Optimation of β-Mannanase Production on Submerged Culture of *Eupenicillium javanicum* as well as pH and Temperature Enzyme Characterizations

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ABSTRAK

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Penelitian telah dilakukan untuk menentukan kadar bungkil kelapa (CM) dan waktu inkubasi optimum untuk memproduksi β-mannanase *Eupenicillium javanicum*. Dua tahap percobaan yang berurutan dirancang dengan pola faktorial. Pada percobaan yang pertama, faktor utama merupakan kadar substrat CM 1, 2, dan 3%, sedangkan sub-faktor adalah waktu inkubasi 1, 2, 3, 4, 5, 6, dan 7 hari. Kedua faktor berinteraksi dengan sangat nyata (P<0,01). Karena aktivitas β-mannanase, kadar protein ekstraselular dan aktivitas sakarifikasi terhadap CM tertinggi diperoleh pada kadar CM 3% dan waktu inkubasi 5 hari (P<0,01), percobaan kedua dirancang untuk kadar CM yang lebih tinggi. Faktor utama tetap merupakan kadar bungkil kelapa pada 3, 4, dan 5%, sedangkan sub-faktor merupakan waktu inkubasi pada 5 dan 6 hari. Kedua faktor tersebut juga berinteraksi dengan sangat nyata (P<0,01) hanya pada aktivitas β-mannanase dan kadar protein, sedangkan aktifitas spesifik tidak berbeda nyata (P>0,05). Aktifitas enzim tertinggi didapatkan pada kadar CM 4% dengan waktu inkubasi lima hari yang tidak berbeda nyata dengan enzim dari CM 3% pada waktu inkubasi yang sama. Dari kedua percobaan dapat disimpulkan bahwa produksi enzim yang paling baik dilakukan pada kadar bungkil kelapa 3% dengan waktu inkubasi 5 hari. Enzim tersebut mempunyai pH optimum 5,4-5,8 sesuai dengan pH duodenum, sedangkan aktivitas pada pH 4,5 relatif rendah. Walaupun aktifitas enzim berkurang pada pH 4,5, enzim masih aktif selama 4 jam. Aktivitas enzim cukup stabil pada pH 5,8 dan 6,5. Suhu optimum aktivitas enzim ialah 50^0C, yang lebih tinggi daripada suhu tubuh unggas (40^0C). Pengurangan aktivitas enzim pada suhu 40^0C dapat diatasi dengan penambahan lebih banyak enzim. Enzim cukup stabil pada inkubasi 4 jam pada suhu ruang 28 dan 40^0C, tetapi aktifitas enzim berkurang banyak setelah inkubasi 60 detik pada suhu 90^0C. Suhu pada alat pencernaan unggas tidak mempengaruhi aktivitas enzim, tetapi dalam proses pembuatan pelet dengan suhu sekitar 90^0C harus dibatasi tidak melebihi 30 detik.

Kata kunci: Bungkil kelapa, β-mannanase, *Eupenicillium javanicum*, karakterisasi pH dan suhu

ABSTRACT

PURWADARIA T., T. HARYATI, E. FREDERICK and B. TANGENDJAJA. 2003. Optimation of β-mannanase production on submerged culture of *Eupenicillium javanicum* as well as pH and temperature enzyme characterizations. *JITV* 8(1): 46-54.

Two successive experiments were conducted to determine the optimal substrate concentration of coconut meal (CM) and incubation time for production of β-mannanase from *Eupenicillium javanicum*. Both experiments were designed based on factorial. In the first experiment, the main factor was substrate concentration of 1, 2, and 3%, while the sub-factor was incubation time of 1, 2, 3, 4, 5, 6, and 7 days. The two factors were interacted highly significantly (P<0.01). Since the highest β-mannanase activity, protein concentration and saccharification activity towards coconut meal were obtained from 3% CM after five day incubation time (P<0.05), the second experiment was designed for higher substrate concentration. The main factor was also substrate concentration of 3, 4 and 5%, while the sub factor was incubation time of 5 and 6 days. The two factors were also interacted highly significantly (P<0.01) for mannanase activity and protein concentration, while specific activity was not significantly different (P>0.05). The best activity was obtained at 4% of coconut meal for five day incubation time, which was not significantly different with that of 3% at the same incubation time. Therefore, it was concluded from both experiments that the best enzyme production was obtained from 3% of coconut meal at incubation time of 5 days. Then, further experiments show that the enzyme had optimum pH at the range of 5.4-5.8, the same pH range in duodenum, while at pH 4.5 the activity was relatively low. Although, at pH 4.5 the enzyme activity was reduced, the enzyme was still active for four hours. At pH 5.8 and 6.5 the enzyme was quite stable. The optimum temperature of the enzyme was at 50^0C, higher than the body temperature of most poultry (40^0C). The reduction of enzyme activity at 40^0C could be overcome by increasing the enzyme concentration. The enzyme was stable after 4 hour incubation at 28 (room temperature) and 40^0C, however, the enzyme activity was considerably reduced at temperature of 90^0C after 60 second incubation. In the poultry digestion system the activity is not affected by temperature, but in the pelleting process where the steam temperature approximately 90^0C has to be limited for not more than 30 seconds.

Key words: Coconut meal, β-mannanase, *Eupenicillium javanicum,* pH and temperature characterization

INTRODUCTION

The needs of feed sources for monogastric in Indonesia are increasing. At present, the insufficiency of feed components especially for protein (soybean meal) and energy sources (corn) are solved by imports. Utilization of agricultural by product such as coconut meal (CM) as a feed component is very possible. However, its digestibility is low. The use of enzymes as feed additive to increase digestibility of feed has been reported (CHESSON, 1987; CAMPBELL and BEDFORD, 1992). The kind of enzyme used is depended on the kind of feed component. Rice bran which contains 1.31% phytate will be more phosphorus digestibility if it was mixed with phytase (RAVINDRAN *et al.,* 1995). While, the use of barley and guar gum will be more effective after the addition of β-glucanase and mannanase respectively (RAY *et al*., 1982; CAMPBELL and BEDFORD, 1992).

The utilization of CM as feedstuff is limited by its high concentration of mannan and galactomannan. Enzymes which digest both compounds are β-mannanase, α-galactosidase and β-mannosidase (MCCLEARY and MATHESON, 1986). The enzymes could be produced by *Aspergillus niger* and *Talaromyces* spp. (ARAUJO and WARD, 1990), *Streptomyces* sp. (ZAMORA *et al.,* 1989) or by *Eupenicillium javanicum* (PURWADARIA *et al.,* 1994). The production of the enzyme in the submerged culture was induced by the substrate containing locust bean gum (JOHNSON, 1990; TORRIE *et al*., 1990; and PURWADARIA *et al.,* 1994) or coconut meal (ZAMORA *et al.,* 1989; IRIANI *et al.,* 1995; and HOSSAIN *et al.,* 1996). The concentration of CM influenced the production of the enzymes, the highest activity of β-mannanase from *Streptomyces sp.* obtained among concentration of 0.5-3.5% was 3% (ZAMORA *et al.,* 1989). The production of the enzymes was also influenced by incubation time. The α-galactosidase, β-mannosidase and β-mannanase activities produced by *E. javanicum* in 3% CM were higher on the incubation time of five days than on four and six day incubations (HARYATI *et al*., 1995). The interaction between substrate concentration and incubation time in the production of *E. javanicum* β-mannanase has not been reported.

The utilization of β-mannanase in the field also related with the characterization of the enzyme such as its optimum pH and temperature and pH and thermal stabilities. The optimum pH and pH stability were important to consider the possibility of the enzyme working on the poultry digestive system. The mean pH of chicken digestive system: duodenum, jejenum, ileum, ceca, rectum, and pancreas are 6.13; 6.29; 6.58; 6.14; 6.82, and 6.58 respectively or are close to pH neutral, while pH of crop, proventriculus and gizzard are in acid condition of 4.67; 4.48 and 2.94 respectively (PATRICK and SCHAIBLE, 1980). Beside for the activity of the enzyme in the digestive system, determination of optimum temperature and thermal stability are also important for enzyme storage and feed processes. The body temperature of chicken is 40.6-41.7^{0}C (PATRICK and SCHAIBLE, 1980), while the poultry pellet formation has to be processed at 60-90^{0}C (SPRING *et al*., 1996) for 60-90 seconds. The reduction of enzyme activity by those temperature condition has to be noticed and solved.

The aim of this experiment is to determine the optimal coconut meal concentration and incubation time for mannanase production by *Eupenicillium javanicum*, and its optimum pH and temperature as well as pH and thermal stabilities.

MATERIALS AND METHODS

Microbe

E. javanicum, a collection of Research Institute for Animal Production was maintained on potato dextrose agar slopes. Stock culture was kept under lyophylization at 4^{0}C, while inoculum was prepared on petri dishes for five days at 28^{0}C.

Enzyme production

The enzyme was produced on the liquid medium (submerged culture) containing minerals in g/l $(NH_4)_2SO_4$ 1.4, KH_2PO_4 2.0, $MgSO_4$ 0.3, urea 0.3, and $CaCl_2$ 0.3 and in ppm $FeSO_4$ 5, $MnSO_4$ 16, $ZnSO_4$ 14, and $COCl_2$ 20 and CM as carbon source (IRIANI *et al.,* 1995). In their experiment (IRIANI *et al.,* 1995) the concentration of coconut meal was only 1% while in present experiment it was varied from 1-5%.

Submerged culture of 100 ml volume was prepared in the 500 ml flasks. The medium was inoculated with 1 cm^2 culture of *E. javanicum* and incubated in the shaker incubator at 28^{0}C and 150 rpm for 1-7 days. The enzyme was extracellular fractions which was separated from the culture by centrifugation for 2 x 20 minutes at 12,000 rpm and 4^{0}C.

Enzyme activities

The assay of β-mannanase was carried out using 0.5% of locust bean gum as a substrate (IRIANI *et al.,* 1995) in sodium acetate buffer (0.1 M, pH 5.8 or otherwise stated). The activity was expressed in unit/ml, where one unit liberated 1μmol of mannose in 1 minute at 50^{0}C (otherwise stated). The concentration of mannose was determined according to DNS method (MILLER, 1959). The calculation of the activity was determined after substraction with the mannose concentration of samples with controls which were

prepared at the same enzyme concentration and inactivated by DNS before the addition of the substrate. Specific activity was calculated in unit/mg extracellular protein.

The assay of saccharification was carried out using suspension of 2% defatted CM as a substrate in the sodium acetate buffer (0.1 M, pH of 5.8) (IRIANI *et al.,* 1995). Two ml of substrate was mixed with 2 ml of appropriate diluted enzyme and incubated at the shaker at 150 rpm., 50^0C for 1 hour. After stopping the enzyme reaction by heating for 5 minutes at 100^0C, the filtrate was separated by centrifugation at 2500 rpm for 15 minutes. The mannose concentration of the enzyme filtrate and control were determined by DNS method. Heating the reaction at 100^0C for the controls was carried out before incubation. Saccharification activity was stated in μmol of mannose produced in 1 minute. Specific activity was calculated in μmol/mg extracellular protein.

Protein assays

Protein was determined by BRADFORD method (1976) and Bovine Serum Albumin (BSA) was used as a standard.

Optimum pH and temperature for β-mannanase activity

The activity of the enzyme at the same temperature of 50^0C and at different pH 4.5; 5.0; 5.4; 5.8, and 6.2 were determined to obtain the pH optimum. While, the temperature optimum of the enzyme was determined at the enzyme activity at pH 5.8 and at different temperatures of 30; 35; 40; 45; 50; 55 and 60^0C. Relative activity was calculated to the optimal pH or temperature activities.

The stabilities of pH and temperature for β-mannanase activity

The pH stability of β-mannanase was investigated by incubating the crude enzyme which was diluted ten times in different pH buffers of 4.5, 5.8 and 6.5 for four hours at 28^0C. The enzyme activity of those enzymes at every hour incubation was determined at every pH and at optimum temperature. Relative activities were determined towards the activity at zero hours of incubation time.

The thermal stability of β-mannanase was investigated by incubating the crude enzyme at 28 and 40^0C for periods of 0-4 hours (samples were collected for every hours) and at 90^0C for 2 minutes (samples were collected for every 30 seconds). The enzyme activity was determined after those incubation periods at pH 5.8 and at optimum temperature. Relative activities were determined towards each temperature activity at zero hours of incubation time.

Statistical analysis

Data from the treatment of CM concentration and incubation time for enzyme production were calculated using analysis variance based on factorial (STEEL and TORRIE, 1980). Firstly, three by seven factorial design was employed to determine the level of CM concentration as a substrate (1, 2, and 3%) and incubation time (1, 2, 3, 4, 5, 6, and 7 days) and their interaction. Data were collected from five replication. Secondly, three by two factorial design was employed in the level of CM concentration (3, 4 and 5%) and incubation time (5 and 6 days). Data were collected from three replication.

RESULTS AND DISCUSSION

Optimation of β-mannanase production

The production of β-mannanase was influenced by concentration of coconut meal on the media and period of incubation time (Table 1, 2 and 3). Statistical analysis of extracellular protein concentration, β-mannanase activity and its specific activity in the first experiment showed that there were interaction between substrate concentration and incubation time (Table 1). The highest extracellular protein and enzyme activity was obtained from 3% substrate concentration at the incubation time of 5 days. In every substrate concentration, the enzyme production, stated as enzyme activity was increasing until optimal incubation time, and there after was stable or decreasing. The enzyme activity of less substrate concentration was shorter declined than that of higher concentration. For example, the enzyme activity of 1% coconut meal started decline after three days, while that of 2 and 3% after five days. The results occur due to the metabolism activity of the mold, which is related to its growth curve and nutrient concentration. It is well known that microbes always have lag phase before the logaritmic period (MOAT and FOSTER, 1988). In the less substrate concentration the adaptation to the environment was faster than in the highest concentration due to oxygen transfer. However, energy supply in the low substrate level was insufficient to support high enzyme production particularly in the late incubation time. The less activity of late incubation time was also related to enzyme instability.

The extracellular protein concentration tends to decrease in the third day of incubation time, then it was increasing again. The results occur due to the protein concentration in the filtrate not only affected by mold excretion, but from the soluble protein of CM.

Table 1. Enzyme activity of β-mannanase produced by *E. javanicum* on the substrate containing 1, 2, or 3% CM

Substrate concentration (%)	Incubation time (days)	Activity (U/ml)	Protein (μg/ml)	Specific activity (U/mg.prot.)
1	1	0.1^{a}	70^{a}	1.7^{a}
	2	7.7^{a}	96^{bc}	80.3^{ab}
	3	39.4^{bc}	42^{a}	1037.9^{d}
	4	36.2^{bc}	87^{bc}	613.5^{bc}
	5	42.8^{bc}	120^{bc}	378.5^{bc}
	6	36.8^{bc}	116^{bc}	330.1^{b}
	7	41.4^{bc}	141^{cd}	291.8^{ab}
2	1	0.5^{a}	122^{c}	4.0^{a}
	2	29.4^{b}	120^{bc}	246.2^{ab}
	3	37.3^{bc}	90^{bc}	424.6^{ab}
	4	49.1^{cd}	98^{bc}	542.2^{bc}
	5	62.0^{d}	106^{bc}	592.4^{bc}
	6	37.2^{bc}	145^{cd}	260.6^{ab}
	7	46.0^{c}	72^{a}	644.2^{c}
3	1	0.4^{a}	211^{d}	2.0^{c}
	2	13.6^{a}	145^{cd}	93.4^{ab}
	3	31.0^{b}	83^{b}	374.4^{bc}
	4	42.0^{bc}	96^{bc}	456.5^{bc}
	5	136.2^{e}	176^{d}	780.7^{cd}
	6	54.8^{cd}	137^{c}	400.4^{bc}
	7	48.2^{cd}	70^{a}	693.6^{c}

Different letters in the same column show significantly different results between treatments ($P<0.05$)

Therefore, for the first day incubation time, the highest concentration of extracellular protein was obtained from 3% CM. The soluble protein is decreasing in the course of incubation time due to the mold metabolism activity. The protein concentration also affected the specific activity of β-mannanase. The highest specific activity was obtained from the concentration of 1% CM at the third day (1037.9 U/mg. protein) which was not significantly different to the one obtained from 3% CM at the fifth day (780.7 U/mg. protein). The non significantly difference occurred due to high variation between replication in the data of 1% CM. From all data, it can be concluded that the 3% CM was better for enzyme production, since it was higher in protein concentration and enzyme activity.

The highest saccharification activity towards CM and its specific activity from the first experiment was also obtained in the substrate concentration of 3% and incubation time of five days (Table 2). Although the composition of complex carbohydrate including mannan should be different between coconut meal and locust bean gum, the changes of saccharification activity towards gum locust bean (β-mannanase activity) and coconut meal along treatments were very similar. Therefore, the activity of β-mannanase on gum locust bean already showed the ability of enzyme to digest CM or the enzyme can be used as an additive for a feed containing high CM ration.

The discovery of the highest enzyme activity in the substrate concentration of 3% and incubation time of five days opened the possibility to increase the enzyme activity at higher substrate concentration. Therefore, in the second experiment the enzyme production was determined at the higher CM concentration of 3, 4 and 5% and at incubation time of five and six days (Table 3). The results showed that higher substrate concentration than 3% did not enhance the enzyme activity. The coconut meal concentration of 4 and 5% might reduce the oxygen transfer which was very important for the metabolism of the mold (MOAT and FOSTER, 1988). Those concentrations also limited the mixture effect of shaker. Longer incubation time did not

overcome the negative effects. Results from the first and second experiments showed that the best production of β-mannanase from *E. javanicum* was obtained at 3% of coconut meal in the incubation time of five days. These results were in agreement with the result of ZAMORA *et al*. (1989) which obtained the optimal CM concentration to produce β-mannanase from *Streptomyces* (an actinomycete instead of mold) at 3%. Five days of incubation time was shorter than the optimal period which was needed to produce mannanase from *Trichoderma harzianum* on the substrate containing locust bean gum (galactomannan) or konjac (glucomannan) that was eight days (TORRIE *et al.,* 1990). The same optimal incubation time of 5 days was also observed in the production of β-glucosidase, β-mannosidase and α-galactosidase from *E. javanicum* (HARYATI *et al.,* 1995). Those results are important, since all enzymes are needed to digest complex mannan (MCCLEARY and MATHESON, 1986).

Table 2. Saccharification activity towards CM of enzyme produced by *E. javanicum* on the substrate containing 1, 2, or 3% CM

Substrate conc. (%)	Incubation time (days)	Sacc. Activity (μmol)	Specific sacc. act.(μmol /mg.prot.)
1	1	0.01^{a}	0.2^{a}
	2	1.91^{c}	20.4^{bc}
	3	1.88^{c}	49.8^{d}
	4	1.84^{c}	31.4^{bc}
	5	4.84^{f}	41.8^{cd}
	6	1.62^{c}	14.7^{ab}
	7	0.46^{b}	3.3^{ab}
2	1	0.08^{a}	0.7^{a}
	2	1.94^{c}	16.3^{b}
	3	2.09^{d}	23.9^{bc}
	4	2.91^{e}	33.0^{c}
	5	5.24^{f}	50.1^{d}
	6	1.46^{c}	10.1^{ab}
	7	0.45^{b}	6.4^{ab}
3	1	0.14^{a}	0.7^{a}
	2	2.31^{de}	16.3^{b}
	3	2.10^{d}	25.3^{bc}
	4	1.83^{c}	19.6^{bc}
	5	7.21^{g}	41.1^{cd}
	6	1.25^{c}	9.1^{ab}
	7	0.50^{bc}	7.2^{ab}

Different letters in the same column shows significantly different results between treatments ($P<0.05$)

Table 3. Enzyme activity of β-mannanase produced by *E. javanicum* on the substrate containing 3, 4, or 5% CM

Substrate concentration (%)	Incubation times (days)	Activity (U/ml)	Protein concentration (μg/ml)	Specific activity (U/mg.prot.)
3	5	172.0^{c}	298^{b}	579^{a}
	6	55.4^{a}	132^{a}	420^{a}
4	5	182.4^{c}	431^{c}	424^{a}
	6	47.5^{a}	154^{a}	361^{a}
5	5	130.6^{b}	400^{c}	329^{a}
	6	48.0^{a}	136^{a}	352^{a}

Different letters in the same column shows significantly different results between treatments ($P<0.05$)

Enzyme characterizations

The optimum pH activity was obtained at pH 5.4-5.8 (Figure 1). The optimum pH of enzyme from *E. javanicum* was lower than the enzyme obtained from other molds such as *Talaromyces* spp. (at pH 6.0-6.6) and higher than that of obtained from *Aspergillus niger* NRRL 337 (at pH 3.2) (ARAUJO and WARD, 1990). Figure 1 showed that small changes at pH of 5.0 from pH 5.4 reduced the activity by 21.2%, while that at pH 6.2 from 5.8 reduced by 50.5%.

Therefore, the activity is much influenced by pH condition. The pH of digestive track in chickens varied from crop to intestines. The pH of crop, proventriculus, gizzard, duodenum, jejenum, and ileum of chicken are 4.39 – 4.82; 4.30 – 4.60; 2.83 – 3.01; 5.95 – 6.40; 6.03-6.62, and 6.36-6.81 respectively (PATRICK and SCHAIBLE, 1980). The optimum pH of β-mannanase was only similar with pH of duodenum, however, low enzyme activity might be performed in other parts of the digestive track. The saccharification activity of enzyme from *E. javanicum* toward coconut meal had maximum activity in one hour incubation (IRIANI *et al*., 1995) or the reaction already starts in less than one hour. That condition is appropriate in a short digestion process in poultry. There is possibility that the digestion starts from the crop and continues to proventriculus. In the gizzard the enzyme might not be working due to its limited activity, but if the enzyme is not completely inactivated by very low pH condition in the gizzard, it will be still working in the duodenum.

The enzyme stability towards different pH condition was performed in Figure 2. The activity was protected at pH 5.8 and 6.5 and reduced at pH 4.5. Although, there is a reduction at pH 4.5, the excess of enzyme addition will improve the digestion. The positive effect of enzyme application from *E. javanicum* in the high ration of CM or soybean meal were reported (TANGENDJAJA *et al*., 1999). SPRING *et al*. (1996) suggested that the protective effect on the enzyme application in low buffer condition might be exerted by other feed components.

The optimum temperature of β-mannanase activity was 50^0C (Figure 3). The body temperature of chicken is 40.6–41.7^0C (PATRICK and SCHAIBLE, 1980). The reduction of enzyme activity by the body temperature has to be considered, the relative activities at 40-45^0C toward optimal temperature (50^0C) were 72.2-75.3%. That reduction could be overcome by higher enzyme addition. That optimum temperature was similar with one fraction of β-mannanases produced by mesophylic *Bacillus* sp. KK01, while other three fractions have optimum temperature at 55-60^0C (HOSSAIN *et al.*, 1996). The pure β-mannanases of *B. subtilis* isolated from Philippine soil has optimum temperature at 55^0C (MENDOZA *et al*., 1994).

The thermal stability of β-mannanase of *E. javanicum* is more related to the storing condition and technology used for mixing the enzyme into feed. The activity of the enzyme was quite stable at 28 and 40^0C, after four hour incubation the activity was more than 100% (Figure 4). Therefore, the enzyme activity in the poultry digestion system was not affected by the body temperature. However, the activity was highly affected at 90^0C. The reduction did not occur in the incubation of 30 seconds, but after 60-120 seconds it was reduced to 66-59.1% (Figure 4).

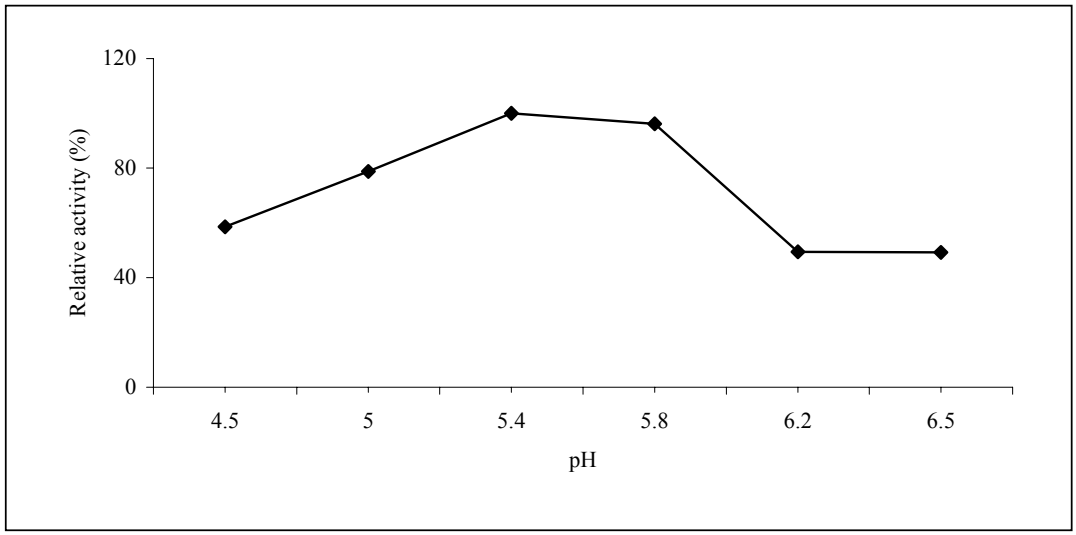

Figure 1. The effect of pH on the activity of β-mannanase produced by *E. javanicum*

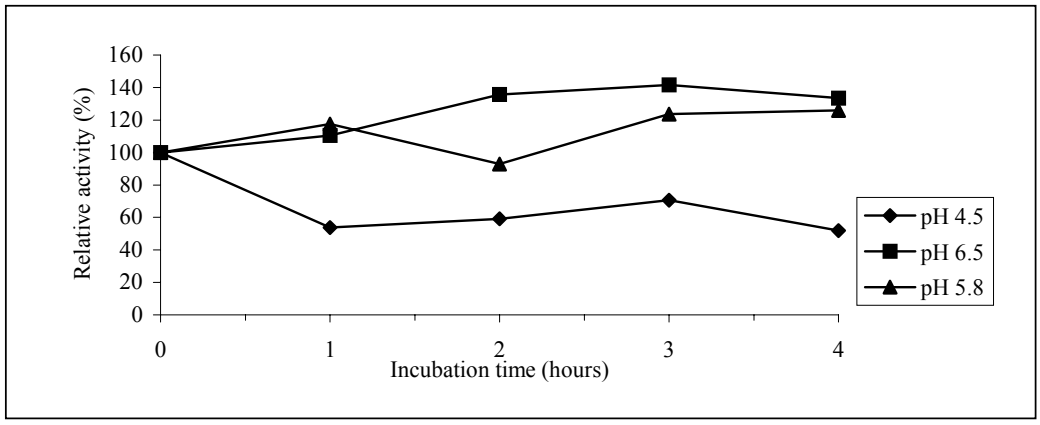

Figure 2. The stability of β-mannanase produced by *E. javanicum* in different pH condition

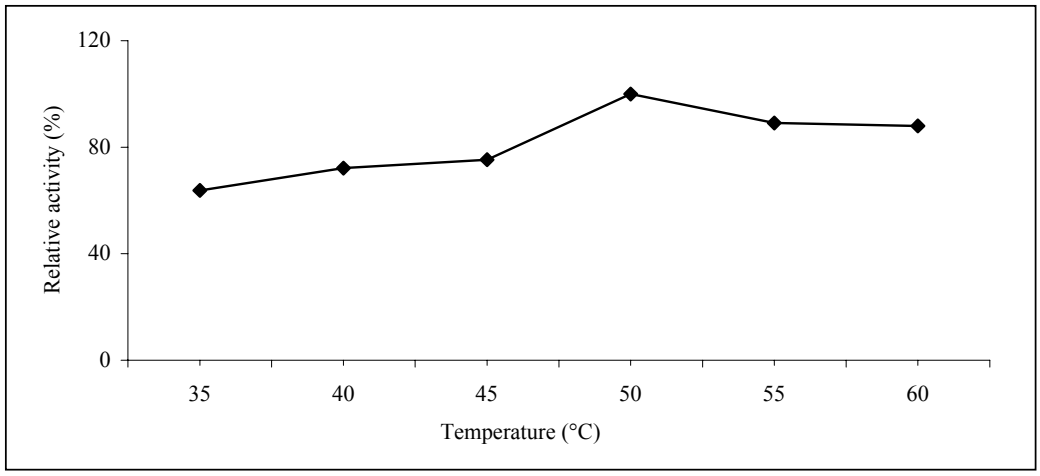

Figure 3. The effect of temperature on the activity of β-mannanase produced by *E. javanicum*

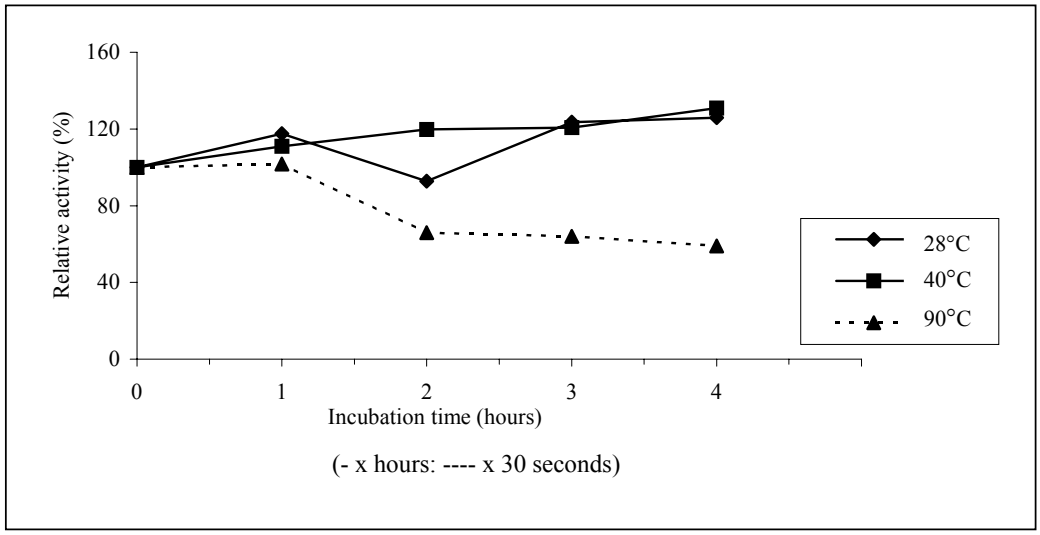

Figure 4. The stability of β-mannanase produced by *E. javanicum* in different temperature condition

SPRING *et al*. (1996) reported that normally, feed is processed in the pelleting equipment for 15 seconds at 60-100^{0}C. In that period of time the activity of β-mannanase produced by *E. javanicum* was not reduced.

The instability of other mannanases towards high temperature was also found from other microorganisms. The mannanases produced by thermophylic molds were more thermostable than by mesophylic molds (ARAUJO and WARD, 1990). The enzymes produced by *Talaromyces* spp. which were thermophylic could be active in the incubation at 75^{0}C, while the one produced by mesophylic mold of *A. niger* NRRL 337 was inactive. The possibility of using the thermotolerant or thermophilic molds to produce enzymes that resistant to high temperature as in the pelleting system, has to consider the body temperature of poultry where the enzymatic process takes place. The low activity in the body temperature causes the loss of benefit. Therefore, the use of mesophylic microorganism to produce hydrolytic enzyme for feed application is still a better choice.

CONCLUSION

The best condition to produce β-mannanase from *E. javanicum* in the submerged culture containing coconut meal was at the concentration of 3% and the incubation time of five days. Although, the optimum pH of the enzyme was at 5.4-5.8, that is only similar with the pH condition of duodenum the enzyme still can work in lower pH. The short time of incubation to digest coconut meal causes the possibility that the enzyme already reacts in the crop. The body temperature of most poultry was less than the optimum temperature (50^{0}C) of enzyme activity, however, the activity reduction could be overcome by higher enzyme addition. The enzyme was unstable at 90^{0}C, therefore feed enzyme processed in the pelleting system should be limited for less than 30 seconds.

REFERENCES

ARAUJO, A., and O.P. WARD. 1990. Extracellular mannanases and galactanases from selected fungi. *J. Ind. Microbiol*. 6:171-178.

BRADFORD, M.M. 1976. A rapid sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. *Anal. Biochem*. 72:248-254.

CAMPBELL, G.L., and M.R. BEDFORD. 1992. Enzyme applications for monogastric feed: A review. *Can. J. Anim. Sci.* 72:449-466.

CHESSON, A. 1987. Supplementary enzymes to improve the utilization of pig and poultry diets. *In*: Recent Advances in Animal Nutrition. W. HARESIGN and D.J.A. COLE (Eds.). Butterworth, London, UK.

HARYATI, T., T. PURWADARIA, J. DARMA and B. TANGENDJAJA. 1995. Production of extracellular glycosidases by *Eupenicillium javanicum* and *Aspergillus niger* NRRl 337 on the coconut meal substrate. Proc. Second Conference on Agricultural Biotechnology. Jakarta, June 13-14, 1995, Indonesia.

HOSSAIN, M.Z., J. ABE and S. HIZUKURI. 1996. Multiple forms of β-mannanase from *Bacillus* sp. KK01. *Enzyme Microbiol. Technol*. 18:95-98.

IRIANI, N., T. PURWADARIA, T. HARYATI and J. DARMA. 1995. Produksi mannanase beberapa isolat kapang mananolitik pada substrat bungkil kelapa (The production of mannanase from mannanolytic molds grown on coconut meal). Simp. Hasil Penelitian dan Pengembangan Bioteknologi II, LIPI-Bioteknologi, Bogor, 6-7 September, 1994, Indonesia. pp: 474-480.

JOHNSON, K.G. 1990. Exocellular β-mannanase from hemicellulolytic fungi. *World. J. Microbiol. Biotechnol*. 6:209-217.

MCCLEARY, B.V. and N.K. MATHESON. 1986. Enzymic analysis of polysaccharide structure. *Adv. Carbohydr. Chem. Biochem*. 44: 147-187.

MENDOZA, N.S., M. ARAI, T. KAWAGUCHI, T. YOSHIDA, and L.M. JOSON. 1994. Purification and properties of mannanase from *Bacillus subtilis*. *World J. Microbiol. Biotechnol*. 10: 551-555.

MILLER, G.L. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal. Chem*. 31: 426-428.

MOAT, A.G. and J.W. FOSTER. 1988. Microbial physiology. Second edition. John Wiley & Sons, Singapore.

PATRICK, H. and P.J. SCHAIBLE. 1980. Poultry: Feeds and nutrition. Second edition. Avi Publishing, Company, Inc, Connecticut, USA.

PURWADARIA, T., T. HARYATI, dan J. DARMA. 1994. Isolasi dan seleksi kapang mesofilik penghasil mananase (Isolation and selection of mesophylic molds producing mannanases). *J. Ilmu dan Peternakan* 7(2): 26-29.

RAVINDRAN, V., W. L. BRYDEN and E.T. KORNEGAY. 1995. Phytates: Occurrence, bioavailability and implications in poultry nutrition. *Poult. Av. Biol. Rev*. 6: 125-143.

RAY, S., M.H. PUBOLS and J. MCGINNIS. 1982. The effect of purified guar degrading enzyme on chick growth. *Poult. Sci*. 61: 488-494.

SPRING, P., K.E. NEWMAN, C. WENK, R. MESSIKOMMER, and M. V. VRANJES. 1996. Effect of pelleting temperature and the activity of different enzymes. *Poult. Sci*. 75: 357-361.

STEEL, R.G.D. and J.H. TORRIE. 1980. Principles and Procedures of Statistics. 2nd Ed. McGraw Hill, New York.

TANGENDJAJA, B., T. PURWADARIA, and M.T. SUHARTONO. 1999. Produksi dan evaluasi selulase, mananase, fitase untuk meningkatkan nilai gizi pakan monogastrik (Production and evaluation of cellulase, mannanase, phytase to improve monogastric nutrient feed). Laporan Riset Unggulan Terpadu Balai penelitian Ternak, Ciawi (Belum seluruhnya dipublikasi).

TORRIE, J.P. D.J. SENIOR and J.N. SADDLER. 1990. Production of β-mannanase by *Thrichoderma harzianum* E58. *Appl. Microbiol. Biotechnol*. 34:303-307.

ZAMORA, A.F., M.R. CALAPARDO, K.P. ROSARIO, E.S. LUIS, and I.F. DALMACIO. 1989. Improvement of copra meal quality for use in animal feeds. *In*: Proc. FAO/CAAS. T.K. MUKHERJEE (Ed.). Workshop on Biotechnology in Animal Production Health in Asia and Latin America, Beijing October 1989. pp. 312-320.