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## **Effort of Increasing Production of Livestock Feed out of Cassava Waste by Identifying the more Suitable Cellulotic Degrading Fungi**

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### Abstract

In the bioethanol production process, as much as 90% of waste was produced. The availability of waste production is very important since waste can be processed to become livestock feed. The solid bioethanol waste contains cyanide (HCN) 5.8177 mg/kg, water 95,21%, ash 0,39%, protein 8,16%, crude fiber 5,45%, crude fat 2.06%, and carbohydrates 83,94%. Processing bioethanol solid waste into livestock feed can be done by utilizing the existing fungi on bioethanol solid waste. Crude fiber (cellulose) and carbohydrates are a source of cellulolytic fungi. Cellulolytic fungi can degrade the role of organic materials contained in bioethanol solid waste, so that it can be made as a source of highly nutritious livestock feed. This study aims to determine the types of cellulolytic fungal isolates contained in bioethanol solid waste which is potentially processed to become livestock wool. Descriptive analysis was employed as a method of the study. Furthermore, *Potato Dextrose Agar* (PDA) was used as a medium for culturing and isolating the fungus. Dilution series and pour plate method were employed to isolate the fungus. And, *Moist Chamber* method was employed to identify it. In addition, *Carboxy Methyl Cellulose* (CMC) was used as medium to identify cellulolytic fungi. The process was carried out up to the level of genus based on macroscopic and microscopic characterization. 10 fungal isolates from the genus of *Aspergillus* sp 1, *Aspergillus* sp 2, *Aspergillus* sp 3, *Aspergillus niger*, *Cladosporium* sp, *Mucor* sp, *Penicillium* sp 1, *Penicillium* sp 2, *Rhizopus* sp and *Trichoderma viride* were yielded in this study. The results of examining cellulose enzyme activity revealed that 9 of 10 isolates of the fungus were capable of degrading cellulose. Isolates yielding the largest cellulose enzyme were *Trichoderma viride*, *Penicillium* sp 1, *Cladosporium* sp and *Aspergillus niger*.

**Keywords:** dilution series, cellulolytic fungi, Moist Chamber, bioethanol solid waste, pour plate

### Introduction

Feed is the most important factor in livestock business. The success of livestock business heavily depends on the kind of wool provided. The effort to utilize waste may improve the availability of feed (Agustini, 2010). In general waste quality for feed is very low. An effort to

improve treatment of livestock feed, therefore, is required. By doing so, the content of nutrition and livestock productivity will increase.

One kind of waste used to livestock wool is bioethanol waste utilized as fibrous wool substance. Bioethanol is one of energy

alternatives using plants as its primary substance and it generally needs fermenting. Bioethanol can be made from cassava. Cassava shares important economic value compared with other kind of tubers. In addition to that, cassava contains high starch which is approximately 25-30% and is suitable for alternative energy (Rikana, Heppy and Adam Risky, 2009).

Bioethanol is produced by fermentation which is then proceeding by distillation. This treatment produces waste which is equal to 90% of the fermentation solution. With a large amount of waste, the process of waste treatment becomes intensely important, because it is able to damage environment when it is not handled well (Wira 2011). Bioethanol solid waste can be utilized as livestock woof, but it previously needs to be fermented. Fermentation is undertaken in order to improve nutritious content of woof substance with low quality and omit anti-nutrition material in the form of cyanide (Hidayat 2010).

Enzyme is protein produced by any living organisms. In the fermentation industry, enzyme is produced by fungi or bacteria (Uusima 2006). One kind of enzymes is cellulose enabling to crack cellulose chemically. It has been revealed that the enzyme of cellulose is one of the enzymes which play an important role in processing organic waste bioconversion to become glucose, one-cell protein, livestock woof ethanol and so on. In line with the advance of industry, the need to cellulose enzyme is getting more increasing recently. In Indonesia the industry utilizing enzyme grows excessively; however, the need to enzyme is not yet met. The enzyme should be imported from other countries (Soeka, Yati S. and Sastraatmajda Dudi D, 1992). One of the possible solutions is to utilize a cellulolytic microorganism. It can produce cellulose enzyme that enable cellulose, produced from bioethanol, to degrade. Microorganism capable of degrading cellulose is fungi. The bioethanol waste of cassava contains many cellulolytic fungi capable of degrading cellulose. The genus of fungi producing cellulose enzyme, among others, are *Aspergillus* sp., *Bulgaria* sp., *Chaetomium* sp., *Helotium* sp., *Myrothecium* sp., *Paecilomyces*

sp., *Penicillium* sp., *Phanerochaeta* sp., *Poria* sp., *Rhizopus* sp., (Irawan, 2008), *Schizophillium* sp., *Serpua* sp., and *Trichoderma* sp. (Ganjar and Syamsuridzal, 2006).

Since fungi capably of degrading cellulose are important, the effort to isolate and identify cellulolytic fungi from bioethanol waste of cassava was carried out through this research. The research is expected to find out cellulose fungus isolate playing a role in processing bioethanol waste of cassava into livestock feed.

## Material and Procedure

### Material and Instruments

The materials used in this research are bioethanol solid waste, PDA (medium Potatoes Dextrose Agar), CMC (Carboxil Methil Cellulose), sterile water, aquades, physiologic NaCl, vaseline, Kongo red, alcohol 95%, alcohol 70%, and cloramfenicol. Meanwhile, the instrument used in this research are autoclave, microwave, scale hot plate and stirrer, erlenmeyer retort, calibrated beaker, petri dish, reaction retort, spatula, pH universal indicator, thermometer, calibrated pipette, filler pipette (rubber bulb), ose needle, dropper pipette, filter paper, object glass and cover glass, pincers, cotton and muslin, microscope, sliding calipers, bunsen burner, reaction retort shelf, rubber, tissue, aluminum foil, plastic seal, board marker, label sheet, and scissors.

### Procedure

#### Preparing bioethanol solid waste

Bioethanol solid waste that will be used as a microorganism isolate resource is gained by taking the solid waste, which is later, put into the bottle. In this case, the room temperature where the bottles are placed is maintained, so that the microorganism inside of the substance remains alive when it is examined.

#### Fungi Isolation

The sample of bioethanol solid waste is weighed as much as 1 gram. Then, it is put into reaction retort containing 9 ml NaCl. Afterward, 1 ml of each diluted material got into petri dish and it is poured PDA medium

(medium of Potatoes Dextrose Agar). Then, it is incubated at room temperature for 72 hours.

### Fungal Colony Purification

The colony with different characteristics is re-isolated and re-inoculated repeatedly so that completely pure culture is produced.

### Fungi Identification

Identifying every colony of fungi microscopically is carried out using a morphological observation. Fungal morphological observation includes color monitoring, colony surface, texture, margin or the edge of colony (Ganjar, *et.al.*, 1999). Meanwhile, to observe microscopic cellulolytic fungal identification is conducted by using moist chamber method. Identifying fungal character is synchronized with its character and morphology based on the identification book.

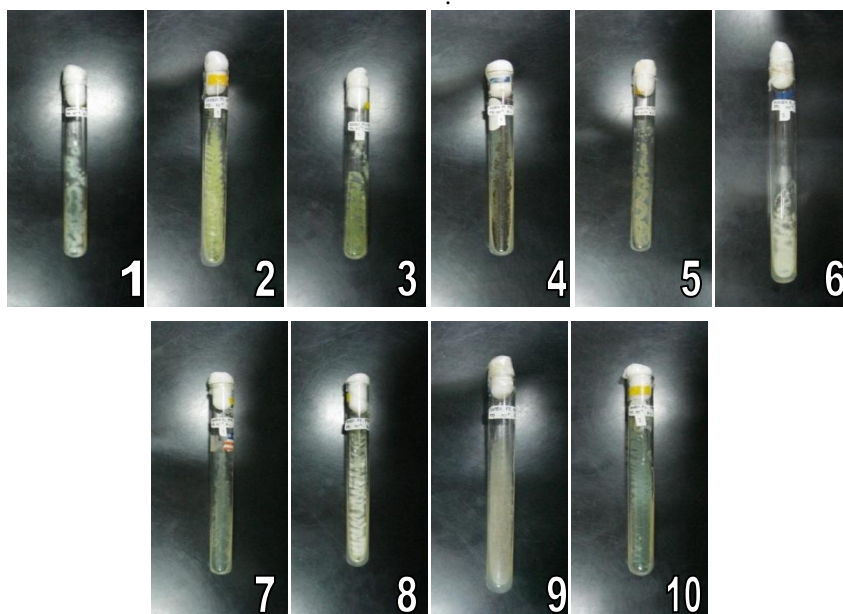
### Cellulose Degradation Activity Test

Fungal isolate is inoculated on PDA medium and incubated by maintaining temperature for 24 hours. After fungal isolate has grown, medium surface is sprinkled by red kongo indicator. It is then flushed by NaCl and observed by measuring the diameter of transparent zone composed around the colony. Ratio between the diameter of transparent zone and that of fungal colony used to know the extent of cellulolytic fungal ability.

### Result and Discussion

#### Cellulolytic Fungal Isolate of Bioethanol Waste of Cassava

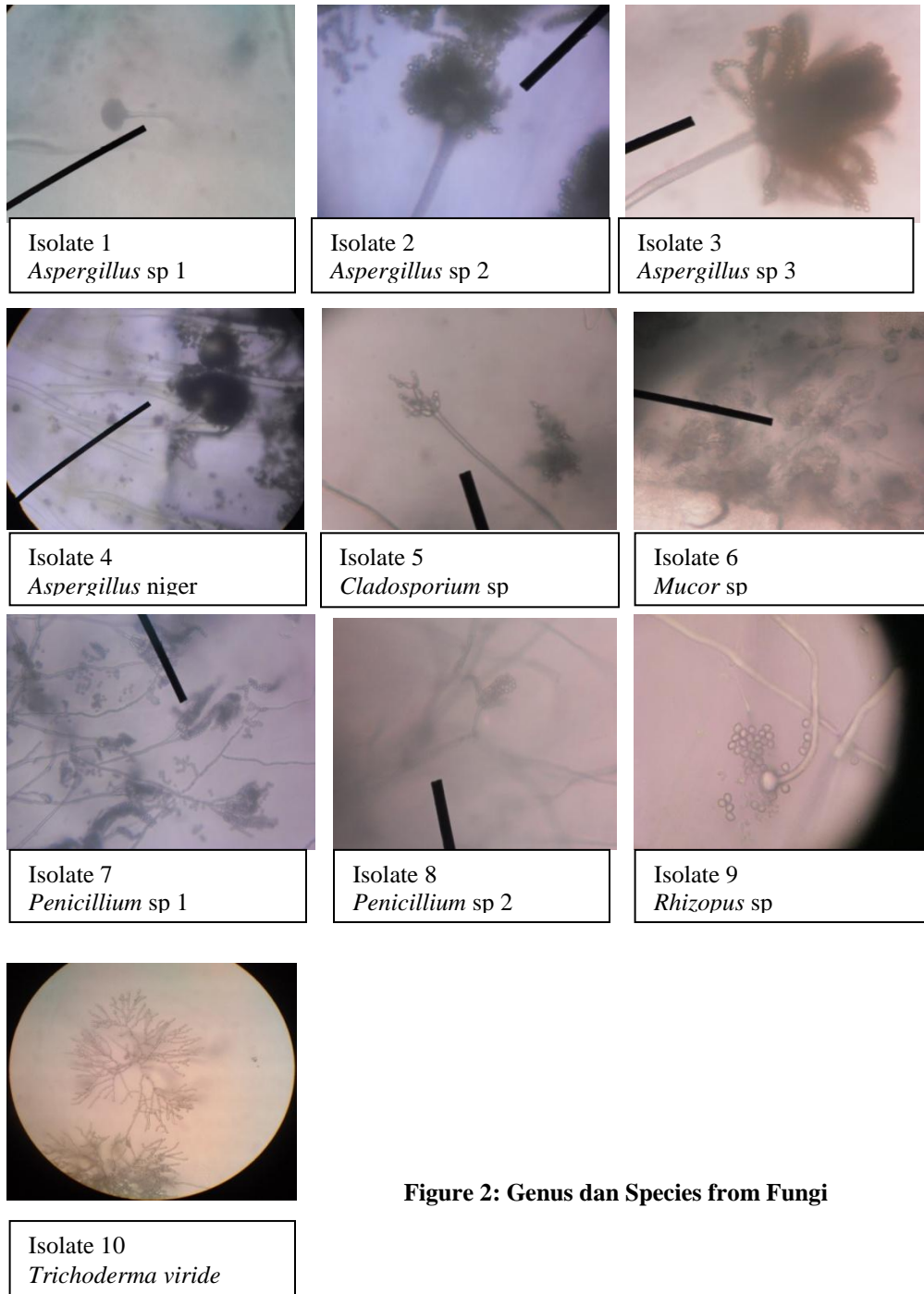
A number of cellulolytic fungi successfully isolated from bioethanol solid waste of cassava are equal to 10 isolates. The result of fungal isolates obtained can be seen on picture 1



**Figure 1: Fungal Isolate**

From the result of identification based on microscopic appearance on moist chamber observation of each fungal isolate, it is

obtained the display of fungal isolate as on picture 2.



**Figure 2: Genus dan Species from Fungi**

**Isolate 1**

Isolate 1 is identified from genus of *Aspergillus* sp 1. The characteristics of macroscopic *Aspergillus* sp 1 isolated on medium of PDA for 7 days with incubation temperature 30<sup>0</sup>C are

dark green color, flat colony surface with crude and granulated texture caused by luxuriance of conidiophores shaped from mycelia on jelly, colorless colony, and inflated margin of colony. *Aspergillus* sp 1 does not share concentric circle

and cannot produce excaudate. Microscopic feature gained is short and one-structured-conidiophores. Outer surface of conidiophores is delicate and its color is hyaline. The tip of conidiophores has vesicular structure resembling wide bludgeon. Upper vesicular surface immediately formed on vesicular is called filial producing conidia. It shapes circle and semicircle.

#### **Isolate 2**

Isolate 2 is identified from genus *Aspergillus* sp 2. The macroscopic features of *Aspergillus* sp 2 isolated on PDA medium on the seventh day with incubation temperature 30°C are yellowish-green color, flat colony surface with crude and granulated texture caused by luxuriant sporulation. By way of contrast, colony has yellow color and inflated texture of margin. *Aspergillus* sp 2 does not have a concentric circle. It produces yellow excaudate. Microscopic features gained are long conidiophores, crude surface of conidiophores with hyaline color, the tip of conidiophores shaping vesicular with semicircular structure, filial structured on vesicular and conidiophores with circular and semicircular structure.

#### **Isolate 3**

Isolate 3 is identified from genus *Aspergillus* sp 3. The macroscopic features of *Aspergillus* sp 3 isolated on PDA medium on the seventh day with incubation temperature 30°C are sort of pale and yellowish-green color, flat colony surface with crude and granulated texture because of luxuriant sporulation. On the contrary, colony has yellow color and has inflated texture of margin. *Aspergillus* sp 3 does not have concentric circle and it produces brownish-yellow excaudate. Microscopic features gained are long conidiophores with hyaline color and crude surface and semicircular shaped vesicular.

#### **Isolate 4**

Isolate 4 is identified from species *Aspergillus niger*. The macroscopic features of *Aspergillus niger* isolated on PDA medium on the seventh day with incubation temperature 30°C are black color caused by luxuriance of structured conidiophores, flat colony surface with crude and granulated texture, and inflated margin of colony. On contrast, colony has black color and

it does not produce excaudate. Meanwhile, microscopic features obtained are long conidiophores with smooth outer surface and brownish-hyaline color, vesicular with circular shape, filial structured on metula with brown color, and circular shaped conidiophores.

#### **Isolate 5**

Isolate 5 is identified from genus *Cladosporium* sp. The macroscopic features of *Cladosporium* sp isolated on PDA medium on the seventh day with incubation temperature 30°C are brownish-dark-green color. On the contrary, colony has blackish-green color, surface resembling a mountain with velvety texture, flat colony margin, no concentric circle and it produces excaudate with hyaline color. In addition to that, microscopic features obtained have a partitioned hypha with multinucleate, conidiophores with lateral shape or terminal on the hypha, conidiophores with chain shape and delicate outer surface. Ramoconodia lies on the basis from septa chain 1 to 2 and has cylindrical shape with delicate outer surface. Conidiophores lie on branching chain with ellipse shape.

#### **Isolate 6**

Isolate 6 is identified from genus *Mucor* sp. The macroscopic features of *Mucor* sp isolated on PDA medium on the seventh day with incubation temperature 30°C are rather creamy white color, flat colony surface with smooth and granulated texture, and flat colony margin. By way of contrast, colony has white color, *Mucor* sp processes no concentric circle, produces no excaudate. Meanwhile, microscopic features acquired are sporangiospora with ellipse shape, calomel with circular shape.

#### **Isolate 7**

Isolate 7 is identified from genus *Penicillium* sp 1. The macroscopic features of *Penicillium* sp 1 isolated on PDA medium on the seventh day with incubation temperature 30°C are rather overcast dark blue color. On contrast, colony has creamy color, flat colony surface with velvety texture and flat colony margin. On the third day, when colony was planted by growing point method shaping like disk, it asserted that *Penicillium* sp 1 owned concentric circle and when it was planted by streak method it had

rather pale-bluish-green color. *Penicillium* sp 1 does not produce excaudate. Microscopic features gained are hyphae having no septa, conidiophores with delicate and transparent shape as well as having many brunches, fialid with cylindrical shape and thick surface, conidiophores with circular and semicircular shape as well as with hyaline color and smooth buttress.

**Isolate 8**

Isolate 8 is identified from genus *Penicillium* sp 2. The macroscopic features of *Penicillium* sp 2 isolated on PDA medium on the seventh day with incubation temperature 30<sup>0</sup>C are white color. On the contrary, colony has white color, crude colony surface, flat colony margin, no concentric circle and excaudate drops. Meanwhile, microscopic features obtained are hyphae having no septa, conidiophores with transparent and delicate shape, fialid with cylindrical shape and thick buttress, conidiophores with circular and semicircular shape as well as with hyaline color and smooth buttress.

**Isolate 9**

Isolate 9 is identified from genus *Rhizopus* sp. The macroscopic features of *Rhizopus* sp isolated on PDA medium on the seventh day with incubation temperature 30<sup>0</sup>C are whitish color then becoming grayish brown caused by brown color from sporangiofor and blackish brown color from sporangia. The surface spreads because of possessing rhizoid and hairy surface. On the contrary, colony has white color. Colony margin is undetermined because *Rhizopus* sp has remarkably rapid growth. On the second day after being inoculated, the diameter reaches 2 cm. on the third day it reaches 7.1 cm and on the following day the petri dish was bursting with *Rhizopus*. *Rhizopus*

does not have a concentric circle and it does not produce excaudate. In addition to that, microscopic features obtained are rhizoid, sporangia with circular and semicircular shape as well as with blackish-brown color, columela with circular shape, sporangiospora with disordered shape. There is one with circular and ellipse shape as well as with line on the surface.

**Isolate 10**

Isolate 10 is identified from genus *Trichoderma viride*. The macroscopic features of *Trichoderma viride* isolated on PDA medium on the seventh day with incubation temperature 30<sup>0</sup>C are dark green color, flat colony surface with delicate and granulated texture and flat colony margin. On the third day of when colony was planted by growing point method shaping like white, yellow, light green and dark green disk, it was conveyed that *Trichoderma viride* had concentric circle and it did not produce excaudate. Meanwhile, microscopic features acquired are flat, partitioned off and branching hyphae shaping braid called mycelium. Conidiophores have branch structuring verticillate. On the tip of conidiophores, it grows cell with structure resembling bottle (fialida).

**Test of cellulose enzyme activity (screening)**

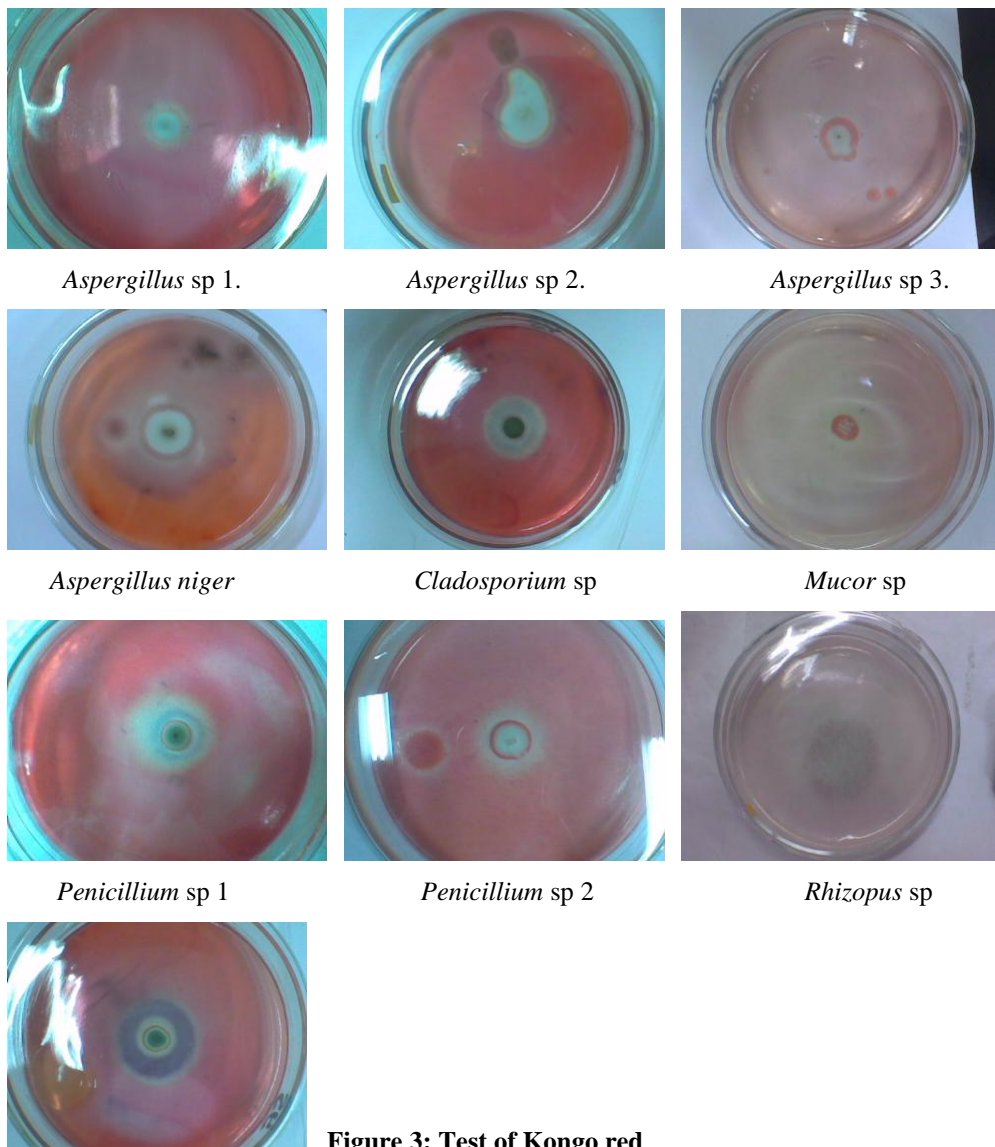
Based on the comparison between transparent zone diameter and colony diameter structured on each isolate, four of ten fungal isolates own huger cellulose degrading ability derived from genus of *Trichoderma viride*, *Penicillium* sp 1, *Cladosporium* sp and *Aspergillus niger*. While one of ten fungal isolates has no cellulose degrading capability. As the comparison between transparent zone diameter and colony diameter formed on each isolate, it can be seen on table 1.

**Table 1: The Result of Testing Cellulose Productability (Screening)**

No	Fungi	DZ (cm)	DK (cm)	IS	Candidate number of Fungal IS
1	<i>Aspergillus</i> sp 1	1.6	1.25	1.28	8
2	<i>Aspergillus</i> sp 2	2.7	2.1	1.28	9
3	<i>Aspergillus</i> sp 3	2.2	1.65	1.3	7
4	<i>Aspergillus niger</i>	4.3	2	2.15	4
5	<i>Cladosporium</i> sp	3	1.25	2.4	3



6	<i>Mucor</i> sp	2.175	1.15	1.89	5
7	<i>Penicillium</i> sp 1	3	1	3	2
8	<i>Penicillium</i> sp 2	2.15	1.35	1.59	6
9	<i>Rhizopus</i> sp	0	7.1	0	10
10	<i>Trichoderma viride</i>	3.25	1	3.25	1



**Figure 3: Test of Kongo red**

*Trichoderma viride*

Fungi are able to degrade cellulose because they can produce cellulose enzyme. The more the production amount of cellulose enzyme on them is, the more rapid cellulose degradation will be (Bagga and Sandhu, 1987 in

Zumrotiningrum *et al.*, 2004). Among four fungal isolates are *Trichoderma viride*, *Penicillium* sp 1, *Cladosporium* sp and *Aspergillus niger*, two of them can be utilized for processing livestock wool from bioethanol

solid waste that is *Trichoderma viride* and *Aspergillus niger*. Because both of them have sufficient high cellulolytic power, it is expected that it can increase protein content, energy, dry material and enables to degrade crude fiber on bioethanol solid waste in order that the waste quality becomes better to consume.

## Conclusion

The research obtained ten fungal isolates growing on bioethanol solid waste. Some of them belong the genus of *Aspergillus* sp 1, *Aspergillus* sp 2, *Aspergillus* sp 3, *Aspergillus niger*, *Cladosporium* sp, *Mucor* sp, *Penicillium* sp 1, *Penicillium* sp 2, *Rhizopus* sp and *Trichoderma viride*. *Trichoderma viride*, *Penicillium* sp 1, *Cladosporium* sp and *Aspergillus niger* are best candidate with the highest ability to degrade cellulose. *Trichoderma viride*, and *Aspergillus niger* can be utilized for processing livestock feed from bioethanol solid waste.

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