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IDENTIFICATION AND EVALUATION OF FIBER HYDROLYTIC ENZYMES IN THE EXTRACT OF TERMITES (*Glyptotermes montanus*) FOR POULTRY FEED APPLICATION

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ABSTRACT

Poultry are not able to digest fiber in the diet. Hydrolytic enzymes including cellulases and hemicellulases have been used as poultry feed supplement. Termites (*Glyptotermes montanus*) have the ability to digest wood that contains high fiber. The purpose of this experiment was to identify the cellulase and hemicellulase of termite extract. The hydrolytic (saccharification) activity of the termite extract on feedstuffs was then evaluated. It contained high endo-β-D-1,4-glucanase (CMCase) activity, but the activities of avicelase, β-D-1,4-mannanase, β-D-1,4-xylanase, and β-D-1,4-glucosidase were very low. The activities of the enzymes were higher in the fresh extract than those extracted after drying at 40°C with blower oven. CMCase (as cellulase), β-D-1,4-mannanase (as hemicellulase), and β-D-1,4-glucosidase (as glycosidase) were reevaluated further to determine the optimum pH and temperatures for maximum activities. The optimum pH for CMCase, β-D-1,4-mannanase, and β-D-1,4-glucosidase were 6.2, 5.0, and 5.8 respectively, while the optimum temperatures were 45-50°C, 50-55°C, and 42-45°C, respectively. The enzyme mixture or cocktail was more appropriate in digesting feedstuffs with high lignocellulose (fiber) such as rice bran and pollard than feedstuffs with more soluble starch such as soybean and corn meals. The extracted enzyme could be immobilized with pollard, but CMCase recovery was low (28.6%), while β-D-1,4-mannanase and β-D-1,4-glucosidase recoveries were 89.2% and 272.9%, respectively. Termite extract contained enzyme cocktails of lignocellulases that potentially be used as feed supplement. However, its use is limited by its low activity.

[*Keywords*: *Glyptotermes montanus,* enzymic activity, feeds, poultry]

INTRODUCTION

Lignocellulose or fiber is the main compound of plant cell wall that consists of cellulose, hemicellulose, and lignin (Haltrich and Steiner 1994). The concentration of fiber and its component varies on kind of plant materials. Plant by-products such as rice bran containing seed coatings have more fiber than rice that contains more starch or soluble carbohydrate. The concentration of crude fiber in the rice bran is 11.6%, while in the polished rice is 1.4% (Hartadi *et al*. 1980). Poultry feed composes mixture of plant materials that certainly contain fibers. The fiber concentration in the broiler ration is limited to 5% due to the absence of hydrolytic enzymes in its digestion system in opposite to ruminants that contain lignocellulolytic microbes that involve in the digestion of fiber. Incorporation of hydrolytic enzymes such as cellulases and hemicellulases (xylanase or mannanase) in the poultry ration enhances feed efficiency (Ray *et al.* 1982; Chesson 1987; Campbell and Bedford 1992; Jackson *et al*. 1999).

Lignocellulases are commercially produced from many kind of microbes, however, they have much lower protein specific activity than amylase. For example, the activity of commercial cellulase produced from *Trichoderma viride* from Sigma-Aldrich Company is 6-10 units per mg protein (product number of C 1794), while that of α-amylase produced from *Bacillus amyloliquefaciens* is 1,000-1,500 units per mg protein (product number of A 0521). The low specific activity of cellulase results the need of large amount of the enzyme in its application which influences the enzyme cost.

Termites (*Glyptotermes montanus*) have the ability to digest wood that contains high fiber, due to the enzyme activity produced by microbes including flagellated protists, yeasts, and bacteria in the termite nest or in its digestive tract (Itakura *et al*. 1997; Brune 1998; Cook and Gold 2000). Recent reference reported that the cellulase gene of termites had been sequenced and the sequence was unlikely from microbial origin (Watanabe *et al*. 1998). This result suggested that the termite itself possibly produces cellulase not only microbes in its digestive tract. However, the function of the gene related to cellulase production was not reported.

Three kind of enzymes involve in the digestion of cellulose, i.e.: (1) endo-β-D-1,4-glucanase which randomly hydrolyzes cellulose into cellodextrin, cellobiose, and glucose; (2) exo-β-D-1,4-glucanase

which removes cellobiose from the non-reducing end of cellulose chain; and (3) β-D-1,4-glucosidase which breakdowns cellobiose into two glucose molecules. Beside the action in the different areas of the cellulose structure, the activity of the enzymes to digest cellulose is much influenced by the structure of cellulose that contains crystalline and amorphous areas (Purwadaria 1995). The activities of amorphous and crystalline cellulases are respectively detected by carboxymethyl cellulase (CMCase) and avicelase.

Enzymes involved in the digestion of hemicellulases are more complex than those of cellulases due to its structure that may contain xylan, galactan, mannan, glucomannan, galactomannan, arabinan, and glucoronoxylan. Endo-β-D-1,4-xylanase and endo-β-D-1,4-mannanase randomly digest the middle chain of xylan and mannan respectively, while glycosidases such as β-D-1,4-xylosidase, α-D-1,6-galactosidase, β-D-1,4-glucosidase, and β-D-1,4-mannosidase remove the end chain of xylan, galactomannan, glucomannan, and mannan (McCleary and Matheson 1986).

The extract of termites contains lignocellulases. Nakashima and Azuma (2000) reported distribution and properties of β-D-1,4 endoglucanase from the digestive tract of *Coptotermes formosanus*. The activity of xylanase could be detected in the termite gut, where xylanolytic bacterium, *Bacillus* sp., was isolated (Shimizu *et al*. 1998). All mixture of bacteria from the guts of *Nasutitermes takasagoenis* could degrade 28% dealkalized lignin, while one of isolated bacterium *Burkheholderia cepacia* KK01 degraded lignin dimmer compounds by 60-95% (Kato *et al*. 1998).

The termite enzyme could be used as poultry feed supplement by directly mixing the dried whole termites in the broiler diet (Ketaren *et al*. 2001; Uhi *et al*. 2001). The inclusion of the termites in the ration increased the feed efficiency resulted by its lignocellulases that could be extracted and used as feed supplement. The use of termite enzyme extract for poultry feed is influenced by its enzyme component, as well as its pH and temperature.

The research was conducted to evaluate all enzyme components of cellulases (CMCase and avicelase for digestion of amorphous and crystalline cellulose respectively), hemicellulases (β-D-1,4-mannanase and β-D-1,4-xylanase) and β-D-1,4-glucosidase of the extract of termites. The effect of all enzyme cocktails in the extract towards materials generally used for poultry ration such as rice bran, wheat pollard, and corn meal is determined. In addition, the possibility of enzyme from termite extract to be preserved in the dry condition through immobilization in pollard is also evaluated.

MATERIALS AND METHODS

Termites and Feed Supply

Worker termites were obtained from rubber plantation from Parungkuda, Sukabumi, West Java. Rice bran, wheat pollard, palm kernel cake (PKC), and dry palm oil mill effluent (POME) were respectively obtained from rice milling (Darmaga, Bogor), wheat milling (PT Bogasari), and palm oil factory in PTP VII, Lampung. Soybean and corn meal were obtained from feed store (PT Indofeed).

Enzyme Extraction

Two kind of extractions were carried out using fresh and dry termites. Worker termites were sorted and kept at -10°C for a night for fresh termites, while for dry termites it was then dried at 40°C in blower oven for a night. Both termites were blended in the McIlvaine buffer at pH 6.2 in the composition of 1:10 (10 g termites in 100 ml buffer). Filtrate or enzyme was separated by centrifugation at 12,000 rpm at 4°C for 15 minutes. It was then added by 0.2% NaN_3 (sodium azide) and kept in -10°C.

Enzyme Activities

The activities of CMCase and avicelase were assayed by determining the reducing sugar produced from CMC and microcrystalline cellulose (Sigmacell-20) as glucose (Haggett *et al*. 1979). Beta-D-1,4-xylanase and β-D-1,4-mannanase (hemicellulases) were determined using Birch Wood xylan and gum locust bean (mannan) as substrates respectively. Xylose or mannose was used as standard for reducing sugar (Rickard and Laughlin 1980; Araujo and Ward 1990). The values are expressed in unit per gram dry matter ($U\ g^{-1}$), where one unit liberates one mmol glucose, xylose or mannose per minute in assay condition (pH 6.2, 45°C other wise stated), while gram dry matter was the dry weight of termites in the extract. The activity of β-D-1,4-glucosidase was assayed using p-nitrophenyl β-D-1,4-glucoside as substrate and one unit liberates one mmol nitrophenol per minute (Ide *et al*. 1983) in assay condition (pH 6.2, 45°C other wise stated). Specific activity of all enzymes was also calculated in unit per gram soluble protein.

Determination of Optimum Temperature and pH

The major activities of enzymes extracted from fresh termites were determined at 37°C (the most optimum temperature for bacterial cellulases) at pH 4.0, 5.0, 5.8,

6.2, 6.5, 7.0, and 7.5 to obtain optimum pH. Two kind of buffers were used, i.e. phosphate buffer for pH 6.2-7.5 and McIlvaine buffer for pH 4.0-6.2. Determination of the optimum temperature for the enzyme assays was carried out at optimum pH and at various temperatures: 27, 32, 37, 39, 42, 45, 50, 55, and 60°C.

The Saccharification Activity on Feedstuffs

The saccharification activities were determined following the determination of avicelase using rice bran, wheat pollard, PKC, dry POME, soybean, and corn meal as substrates. The optimum incubation time of the reaction was firstly determined for 1, 2, 4, 8, 14, 24, and 48 hours and reducing sugar produced was determined with DNS method (Haggett *et al*. 1979) at pH 6.2, 45°C. The activity is stated in µmol glucose produced per one minute in assay condition (pH 6.2, 45°C)

Determination of Soluble Protein Concentration

Protein concentration in the extract enzyme was determined by Bradford method (Bradford 1976) using the dye solution, *coomassie blue* G 250 and bovine serum albumin was used as a standard. The concentration was detected by mixing 0.1 ml of the sample with 5 ml dye solution and the absorbance was read at 595 nm.

Enzyme Immobilization with Pollard

The fresh enzyme of 4 ml from the extract of 1:10 (w:v) was mixed with 4 g finely ground pollard (0.5 mm). The mixture was then frozen and dried with vacuum dryer. Enzyme was extracted again with McIlvaine buffer pH 6.2 in the way like termite extract. Activity recovery of immobilized enzymes was determined using ratio of activity obtained from the immobilized enzyme extract with the activity of enzyme added.

RESULTS AND DISCUSSION

Identification of Enzyme Activity

The fresh extract of termites using McIlvaine buffer pH 6.2 contained all kind of enzymes assayed, those were CMCase, avicelase, β-D-1,4-mannanase, β-D-1,4-xylanase, and β-D-1,4-glucosidase (Table 1). The highest enzyme activity was observed on CMCase or endo-β-D-1,4-glucanase that digested amorphous cellulose, while the enzyme that responsible in digesting crystalline cellulose (avicelase) was very low. These data suggest that the extract was not suitable for fiber digestion since most natural fiber contain high crystalline cellulose.

Table 1. Activities of enzymes extracted from fresh termites at pH 6.2 and 45°C.

Kind of enzymes	Enzyme activity (U g^{-1} DM)	Specific activity (U g^{-1} soluble protein)
CMCase	535.15	4,932
Avicelase	0.14	1
β-D-1,4-mannanase	8.84	82
β-D-1,4-xylanase	4.19	39
β-D-1,4-glucosidase	0.47	4

DM is dry matter of termites.

The ability of termites to digest wood might be related to the activity of microbes in the nest, or the ligninase in the digestive tract (Sands 1970; Kato *et al*. 1998). Activity of endo-β-D-1,4-glucanase (CMCase) of the fresh termite extract was higher than other enzymes. The similar result was also reported in *C. formosanus* (Nakashima and Azuma 2000). Five kind of CMCases were isolated from the extract of digestive tract of the termites, i.e. EG-A, B, C, D, and E. The EG-A, B, and E were isolated from salivary glands, while EG-C and D might be produced by protozoa in the hindgut (Nakashima and Azuma 2000). They also determined the digestion mechanism of pure EG-E on amorphous cellulose and reported that the mechanism was similar to CMCase collected from other termites and roach.

Nakashima and Azuma (2000) also reported that the specific CMCase activities of salivary glands, foregut, midgut, and hindgut of termites varied from 326 to 8,420 U mg^{-1} protein. Less high specific activity obtained from termites as the enzyme was extracted from the whole body that contains other soluble protein, while that from *C. formosanus* was extracted from digestive tract, where the enzyme primarily produced. The soluble protein from digestive tract was mostly related to enzyme protein.

The less activity or amount of avicelase might be caused by the microbial population in the digestive tract which did not produce sufficient enzyme for crystalline cellulose digestion. Odelson and Breznak (1985) reported that *Trichomitopsis termopsidis* isolated from the gut of *Zootermopsis* produced the specific activity of CMCase (8.4 mU mg^{-1} protein) that was 11.5 times higher than avicelase (0.73 mU mg^{-1} protein). Digestion towards high crystalline activity of

fiber (wood) might be performed in the nest by fungi and molds (Sands 1970). In our other experiment, a mold producing high crystalline cellulase, *Penicillium nalgiovense* S11, was isolated from the nest of a Termitidae in Ciawi, Bogor (Nurbayti 2002).

The activity of β-mannanase from the extract was higher than β-xylanase, another hemicellulase, however, both activities were much less than that of CMCase. The lower amount of β-D-1,4-xylanase than CMCase was also observed in the extract of digestive tract of *Reticulitermes speratus* (Inoue *et al.* 1997). They also reported that cellulolytic ptotozoa was higher than xylanolytic protozoa in the digestive tract. Feeding termites with xylan reduced some protozoa population in the gut and decreased the metabolism activity. In the opposite the activity increased when cellulose was fed to the termites. The correlation between microbial population and kind of enzymes is related to the inducer and repression regulation in the lignocellulase production. The detection of β-D-1,4-mannanase in the termites has not been reported, but its presence in the termites might be related to the environment containing high mannan. Beside xylanolytic protozoa, xylanolytic bacteria might produce xylanase in the digestive tract of termites. *Bacillus* sp. and *B. pumilus* were isolated from termite gut (Shimizu *et al*. 1998; Ardiningsih 2002). The activity of β-glucosidase was also detected in the extract of *C. formasanus* (Asada *et al*. 1999). The enzyme was distributed to salivary glands (17.5%), foregut (1.3%), hindgut (2.4%), and remaining other body parts (21.6%). It was suggested that the enzyme participates in the energy metabolism of the worker termites.

The activities of CMCase (as cellulase), β- D-1,4-mannanase (as hemicellulase), and β-D-1,4- glucosidase (as glycosidase) were selected for further characterization. Avicelase and β-D-1,4-xylanase were not continued studying due to low activities. Firstly, the optimum pH and temperature for the three enzymes were detected. The optimum pH for CMCase, β-D-1,4-mannanase, and β-D-1,4-glucosidase were 6.2, 5.0, and 5.8, respectively (Fig. 1), while the optimum temperatures were 45-50°C, 50-55°C, and 42-45°C, respectively (Fig. 2). The optimum pH and temperature were specific for each enzyme although produced from the same microbe or had the same substrate activity (isoenzyme). The optimum pH range of the three enzymes was similar with the pH of foregut and the end part of the hindgut of *Thoracotermes macrothorax* (Brune 1998).

The higher amount of CMCase and β-D-1,4-glucosidase in the salivary gland near the foregut (Asada *et al*. 1999; Nakashima and Azuma 2000) might influence their optimum pH. The optimum pH of the highest CMCase activity, EG-E, was 6.0, while that of β-glucosidase was 5.0. Both enzymes were collected from salivary glands of *C. formasanus* (Asada *et al*. 1999; Nakashima and Azuma 2000). The optimum temperatures of the three enzymes from termites were 42-50°C, which were favorable to the environment of the termites including the microbial population in the digestive tract where the enzymes produced. Asada *et al*. (1999) and Nakashima and Azuma (2000) reported

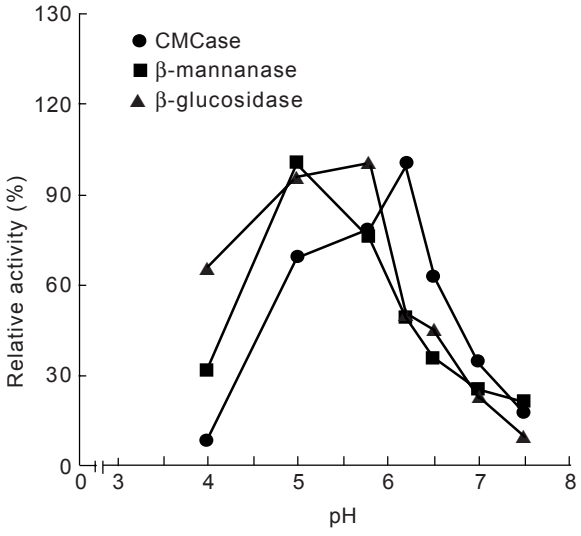

Fig. 1. Optimum pH for CMCase, β-D-1,4-mannanase, and β-D-1,4-glucosidase activities extracted from fresh termites. Each enzyme was assayed at 37°C.

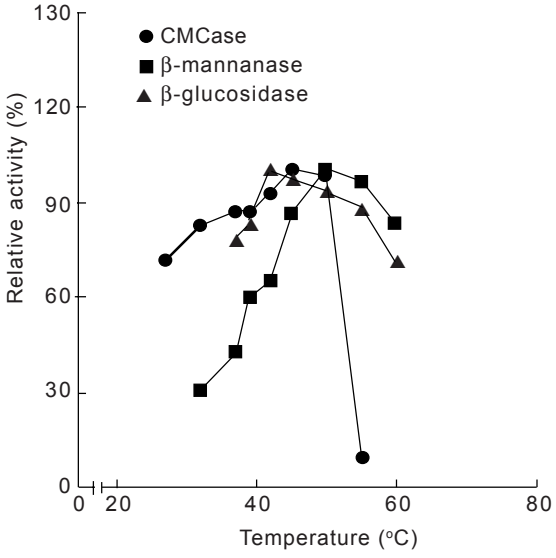

Fig. 2. Optimum temperature for CMCase, β-D-1,4 mannanase, and β-D-1,4-glucosidase activities extracted from fresh termites. Each enzyme was assayed at optimum pH.

that the optimum temperature of EG-E and β-glucosidase was 50°C.

The optimum pH activities of CMCase, β-D-1,4-mannanase, and β-D-1,4-glucosidase from termites were similar to the poultry intestines including duodenum, jejunum and ileum, i.e. 5.95-6.81 (Patrick and Schaible 1980). Although the activities were very low in the lower pH, the enzymes were still active at pH 4 (Fig. 1), or very possible the enzymes especially for CMCase start digesting process in the crop and proventriculus (pH 4.3-4.6). The low pH in the gizzard (2.94) will cause problem for enzyme activities. However, it was indicated that some feed components might protect enzyme activities (Spring *et al*. 1996).

The optimum temperature of CMCase, β-D-1,4-mannanase, and β-D-1,4-glucosidase from termites was appropriate for poultry body temperature (Fig. 2). Except for β-D-1,4-mannanase (64.7%), the relative activities of CMCase and β-D-1,4-glucosidase at poultry body temperature of 40.6-41.7°C are 91.9% and almost 100%, respectively.

The use of dry termites in poultry feed is thought to be more convenient than fresh termites due to better preservation and longer storage. Therefore, the activities were compared between enzymes extracted from fresh and dry termites (Table 2). Although the extract of enzyme had optimum activity at 40°C, drying with blower oven at 40°C reduced activities of all enzyme components. The incubation time of enzyme assays was 30-60 minutes, while drying was kept over the night (+ 24 hours). The long period of time affected the enzyme stability and reduced enzyme activity. The highest reduction was observed in CMCase that reached 70%. It is possible that the temperature (40°C) denatured protein in the extract including the enzymes and reduced the soluble protein concentration in the extract. However, the enzyme denaturation was much less than other protein. This is shown by the reduction of specific activities based on the protein concentration which were less than the reduction of activities based on the dry matter (Table 2).

Although the enzyme activities were higher in the fresh extract, *in vivo* evaluation shows that better digestion was obtained from the dry termites (Ketaren *et al*. 2001). The authors could not explain the reason for the differences. Present results explained that the fresh termites might have excess the optimum levels. According to Michaelis-Menten coefficient, too high enzyme activities or too low concentration of subtrates reduce the digestion capacity of enzymes (Suelter 1985).

The Saccharification (Hydrolytic) Activity of Enzymes on Different Feedstuffs

The effectiveness of enzyme application as feed supplement is influenced by period of incubation time for hydrolytic action. The action related to the fiber structure and concentration of the feed as well as the activity of enzyme components. The period of incubation is limited by the poultry digestion process in the digestive tract, which takes approximately 3-4 hours. The extract of termites was capable digesting the fiber (carbohydrate) of rice bran, wheat pollard, PKC, and POME into reducing sugar (Fig. 3). The formation of reducing sugar was increasing in the course of incubation time. For all substrates (feedstuffs), the formation of reducing sugar started from 1 hour and reduced after 14 hours. It could be concluded that enzymes of the extract or whole termite application might take part in the digestion in poultry gastro intestinal tract.

The amount of reducing sugar was influenced by kind of substrates (Fig. 3). Determination of reducing sugar produced towards time of incubation (saccharification activity) is performed in Table 3. The most appropriate substrate for the enzyme was rice bran followed by wheat pollard, POME, soybean meal, corn meal, and PKC. Although avicelase activity of termite extract was low (Table 1), the higher saccharification activities were obtained when using rice bran and wheat pollard that contain more fiber than soybean and corn meal containing more soluble carbohydrate such as starch. These data showed the possibility of termite extract having more lignocellulases (including high CMCase and ligninase) than amylases. The low

Table 2. Activities of CMCase, β-D-1,4-mannanase, and β-D-1,4-glucosidase extracted from fresh and dry termites at the optimum pH and temperatures.

Enzymes	Activities (U g^{-1} DM)		Specific activity (U g^{-1} protein)		Optimum condition	
	Fresh	Dry	Fresh	Dry	pH	Temperature (°C)
CMCase	535.15	159.00	4,932	3,057	6.2	45
β-D-1,4-mannanase	10.26	4.66	95	90	5.0	50
β-D-1,4-glucosidase	0.92	0.55	9	11	5.8	42

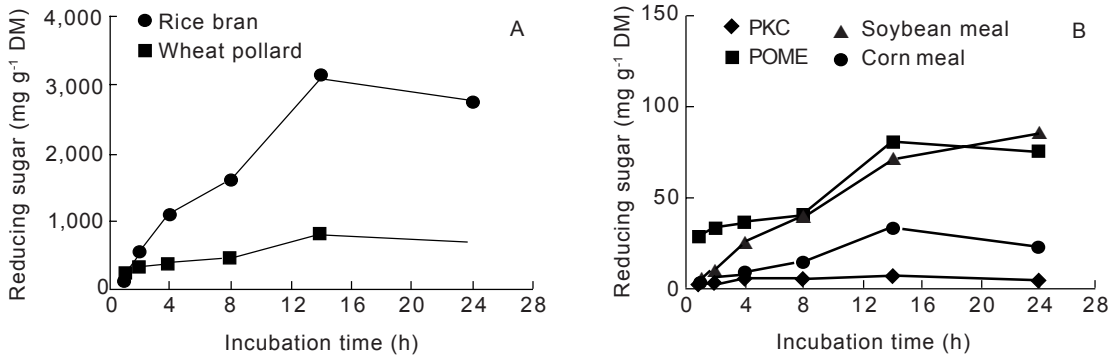

Fig. 3. Reducing sugar produced in the saccharification of feedstuffs by fresh enzyme extract of termites; A = rice bran and wheat pollard, B = palm kernel cake (PKC), palm oil mill effluent (POME), soybean, and corn meals. All reactions were calculated to the same concentration of enzyme addition. DM is dry matter of termites.

Table 3. Saccharification activities of enzyme extracted from fresh termites on feedstuffs at pH 6.2 and 45°C and for 4-hour incubation.

Feedstuffs	Activity (μmol g^{-1} DM)
Rice bran	25.30
Wheat pollard	8.32
Palm kernel cake	0.11
Palm oil mill effluent	0.78
Soybean meal	0.56
Corn meal	0.17

DM is dry matter of termites.

saccharification activity in the more soluble material might be also influenced by the repression effect of reducing sugar in the more soluble carbohydrate materials. Surprisingly, the saccharification activity on PKC was lower than that on POME. It was reported that POME was more difficult to be digested than PKC by enzyme produced from *Eupenicillium javanicum*, a mold isolated from palm oil seed (Purwadaria *et al*. 2003). The termites might contain more specific enzyme for the fiber of POME, while *E. javanicum* had high β-D-1,4-mannanase that was more specific for the digestion of mannan and galactomannan in PKC. The cellulose and lignin contents of PKC were 14.2% and 20.5% respectively, while those of POME were 20.8% and 25.6% (Purwadaria *et al*. 2003).

Immobilization of Extract Enzyme with Wheat Pollard

The termite extract enzyme could be immobilized with pollard, however, the recovery of CMCase was very low (28.6%), while the recoveries of β-D-1,4-mannanase and β-D-1,4-glucosidase were 89.2% and 272.9%, respectively (Table 4). The low CMCase recovery activity occurred due to the strong association between enzyme and the carrier (pollard). Pollard contains 10% crude fiber or quite high cellulose, while the concentration of mannan should be less than the cellulose. Therefore, association between CMCase and pollard is stronger than that of β-D-1,4-mannanase and β-D-1,4-glucosidase. The strong association might reduce the enzyme dissociation from the carrier, acted as substrate competition, and reduce the enzyme recovery. The substrate of β-D-1,4-glucosidase is short oligosaccharides especially cellobiose, that is soluble affecting more dissociation. Although the dissociation of β-D-1,4-glucosidase from pollard was high, the recovery that would be impossible more than 100%. The cations in the pollard might work as coenzyme and enhance the activity and

Table 4. Activity recoveries of CMCase, β-D-1,4-mannanase, and β-D-1,4-glucosidase in the pollard immobilization.

Steps of treatment	CMCase activity (U g^{-1} pollard DM)	β-D-1,4-mannanase (U g^{-1} pollard DM)	β-D-1,4-glucosidase (mU g^{-1} pollard DM)
Extract enzyme	16.87	0.31	34.30
Immobilized enzyme	4.82	0.28	93.60
Activity recovery (%)	28.60	89.20	272.90

increase the recovery. It could be concluded that the immobilization with pollard could be used to preserve enzyme in the dry condition, however, for application it should be further evaluated. The same method has been done for β-D-1,4-xylanase of *Bacillus pumilus* PU 4-2 (Marbun 2003) and β-D-1,4-mannanase of *E. javanicum* (Tangendjaja *et al*. 1997).

Although the termite extract enzyme contains cocktails of lignocellulases, the activity of crystalline cellulose (avicelase) and hemicellulases per termite mass was not high. However, this evaluation could answer the positive effect of termite supplement in the poultry ration (Ketaren *et al*. 2001; Uhi *et al*. 2001). The lignocellulolytic microbes in the digestive tract and nest are more appropriate to be isolated and selected to produce enzymes. The production of microbes will be faster and easier than that of termites (insects). Certainly, many kind of microbes need to be selected to get the activity as complete as termite extract. For advanced facilities, the DNA of the microbes in the digestive tract or termites might be cloned for maximum enzyme production.

CONCLUSION

The extract of termites contained high endo-β-D-1,4-glucanase (CMCase) activity, but the activities of avicelase, β-D-1,4-mannanase, β-D-1,4-xylanase, and β-D-1,4-glucosidase were very low. The activities of the enzymes were higher in the fresh extract than those extracted after dried at 40^{o}C with blower oven. The optimum pH for the activities of CMCase, β-D-1,4-mannanase, and β-D-1,4-glucosidase were 6.2, 5.0, and 5.8 respectively, while the optimum temperatures were 45-50^{o}C, 50-55^{o}C, and 42-45^{o}C, respectively. The optimum pH of the enzymes are similar to the pH in poultry intestine. The body temperature of poultry is suitable for CMCase and β-D-1,4-glucosidase, while β-D-1,4-mannanase was reduced to 64.7% from the optimum temperature at 50-55^{o}C .

The enzyme could increase the digestion of poultry feedstuffs containing high lignocellulose. The enzymes digested rice bran better than wheat pollard, POME, PKC, corn, and soybean meals. Immobilization of enzymes with pollard reduced the activity of CMCase and β-D-1,4-mannanase, but increased β-D-1,4-glucosidase. The reduction limits the effect of digestion activity. The possibility to use the termites and enzyme extract for poultry feed additive is depending on culturing the termites. A large amount of termites are needed to obtain the significant units of activity.

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