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Optimization of Cassava Waste from Bioethanol Post-Production through Bioactivity Process Consortium of *Saccharomyces cerevisiae*, *Trichoderma viride* and *Aspergillus niger*

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Citation: Yani Suryani (2013) “Optimization of Cassava Waste from Bioethanol Post-Production through Bioactivity Process Consortium of *Saccharomyces cerevisiae*, *Trichoderma viride* and *Aspergillus niger*”, Asian Journal of Agriculture and Rural Development, Vol. 3, No. 4, pp. 154-162.

Acknowledgements: With the completion of this scientific article, the author would like to thank profusely to Institution of Research and Community Services Universitas Padjadjaran that had provided research funding through research program PPM PRODUCTIVE UNPAD 2012.



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Abstract

The result of laboratory analysis on bioethanol waste shows nutritional and anti-nutritional content (HCN). Based on it, in order to prevent environment damage then performed a bioconversion of bioethanol waste for base material of sheep forage by fermented previously using consortium of *Saccharomyces cerevisiae*, *Trichoderma viride*, and *Aspergillus niger* to eliminate the waste anti-nutritional content and increase nutritional content. This research used Completely Randomized Design (CRD) factorial pattern with two factors and three replications. The first factor was inoculum dose (D) and the second factor was variation of tested microorganisms (M). The variables measured were HCN content and nutrient content of fermented products, that were water content, protein content and crude fiber content by proximate analysis. Furthermore, the data was statistically analyzed by analysis of variance (ANOVA), and was there significant difference analyzed further by Duncan's multiple range test of 5% level. Nutritional compositions of bioethanol waste from cassava that had been fermented by consortium of *Saccharomyces cerevisiae*, *Trichoderma viride*, and *Aspergillus niger* experienced changes compared to before fermentation. The results showed an increase in protein level, while fiber content, water content, and HCN content were decrease. Bioethanol waste from cassava fermented by *Aspergillus niger* and *Saccharomyces cerevisiae* (k_3) with 2% inoculum dose had the highest protein content increase from 11.79% to 25.41% and had the lowest crude fiber content decrease from 16.4% to 12.84 %. The highest HCN decrease obtained from the fermentation of consortium *Aspergillus niger* and *Trichoderma viride*.

Keywords: *Aspergillus niger*, Bioactivity, Bioethanol waste, Consortium, Optimization, *Saccharomyces cerevisiae*, *Trichoderma viride*

Introduction

Bioethanol is a petroleum alternative energy that is produced in the world thus far (Anindyawati, 2009). The results of the purification and separation on bioethanol-manufacturing process are ethanol, bioethanol solid waste and liquid waste (Endah *et al.*, 2007).

In 2012, it is projected that 15 million kiloliters of premium will be replaced about

20% by the BE-10 gasohol. If the yield produced from 1 ton of cassava biomass can be converted to 166.6 liters bioethanol, the waste that will be produced from bioethanol production is about 15 million tons of waste per year. Until now, cassava waste from bioethanol post-production is untapped well, and even tends to be left. If this is allowed to prolong, it will have a negative impact on the environment, one of the worst impacts is water quality decrease (Sukmaji, 2008).

One of potential utilization is converting waste into sheep forage, by previously improving nutrient and eliminating HCN contained in waste through fermentation process. It is useful to overcome the problems due to the disruption of sheep productivity for limited forage availability (Tarmidi, 1999). Fungal groups which are potential to be used as inoculums in waste bioconversion process of bioethanol from cassava are *Saccharomyces cerevisiae*, *Trichoderma viride*, *Aspergillus niger*, and its consortium.

The fermentation process can improve nutritional value, harmless, does not cause pollution and relatively low cost (Doyle *et al.*, 1986). Through the fermentation process, it is possible to derive benefits on reducing anti-nutrients and toxins contained in foodstuffs. Based on research conducted by Abdulah (2009), HCN content contained in cassava peel decreased from 0.024% to 0.009% after 5 days fermentation. Fermentation is also one of the numerous ways to lower crude fiber content and reduce toxic content contained in forage material, also has more value in improving the nutrient quality and livestock palatability improvement.

In the implementation of fermentation, potential fungi are essential, such *Aspergillus niger*, *Trichoderma viride*, *Saccharomyces cerevisiae* (Soeka, 1992). Utilization of *Saccharomyces cerevisiae* in fermentation is based on ability of the microbe to produce vitamins, enzymes, nutrients, and other cofactors (Dawson & Alison, 1993). *Saccharomyces cerevisiae* contains glutamic acid which can reform forage palatability, assimilate protein and secrete essential amino acids, also contains vitamin B complex (Fardiaz, 1992).

Trichoderma viride is saprophyte, aerobic, grow at temperature of 30°C and pH 5. This fungus is a cellulase enzymes producing microbe that can degrade cellulose. Cellulase enzymes produced by *Trichoderma viride* can break down starch or non-polysaccharides starch into monomeric sugars which are more easily metabolized (Iyayi and Aderolu, 2004).

Aspergillus niger is a mold type microbe that can grow rapidly and harmless because it does not produce mycotoxins. In addition, it use is

easy because many are used commercially in the production of citric acid, gluconic acid and some enzymes such as amylase, pectinase, amilo-glucosidase and cellulase. *Aspergillus niger* has good amylolytic and proteolytic capacities, and it can produce the extra-cellular phytase enzyme. The result of fermentation can be used as a source of single cell protein (SCP) and the culture media as potential energy source.

This research aimed to obtain the best inoculum type and optimum dose from fungi types used to improve nutritional content and able to reduce HCN antinutrient contained in cassava waste from bioethanol post-production.

Results from this research are expected to overcome two important social issues, namely issues of bioethanol post-production cassava waste which disturbs and threatens environment health, it can also undertake the problem of declining sheep productivity as the consequence of minimal and limited forage availability.

Materials and Methods

The research method used was experimental method using Completely Randomized Design (CRD) 3x3 factorial pattern with three replications (Gaspersz, 1989). The first factor was microorganism consortium (M) and the second factor was inoculum dose (D). Microorganism consortium consisted in three levels, namely:

Consortium 1 (m_1): *Trichoderma viride* and *Saccharomyces cerevisiae*

Consortium 2 (m_2): *Aspergillus niger* and *Trichoderma viride*

Consortium 3 (m_3): *Aspergillus niger* and *Saccharomyces cerevisiae*

Inoculum dose (D), respectively: $d_1 = 2g$, $d_2 = 3g$ and $d_3 = 4g$. Parameters measured were crude protein content, crude fiber content and moisture content of fermentation products through proximate analysis, also HCN content of fermentation products. Data were analyzed by Analysis of Variance and continued with Duncan's Multiple Range Test (Gaspersz, 1989).

Stages in this research were: (1) inoculums preparation; (2) microbial inoculums production; (3) bioethanol waste fermentation. Inoculums preparation of pure culture of *Aspergillus niger*, *Trichoderma viride* and *Saccharomyces cerevisiae* was inoculated on slant agar media, then incubated at 30°C for 3 days. Producing of microbial inoculums used about 900 grams of rice added with 100 grams of bioethanol waste flour and mixed with 1 liter distilled water, then steamed for 1 hour. After that, it was cooled (temperature 30-35°C), then put into a plastic bag. Every 1000 grams of substrate was inoculated with 100 ml of suspension, then stirred until homogeneous, and perforated using a needle and then incubated at 30-35°C (Gandjar, 1999) for 72 hours in incubator (Shang, 1987). After the substrate was fulfilled with the mold, then it was dried using light oven at 45-45°C and milled into powder, then used as inoculants. Furthermore, fermentation of bioethanol waste was 400 grams of bioethanol waste put into heat-resistant plastic bag and added 400 mL water, then sterilized at temperature of 121°C,

2 atm for 15 minutes. After the bioethanol waste got cold, it was inoculated with previously produced inoculums as much as 2%, 3%, and 4% (w/v) bioethanol waste dry matter. Then the material was stirred until homogeneous. After that, the plastic bags were perforated to get aerobic conditions. Each plastic bag incubated at 30°C for 3 days. If fermentation time had been completed, then did the proximate analysis which included the calculation of crude protein content, crude fiber content, water content, and HCN content.

Results and Discussion

Effect of Microbial Consortium Type and Inoculum Dose on Water Content of Bioethanol Waste from Cassava Fermentation Result

Water content averages of cassava bioethanol waste resulted from fermentation through proximate analysis are presented in Table 1.1., while the decrease in water content chart is presented in Figure 1.1.

Table 1.1: Water Content (%) of Bioethanol Waste from Cassava Fermentation Result based on Microbial Consortium Type and Inoculum Dose

Consortium Types	Inoculum Doses (%)			Average
	2	3	4	
<i>Trichoderma viridae</i> and <i>Saccharomyces cerevisiae</i>	34.48	28.12	27.04	29.88
<i>Aspergillus niger</i> and <i>Trichoderma viridae</i>	32.55	29.41	27.74	29.9
<i>Aspergillus niger</i> and <i>Saccharomyces cerevisiae</i>	34.17	28.94	26.51	30.21
Average	33.74	28.82	26.09	

From the data listed in Table 1.1., it shows that the water content of cassava bioethanol waste resulted from fermentation decreased during the fermentation appropriate with inoculum dose on all treatments. Analysis of variance showed that only inoculum dose gave significant effect on water content decrease, while consortium type did not perform

significant difference at 5% confidence level. This meant that the effect of consortium type on water content decrease during fermentation was same for each treatment. To determine the further difference of each inoculum dose treatments, Duncan's Multiple Range Test was carried out which results are presented in Table 1.2.

Table 1.2: Duncan's Multiple Range Test of Inoculum Dose Effect on Water Content

Inoculum Doses (%)	Water Content (%)	Significance ($\alpha = 5\%$)
2	33.74	C
3	28.82	B
4	26.09	A

Exp: Numbers followed by the same letters are not significantly different on $\alpha = 5\%$ DMRT

The data in Table 1.2 show that every different inoculum dose produced different water content in bioethanol waste from cassava fermentation result, with the highest decrease of water content obtained at 4% inoculum dose. The decrease of water content in fermentation substrate simultaneously with the increase dose of inoculum associated to water utilization by microbes for growth and due to evaporation during the fermentation process.

Water is necessary for microbial growth. Microbes will grow well if the need for water and nutrients are met. Water acts as a solvent in metabolic activity in the microorganism cells and serves as a catalyst directly involved in some enzymatic reactions (Sastramihardja, 1989). Fardiaz (1988) stated that water besides functioning as a reactant in hydrolysis reaction also as product of oxygen reduction in electron transfer system. In line with the growth of microbes during fermentation, water demand will increase, consequently the water content in substrate will decrease.

The decrease in water content of cassava bioethanol waste resulted from fermentation was also associated with evaporation during fermentation process. The longer the fermentation, the more water was evaporated, consequently water content of cassava bioethanol waste resulted from fermentation was lowered. Sastramihardja (1989) stated that during aerobic fermentation process, it will produce carbon dioxide and water vapor which causes water content decrease in fermentation media. Similarly, according to Sofyan (2003) stated that the energy generated during the fermentation process is in heat form. The heat generated causes the substrate temperature rises and the water produced during fermentation process will evaporate which

results water content decrease in the substrate. The water content in cassava bioethanol waste resulted from fermentation was still higher than the water content should be contained in forage material maximum 12%.

Effect of Microbial Consortium Type and Inoculum Dose on Crude Protein Content of Bioethanol Waste from Cassava Fermentation Result

After the fermentation, it was identified that crude protein content of bioethanol waste from cassava had increased for all treatments compared with crude protein content in bioethanol waste from cassava before fermentation. It was also identified that crude protein content in cassava bioethanol waste resulted from fermentation decreased with fermentation time accretion.

Analysis of variance result showed that both consortium type and inoculum dose gave significantly different effect on crude protein content of cassava bioethanol waste resulted from fermentation, but there was no interaction between inoculum dose and consortium type on crude protein content increase. This meant that the effect of inoculum dose factor was same for every type of consortium, and consortium type effect was same for each inoculum dose on crude protein content change of cassava bioethanol waste from fermentation result. Further, Duncan's Multiple Range Test was carried out to determine treatment differences among inoculum doses on crude protein content change presented in Table 2.1. While Duncan's Multiple Range Test result to determine treatment differences in consortium type on crude protein content change, it can be seen in Table 2.2.

Table 2.1: Duncan's Multiple Range Test of Inoculum Dose Effect on Crude Protein Content

Inoculum Doses (%)	Crude Protein Content (%)	Significance ($\alpha = 5\%$)
2	22.64	c
3	21.25	b
4	19.14	a

Exp: Numbers followed by the same letters are not significantly different on $\alpha = 5\%$ DMRT

Table 2.2: Duncan's Multiple Range Test of Consortium Type Effect on Crude Protein Content

Consortium Types	Crude Protein Content (%)	Significance ($\alpha = 5\%$)
<i>Trichoderma viride</i> and <i>Saccharomyces cerevisiae</i>	16.71	a

<i>Aspergillus niger</i> and <i>Trichoderma viride</i>	22.48	b
<i>Aspergillus niger</i> and <i>Saccharomyces cerevisiae</i>	23.85	c

Exp: Numbers followed by the same letters are not significantly different on $\alpha = 5\%$ DMRT

Data in Table 2.1 show that each inoculum dose produced different crude protein content with the highest average increase in crude protein content obtained at 2% inoculum dose. The increase in crude protein content of cassava bioethanol waste resulted from fermentation associated with the increase of microbial cell number and enzymes released during fermentation. Increasing of microbial cells number during fermentation causes an increase in nucleic acids and protein contents. Brock *et al.* (1994) stated that the higher the growth rate, the larger the cell size and the higher the amount of nucleic acid in cell. In addition, microbe itself is a single cell protein that can increase protein content in the substrate. Pederson (1971) stated that every microbial cell contains many proteins which levels are varied, depend to the species. Oboh *et al.* (2002) stated that the growth of microbes during fermentation can increase protein content in the fermented substrate.

Data in Table 2.1 show that microbial consortium *Aspergillus niger* and *Saccharomyces cerevisiae* (k_3) gave the highest average in protein content increase than other consortiums. Higher increase of protein by microbial consortium *Aspergillus niger* and *Saccharomyces cerevisiae* (k_3) showed that consortium 3 had higher potential to increase crude protein on cassava bioethanol waste substrate than other consortiums.

From fermented yield of cassava bioethanol waste by three types of microbes with different inoculum doses in this research, it can be concluded that the consortium of *Aspergillus niger* and *Saccharomyces cerevisiae* (k_3) with

2% inoculum dose gave the highest effect on crude protein content increase of cassava bioethanol waste.

Effect of Microbial Consortium Type and Inoculum Dose on Crude Fiber Content of Bioethanol Waste from Cassava Fermentation Result

Crude fiber content of cassava bioethanol waste fermentation result at 2% inoculum dose for all treatments experienced varied decrease if compared to crude fiber content of cassava bioethanol waste before fermentation. Along with the fermentation, crude fiber content experienced re-increase.

Variance analysis showed that both consortium type and inoculum dose gave significantly different effect on fiber content of cassava bioethanol waste from fermentation result, although there was no interaction between inoculum dose and consortium type on fiber content change. This meant that inoculum dose and consortium type respectively influenced on crude fiber content change from cassava bioethanol waste resulted from fermentation, and also meant that the effect of inoculum dose factor was same for every consortium type, and consortium type effect was same for each inoculum dose on fiber content change. Further, Duncan's Multiple Range Test was done to find out treatment differences among inoculum doses on crude fiber content change presented in Table 3.1. While, Duncan's Multiple Range Test result to determine treatment differences in consortium type on crude fiber content change can be seen in Table 3.2.

Table 3.1: Duncan's Multiple Range test of Inoculum Dose Effect on Crude Fiber Content

Inoculum Doses (%)	Crude Fiber Content (%)	Significance ($\alpha = 5\%$)
2	13.99	a
3	15.51	b
4	16.87	c

Exp: Numbers followed by the same letters are not significantly different on $\alpha = 5\%$ DMRT

Table 3.2. Duncan's Multiple Range Test of Consortium Type Effect on Crude Fiber Content

Consortium Types	Crude Protein Content (%)	Significance ($\alpha = 5\%$)
<i>Trichoderma viride</i> dan <i>Saccharomyces cerevisiae</i>	15.91	b
<i>Aspergillus niger</i> dan <i>Trichoderma viride</i>	16.19	b
<i>Aspergillus niger</i> dan <i>Saccharomyces cerevisiae</i>	14.27	a

Exp: Numbers followed by the same letters are not significantly different on $\alpha = 5\%$ DMRT

Data in Table 3.1 show that 2% inoculum dose could lower the crude fiber as the highest compared to other inoculum doses. Change in fiber content in cassava bioethanol waste fermentation result was influenced by mold mycelia growth intensity and mold ability to break down crude fiber to fulfill energy need with cellulase enzyme help (Mirwandhono and Siregar, 2004). Decrease in crude fiber content of cassava bioethanol waste resulted from fermentation was influenced by cellulase enzyme activity produced by *Aspergillus oryzae* and *Trichoderma viridae* (Tortora *et al.* 2001; Yu-ichi *et al.* 2002). Cellulase enzyme plays role in degrading cellulose on fermentation substrates into simple carbohydrate (glucose), which can be utilized by microbes as an energy source for growth. The highest cellulase enzyme activity was assessed in 2% inoculum dose.

Crude fiber content increase at 3% and 4% inoculum doses associated with increase in microbes amount. The increase in crude fiber content was mainly due to mold mycelia growth in fermentation substrate. Moore-Landecker (1990) stated that main components of cell wall of mold mycelium and spores are chitin and cellulose which have same function with cellulose in plants. The components are polysaccharide commonly classified as crude fiber component (Anggorodi, 1990). Similarly with Winarno opinion (1983) explained that fungi cell wall chemically consists of carbohydrates, such as cellulose, hemicellulose, pectin, and non-carbohydrates part. The increase in crude fiber content after fermentation can be seen on soybean fermentation into tempeh. Crude fiber content in tempeh is higher than in pre-fermented soybean. This is caused by *Rhizopus oligosporus* mold mycelium growth in tempeh (Hidayat *et al.* 2006).

From the data listed in Table 3.2, it can be seen that microbial consortium of *Aspergillus niger* and *Saccharomyces cerevisiae* could decrease a highest crude fiber content compared with other microbial consortiums. Higher decline of crude fiber by microbial consortium *Aspergillus niger* and *Saccharomyces cerevisiae* (k_3) than other microbial consortiums indicated that consortium 3 (k_3) had higher potential to lower crude fiber on substrate of bioethanol waste from cassava. This meant that the microbes in consortium 3 could cooperate well in reducing crude fiber in cassava bioethanol waste.

Another study which showed crude fiber content decrease was Iyayi and Aderolu research (2004), they reported that crude fiber content of hay, rice bran, corn cobs, and palm kernel flour fermented by *Trichoderma viride* for 14 days decreased by 35%, 40%, 36%, and 37.50%. Another study showing crude fiber content increase was Tjakradidjaja *et al.* (2007) reported that *Jatropha* seed residue fermented by *Rhizopus oligosporus* and *Trichoderma viride* had increase in fiber content from 38.49% to 47.45% after fermented by *Rhizopus oligosporus* and became 45.72% after fermented by *Trichoderma viridae*.

From fermentation result of cassava bioethanol waste by three microbe types with three different doses in this research, it could be concluded that consortium of *Aspergillus niger* and *Saccharomyces cerevisiae* (k_3) at 2% inoculum dose gave the highest influence on crude fiber content decrease of cassava bioethanol waste resulted from fermentation.

Effect of Microbial Consortium Type and Inoculum Dose on HCN Content of Bioethanol Waste from Cassava Fermentation Result

The average HCN content of bioethanol waste from cassava fermentation result is presented in Table 4.1.

Table 4.1: HCN Content (%) of Bioethanol Waste from Cassava Fermentation Result based on Microbe Consortium Type and Inoculum Dose

Consortium Types	Inoculum Doses (%)			Average
	2	3	4	
<i>Trichoderma viridae</i> dan <i>Saccharomyces cerevisiae</i>	4.28	3.52	3.31	3.7
<i>Aspergillus niger</i> dan <i>Trichoderma viridae</i>	2.59	1.89	1.43	1.97
<i>Aspergillus niger</i> dan <i>Saccharomyces cerevisiae</i>	2.90	2.20	1.18	2.09
Average	3.25	2.53	1.97	

Data in Table 4.1. show decreasing in HCN content of bioethanol waste from cassava fermentation result along with inoculum doses.

Analysis of variance result showed that both consortium type and inoculum dose gave significant effect on HCN content decrease of cassava bioethanol waste resulted from fermentation, but there was no interaction between inoculum dose and consortium type on HCN content decrease. This meant that

effect of inoculum dose factor was same for every type of consortium, and consortium type effect was same for each dose inoculum on HCN content decrease. To find out the treatment differences among inoculum doses on HCN content decrease, conducted Duncan's Multiple Range Test which result can be seen in Table 4.2, while the Duncan's Multiple Range Test to identify the treatment difference among consortium types on HCN content decrease can be seen in Table 4.3.

Table 4.2: Duncan's Multiple Range Test of Inoculum Dose Effect on HCN Content

Inoculum Doses (%)	HCN Content (%)	Significance ($\alpha = 5\%$)
2	3.25	C
3	2.53	b
4	1.97	a

Exp: Numbers followed by the same letters are not significantly different on $\alpha = 5\%$ DMRT

Table 4.3: Duncan's Multiple Range Test of Consortium Type Effect on HCN Content

Consortium Types	HCN Content (%)	Significance ($\alpha = 5\%$)
<i>Trichoderma viridae</i> dan <i>Saccharomyces cerevisiae</i>	3.7	B
<i>Aspergillus niger</i> dan <i>Trichoderma viridae</i>	1.97	A
<i>Aspergillus niger</i> dan <i>Saccharomyces cerevisiae</i>	2.09	A

Exp: Numbers followed by the same letters are not significantly different on $\alpha = 5\%$ DMRT

Data in Table 4.2 show that HCN decrease at 4% inoculum dose was higher compared to other inoculum doses. HCN decrease was assumed that HCN was degraded by enzymes produced by *Aspergillus niger* and *Trichoderma viride*. Rusdi (1992) stated that fermentation process can reduce material containing toxins contained in fermented

material. Data listed in Table 4.3 show that the microbial consortium of *Trichoderma viride* and *Saccharomyces cerevisiae* (k_1) gave different effect on HCN decrease of cassava bioethanol waste fermentation result compared with microbe consortium of *Aspergillus niger* and *Trichoderma viridae* (k_2) and microbe consortium of *Aspergillus niger* and

Saccharomyces cerevisiae (k_3). The difference effect among consortiums was assumed to be related to the difference amount of microbes among consortium of *Trichoderma viride* and *Saccharomyces cerevisiae* (k_1), *Aspergillus niger* and *Trichoderma viridae* (k_2), also *Aspergillus niger* and *Saccharomyces cerevisiae* (k_3) which caused different HCN decrease amount.

Conclusions and Recommendations

Conclusions

Nutritional composition of bioethanol waste from cassava that had been fermented by *Aspergillus niger*, *Trichoderma viride* and *Saccharomyces cerevisiae* experienced changes compared to before fermentation. The result showed increase in protein content, while the fiber content, water content, and HCN content were decrease.

Waste of bioethanol from cassava fermented by *Aspergillus niger* and *Saccharomyces cerevisiae* (k_3) with 2% inoculum dose had the highest protein content increase from 21.79% to 35.41% and the lowest crude fiber content decrease from 16.4% to 12.84 %. The most HCN content decrease obtained from fermentation of consortium *Aspergillus niger* and *Trichoderma viride*.

Suggestions

Further research should be conducted to determine the effect of single microbe consortium used in fermenting waste of biethanol from cassava to increase nutrient and decrease HCN of cassava bioethanol waste. If the results of this research will be used, it should be taken microbial consortium *Aspergillus niger* and *Saccharomyces cerevisiae* with 2% inoculum dose.

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