ASSESSMENT ON COW RUMEN FLUID CELLULOSE-AMYLASE ENZYME ACTIVITY AS AN ALTERNATIVE SOURCE OF CRUDE FIBER DEGRADING ENZYME IN FISH FEED MATERIALS

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Abstract

A study to assess Cow Rumen Fluid Microorganism Cellulose-Amylase as an Alternative Source of Crude Fiber Degrading Enzyme in Fish Food was performed at the Microbiology Laboratory of the Biology Department Unpad and Chemistry Research Laboratory at the Chemistry Department Unpad. This study was aimed to reveal activities of cellulose and amylase enzymes produced by microorganisms in cow rumen fluid, both in the fresh and crude enzyme extract forms, and to compare them to commercial cellulose and amylase enzymes. The design applied for this study was the descriptive method. The parameter observed at the end of this study was the cellulose and amylase enzyme activities in cow rumen fluid, crude enzyme extract from cow rumen fluid, as well as the commercial cellulose and amylase enzymes. The results showed that the cellulose enzyme activity in cow rumen fluid, crude enzyme extract from cow rumen fluid and commercial cellulose enzyme with Novozymes brand were 2.43, 3.06, and 511.49 U/ml, respectively; whereas, the amylase enzyme activity in cow rumen fluid, crude enzyme extract from cow rumen fluid, and Novozymes commercial amylase enzyme were 3.67, 7.08, and 283,819.62 U/m, respectively.

Key words: cow rumen fluid, crude extract enzyme, amylase and cellulose enzymes

INTRODUCTION

Crude fiber is one of the non-starch polysaccharides than is difficult to digest by fish due to the limited amount of digestive enzymes produced by fish, i.e. only from the digestive tract and microflora in the intestine [1]. According standard for fish feed, fish is only able to tolerate less than 10% crude fibers in its feed [6].

The improvement in agricultural waste quality as the source of fish feed can be attained through biological treatments using microorganism or enzyme [8]. The use of external commercial enzyme is one of the efforts applied by the fish feed industry to support the pre-digestion process of crude fibers in the form of cellulose, hemicellulose, and lignin, to convert the crude fibers into a more easily digested form in the fish body, e.g. in the form of poli- or oligosaccharide. However, almost all enzymes used are imported products which are also expensive. Furthermore, there is no certain guarantee that the commercial enzymes will work optimally in the agricultural waste-based fish feed due to the variation of its chemical structures.

Cow rumen fluid is a potential enzyme source because it contains various types of enzymes that can break almost all complex structures in fish food. In addition, rumen is a source of polysaccharide degrading enzyme, especially cellulose and xylanase [9]. Rumen fluid contains α-amylase, galactosidase, hemicellulose, cellulose and xylanase enzymes that are active in xylan and arabinosa [2].

Enzyme has a specific ability to degrade materials according to its type. This ability can be measured through an enzyme activity assessment. The use of enzyme produced by microorganism from cow rumen fluid is also needed to be tested beforehand, so that the ability in to degrade fish feed with high crude fibers will be measurable, especially for cellulose. The assessment will then be the

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The manuscript was received: 16.02.2015
Accepted for publication: 31.03.2015

basic reason for using a more economical natural enzyme as the substitute for the commercial enzymes in the in-vivo agricultural waste pre-digestion process with the purpose of reducing crude fiber, especially cellulose, in agricultural waste-based fish feed.

METHODS

Time and Location

The study was performed at the Basic Microbiology Laboratory of the Biology Department, and the Research Laboratory of the Chemistry Department, Faculty of Mathematics and Natural Sciences, Padjadjaran University. This study is using descriptive methods.

Tools and Materials

The tools and materials used in this research include incubator oven, UV-Vis spectrophotometer, centrifugation device, vortex, autoclave, shaker incubator, air flow laminar, weigh balance, water heater, micro pipette, micro pipette tip, polypropylene tube, eppendorf tube, and glass equipments. The samples used consisted of cow rumen fluid collected from an animal slaughter house in Ciwastra, Bandung, crude enzyme extracts from cow rumen fluid, and Novozymes commercial cellulose and amylase enzymes. The chemicals used are glucose, aquadest, dissolvable starch, CMC (Carboxy Methyl Cellulose), phosphate buffer, 3,5-Dinitrosalycyclic Acid (DNS) reagent.

Procedure

1. Sample Preparation

The rumen fluid was obtained by collecting the whole cow rumen which then stored in its fresh form. Some of the rumen was converted into crude enzyme extract by separating the fluid and solid matters using gauze. Furthermore, the cow rumen fluid was poured into a plastic bottle and stored in a cooler box with a temperature of 39-40^{o}C. This temperature was adjusted to the rumen natural temperature so that the microorganism inside will survive until the time for observation.

2. Establishing the Glucose Standard Curve

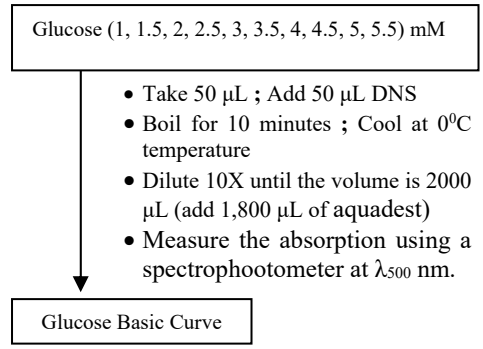

Figure 1. Establishing Glucose Basic Curve

3. α-amylase Activity Assessment

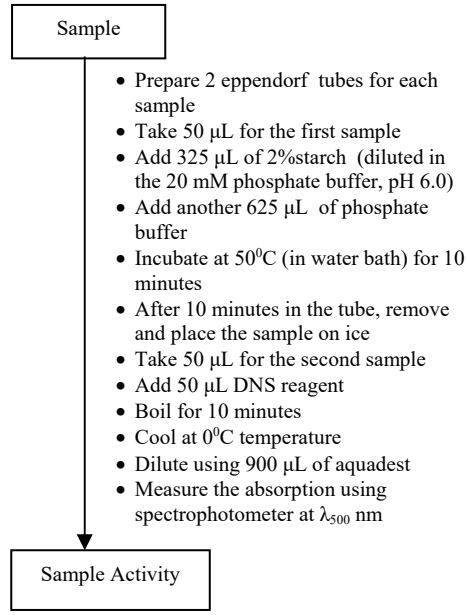

Figure 2. Amylase Enzyme Activity Measurement Chart

4. Cellulose Enzyme Activity Assessment

The determination of cellulose enzyme activity (FP-ase) value was performed through mixing 0.5 ml of enzyme using a piece of 50 mg filter paper with 1 ml 0.05 M citrate phosphate buffer pH 4,8. The mixture was then incubated at 50°C for 1 hour. The reaction was stopped by adding 3 ml DNS (3,5 dinitrosalicylic acid). It was then heated in boiling water for 5 minutes. After cooling

off, it underwent centrifugation t 3,000 rpm for 15 minutes. The reducing sugar was then performed using spectrophotometer at the wave length of 575 nm.

Observation Parameter

The parameters tested in this study were cellulose and amylase enzymes in cow rumen fluid, crude extract enzyme, and cellulose and amylase commercial enzyme activities.

Enzyme activity calculation:

$$\text{Activity (µmol)} = \frac{(glucose)}{10\ minutes}\ x\ \frac{1000}{Venzim} \quad \ldots (1)$$

Notes:

[Glucose] = Glucose concentration from enzyme cutting obtained from sample absorption plot in the glucose basic curve (µmol).

VE = Tested enzyme (µL) Volume

FP = Dilution factor

RESULTS AND DISCUSSION

According to the enzyme activity assessment result, it was revealed that the cellulose enzyme activity in cow rumen fluid, cow rumen fluid crude enzyme extract, and Novozymes commercial cellulose were 2.43, 3.06, and 511.49 U/ml, respectively while the amylase enzyme activity in cow rumen fluid, crude enzyme extract of cow rumen fluid, and Novozymes commercial amylase enzyme of were 3.67, 7.08, and 283,819.62 U/ml (Table 1), respectively.

Table 1 Crude Extract Enzyme, Cow Rumen fluid, Commercial Cellulose (Novozymes) Activities

No	Type	Enzyme Activity (u/ml)	
		Cellulose	Amylase
1	Cow Rumen fluid	2.43	3.67
2	Crude Extract Enzyme	3.06	7.08
3	Commercial Amylase (100000 x dilution)	-	283819.62
4	Commercial Cellulose (10 x dilution)	511.49	-

The cellulose and amylase enzyme activities assessment results for rumen fluid and its crude enzyme extract show that there are internal cellulose and amylase enzyme activities. These activities derive from microorganism secondary metabolite secretion inside the cow rumen fluid and the crude enzyme extract. More than 200 bacterial and protozoa species have been identified from rumen fluid [3]. However, the most important among these microorganisms are cellulose and hemicelluloses digesting-microbes, starch digesting-microbes, sugar digesting-microbes, protein digesting-microbes, lactate users, and methane generators.

The result of cellulose enzyme measurement in the crude enzyme extract from rumen fluid in this study was 3.06 U/ml. This value is higher than that of a study by Murni [5]. i.e. 2.43 U/ml. This finding is related to the fact that rumen microorganism composition is different in each cow. Cow rumen is a complex ecosystem and occupied by various microorganism which amount and type depend on the fish feed [4]. Furthermore, the different of fish feed composition given to the cow will affect the enzyme activity in the rumen. A study by Pantaya [7] showed the same result regarding the xylanase enzyme assessment. The xylanase enzyme activity in cow that is given 100% of alfalfa grass is 528 U/ml [4]. Meanwhile, cows that are given concentrates show an activity of 1.085 U/ml [7].

The cellulose and amylase enzyme activities in cow rumen fluid and crude enzyme extract have different values. The cellulose enzyme activity in cow rumen fluid is 2.43 U/ml while the activity in the crude enzyme extract is 3.06 U/ml. Furthermore, the amylase enzyme activity in cow rumen fluid is 3.67U/ml and the activity in the crude enzyme extract is 7.08 U/ml The cellulose and amylase activity values in the crude enzyme extract is higher than in the cow rumen fluid. This condition is caused by the thickening in the crude enzyme extract preparation process that includes centrifugation and ammonium sulfate

addition. The ammonium sulfate addition results in salting out, where the enzyme is condensed by the high concentration neutral salt. Ammonium sulfate salt is often used in the salting out process due to its high solubility, non toxic nature, and ability to stabilize enzymes [7]. In the end of the salting out process, the enzyme sediment will be formed and the concentration is thicker than that of the cow rumen fluid. Lee [4] stated that in addition to xylanase, the enzymes that are condensed in an ammonium sulfate concentration with 70% saturation are α-amylase and cellulose.

The use of cow rumen fluid and crude extract enzyme as the source of enzyme that degrades cellulose and amylase crude fiber is possible according to the assessment in this study. To use it as an enzyme, an adjustment in the amount and doses is needed, i.e. by comparing its ability to the commercial enzyme that has pure characteristics. The commercial enzymes that are used as comparators in this study are pure cellulose and amylase enzymes from Novozymes. The results of cellulose and amylase activities assessment on the commercial enzyme show that the value of cellulose activity is 511.49 U/ml, while the value of amylase activity is 283819.62 U/ml. Based on these values, 425 ml/gram of cow rumen fluid is needed to degrade cellulose and 394.408 ml/gram to degrade amylum in 1 gram substrate whereas for crude enzyme extract, 91.33 ml is needed to degrade cellulose and 40.088 ml is needed to degrade amylum in 1 gram substrate.

CONCLUSION AND RECOMMENDATION

Conclusion

1. The microorganisms in cow rumen fluid and crude enzyme extract have cellulose and amylase enzyme activities that are sufficient to be used for hydrolysis of fish feed. The cellulose enzyme activities in cow rumen fluid and crude enzyme extract are 2.43 and 3.06 U/ml, respectively. Meanwhile the amylase enzymes activities in cow rumen fluid and crude extract enzyme are 3. 67 and 7.08 U/ml, respectively.

2. Compared to commercial cellulose and amylase enzymes with pure characteristics, the value of cellulose enzyme in cow rumen fluid is 1 : 210 and for amylase is 1 : 166. The value of cellulose enzyme activity in cow rumen fluid crude extract is 1 : 77,335 and for amylase is 1 : 400,875.

Recommendation

Follow up studies on the assessment ofn the ability of the cellulose and amylase enzymes produced by microorganisms in cow rumen fluid and the crude enzyme extract towards high-crude fiber substrateare needed for more accurate measures of their cellulolytic and amylolytic abilities.

ACKNOWLEDGMENT

The authors would like to thank the Rector of Padjadjaran University who have provided funding for implementing and supporting this study through DIPA of Padjadjaran University.

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