CHAPTER 5
STUDY ON ENDOCELLULASE-FREE MULTIENZYME COMPLEX LIKE XYLANOSOME

5.1 Introduction

The strain NOI-1 was isolated from a biomass degrading community, NKP and was identified as *T. thermosaccharolyticum.* This bacterium could not grow on cellulose as sole a carbon source but can grow on xylan very well. This strain could produce endocellulase-free-xylanolytic enzyme. Xylan-degrading enzyme have attracted much attention because of their applications in industrial processes such as pre-bleaching of paper pulps, xylooligosaccharide production, modification of cereal-based food stuffs, improving the digestibility of animal feed stocks, and bioconversion of lignocellulosic materials into fermentable products (Beg et al., 2001). In these applications, it is important for the enzyme preparation to be cellulase-free, in order to retain the integrity of the pulps or fibers for cellulose pulps preparation and textile fibers liberation (De Oliveira da Silva and Carmona, 2008). Aerobic and anaerobic mesophilic microorganisms are known to produce xylan-degrading enzymes, however, most studies have focused on aerobic bacteria and fungi (Coughlan and Hazlewood, 1993). However, most industrial applications were carried out at high temperature, thus, thermostable enzymes will be more preferable (Maheshwari and Kamalam, 1985). Recently, thermophilic microorganisms have been recognized for production of thermostable enzymes, with few reports on the production of endocellulase-free thermostable xylanases (Maalej et al., 2009). Multiple forms of cellulases and xylanases are organized into such complexes which are dedicated to hydrolyze lignocellulosic substances effectively because of their ability to bind to insoluble cellulose and/or xylan via cellulose-binding and xylan-binding modules, respectively (Bayer et al., 2004). Thus, the arrangement of cell wall-degrading enzymes into a multienzyme complex provides advantages over single enzyme systems (Shoham et al., 1999). *T. thermosacharolyticum* are thermophilic anaerobic bacteria that are of considerable interest for hydrogen production and thermostable amylolytic enzymes production (Ganghofner et al., 1998; O-Thong et al., 2008). Furthermore, the enzymatic system of *Thermoanaerobacterium* has not been reported in term of their multienzyme complexes.

Therefore, in this study, *T. thermosaccharolyticum* strain NOI-1 and its enzyme was characterized.

5.2 Results and Discussion

5.2.1 Characterization of bacterium, the strain NOI-1

The strain NOI-1 was identified by sequence similarity with the sequences deposited in NCBI databases. Results show that strain NOI-1 shows high similarity (99%) with *T. thermosaccharolyticum* and will be refered as *T. thermosaccharolyticum* NOI-1 here after. The morphology of strain NOI-1 cell was observed by scanning electron microscopy (Fig. 5.1). Results indicates that *T. thermosaccharolyticum* NOI-1 is a rod-shaped (0.2-0.5 µm in width and 2.0-5.0 µm in length), Gram-positive, strictly anaerobic, and endospore-forming bacteria. The adhesions of the cells of strain NOI-1 to insoluble materials was investigated by growing strain NOI-1 in BM medium containing OSX. The result showed that the strain NOI-1 cells could adhere to both insoluble xylan and Avicel at 71.2% and 63.0%, respectively. SEM observation of cell adhesion to insoluble xylan was shown in Fig. 5.1.

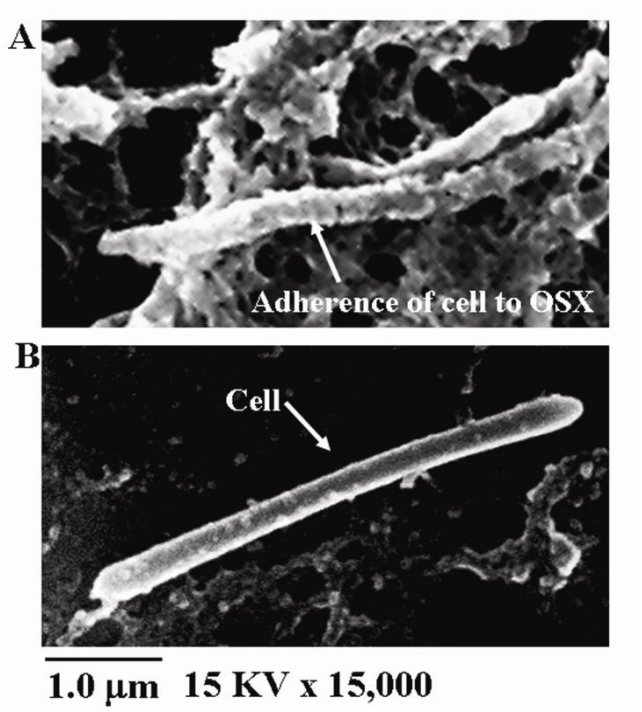

Figure 5.1 SEM of morphology and cell surface structure of *T. thermosaccharolyticum* strain NOI-1 harvested at (A) the exponential growth phase showing the adhesion of cell to OSX and (B) early declining growth phase showing no adhesion of cell to OSX.

SEM observation of cell surfaces of cell harvested during the exponential growth phase (Fig 5.1A) shows that the surfaces of cells were adhered to xylan, which was similar those observe by the multienzyme complex-bound cell surface such as *C. thermocellum* (Bayer and Lamed, 1986), *Fibrobacter succinogens* (Morris, 1988), *Ruminococcus albus* (Rincon et al., 2003), *Paenibacillus curdlanolyticus* (Pason et al., 2006) and *Tepidimicrobium xylanilyticum* (Phitsuwan et al., 2010) to insoluble substances. In contrast, no adhesion of xylan to cell surface was observed on cells harvested at the declining growth phase (Fig. 5.1B). The releasing of multienzyme complex from the cell surface into the culture medium is probably due to the presenceof less amount of xylan, the cells deteriorated and occurrence of some hydrolysis products and/or metabolic products during cultivation (Waeonukul et al., 2009). The strain NOI-1 cell was found to adhere to insoluble xylan and Avicel as the same as multienzyme complex-producing strain cells (Pason et al., 2006). These results suggested that the strain NOI-1 has an essential component responsible for anchoring the cellulose and xylan present on the cell surfaces.

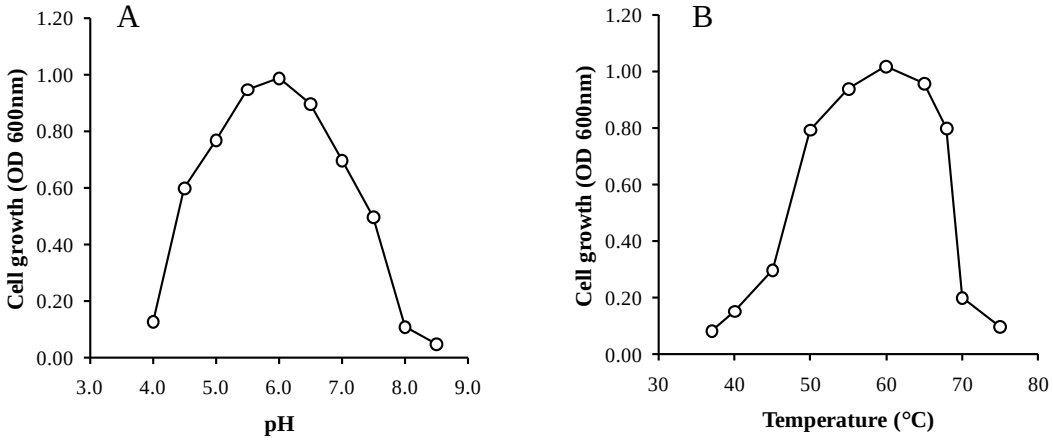

Figure 5.2 Effect of pH (A) and temperature (B) on cell growth using OSX as sole a carbon source.

The effect of temperature and pH on cell growth of the *T. thermosaccharolyticum* NOI-1 was studied. Results indicate that it was able to grow at various temperatures, ranging from 50°C to 68°C and at pHs, ranging from 4.5 to 7.5. Temperature and pH optima of this were observed at 60°C and 6.0, respectively (Fig. 5.2), similar to several other report for the genus *Thermoanaerobacterium* such as *T. thermosaccharolyticum* strain FH1 (Hoster et al., 2001), *T. saccharolyticum* (Lee et al., 1993) and *T. xylanolyticum* (Zhang et al., 2003). Carbon utilization of strain NOI-1 was shown in Table 5.1. Results indicates that the strain NOI-1 can utilized a variety of the carbon sources such as L-arabinose, D-fructose, D-galactose, D-glucose, D-mannose, D-xylose, cellobiose, lactose, maltose, sucrose, trehalose, raffinose, starch, xylan and manitol. However, it could not utilize D-rhamnose, dextran, pectin, cellulose powder and glycerol. According to the carbon source utilization, the strain NOI-1 were found to be different when compared with other strains of *T. thermosaccharolyticum* such as DSM571 (Lee et al., 1993) , FH1 (Hoster et al., 2001) and PSU-2 (O-Thong et al., 2008). The strain PSU-2 can utilize D-rhamnose but the strains NOI-1 and DSM571 can not utilize. For dextran utilization, the strains FH1 and PSU-2 can utilize but the strains DSM571 and NOI-1 not utilize. Furthermore, the strain NOI-1 can utilize manitol but not for the other strains. Thus, it can be concluded on the basis of the 16S rRNA gene analysis and the carbon sources utilization that the strain NOI-1 represents a new strain within the species of *T. thermosaccharolyticum.*

Table 5.1 Utilization of carbon sources for the strain NOI-1 compared with other strains of *T. thermosaccharolyticum*, DSM571 (Lee et al., 1993), FH1 (Hoster et al., 2001), and PSU-2 (O-Thong et al., 2008).

Carbon sources	**DSM571**	**FH1**	**PSU-2**	**NOI-1**
L-Arabinose	+	+	+	+
D-Fructose	+	+	+	+
D-Galactose	+	ND	+	+
D-Glucose	+	+	+	+
D-Mannose	+	ND	+	+
D-Rhamnose	-	ND	+	-
D-Xylose	+	+	+	+
Cellobiose	+	+	+	+
Lactose	+	+	+	+
Maltose	+	+	+	+
Sucrose	+	+	+	+
Trehalose	+	ND	+	+
Raffinose	ND	+	+	+
Dextran	-	+	+	-
Pectin	-	-	-	-
Starch	+	+	+	+
Xylan	+	+	+	+
Cellulose	ND	ND	+	-
Manitol	-	-	-	+
Glycerol	-	-	-	-

+, growth; -, no growth; ND, not determined

The fermentation products were determined at stationary phase (Fig. 5.3). Ethanol, acetic acid and butyric acid were produced as main fermentation products when strain NOI-1 was grown on OSX at 60 °C under anaerobic condition. The result showed that ethanol (58.34 % of total products) was the major product which was converted from OSX, which were similar to the fermentation product obtain through fermentation by other *Thermoanaerobacterium* species such as *T. polysaccharolyticum* and *T. zeae*

(Cann et al., 2001). This shows that ethanol was the major fermentation product, indicating that the strain NOI-1 could convert xylan to ethanol directly.

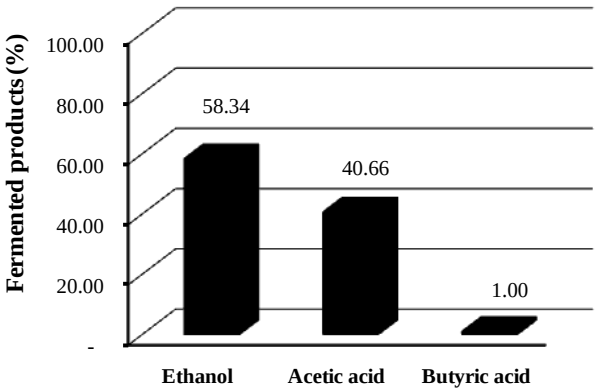

Figure 5.3 Fermented products in culture medium of OSX-grown cells at stationary phase (3 days)

5.2.2 Characterization of crude enzymes from *T. thermosaccharolyticum* strain NOI-1

When *T. thermosaccharolyticum* strain NOI-1 was grown in BM medium containing OSX as a carbon source, it produces endocellulase-free xylanolytic enzymes under anaerobic and thermophilic conditions.Time courses for production of xylanase in pellet and culture supernatant are shown in Fig.5.4. In the pellet, the highest xylanase activity was observed at the exponential growth phase (1 day), and then rapidly decreased, while xylanase activity in the culture supernatant rapidly increased at the exponential growth phase and slightly increases thereafter (2-3 days) and remained nearly constant at the stationary growth phase (3 days).

At the beginning of cultivation, xylanase activity could be detected in the pellet more than culture supernatant. At the stationary growth phase, as the enzyme was released from the pellet into culture supernatant, most of the enzyme was found in culture supernatant. The result was similar to those of bacteria which produced multienzyme complex around the cell surfaces and adhere to these substrates and secreted to culture supernatant at the late stationary growth phase (Doi et al., 2003; Pason et al., 2006; Van Dyk et al., 2009).

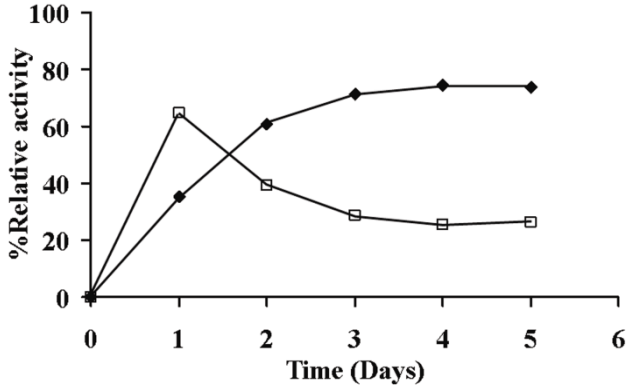

Figure 5.4 Time courses of xylanase production of the strain NOI-1 in pellet (□) and culture supernatant (■).

After the bacterium was cultivated on xylan, at the stationary growth phase (3 days), enzyme activities were determined in the culture supernatant (extracellular protein) and pellet-bound protein. Enzyme activities of the extracellular and pellet-bound protein are shown in Table 5.2. The result showed that xylanase, β-xylosidase, α-L-arabinofuranosidase, acetyl esterase, β-glucosidase and cellobiohydrolase were detected in both fractions whereas avicelase and CMCase were not detected in both fractions. Additionally, the hydrolytic activities on other polysaccharides such as mannan and starch were detected in the extracellular fraction, but only starch hydrolysis were detected in the pellet bound fraction. Thermophilic xylanases have been reported from *Thermotoga maritime*, *Clostridium thermohydrosulfuricu* (Sonne-Hansen et al., 1993), *Clostridium stercorarium* (Sakka et al., 1994), *Cellulomonas fimi* (Nikolova et al., 1997) etc., but there are only a few reports on endocellulase-free thermostable xylanases from bacteria and fungi such as *Thermomyces lanuginosus* (Gomes et al., 1993), *Saccharomonospora viridis* (Roberts et al., 1990), *Bacillus* sp. (Dey et al., 1992) and *Chainia* sp. (Bandivadekar and Deshpande, 1994). The strain NOI-1 exhibited significant amounts of xylanolytic enzymes production at 60°C under anaerobic conditions and this enzyme was endocellulase-free xylanolytic enzymes. The endocellulase-free xylanolytic enzymes produced from this strain may potentially be applied in enzymatic hydrolysis of xylan of lignocellulosic materials, kraft pulps for paper industries, textile processes and the food and feed industries (Gomes, 1994). Furthermore, the strain NOI-1 exhibited significant amounts of xylanolytic enzymes

production at 60°C under anaerobic conditions with lack of CMCase and avicelase activities. However, most of enzyme activities were detected in the extracellular fraction at strationary phase. Thus, the extracellular protein was used for further study.

Table 5.2 Enzymatic activities of culture supernatant (extracellular protein) and pellet-bound protein from *T. thermosacharolyticum* strain NOI-1

Enzymes	**Specific activities (U/mg Protein)**	
	Extracellular	**Pellet-bound**
Xylanase	2.55	0.69
β-Xylosidase	2.00	0.67
α-L-arabinofuranosidase	2.44	2.17
Acetyl esterase	0.01	0.01
β-Glucosidase	0.49	0.41
Cellobiohydrolase	0.39	0.36
Avicelase	ND	ND
CMCase	ND	ND
Amylase	0.90	0.5
Mannanase	0.21	ND

ND, were not be able to detect under the assay condition

Binding ability of crude enzyme from extracellular protein obtained at stationary phase was explained. Since the strain NOI-1 cells could adhere to insoluble polysaccharides, which were similar to those of the multienzyme complex-bound cells, the ability of the crude enzyme to bind to insoluble polysaccharides was investigated. The results showed that crude enzyme was able to bind to OSX and Avicel at 40.7% and 35.2%, respectively, and exhibited stronger affinity to OSX than to Avicel (Fig. 5.5). The binding ability of enzymes to insoluble substrates plays an important role in the efficiency of enzymatic hydrolysis of the insoluble substrates (Bayer et al., 2004). Many investigators have reported that the multienzyme complex contained catalytic and non-catalytic domains such as cellulose-binding domains (CBDs) and/or xylan-binding domains (XBDs) (Kulkarni et al., 1999). This may indicate that the multienzyme complex produced by the strain NOI-1 might have XBD and CBD.

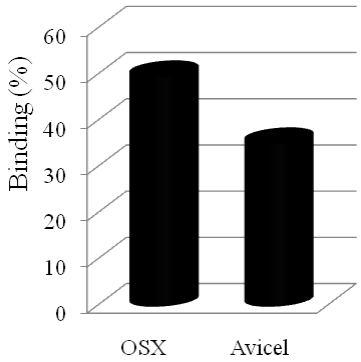

Figure 5.5 Binding abilities of crude enzyme from the strain NOI-1at stationary phase to insoluble polysaccharide

The extracellular and pellet-bound proteins were analyzed by Native-zymograms, and SDS-zymograms, using soluble xylan as substrate to determine the activity of xylanase subunits. Native-zymograms showed one band of high molecular mass (Fig 5.6, 1A and 1B) for both pellet bound and extracellular proteins whereas SDS-zymograms, showed 6 subunits of xylanase activity in the pellet bound protein (Fig 5.6, 2A) and 9 subunits of xylanase activity in extracellularprotein(Fig 5.6, 2B). Among them, 6 proteins with molecular-mass of 115, 100, 91, 84, 74 and 59 kDa showed xylanase activity in both the pellet bound and extracellular proteins. These results indicated that many proteins possessing xylanase activities were assembled into a multienzyme complex (xylanosome) in the extracellular and pellet bound fraction. At stationary growth phase of the strain NOI-1, xylanase subunit in extracellular protein exhibits higher activity than those of pellet bound protein which matched the result to determination of xylanase activity so showed higher xylanase activity in extracellular protein than pellet bound protein (Table 5.2). Thus, extracellular protein was used future study.

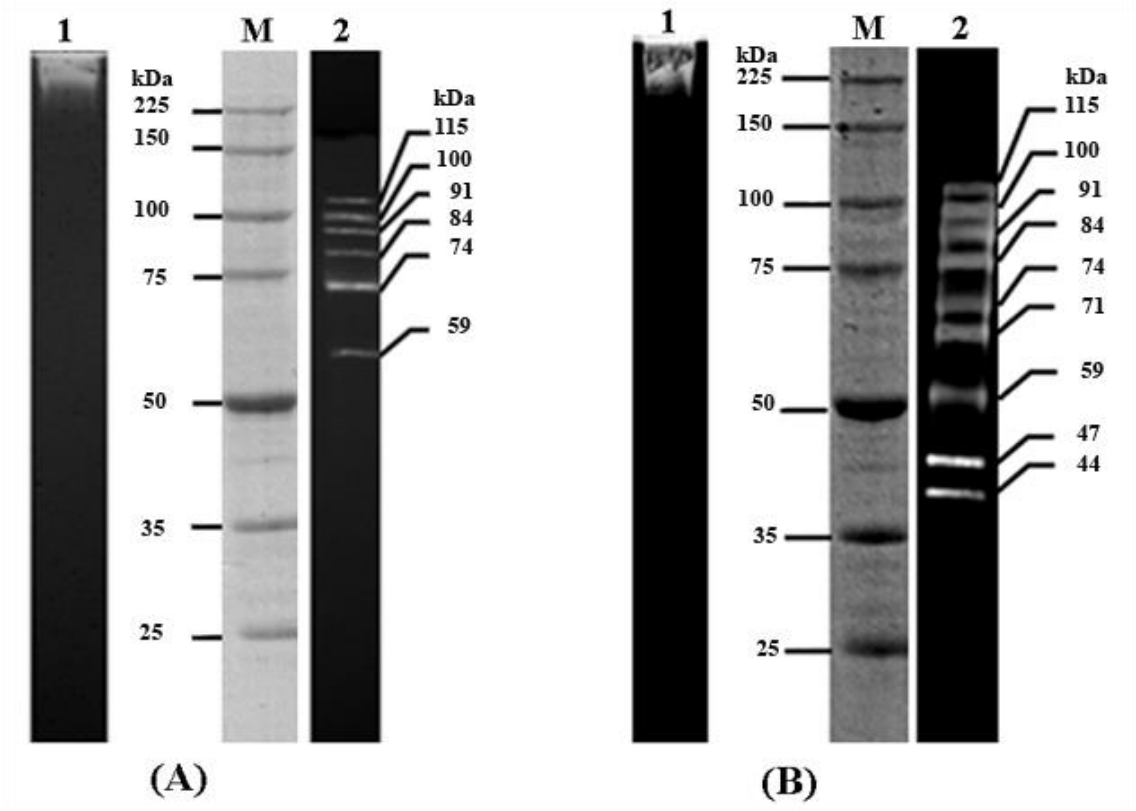

Figure 5.6 Patterns of xylanase activities by zymogarm gel electrophoresis from (A) pellet-bound protein and (B) extracellular protein. Lane 1, Native-zymogram; Lane 2, zymogram analysis of xylanase activity; M, Molecular mass in kDa

Crude enzyme from extracellular protein obtained from stationary growth phase of the strain NOI-1 was studied for the effect of pH and temperature on xylanase activity and stability. Xylanase activity was tested from pH 4.0 to 12.0 (Fig. 5.7A) and the optimum pH was found to be 6.0. However, a relatively broad pH plateau was observed between pH 4.0 to 10.0, exhibiting a relative activity close to the optimal value. The pH stability of the xylanase of the strain NOI-1 was assayed from pH 4.0 to12.0 (Fig. 5.7A). Results concluded that it has high stability (above 80%) in the pH range of 5.0 to 10.0, and relatively low stability (below 80%) below pH 4.0 and above pH 11.0. This means that this xylanase exhibits a broad pH stability, in agreement with the general observation of *Bacillus* spp. and other bacteria xylanases which are also active over a broad pH range (Kulkarni et al., 1999).

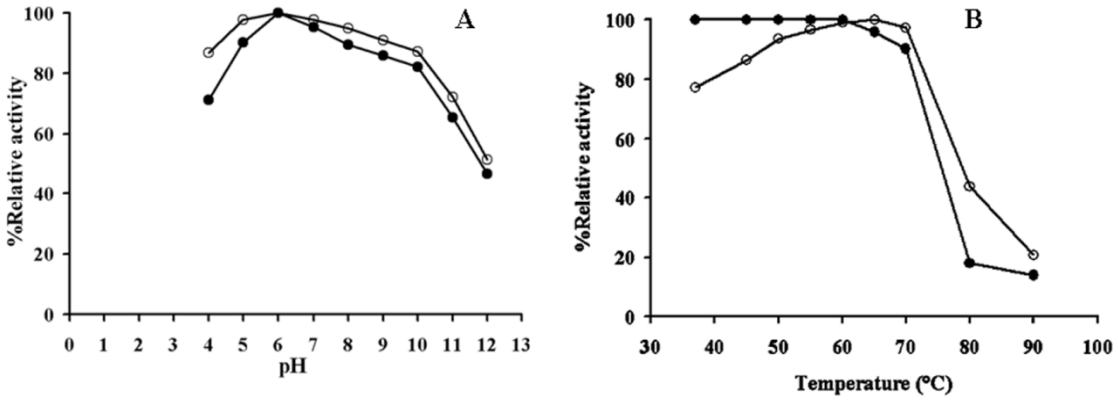

Figure 5.7 Effect of pH on activity (○) and stability (●) of xylanase (A) and effect of temperature on activity (○) and stability (●) of xylanase (B)

In order to determine the effect of temperature on enzyme stability and optimum temperature for this enzyme, enzymatic assay was performed at the temperature range of 37-90 °C. Xylanase from the strain NOI-1 exhibited its optimum activity at 60 °C, and was stable up to 70 °C for 60 min (Fig. 5.7B). Optimal condition (pH and temperature) as well as the stability of enzyme represent an important properties for industrial enzymes (Gattinger et al., 1990). Xylanases with high thermostability and pH are required in the pulp and paper industry (Karlsson et al., 1998). Since the xylanase produces the strain NOI-1 could be active in the broad ranges of pH and temperature, it would be suitable for application in paper industry.

Xylan-hydrolysis efficiency of endocellulase-free xylanolytic enzyme from the strain NOI-1 was explained. The crude enzyme from *T. thermosaccharolyticum* NOI-1 was incubated with 1% (dry weight) xylans (OSX, BWX and LWX) and the amount of reducing sugar released was determined at 0, 5, 10, 15, 20, 45 and 60 min. Results shows that hydrolysis of xylan increase rapidly during the first 10 min of fermentation as indicated by the rapid increase in reducing sugar amount in the fermentation medium (Fig. 5.8). In addition, the crude enzyme from *T. thermosaccharolyticum* NOI-1 was able to hydrolyze LWX better BWX and OSX. These results indicated that this crude enzyme is more efficient in degradation of hard wood (LWX and BWX) than soft wood (OSX) because softwood xylans are shorter and less branched (degree of polymerization

between 70 and 130) than hardwood xylans. Furthermore, the content, position, and structure of xylan are one of the factors affecting the hydrolysis resistance of biomass (Liab et al., 2000).

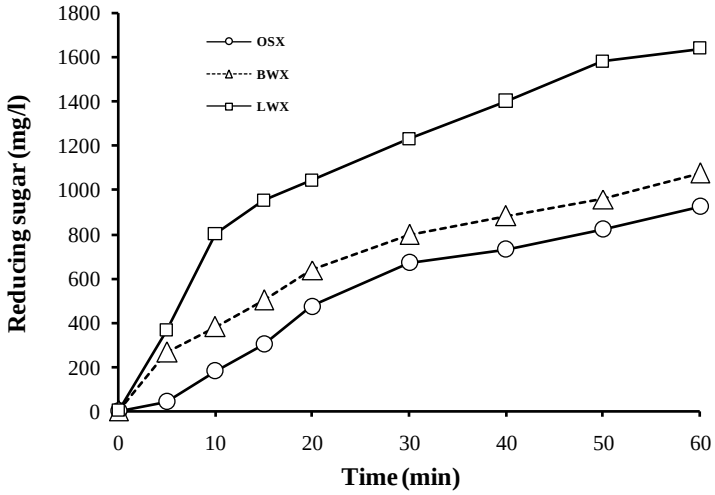

Figure 5.8 Time course of hydrolysis of pure xylans (OSX, BWX and LWX) by crude enzyme (1.0 U xylanase) from *T. thermosaccharolyticum* NOI-1(A); OSX, (o); LWX, (□); BWX, (Δ)

The hydrolysis of various xylan substrates by crude enzyme from *T. thermosaccharolyticum* NOI-1 was examined by analysis of products using TLC (Fig. 5.5B). The hydrolysis products of OSX, BWX and LWX were xylose and a series of XOs larger than xylobiose. The results showed that xylanolytic enzymes from the strain NOI-1 may be used in producing XOs. XOs showed a remarkable potential for utilization in many fields, including pharmaceuticals, feed formulations, and agricultural application (Vazquez et al., 2001).

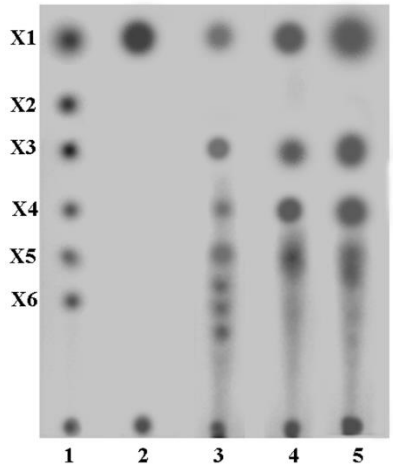

Figure 5.9 Thin layer chromatography of the hydrolysis products of xylan by crudexylanase (1.0 U) for 60 min. Lane 1, standard xylooligosaccharides (X1: xylose, X2: xylobiose, X3: xylotriose, X4: xylotetraose, X5: xylopentaose and X6: xylohexaose); Lane 2, xylose; Lane 3-5, the hydrolysis products from OSX, BWX and LWX, respectively.

5.2.3 Isolation, purification and characterization of endocellulase-free multienzyme complex from *T. thermosaccharolyticum* strain NOI-1

In previously studied, a new thermophilic anaerobic endocellulase-free xylanolytic enzymes producing bacterium, *T. thermosaccharolyticum* strain NOI-1 was isolated. The properties of cell and protein binding ability, and subunits of xylanase in extracellular protein were determined and explained. Results indicates that this isolated enzyme possess a multienzyme complex properties. Thus, it is to our advantage to isolate, purify and characterize this multienzyme complex from crude enzyme in the extracellular protein fraction that produced by *T. thermosaccharolyticum* strain NOI-1.

Isolation and purification of this multienzyme complex from crude enzyme in extracellular protein was performed by using affinity purification on cellulose (Avicel) and gel filtration chromatography. Although, crude enzyme from the strain NOI-1 had ability to bind to OSX more than Avicel, Avicel was selected to purify the multienzyme complex for affinity column because Avicel gave a flow rate higher than xylan. The cellulose-binding proteins (mainly multienzyme complex) were collected, which was then applied onto a Sephacryl S-300 column. Only one peak was observed and the molecular mass was estimated to be 1,200 kDa (Fig. 5.10).This purified, high molecular

mass protein shows to exhibits high xylanase activity. Therefore, the property of this protein (namely isolated multienzyme complex) was characterized.

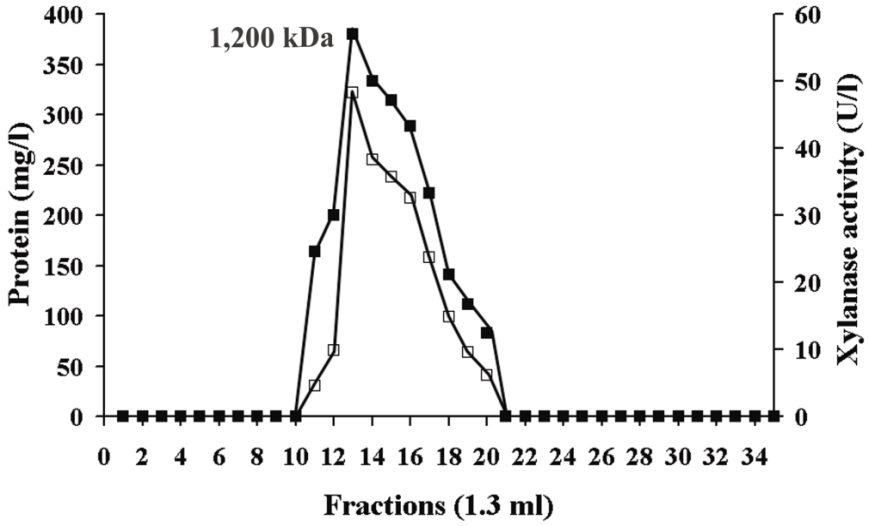

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Figure 5.10 Gel filtration chromatography on Sephacryl S-300 column of the isolatedmultienzyme complex of thestrain NOI-1, Symbols: ■, protein (mg/l); □, xylanase activity (U/l).

The isolated multienzyme complex from the strain NOI-1 was determined enzymatic activities and then compared with crude enzyme from NOI-1 and *Bacillus firmus* K-1 (Table 5.3). Generally, the isolated enzyme displayed an endocellulase-free multienzyme complex property. Compared to the crude enzyme which consist of 8 enzymatic activities, isolated the multienzyme complex only shows to have 5 enzymatic activities, with no acetyl esterase, amylase and mannanase activity detected.

Table 5.3 Enzymatic activities of crude enzyme and isolated multienzyme complex from the strain NOI-1 and crude enzyme from *Bacillus firmus* K-1

Enzymes	Specific Activity (U/mg Protein)		
	Crude enzymeof the strain NOI-1	**Isolated multienzyme complexof the strain NOI-1**	***Crude enzymeof the strain K-1**
Xylanase	2.55	9.07	4.80
β-Xylosidase	2.00	9.35	0.21
α-L-Arabinofuranosidase	2.44	11.26	0.15
Acetyl esterase	0.01	-	0.24
β-Glucosidase	0.49	0.24	-
Cellobiohydrolase	0.39	0.05	ND
Avicelase	-	-	ND
CMCase	-	-	-
Amylase	0.90	-	ND
Mannanase	0.21	-	ND

-, not able to detect under the assay condition

ND, not detected

*, (Ratanakhanokchai et al., 1999)

The hydrolysis efficiency of pure xylan (OSX) and biomass (corn hull) with three sources of xylanolytic enzyme was determined and compared. The three sources of enzyme used are: (i) isolated endocellulase-free multienzyme complex of strain NOI-1, (ii) crude enzyme of strain NOI-1, and (iii) endocellulase-free xylanolytic enzyme of *B. firmus* stain K-1 (non multienzyme complex) (Ratanakhanokchai et al., 1999). Results of the hydrolysis efficiency based on the amount of reducing sugar released were shown in Fig. 5.11. It can be seen that the isolated multienzyme complex and the crude enzyme of the strain NOI-1 hydrolyzed OSX more efficiently than enzymes from the strain K-1. In addition, the isolated multienzyme complex and crude enzyme of strain NOI-1 shows a rapid hydrolysis of OSX within the first 5 min of incubation, which slightly increased and then remained nearly constant thereafter. Contrariwise, the amount of reducing sugars released by incubation with enzymes of the strain K-1 showed slight increased along the 60 min of incubation and remained relatively constant thereafter. These might be due to the stability of the enzymes and/or end products inhibition (Bachmann and

McCarthy, 1991). Additionally, the enzyme hydrolyzed not only pure xylan but also xylan of agricultural residue such as corn hull even though without any pretreatment. The result showed that the amount of reducing sugars released from corn hull after enzyme treatment with crude enzyme, isolated multienzyme complex from the strain NOI-1, and the crude enzyme from the strainK-1 were 675.2, 1,475.1, and 250.2 μg/U enzyme, respectively. This means that the multienzyme complex of the strain NOI-1 hydrolyzed xylans more efficiently than the other two enzyme investigated.This might be due to the presence of XBD, CBD, as well as the enzymes exist as a complex of enzyme that acted synergistically during the hydrolysis of xylans.

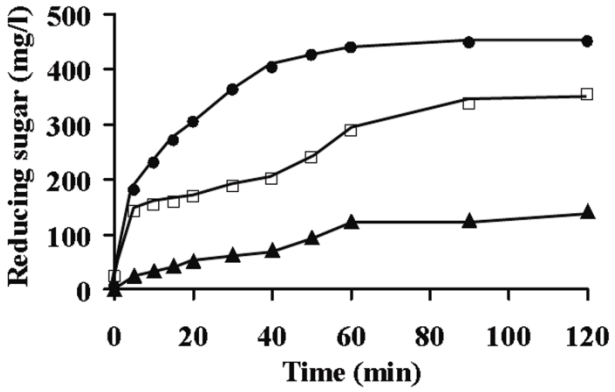

Figure 5.11 Time courses of the hydrolysis of OSX by crude enzyme (□) and the isolated multienzyme complex (●) from the strain NOI-1 and crude enzyme from *B. firmus* K-1 (▲)

The effect of pH and temperature on enzyme activity and stability (isolated multienzyme complex) was determined. The effect of pH on xylanase activity and stability of the isolated multienzyme complex from the strain NOI-1 was studied in the range of pH 4.0 to 12.0 (Fig. 5.12 A). Optimum pH was found to be 6.0 with a broad pH range between 4.0 and 10.0, exhibiting more than 85% relative activity. The pH stability of the xylanase showed high stability (above 80%) over a wide range from pH 5.0 to 10.0. Thus, isolated multienzyme complex from the strain NOI-1 could be active and stable in broad ranges of pH. The isolated multienzyme complex from the strain NOI-1 shows maximum enzyme activity at 60°C and a marked decrease in enzyme activity above 70°C for 60 min (Fig. 5.12 B). Therefore, crude enzyme from the strain NOI-1

can be applied in various industries such as pulp and paper,food/feed and enzymatic hydrolysis of xylan in lignocellulosic materials (De Oliveira da Silva and Carmona, 2008).

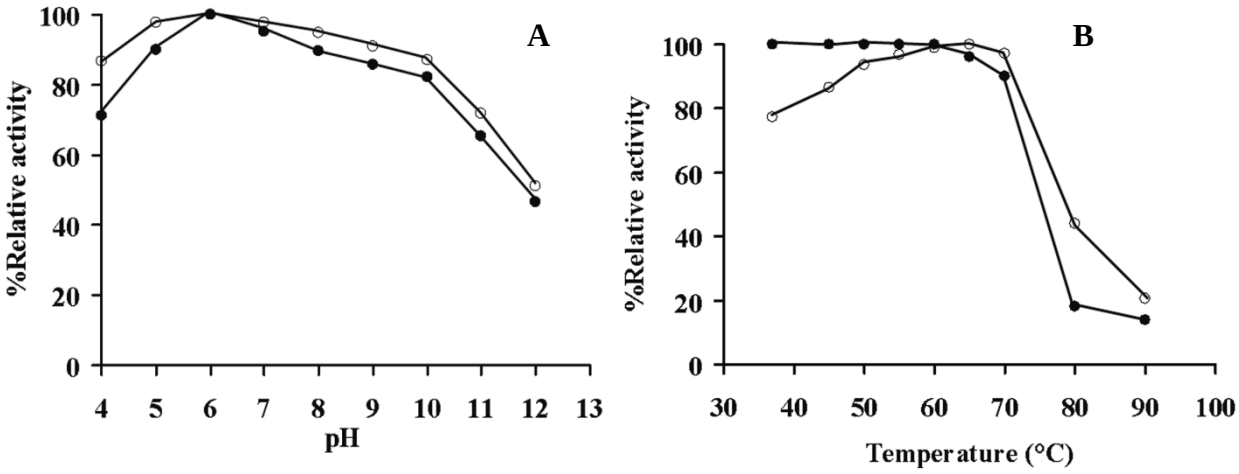

Figure 5.12 Effect of pH (A) and temperature (B) on the activity (○) and stability (●) of the isolated multienzyme complex (xylanase)

Pattern of the components of isolated multienzyme complex from the strain NOI-1 was investigated by gel-electrophoresis and zymograms and compared with those of crude enzyme. The crude enzyme exhibited 3 proteins bands on native-PAGE (Fig. 5. 13, lane 1), whereas the isolated multienzyme complex showed a single band on both native-PAGE (Fig. 5.13, lane 2) and native zymogram for xylanase activity (Fig. 5.13, lane 3), but no CMCase activity on the CMC-zymogram (Fig. 5.13, lane 4). At least 21 bands were observed on the SDS-PAGE of the isolated multienzyme complex (Fig. 5.13, lane 5) with a molecular mass range of 21 to 150 kDa. SDS-zymogram revealed 5 proteins bands (82, 104, 120, 136 and 150 kDa) having xylanase activity (Fig. 5.13, lane 6) but no CMCase activity on the CMC-zymogram (Fig. 5.13, lane 7). Moreover, the isolated multienzyme complex contained activities of β-xylosidase, α-L-arabinofuranosidase, β-glucosidase, and cellobiohydrolase (Table 5.3). Thus, the multienzyme complex of the strain NOI-1 contains xylanases associated with these enzymes. The multienzyme complex with lack of CMCase and avicelase activities has not been reported before

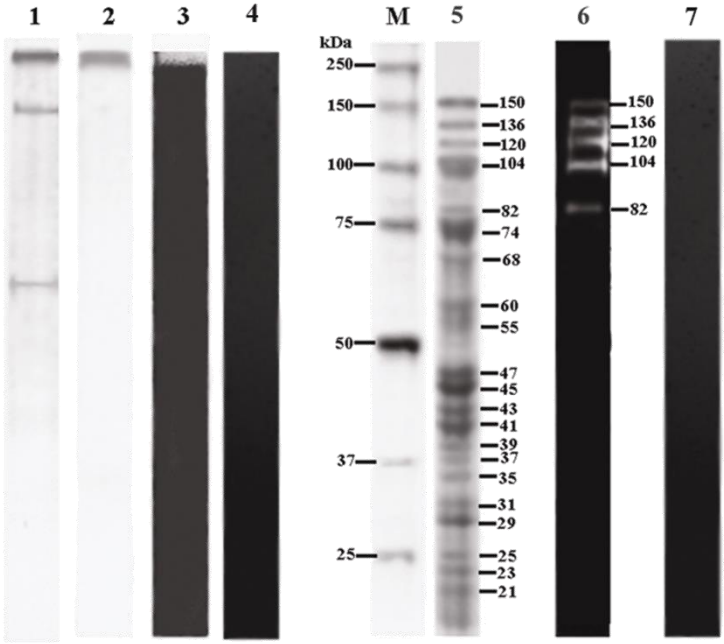

Figure 5.13 Patterns of proteins and xylanase and CMCase activities by gel electrophoresis. Lane 1, native-PAGE of crude enzyme; lanes 2-7, native–PAGE, native zymogram for xylanase and CMCase activities, SDS–PAGE, SDS-zymograms for xylanase and CMCase activities of the isolated multienzyme complex of the strain NOI-1, respectively. Molecular mass in kilodaltons is indicated on lane M.

In conclusion, the strain NOI-1 was a thermophilic, anaerobic bacterium, identified as *T. thermosaccharolyticum* strain NOI-1. The endocellulase-free multienzyme complex of this bacterium showed a molecular mass of 1,200 kDa and consists of xylanases, β-xylosidase, α-L-arabinofuranosidase, β-glucosidase and cellobiohydrolase. Moreover, it was found to hydrolyze xylan and corn hull efficiently. This is the first report of an endocellulase-free multienzyme complex produced by *Thermoanaerobacterium* species. This enzyme indicated the possible application of such enzymatic approach in some industrial processes, which require enzyme to retain its activity at high temperature, wide range of pH and with lack of endocellulase activity. Multienzyme complex, cellulosome was initially identified and described in an anaerobic, thermophilic, cellulolytic bacterium, *C. thermocellum*. The major enzyme in the cellulosome was cellulase (Lamed et al., 1983). On the other hand, xylanosomes, having a structure analogous to the cellulosomes, are discrete, multifunctional, and multienzyme

complexes that can be found in several microorganisms. These complexes play an important role in the degradation of hemicellulases (Sunna and Antranikian, 1997). Thus, the endocellulase-free multienzyme complex produced from *T. thermosaccharolyticum* strain NOI-1 like xylanosome.