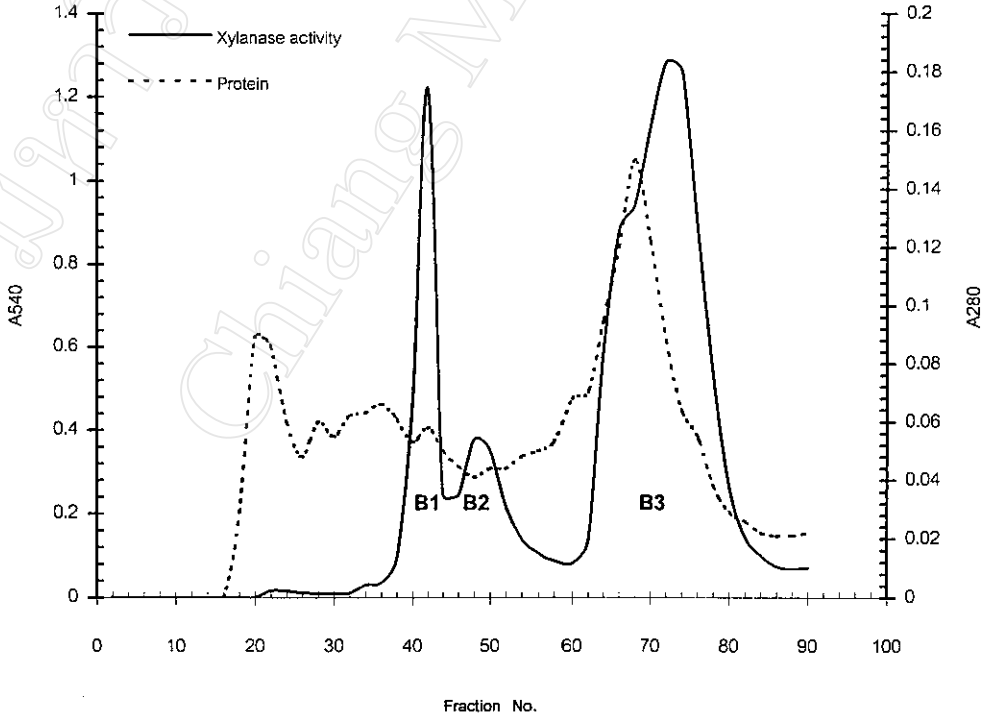

Figure 3.7 Chromatogram of Sephadex G-100 column chromatography of Fraction B

Multiplicity of xylanases were depended on the regulation, the substrate cross-specificity, and the post-translational modifications of xylanases. The substrate cross-specificity was reported in numerous lignocellulolytic enzymes, some of them had very wide specificity, and some had high secondary activities (Wong *et al.*,1988). Many xylanases are apparently glycosylated, such as *Bacillus* xylanases. Some are apparently translated as precursors with peptide signal sequences. It is possible that some of the xylanases detected in culture medium are precursors of/or truncated form of other xylanases (Wong *et al.,* 1998, Ruiz-Arribas *et al.,* 1997). The other possible of multiplicity of xylanase is that they are products from different genes.

Low MW xylanase with basic pI and High MW xylanase with acidic pI are the most commonly produced by microorganisms such as *Clostridium* and *Streptomyces*. The high MW xylanases in *Streptomyces* spp. have relatively neutral pI (5.2 to 7.3).

3.4 Some properties of xylanases

3.4.1 Optimum temperature for xylanase

The optimum temperature profiles of *Streptomyces* Ab106.3 xylanases were shown in Figure 3.8, 3.9, and 3.10. They were also summarized in table 3.1 and 3.2

The crude xylanase showed the highest activity at 65 °C. It retained 85% of the enzyme activity at 75 °C. Partial purified fraction A xylanase had broad optimum temperature of 55°C -65 °C. It retained 91% of the enzyme activity at 70 °C, but the enzyme activity rapidly declined to 60% at 75 °C and lost most of enzyme activity at 80 °C. Partial purified fraction B xylanase showed the optimum temperature at 70 °C, but the enzyme activity declined rapidly to 47% at 75 °C and lost all the activity at 80 °C.

Fraction A and Fraction B had slightly different optimum temperature profiles compared with the crude enzyme. The optimum temperature of Fraction B was 5 °C higher than that of fraction A. Fraction A1 had the optimum temperature in the range of 60°C -70 °C compared to fraction A2 and A3, which had optimum

temperature at 60 °C. All of them rapidly lost their activities at 75 °C and did not have the activity at 80 °C.

Fraction B1, and B3 had the optimum temperature profiles similar to fraction B, but fraction B2 was different. It had optimum temperature of 65 °C. Nonetheless, all of them lost their activities at 75 °C. However, fraction B1 and B2 retained only 30% of their activities at 80 °C, which were different from fraction B and B3. From table 3.1 xylanase obtained from *Streptomyces* Ab106.3 could work at high temperature up to 70 °C.

Generally, endoxylanases produced by genus *Streptomyces,* are optimally active at temperature ranges between 45°C and 75 °C as shown in table 3.2 (Sunna and Antranikian, 1997). Xylanases produced by *Streptomyces* Ab106.3 had quite high optimum temperature of 55°C -70 °C, which were compatible to *Streptomyces cyaneus* MT 813 and *Streptomyces thermoviolaceus* OPC-520.

Table 3.1 The optimum temperature for xylanase activities from *Streptomyces* Ab106.3

Xylanase	Optimum temperature (°C)
Crude	65
Fraction A	55-65
Fraction A1	70
Fraction A2	60
Fraction A3	60
Fraction B	70
Fraction B1	65-70
Fraction B2	65
Fraction B3	70

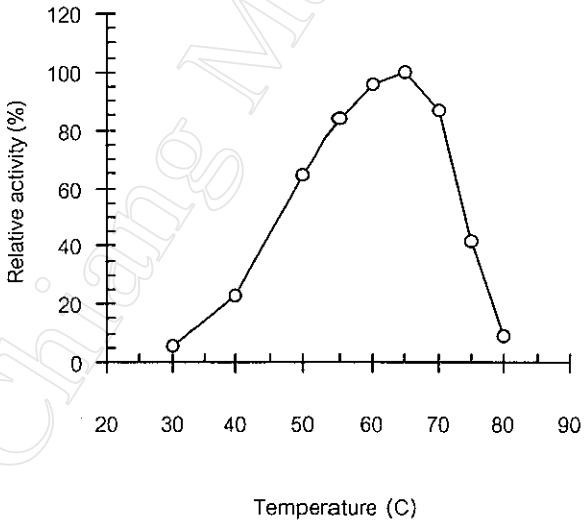

Figure 3.8 The optimum temperature for crude xylanases.

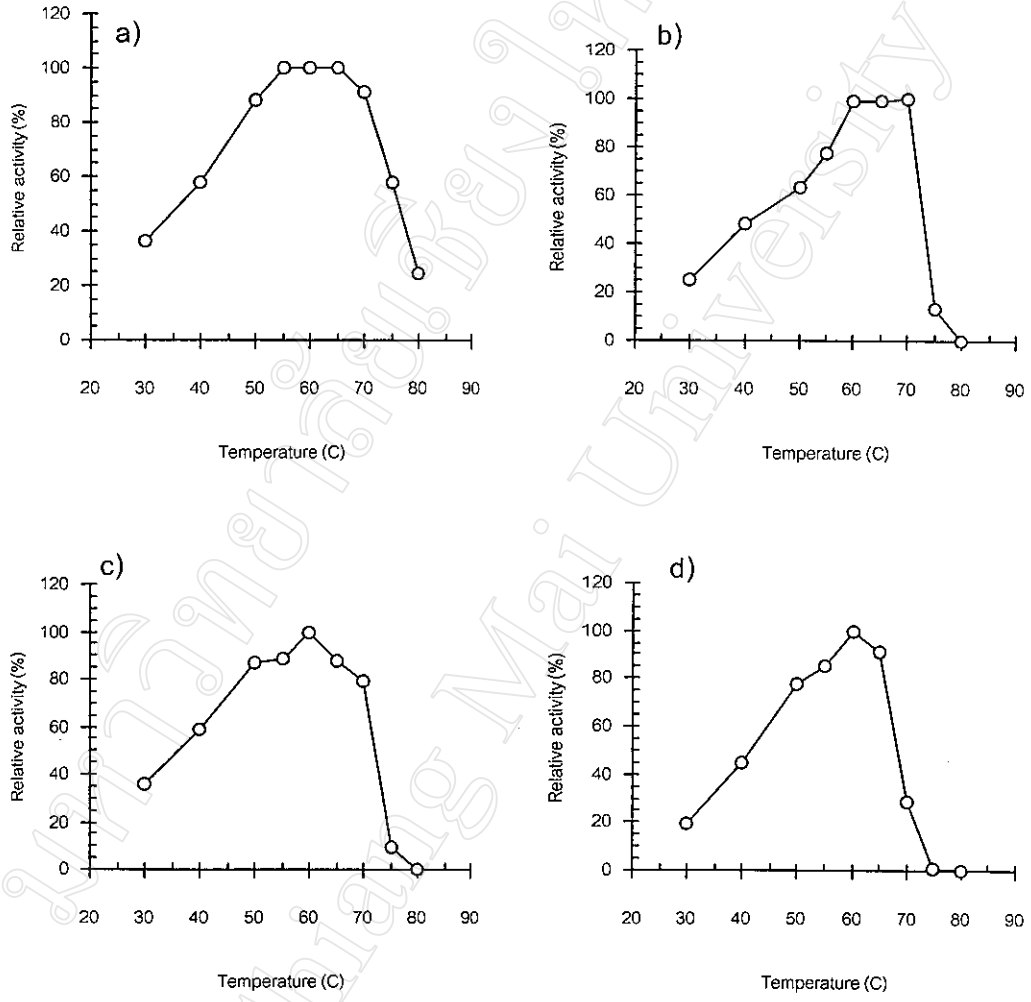

Figure 3.9 The optimum temperature for partial purified xylanases:
a) Fraction A, b) Fraction A1, c) Fraction A2, and d) Fraction A3

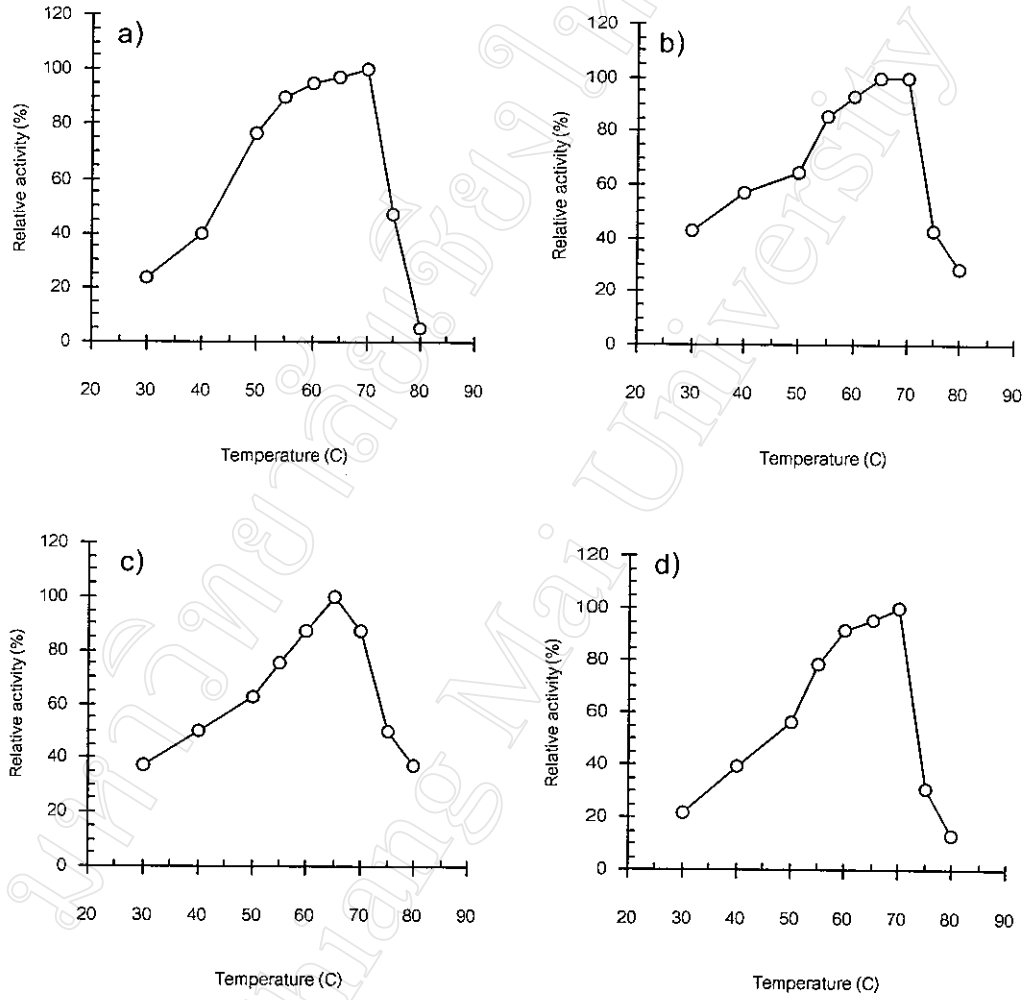

Figure 3.10 The optimum temperature for partial purified xylanases:
a) Fraction B, b) Fraction B1, c) Fraction B2, and d) Fraction B3

Table 3.2 The optimum temperature of some xylanases obtained from *Streptomyces*

Organisms	Xylanase	Optimum temperature (°C)
Streptomyces sp. T_7		60
	X-I	60-65
Streptomyces sp. 3137	X-II-A	60-65
	X-II-B	60-65
	X_{IA}	60
Streptomyces sp. EC10	X_{IB}	60
	X_{II}	60
	I	50
Streptomyces sp. A451	II	50
	1a	55
	1b	60
Streptomyces sp B-12-2	2	60
	3	60
	4	60
Streptomyces cyaneus	I	72
MT 813	II	65
	II	55
Streptomyces exfoliates MC_1	Ib	50
	Id	55
Streptomyces thermoviolaceus	I	70
OPC-520	II	60
Streptomyces sp. E-86		55-65
	Ib	50
Streptomyces exfoliates MC_1	Id	55
	II	55
Streptomyces lividan		60
Streptomyces xylophagus		55-60

Source: Sunna and Antranikian, 1997.

3.4.2 Optimum pH for xylanase activity

The optimum pH profiles of xylanases from *Streptomyces* Ab106.3 were shown in Figure 3.11, 3.12, and 3.13. They were summarized in table 3.3.

The crude xylanase showed the highest activity at pH 6.0. It retained 50% of the activity at pH 7.0. Partial purified fraction A xylanase also showed the highest activity at pH 6.0. It retained 70% of activity at pH 7.0 and lost 50% of the enzyme activity at pH 8.0. Partial purified fraction B xylanase showed broad optimum pH in the range of 5.0-7.0. It retained 75% and 65% of the activity at pH 8.0 and 9.0, respectively.

Fraction A had the similar pH optimum profile to the crude xylanase. Fraction A1 had the optimum pH higher that those of fraction A2 and A3. At pH 8.0, fraction A1 was more active than those of fraction A2 and A3. However, all of them had the similar pH optimum profiles compared to fraction A.

Fraction B was different from fraction A, it active at acidic to neutral pH and retained about 60-70 at basic pH, 8.0-9.0.Fraction B1, B2 and B3 showed the similar pH optimum profiles. All of them had the optimum pH at 7.0. Fraction B3 had 86% of the activity at pH 8.0, which was higher than those of fraction B1 and B2. All of them had similar optimum pH profiles compared to fraction B.

It was interesting to know that the pH optimum of xylanases produced by *Streptomyces* Ab106.3 was near neutral, pH 5.0-7.0, which was similar to *Streptomyces* coded in table 3.3.

Fungal xylanases usually had the optimum pH in acidic range. Bacterial xylanases had the optimum pH in near neutral, but some alkali tolerant strain of genus *Bacillus* produced xylanases that could work at basic pH ranges from pH 8-10. An alkalophilic *Bacillus* sp. Sam-3, had the optimum pH at 8.0 (Shah *et al.,* 1999*), Bacillus* sp. AR-009 had the optimum pH at 9-10 (Gessesse, 1998). Generally, the optimum pH of xylanases produced by genus *Streptomyces* was near neutral as shown in table 3.4. However, some of them produced xylanases, which were active at basic pH, for example *Streptomyces cyaneus* MT 813 and *Streptomyces* EC10. Xylanases produced by *Thermomyces lanuginosus* exhibited the highest activity at pH 7.0 (Cesar and Mrsa, 1996).

Table 3.3 The optimum pH for xylanase activities from *Streptomyces* Ab106.3

Xylanase	Optimum pH
Crude	6.0
Fraction A	6.0
Fraction A1	7.0
Fraction A2	6.0
Fraction A3	6.0
Fraction B	5.0-7.0
Fraction B1	7.0
Fraction B2	7.0
Fraction B3	7.0

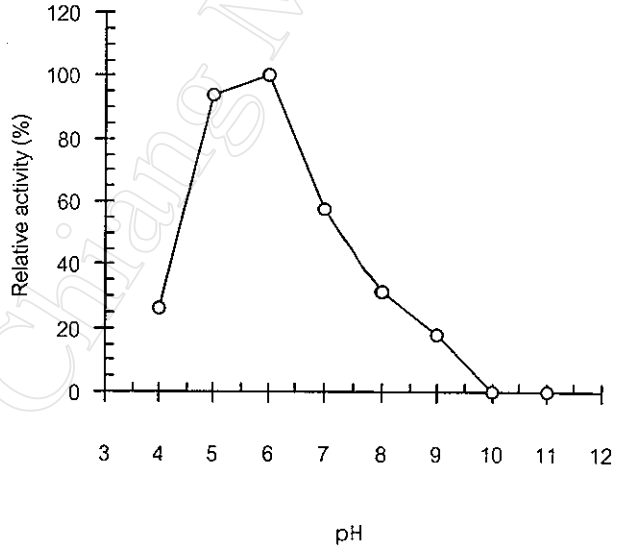

Figure 3.11 The optimum pH for crude xylanase

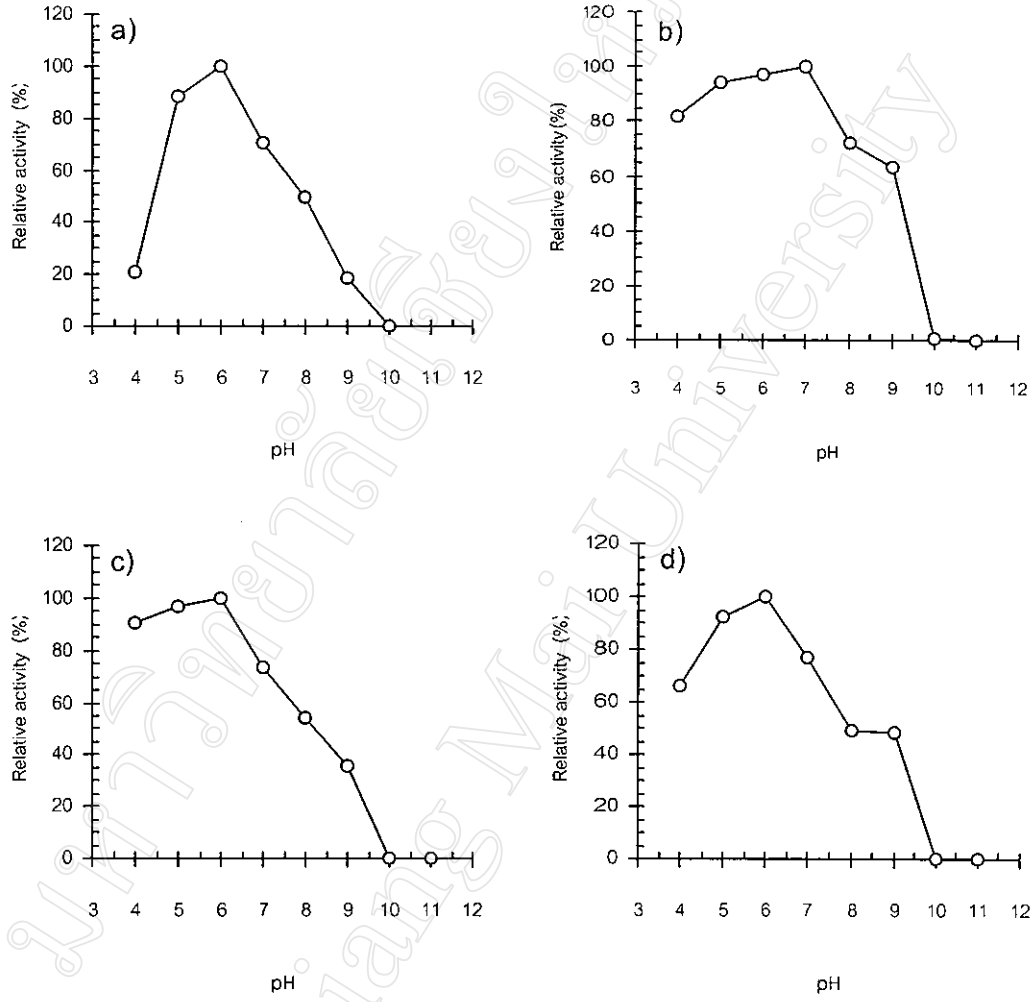

Figure 3.12 The optimum pH for partial purified xylanases:
a) Fraction A, b) Fraction A1, c) Fraction A2, and d) Fraction A3

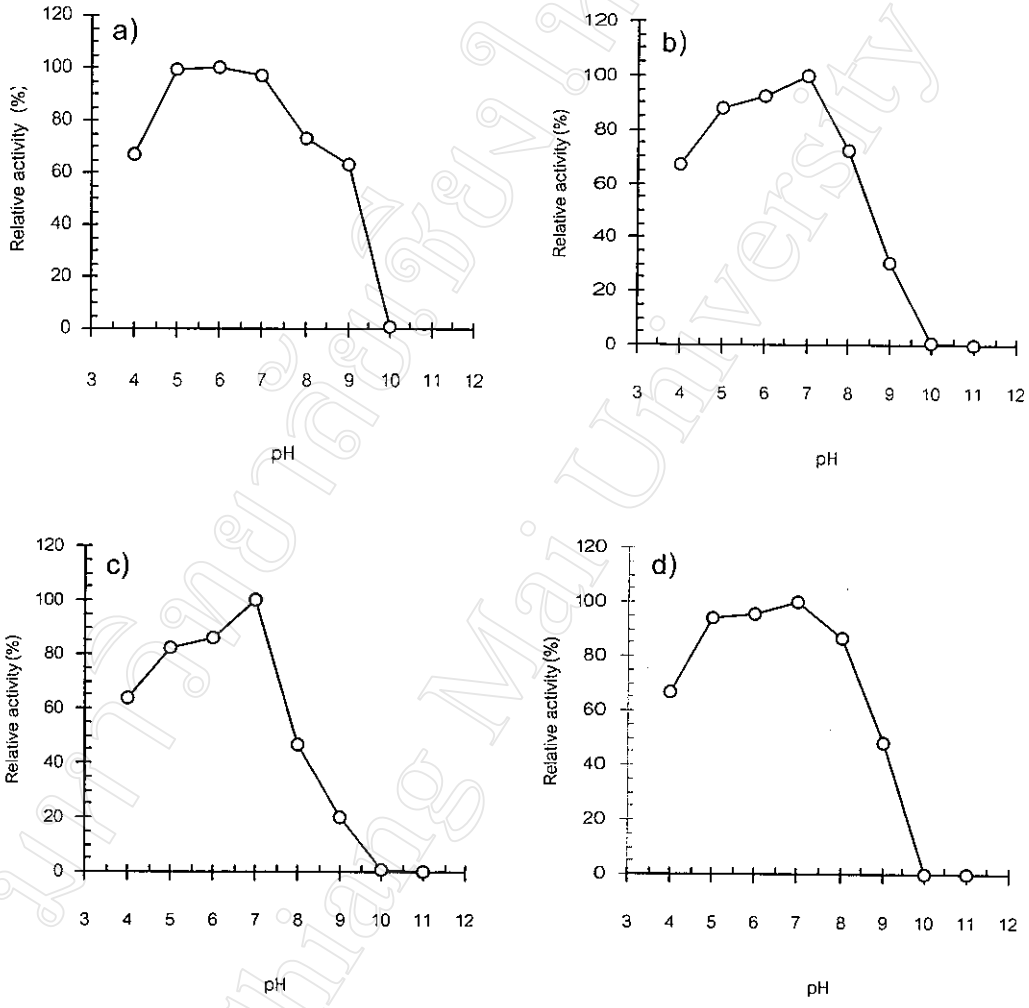

Figure 3.13 The optimum pH for partial purified xylanases:
a) Fraction B, b) Fraction B1, c) Fraction B2, and d) Fraction B3

Table 3.4 The optimum pH of some xylanases from *Streptomyces*

Organisms	Xylanase	Optimum pH
Streptomyces cyaneus MT 813	I	8.0
	II	6.5
Streptomyces exfoliatus	Ib	5.5
	Id	7.0
Streptomyces exfoliates MC_1	II	5.5
	II	5.5
	Ib	5.5
	Id	7.0
Streptomyces flavogriseus	X_1	5-7.5
	X_2	5.5
Streptomyces lividans		6.0
Streptomyces sp B-12-2	1a	6.0
	1b	7.0
	2	7.0
	3	7.0
	4	6.0
Streptomyces sp. 3137	X-I	5.5-6.5
	X-II-A	5.0-6.0
	X-II-B	5.0-6.0
Streptomyces sp. A451	I	5.6
	II	5.4
Streptomyces sp. E-86	X-II-B	5.0-6.0
	X-II-A	5.5-6.5
	X-I	5.5-6.5
Streptomyces sp. EC10	X_{IA}	7.0-8.0
	X_{IB}	7.0-8.0
	X_{II}	7.0-8.0
Streptomyces sp. KT-23		5.5
Streptomyces sp. T_7		4.5-5.5
Streptomyces thermoviolaceus OPC-520	I	7.0
	II	7.0
Streptomyces xylophagus		6.2

Source: Sunna and Antranikian, 1997 and Wong *et al.*, 1988

3.4.3 Thermal stability of xylanases

The temperature stability profiles of xylanases from *Streptomyces* Ab106.3 were shown in Figure 3.14, 3.15, and 3.16 and also summarized in table 3.5. Crude xylanases retained 90% of the activity at 72 h at 50 °C. The half live was 17 h, at 70°C and 80 °C. Partial purified fraction A xylanase retained 80% of the activity at 72 h, 50 °C. It's half live was 72 h at 60 °C. Partial purified fraction B xylanase retained 90% of the activity at 72 h, 50 °C. It's half live was 1 h at 70°C. So, fraction A xylanase was comparatively more stable than that of fraction B xylanase.

Fraction A1, A2, and A3 showed the different temperature stability profiles. Fraction A1 and A2 were quite stable at 50°C and 60 °C. However, fraction A2 was more stable that that of fraction A1 at 70 °C. Fraction A2 had half live of 6.5 h at 70 °C, but fraction A1 had only 30 min. Fraction A3 was less thermo stable than those of fraction A1 and A2. It lost the activity at 70 °C and 80 °C. Moreover, it retained only 60% of the activity at 50 °C, and had half live of 8 h at 60 °C. fraction A3 seemed to be less thermo stable among other fractions.

Fraction B1, B2, and B3 showed the similar temperature stability profiles at 50°C and 60°C. They retained 90% and 80% of the activity, respectively. At 70°C, fraction B1, B2, and B3 had half lives of 48 h, 18 min, and 13 min, respectively. At 80 °C, fraction B2 lost the activity, fraction B1 and B3 had half lives of 30 min and 6 min, at 50°C respectively.

From table 3.5 xylanases from *Streptomyces* Ab106.3 had the half life of 60 °C for approximately 72 h. Xylanases from *Streptomyces thermoviolaceus* were also stable at 65 °C (Grag *et al.*, 1998).

Table 3.5 Half-life of xylanases from *Streptomyces* Ab106.3 at various temperatures.

Enzyme	Half life (T ½)			
	50°C	60°C	70°C	80°C
Crude enzyme	>72 h	>72 h	17 h	18 min
Fraction A	>72 h	~72 h	19 h	30 min
Fraction A1	>72 h	>72 h	30 min	18 min
Fraction A2	>72 h	>72 h	6.5 h	12 min
Fraction A3	>72 h	8 h	-	-
Fraction B	>72 h	>72 h	1 h	42 min
Fraction B1	>72 h	>72 h	48 min	30 min
Fraction B2	70 h	60 h	18 min	-
Fraction B3	>72 h	>72 h	13 min	6min

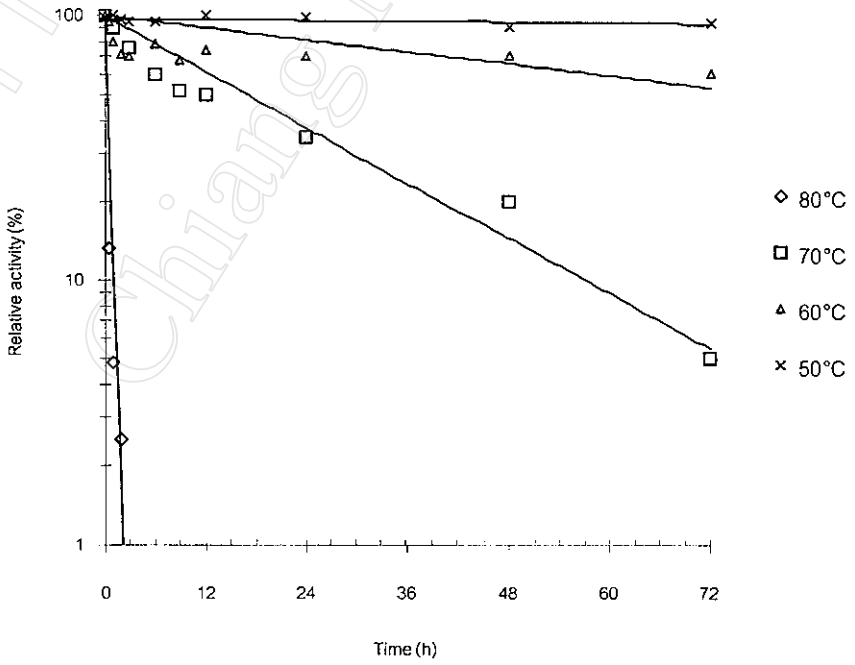

Figure 3.14 Temperature stability profile of crude xylanase

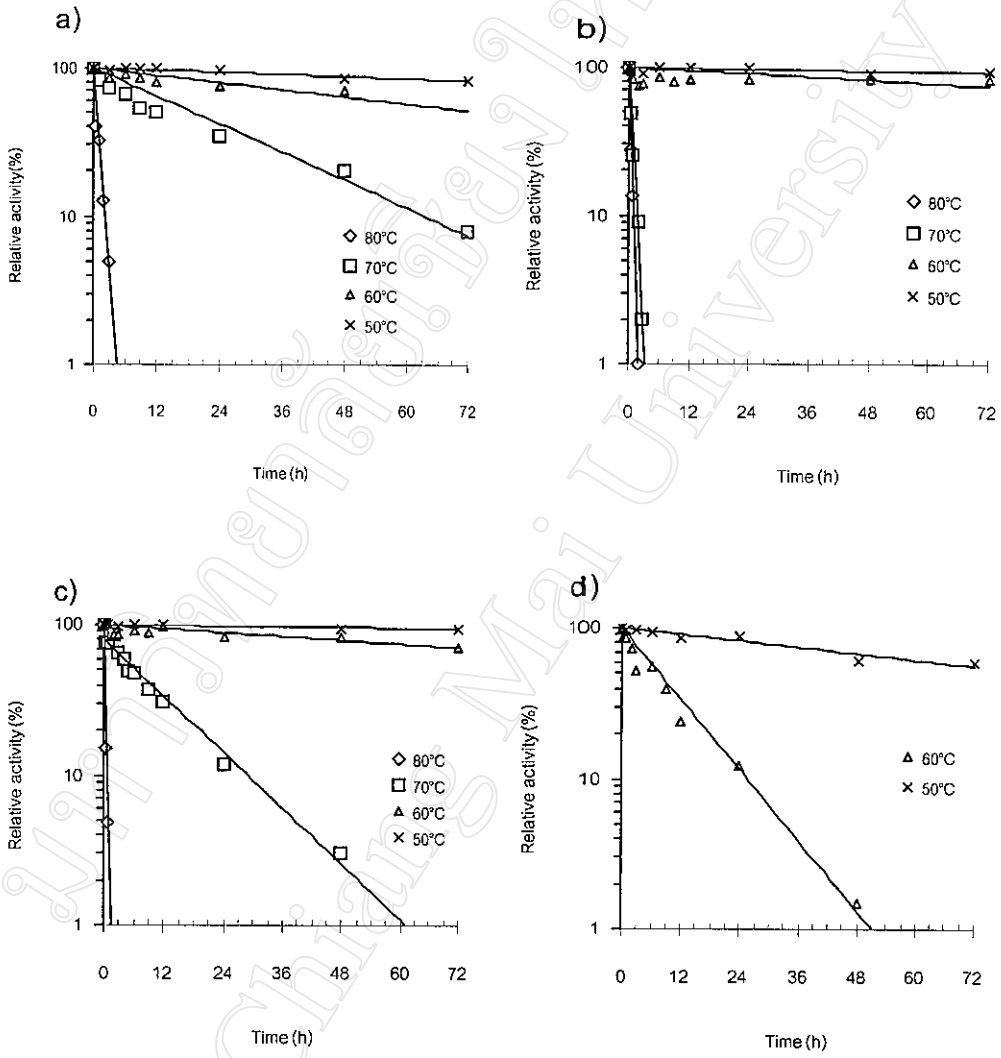

Figure 3.15 Temperature stability profiles of partial purified xylanase
a) Fraction A, b) Fraction A1, c) Fraction A2, and d) Fraction A3

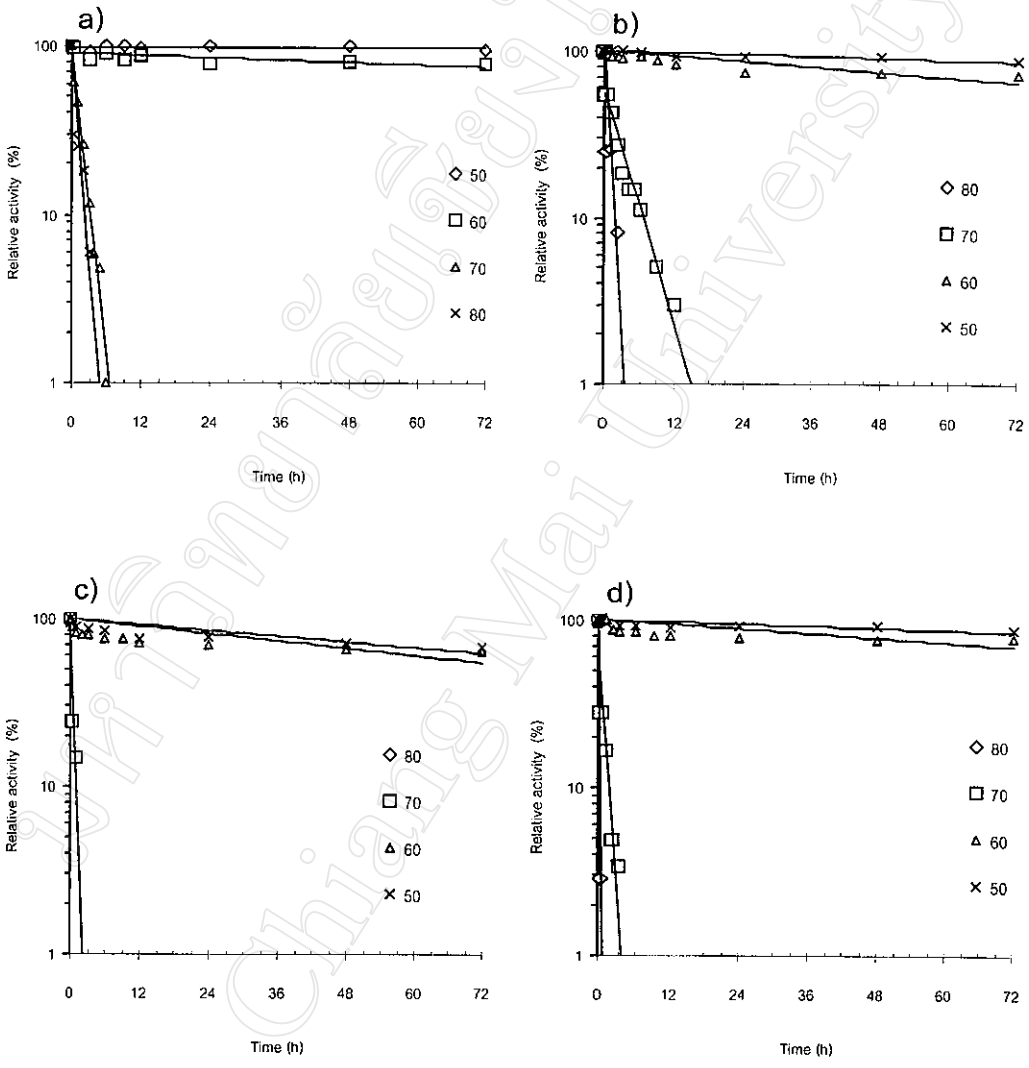

Figure 3.16 Temperature stability profiles of partial purified xylanase:
a) Fraction B, b) Fraction B1, c) Fraction B2, and d) Fraction B3

Xylanases obtained from *Streptomyces* Ab106.3 showed the similar temperature stability profile to *Thermomyces lanuginosus* as described elsewhere (Singh *et al.*, 2000b).

3.4.4 pH stability of xylanase

The pH stability profiles of xylanases obtained from *Streptomyces* Ab106.3, after 24 h of incubation at 4 ^{o}C, were shown in Figure 3.17, 3.18, and 3.19. They were also summarized in table 3.6.

From figure 3.17, after 24 h of incubation at 4 ^{o}C, the crude enzyme retained 98-100% of the enzyme activity at pH range of 5.0-6.0. It retained 65% and 60 % of the enzyme activity at pH 4.0 and 7.0, respectively. It seemed to be stable at near neutral pH. It was not stable at basic pH range, 8.0 to 11.0.

Partial purified fraction A xylanase retained 98 to 100% of the activity at pH range of 5.0-6.0. Fraction A was not stable at pH 4.0. It retained only 40% of the enzyme activity. In addition, at pH 7.0 and 8.0, it retained 80% and 70% of the activity, respectively.

Partial purified fraction B xylanase seemed to has broad range of pH stability. It retained 93-100% of activity at the range of acid to neutral pH, 4.0-7.0. In addition, it retained 85% and 73% of the enzyme activity at pH 8.0 and 9.0, respectively.

From pH stability profiles, fraction A had closely pH stability profiles to the crude enzyme than that of fraction B. Compared with fraction A, Fraction A1 had the different pH stability profile. It had broader pH stability, 5.0-7.0. It retained 80% and 65% of the enzyme activity at pH 8.0 and pH 9.0, respectively. The pH stability profile of fraction A2 was similar to fraction A. Fraction A3 had the different pH stability profile compared with fraction A. It retained 100% and 84% of the enzyme activity at the pH range of 6.0-7.0, and 8.0, respectively. From pH stability profiles, fraction A1, A2, and A3 seemed to be different to each other.

Fraction B1 and B2 had the similar pH stability profiles, but the latter one had less stability at pH 9.0. The pH stability profile of fraction B3 seemed to be the same as fraction B1, but it was more stable at pH 5.0 than that of fraction B1 was.

From pH stability profiles, fraction B1, B2 and B3 were slightly different from each other.

Xylanase produced by *Thermomyces lanuginosus* was stable for 96 h at pH between 5.0-9.0 (Cesar and Mrsa, 1996). After 24 h of incubation at 4 °C, fractions A , A1, A2, and A3 xylanases obtained from *Streptomyces* Ab106.3 were stable at pH between 5.0-8.0, which was similar to xylanase obtained from *Fusarium oxysporum* (Kuhad *et al.*, 1998).

Table 3.6 Summary of pH stability profiles of xylanases obtained from *Streptomyces* Ab106.3.

Xylanases	pH stability
Crude	5.0-6.0
Fraction A	5.0-6.0
Fraction A1	5.0-7.0
Fraction A2	5.0-6.0
Fraction A3	6.0-7.0
Fraction B	6.0-7.0
Fraction B1	7.0
Fraction B2	7.0
Fraction B3	5.0-7.0

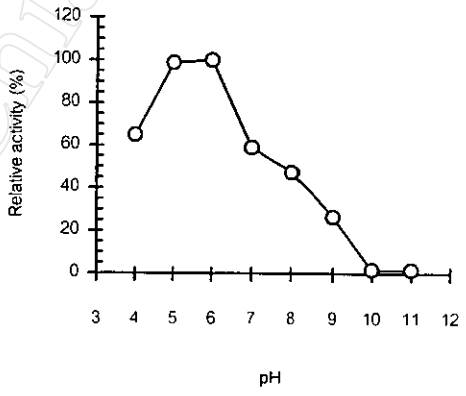

Figure 3.17 The pH stability profile of crude xylanase

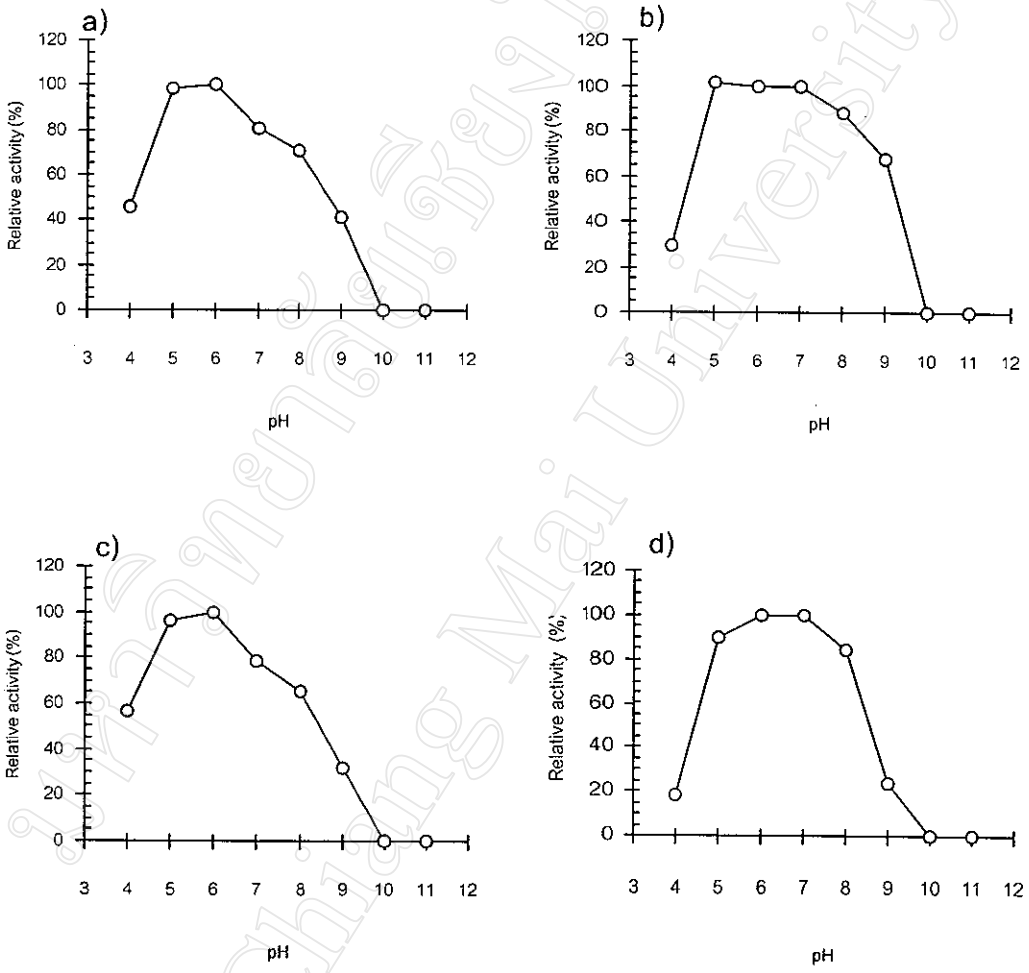

Figure 3.18 The pH stability profiles of partial purified xylanases
a) Fraction A, b) Fraction A1, c) Fraction A2, and d) Fraction A3

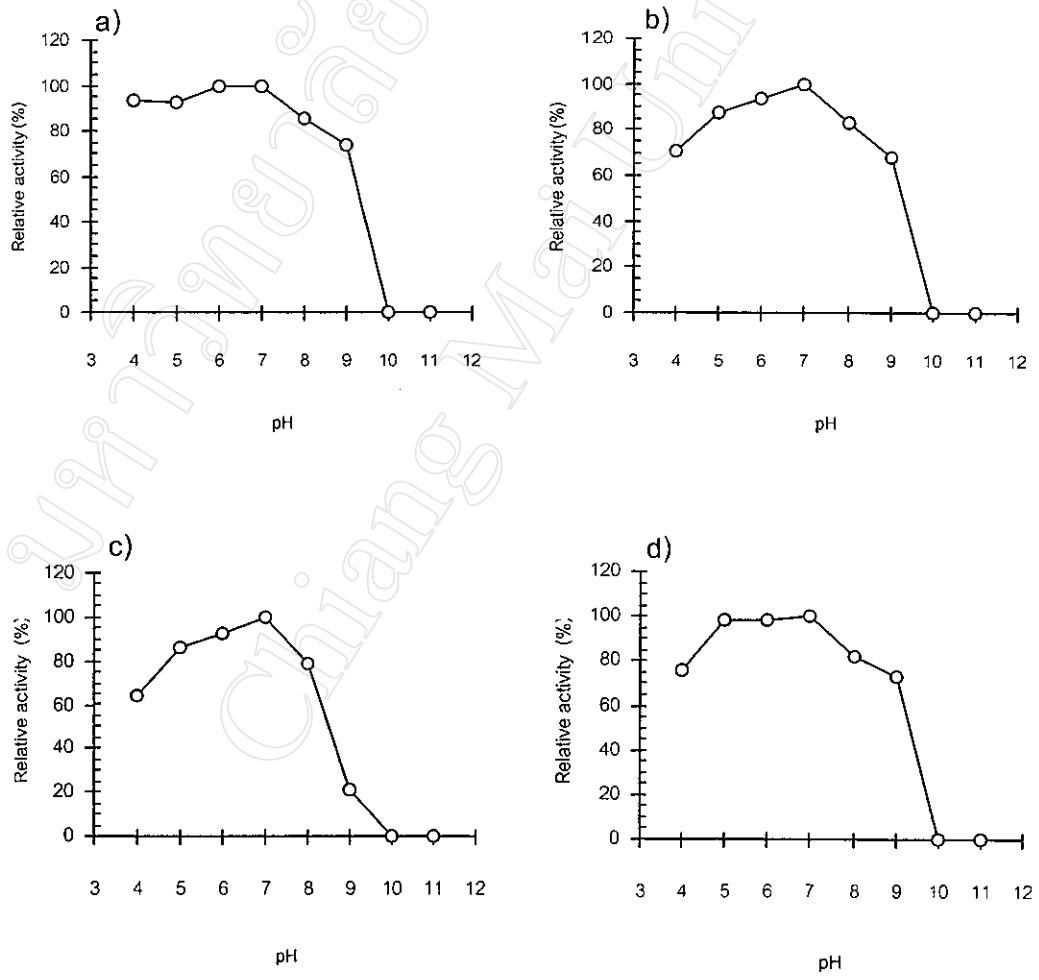

Figure 3.19 The pH stability profiles of partial purified xylanase
a) fraction B, b) fraction B1, c) fraction B2,and d) fraction B3

3.4.5 SDS-PAGE and Zymogram

The SDS-PAGE was prepared by using 12% resolving gel, 100 V, 120 min. SDS-PAGE and zymogram of the crude xylanase and partial purified xylanases obtained from *Streptomyces* Ab106.3 were shown in figure 3.20, 3.21, and 3.22. DEAE-cellulose column chromatography could separate xylanases into 2 groups. Both groups contained various proteins with different molecular weights, but fraction A might contain more proteins than those of Fraction B, indicated by more protein bands.

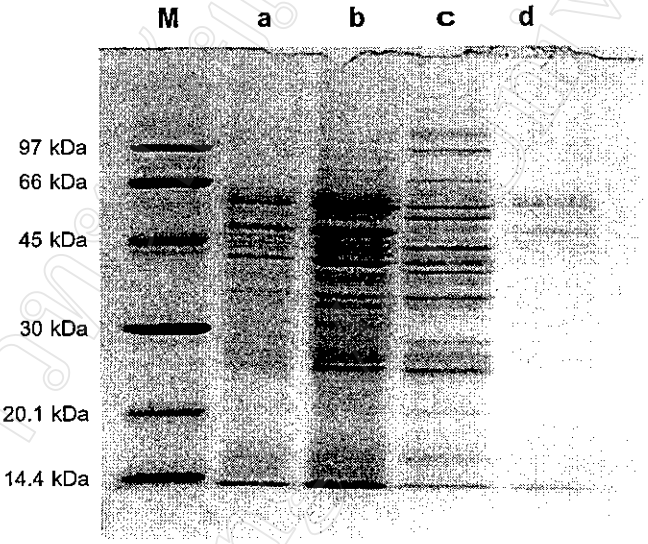

Figure 3.20 SDS-PAGE of a) crude enzyme, b) Ammonium precipitate, c) fraction A, d) fraction B, and M) molecular weight marker

The partial purified enzyme by ammonium sulfate precipitation and dialysis contained more bands of protein in lane b compared to lane a, which was the original crude enzyme. After DEAE-cellulose column chromatography, the crude enzyme was separated into 2 groups as previously described. Although, A_{280} of fraction B seemed to be higher than that of fraction A, but the SDS-PAGE showed that fraction B might contain lower protein concentration than fraction A did. After gel filtration chromatography, fraction A was separated into 3 fractions, as shown in figure 3.6. SDS-PAGE and chromatogram also showed an agreement in result. Fraction A3

contained high amount of protein as shown in figure 3.21 lane c, fraction A1 and A2 contained lower amount of protein compared to fraction A3. From the chromatogram, fraction A2 contained at least 2 xylanase activities; the intense one and the faint one with lower molecular weight. Fraction A1 might contain the same xylanase as fraction A2, because the halo of the xylanase activity was in the same position. Fraction A3 showed very faint halo of xylanase activity, suggested that fraction A1 and A3 might be derivatives of fraction A2.

Gel filtration chromatogram of fraction B showed that fraction B might have 3 derivatives. The major peek was fraction B3, which showed the most intense halo of the xylanase activity. From the zymogram, fraction B3 contained high molecular weight xylanase. Fraction B2 also showed the halo of the xylanase activity at the same position as fraction B3 was, suggested that xylanase in fraction B2 might be the same xylanase as in fraction B3, but it was a little size different. Fraction B1 might contain the mixture of xylanases from fraction A2 and fraction B3, because it showed 2 halos of the xylanase activity in the same position as xylanases of fraction A2 and fraction B3 was.

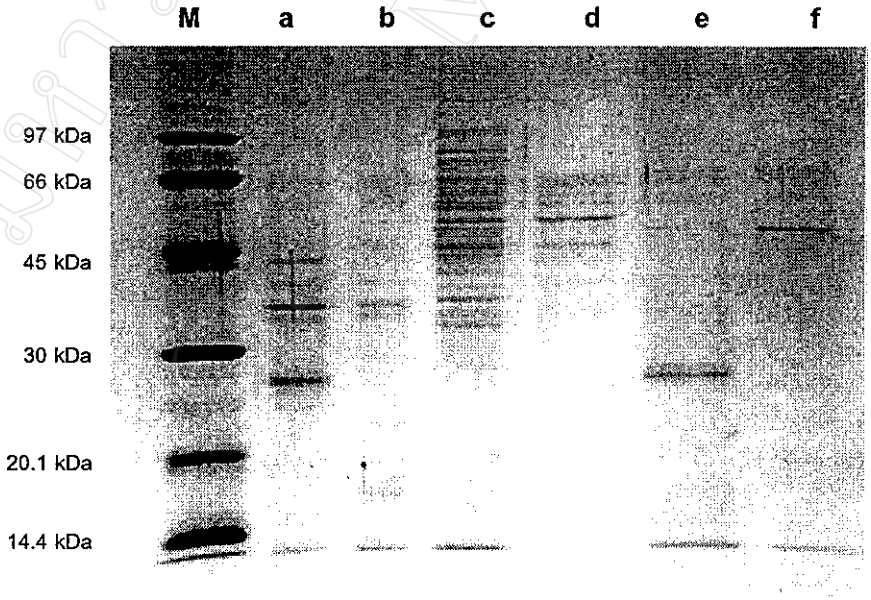

Figure 3.21 SDS-PAGE of a) fraction A1, b) fraction A2, c) fraction A3, d) fraction B1, e) fraction B2, f) fraction B3, and M) molecular weight marker

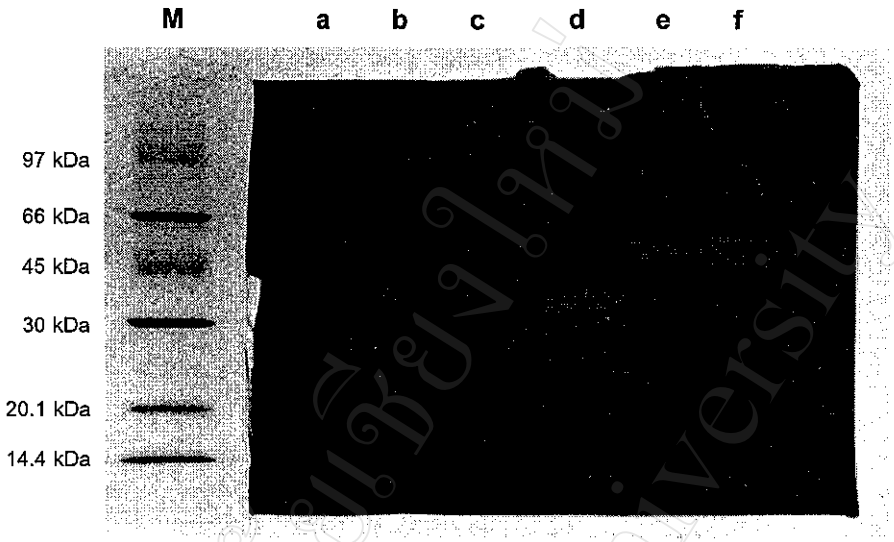

Figure 3.22 Zymogram of a) fraction A1, b) fraction A2, c) fraction A3, d) fraction B1, e) fraction B2, f) fraction B3, and M) molecular weight marker

Thus, from chromatogram and zymogram results, *Streptomyces* Ab106.3 might produce at least 3 forms of xylanases, first xylanase had the largest molecular weight in the ranges of 45-66 kDa, second xylanase had the medium molecular weight in the ranges of 30-45 kDa, the last one had the smallest molecular weight in the ranges of 20.1-30 kDa.

Table 3.7 Xylanase purification

	Xylanase activity (U)	Protein (mg)	Specific activity (U/mg)	Yield (%)	Purification fold
Crude enzyme	5.21	0.90	5.79	100.00	1.00
Ammomium sulfate precipitation	4.24	0.37	11.45	81.25	1.98
DEAE-cellulose					
fraction A	2.20	0.17	13.26	42.13	2.29
fraction B	1.20	0.12	10.13	23.10	1.75
total activity	3.40			0.16	
Sephadex G-100					
fraction A1	0.26	0.005	54.25	5.01	9.37
fraction A2	1.37	0.006	235.13	26.24	40.61
fraction A3	0.30	0.055	5.36	5.70	0.93
total activity	1.93			0.09	
fraction B1	0.15	0.016	9.64	2.89	1.66
fraction B2	0.10	0.011	8.66	1.84	1.50
fraction B3	0.46	0.018	25.81	8.77	4.46
total activity	0.70			0.03	

3.4.6 Conclusions

1. Baggase was the best carbon source for xylanase production by *Streptomyces* Ab106.3
2. Xylanases of 15.9 U/ml could be obtained from a 5-L fermentor. It was about 2 folds compared to shaked flask production.
3. Tween 80 enhanced enzyme production, but it caused purification difficulty.
4. DEAE-cellulose could separate the crude xylanases into 2 fractions, fraction A and fraction B, which were different in enzyme characteristics.
5. Gel filtration chromatography could separate fraction A into 3 fractions, the major fraction was fraction A2 which contained at least 2 xylanases, the other fractions could be the derivatives of fraction A2, they had the similar enzyme characteristics as fraction A2. Fraction B also had 3 derivatives, the major fraction was fraction B3, which contained 2 more derivatives, all enzymes had the similar characteristics, as shown in table 3.8
6. From zymogram, *Streptomyces* Ab106.3 might produce 3 forms of xylanases with different in enzyme characteristics.
7. The purification steps used in this experiment might be suitable to separate xylanases in fraction A2 from others, because the purification yield of this fraction was the highest, 40 folds of purification were archived.
8. Excess oxygen supply may have an inhibition effect on xylanase production.

Table 3.8 Summary of some properties of xylanases obtained from *Streptomyces* Ab106.3

Enzyme	Opt. pH	Opt. Temp. (°C)	pH stability	Half life (T ½)			
				50°C	60°C	70°C	80°C
Crude enzyme	6.0	65	5.0-6.0	>72 h	>72 h	17 h	18 min
Fraction A	6.0	55-65	5.0-6.0	>72 h	~72 h	19 h	30 min
Fraction A1	7.0	60-70	5.0-7.0	>72 h	>72 h	30 min	18 min
Fraction A2	6.0	60	5.0-6.0	>72 h	>72 h	6.5 h	12 min
Fraction A3	6.0	60	6.0-7.0	>72 h	8 h	-	-
Fraction B	5.0-7.0	70	6.0-7.0	>72 h	>72 h	1 h	42 min
Fraction B1	7.0	70	7.0	>72 h	>72 h	48 min	30 min
Fraction B2	7.0	65	7.0	70 h	60 h	18 min	-
Fraction B3	7.0	70	5.0-7.0	>72 h	>72 h	13 min	6min