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The Effect of Incubation Time on Fermentation of Cassava Bagasse by *Trichoderma Mutant*

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***Abstract*—This study aimed to study the effect of incubation time on fermented onggok using Trichoderma AA1 mutants. The four levels of fermentation duration, including 0 (control), 1, 2, and 3 weeks were treated for onggok fermentation. The experiment was designed with a One-way classification of Completely Randomized Design (CRD). Fermentation medium is made by mixing 1000g onggok, 2g molasses, 2g ammonium sulphate, 30g bran and 1500ml aquades. The fermentation medium was inoculated with 2g Trichoderma AA1 Mutant inoculum, then incubated for 3 weeks. During incubation weeks 0, 1, and 3, observations of temperature, pH, and digestibility value of onggok-fermented dry matter were carried out. To measure dry matter digestibility, onggok-fermented sampling was carried out as much as 20g, then dried for 12 hours at 70°C. The measurement of dry matter digestibility using the gas-test method. The results showed that the fermentation temperature increased very significantly after fermentation lasted one week, then decreased, but still higher than the temperature of the control treatment. pH simultaneously experienced a significant decrease after fermentation lasted for 1, 2 and 3 weeks. While the value of dry matter digestibility was not significantly changed in onggok-fermented 1 and 2 weeks. But the digestibility value decreases in onggok-fermented 3 weeks.**

***Keywords*—*cassava bagasse, fermentation, incubation time, Trichoderma AA1 mutants*.**

I. Introduction

Cassava bagasse is a solid waste that results from the processing of cassava into tapioca. Cassava-bagasse is quite high availability in Indonesia and has nutritive potential for livestock [17]. Most cassava production is absorbed by tapioca industry, so that more than 1.2 million tons of *onggok* are produced every year [1].

Besides production potential, cassava-bagasse has nutritive constraints, namely high cellulose content of 29% and low protein of 1.33% [2]. Cellulose is a typical nutrient that makes up the plant cell wall in the form of D-glucose polymer with bond β-1,4 glycosidic [3]. Cellulose cannot be digested by several types of livestock, including poultry [4].

The fermentation using a cellulolytic microbe in *onggok* is a way to overcome these obstacles. *Trichoderma* AA1 mutants are microbe producing enzyme cellulase and resistant to repression of catabolites in the medium [5]. Several studies have shown that *onggok* fermentation can increase protein content and digestibility [6]; amino acid content, nutrient digestibility and metabolized energy [7]. On the contrary, *onggok* fermentation using *Trichoderma* AA1 mutants can reduce cellulose and crude fiber content [7]. The study aims to study the effect of incubation time on *onggok* fermentation using *Trichoderma* AA1 mutants.

II. Material and Method

A. Preparation of Microbe and Fermentation Medium

Microbes used in this study were *Trichoderma* AA1 mutants, TrichoStar brand in the form of mash produced by the Faculty of Agriculture, Veteran Bangun Nusantara University. Microbe inoculum is made by dissolving 2 g molasses in 200 ml of boiling distilled water in a 500 ml elemeyer tube, then cooled. After that 2 g of *Trichoderma* AA1 mutant are added to molasses solution, then incubated for 24 hours in a rotary shaker incubator.

B. Fermentation

The fermentation medium was inoculated with *Trichoderma* AA1 Mutant inoculum, then incubated in a bioreactor for 3 weeks in aerobic conditions. Every week (starting from week 0 to week 3, according to the treatment applied) an observation of temperature, pH, and digestibility value of dry matter was observed fermented cassava-bagasse. To measure the dry matter digestibility, a sampling of 20g fermented cassava-bagasse was carried out, then dried in an oven for 12 hours at 70oC. The sample is then used to test the value of dry matter digestibility.

C. Measurement technique and statistical analysis

PH measurement is carried out on fermented cassava-bagasse samples in wet conditions using a pH-meter. The temperature measurement is done using a thermometer that has been installed in the bioreactor. The measurements of dry matter digestibility were carried out in vitro using a gas-test method. To determine the effect of time of incubation treatment (week 0, 1, 2 and 3) on pH, temperature, and digestibility of dry matter fermented cassava-bagasse carried out One-way Analysis of Variance (One-way Anova) by Duncan's follow-up test Multiple Range Test (DMRT) [8].

III. Result and Discussion

The results of measurements of temperature, pH and dry matter digestibility values in vitro (KcBK) and the results of statistical analysis can be seen in Table 1.

TABLE 1. AVERAGE TEMPERATURE, PH, AND KCBK FERMENTED CASSAVA-BAGASSE ON A VARIETY OF LONG INCUBATION TREATMENT.

Variables	Time of Incubation (week)			
	0	1	2	3
Temperature, °C	29.00 [a]	37.26[d]	33.52[c]	32.57[b]
pH	5.00 [c]	4.57[b]	4.46[ab]	4.32[a]
KcBK, %	71.52[b]	70.12[b]	62.53 [ab]	57.81[a]

abc on the same line shows the difference Significantly (P<0.05).

A. *Temperature*

The mean temperature in the fermentation bioreactor at week 0 was 29° C, at which time the room temperature was 28-29° C. After one week of fermentation, there was a significant increase in temperature (weeks 1, 2 and 3). From Table 1, it can be seen that the fermentation temperature increased in the first week and then dropped in the second and third weeks. This indicated that the incubation time for 1 week was the most optimal time for cassava bagasse fermentation.

The increase in temperature is thought to be related to the microbe's metabolic process. The microbe's activity in decomposing organic matter, which produces energy in the form of heat, CO_2 and water vapor, causes an increase in temperature. The decrease in fermentation temperature (week 2 and 3) indicates a decrease in microbe activity. Although there was a decrease in fermentation temperature in the 2nd to 3rd week, the fermentation process was still going on. This can be seen from the fermentation temperatures of the 2^{nd} and 3^{rd} weeks, which are still significantly higher than the 0th week.

The length of incubation in fermentation is related to the microbe growth phase. [9] stated that a short incubation time resulted in limited microbe opportunities to continue to grow and multiply, so that the number of substrate components that can be converted into cell mass is also small. On the contrary, with a longer incubation time, the microbe will have more opportunities to grow and multiply until the stationary phase is reached, namely the growth rate equals zero and the total number of total cell-mass is constant.

The fermentation process occurs through a series of biochemical reactions that convert dry matter into energy (heat), water molecules (H_2O) and CO_2 [10]. The results of research by [11] also showed an increase in temperature during fermentation, which is a change in temperature from 28° C to 53° C on the 7th day and then dropped to 11th to 37.5° C.[12] stated that temperature greatly affects the growth rate of microorganisms, the rate of enzyme synthesis and the rate of enzyme inactivation.

B. *Degree of acidity (pH)*

The longer incubation time for cassava bagasse fermentation using *Trichoderma* AA1 mutants resulted in a mean pH that decreased significantly from week 0 to week 3 (Table 1). This shows that the acidity of the material in the bioreactor is increasing. The value of HP is affected by the chemical compounds produced during the fermentation process. The fermentation process goes well marked by a decreased pH [13].

The decrease in pH in fermentation is a result of the formation of H + ions from microbe activity in breaking down organic matter [14]. The simultaneous decrease in pH value from weeks to weeks of fermentation is thought to be due to the accumulation of simple reducing sugars produced from hydrolysis of cellulose randomly on 1.4-D-glycosidic bonds. Changes in pH can affect the work of enzymes because of changes in the catalytic region and conformation of the carboxyl groups and aminoenzyme groups.

A certain pH value that allows the enzyme to work optimally is called the optimum pH [15]. Each enzyme also has a different optimum pH. The pH produced in this study strongly supports the high cellulose enzyme activity. Cellulose enzymes, especially CMCase (Endo-β-1,4-glucanase) tend to be optimum at acidic pH, ie in the pH range 4-6,5 [16].

C. *Digestibility of Dry Materials*

The incubation time for cassava bagasse fermentation using Trichoderma AA1 mutants significantly (P 0.05) on the KcBK value fermented cassava-bagasse. However, this effect began to occur during the incubation of the 3rd week. That is, the incubation period of week 0 to week 2 did not change the digestibility value of fermented cassava-bagasse are significant. This indicates that the cassava-bagasse fermentation process using *Trichoderma* AA1 does not require a long incubation time to get the KcBK value optimal, which is just 1 week.

IV. Conclusion

The study concluded that cassava-bagasse fermentation using *Trichoderma* AA1 mutants can increase the temperature and reduce the pH of the incubation period 1 to 3 weeks. Meanwhile, the value of dry matter fermented cassava-bagasse can be sustained on a long incubation week 1 and 2.

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