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High performance of enzymatic bioprocess for production of biomass-based bioethanol of sago palm fiber waste

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A B S T R A C T

Biomass waste in the form of fiber dregs contains many components of lignocellulose and hemicellulose. Lignocellulose can be used to produce ethanol through enzymatic biotechnology processes. Sago palm fiber industry is one potential industry producing biomass waste in the form of solid waste of fiber dregs (about 30% from the weight of processed raw materials). Solid fiber waste contains crude fiber and lignocellulose compound consists of cellulose (35-50%), hemicellulose (20-35%) and lignin (12-20%). This study aimed to utilize solid waste of sago palm fiber as a raw material of bioethanol production through enzymatic biotechnology processes of delignification, saccharification and fermentation which was then purified by distillation process to get ethanol. Delignification, saccharification, and fermentation stages are conducted using Phanerochaete chrysosporus Mushroom, Trichoderma viride fungus, and saccharomyces cerevisiae, respectively and then purified by distillation process (one level) to produce ethanol. The process of saccharification and fermentation process were done in an integrated manner (addition of Trichoderma viride and yeast saccharomyces cereviceae fungi done simultaneously). The weight of raw materials of solid waste fiber treated was about 6 kgs. Alcohol content obtained was about ± 4% (distillation). No water, air, and soil pollution inflicted, more added value, and sustainable were the main benefits of biotech process or bioprocess.

1. INTRODUCTION

Sago palm fiber industries are spread over places in Central Java, such as in Jepara, Pati, Kendal and Klaten. Identification result has indicated that sago palm fiber industrial centers in Daleman village of Klaten are about 52 of 1320 factories with production capacity and solid waste generated of 660 ton/month and of ± 240 ton, respectively.

Sago palm fiber industry is one of the industries that has the potential to produce solid waste biomass as fiber pulp. Carbohydrate and crude fiber contents in sago palm fiber are 19.69% and 29.28%, respectively (Rame & Nani, 2016). Solid waste biomass as fiber pulp contains many components of lignocellulose and hemicellulose. Palm pulp contains Lignocellulose crude fiber compounds of cellulose (35-50%), hemicellulose (20-35%) and lignin (12-20%) (Mosier et al., 2005). Solid waste used for livestock fodder and mushroom growing media other they throw it in roadside and the side of the river. Problems accumulate increasingly so that it's will disrupt activities and comfortness people in long-term and can cause

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environmental pollution because the sago palm fiber still contains organic substances that will decompose into a simple compound that has a bad odor (Jin, 2015).

The alternative of solid waste management for sago palm fiber is utilized as raw material in bioethanol manufacture. Factors that encourage the research use of lignocellulosic materials into bioethanol to energy source caused 1) energy needs increase every year, 2) energy sources from natural resources such as oil, gas, and coal are decreasing, 3) bioethanol has characteristics that can increase efficiency combustion 4) reduce greenhouse gas emissions and global warming, and 5) lignocellulosic materials are abundant and are not used as foodstuffs, so energy source does not interfere with the food supply.

The aim of this study was to utilize solid waste fiber/ sago palm fiber as the raw material of bioethanol manufacture through biotechnology process with stages were deligninfication, saccharification and fermentation process technology to obtain ethanol by the distillation process.

Biomass is the raw materials of alternative energy sources that possible for a substitute for fossil fuels. Sago fiber biomass generated from the extracting process can be made into bioethanol (Awg-Adeni, Bujang, Hassan, & Abd-Aziz, 2013). Several studies suggested that bioethanol production were not feasible economically and also caused an environmental effect. Ethanol production by integration and separation using solid waste of sago palm fiber fiber as raw material, and *Phanerochaete chrysosporus* in delignification process (Saritha et al., 2012), *Trichoderma viride* in saccharification process (Ko et al., 2009) and *Saccharomyces Cerevisiae* in fermentation process are expected to produce ethanol without causing environmental effect (Mussatto et al., 2010).

Conversion of lignocellulose material into ethanol is basically done through several stages that are stimulated by enzymes according to their activities including delignification, saccharification and fermentation stages (Sun & Cheng, 2002). Previous research executed the delignification process using diluted strong acid, but this method may produce furfural and hydroxymethylfurfural byproducts which can inhibit fermentation process (Harmsen, 2010) and the wastewater will pollute the environment and requires equipment that resistant for acid and high-temperature.

According to (Hermiati et al., 2010), utilization of white fungus in the delignification process has been reported by some researchers and is a better method than chemical because of its selectivity, energy-efficiency, and environmental-friendly. However, enzymatic degradation of cellulose generally does not lead into high conversion, due to the presence of complex cellulose in the lignocellulose structure (biomass), a crystalline structure in cellulose and the strong bond between cellulose and lignin, so that cellulose degradation cannot be easily (Walker & Stewart, 2016). To find out how far the enzymatic bioprocess of lignocellulose into bioethanol as a source of renewable alternative energy from palm sugar fiber bedding, it is necessary to do this research (Nigam & Singh, 2011).

2. METHODS

The material for this research was a solid waste of palm fiber taken from the small industry of starch palm in Daleman, Tulung, Klaten, Central Java Province. Microbes producing Enzymes such as *Phanerochaete chrysosporus*, *Trichoderma viride*, *Saccharomyces Cerevisiae* were obtained from PAU Center for Nutrition Studies and Nutrition- UGM. Potato Dextro agar media and Malt Agar extract, chemicals for microbial nutrition consist of KH_2PO_4; $(NH_4)\ 2SO_4$; $MgSO_4.7H2O$; $CaCl_2.2H_2O$; $FeCl_3.6H_2O$; $ZnSO_4.7H_2O$; Urea; $MnSO_4.H_2O$; $NaNO_3$; HCl and chemicals for carbohydrate, glucose analysis.

The equipment used were a plastic box, distillator, glass equipment, autoclave, incubator, oven, pH meter, thermometer, and blender. The research steps were microbial regeneration; microbes used were a phanerochaete chrypsosporus mushroom (white fungus mushroom) for the delignification process, *Trichoderma viride* fungus for saccharification process, and yeast *Saccharomyces Cerevisiae* for fermentation process. Each type of microbe was regenerated in accordance to the stage of the process to

be done. Preparation of nutrient solution from chemical compounds was dissolved in aquadest up to 5 liters volume.

The sterilization of materials sago palm fiber and nutrient solution were done using autoclave at 121°C for 15 minutes. The container for experiments was sterilized using alcohol 70 %. Raw materials of sago palm fibers were prepared out by drying, cutting, grinding sifting, and weighing. Sago palm fiber was dried until water content ≤ 10%, then cut and ground to the size of ± 0.5 cm to get similar shapes and facilitate fermentation process. The quality of the material was measured based on the analysis of cellulose, hemicellulose and lignin parameter (SNI 0492, 2008) (SNI 2891, 1992). The experiment was started with delignification process using *Phanerochaete chrysosporus* fungus/ white filaments varied of 2 tubes, 4 tubes, 6 tubes, and 8 tubes. The performance tests of the content were lignin, cellulose, hemicellulose. The results of analysis were evaluated and the smallest and biggest lignin and cellulose contents were used as the basic for experiments. The delignification process was done at of 30-35 °C for 25 days.

The microbial cultures preparation for saccharification process by microbial inoculation was done with the addition of *Trichoderma viride* at 30-40 °C and pH 5. The experiments were carried out with ripening of 3 days, 4 days, 5 days, 6 days. The term of saccharification refer to the analysis of glucose level (SNI 2891, 1992) and selected highest glucose content was used as the basic for the next experiments.

The preparation culture of *Saccharomyces Cerevisiae* for fermentation processes.

The microbial inoculation with ripening for 3-7 days to convert glucose to ethanol with addition of *Saccharomyces Cerevisiae* 3 tubes per 1 kg of delignified powder. Fermentation was done at 30 °C with ripening times were 3, 5 and 7 days. The fermentation time was reached, filtrate that was purified by distillation processes to get ethanol as product. The distillation process carried out by one stage and setting temperature was 78°C (Ko et al., 2009). The result of ethanol was analyzed using Gas Chromatography. Flow diagram of bioprocess stages is shown in **Figure 1**.

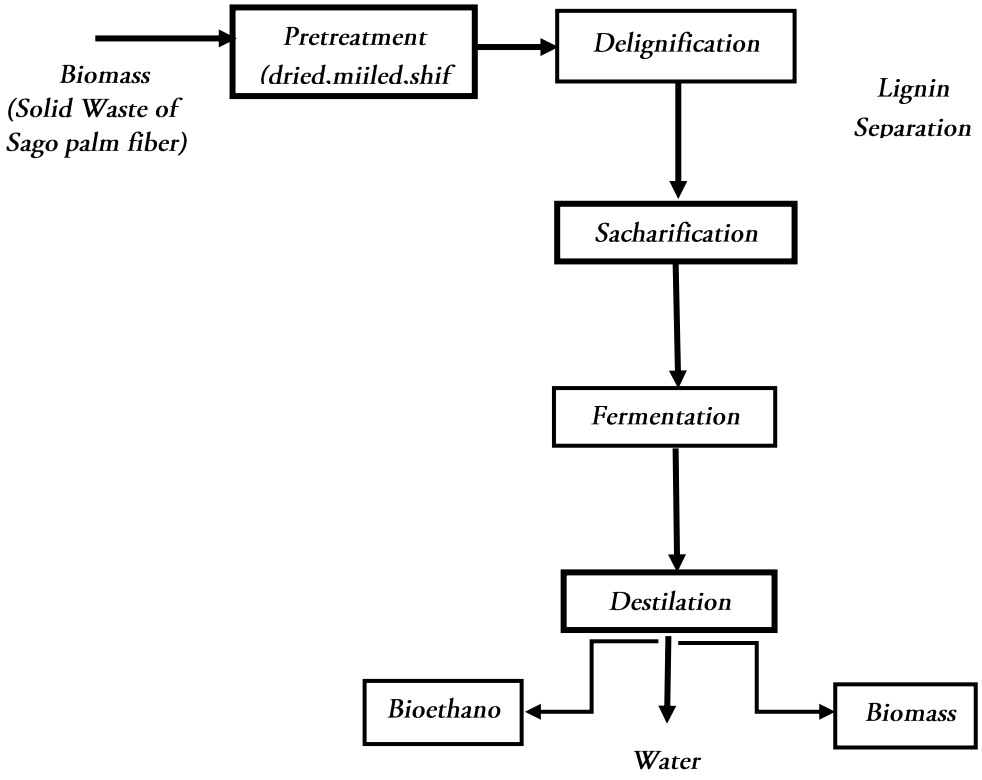

Figure 1. Flow diagram to produce bioethanol with raw material solid waste of sago palm fiber by enzymatic processes

The venue of activity of the Research was conducted at BBTPPI in Biotechnology Laboratory, Jalan Ki Mangunsarkoro No. 6 Semarang and testing laboratory in PAU UGM Jogyakarta.

3. RESULT AND DISCUSSION

3.1. Characterization of the analysis result

Characterization of analysis result from solid waste of sago palm fiber after pretreatment is tabulated in **Table 1**.

From characterization result of raw materials mentioned above, sample number 3 was selected (milled mixture 40 mesh) as the research raw materials based.

3.2. Delignification Process of Results

From **Table 2** obtained the best delignifcation process on DG3 formula, which then used as delignification process based using solid waste 6 kg.

3.3. The result of analysis Saccharification Processes

Saccharification Process of results can be seen in **Table 3**.

Results of the saccharification process on 4 (four) treatments, obtained as in **Table 3** above. The highest glucose level was obtained in the saccharification process by using 1 tube *Trichoderma viride* / 0,5 kg powder delignified tube with 3 days ripening time and 1.95% glucose level was obtained. It is used for reference of the next process.

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Table 1. The result of analysis organic solid waste compound of sago palm fiber

No	Sample Code	Lignin (%)	cellulose (%)	HemicelIulosa (%)	Carbohydrate (%)	Water (%)
1.	Dregs of Flour	9,77	19,48	35,94	53,99	15,66
2.	Dregs of Fiber	15,50	43,74	20,77	31,94	11,57
3.	Dregs of Mixture milled 40 mesh	14,04	30,72	33,23	50,15	9,5

Table 2. Result of analysis delignification processes

Sample Code	Lignin (%)	Cellulose (%)	Hemicellulosa (%)	Carbohydrate (%)	Water (%)
DG 1	6,89	22,80	33,94	55,23	9,54
DG 2	7,75	28,05	33,28	54,42	9,54
DG 3	6,32	30,49	31,67	56,75	9,12
DG 4	9,22	27,98	27,86	53,63	9,35

Where :

DG 1 =Delignification Using *Phanerochaete Chrys* 1 Tube/0,5 kg solid waste

DG 2 =Delignification Using *Phanerochaete Chrys* 2 Tube/0,5 kg solid waste

DG 3 =Delignification Using *Phanerochaete Chrys* 3 Tube/0,5 kg solid waste

DG 4 =Delignification Using *Phanerochaete Chrys* 4 tabung/0,5 kg solid waste

Table 3. The result of glucose analysis at Saccharification processes

Saccharification	Glucose Content in Ripening Time				
	3 days (%)	4 days (%)	5 days (%)	6 days (%)	7 days (%)
S 1	1,95	1,50	O,90	0,31	0,53
S 2	0,94	1,07	0,67	0,41	0,55
S 3	0,70	0,78	0,74	0,33	0,38
S 4	1,10	1,04	0,72	0,39	0,48

Where :

S 1 = Saccharification using 1 tube *Trichoderma viride* / 0,5 kg powder delignified

S 2 = Saccharification using 2 tube *Trichoderma viride* / 0,5 kg powder delignified

S 3 = Saccharification using 3 tube *Trichoderma viride* / 0,5 kg powder delignified

S 4 = Saccharification using 4 tube *Trichoderma viride* / 0,5 kg powder delignified

3.4. Fermentation Process Results

Fermentation process followed by the purification process with distillation obtained the results as in table 4 below:

Table 4. Ethanol Results of Fermentation Processes at Variation Times

Number	Code Process	Content of Ethanol (%)
1	Fermentation 3 days	8,92
2	Fermentation 5 days	1,48
3	Fermentation 7 days	0,13

Regarding **Table 4** above on 3 days fermentation, the highest ethanol contain was 8,92 %. Sago palm fiber Solid waste is a by-product resulting from the extraction of sugar sago palm fiber. Solid waste used obtained from industrial centers of sago palm fiber in Klaten.

Based on the test results, waste contains cellulose 19,48%; hemicellulose 35.94%, and carbohydrates 53.94%. While the waste fiber form has a content of cellulose 43.74%; hemicellulose 20.77%, and carbohydrates 31.94. Since the cellulose content is relatively high then the solid waste of sago palm fiber is feasible to be used as biomass for bioethanol bioprocess. Visualization of solid waste of sago palm fiber in **Figure 2** as below:

Figure 2. Solid Waste of Sago palm fiber dregs

Figure 3. Solid Waste after pretreatment

Pretreatment on solid waste of sago palm fiber by grinding was done to increase the surface area of waste so that microbe may easily penetrate into waste powder of sago palm fiber. Solid waste was not directly delegated because of the high water content of > 60%. Waste was aerated to dry to avoid damage to carbohydrate structure. Dried waste was mashed up to 40 mesh and powder-shaped to enlarge the

surface area. It was expected to enlarge the field of waste contact with microorganisms. Next, waste that has been smoothed, then added water and nutrients, and put into heat-resistant plastic and sterilized.

Solid waste samples were initially delignified. This process is important because it can affect the bioethanol rendement. Lignocellulose is an organic component in nature that consists of cellulose, hemicellulose, lignin, extractive, and ash (Louren, 2015). The delignification process is intended to decompose lignin from waste using *Phanerochaete chrysosporus* to obtain free cellulose used saccharification process (Saritha et al., 2012). Waste that still contains lignin can inhibit the fermentation process. Lignin was separated by adding *Phanerochaete chrysosporus* from the medium of PDA (stock) and inoculated into the waste then incubated for 25 days at room temperature (27° C) until the *Phanerochaete chrysosporus* mycelium enveloped it as shown in Figure 3, after which it was dried in the sun.

Delignification results above show that the smallest lignin content with cellulose was relatively high using 3 tubes / 0.5 kg of waste. The next experiment using 6 tubes/ kg of Starch palm solid waste. Probably, *Phanerochaete chrysosporus* fungus 3 tubes / 0.5 kg of waste, peroxidase enzyme activity is maximum so that lignin decomposes higher (You & Percival Zhang, 2017).

Figure 4. Solid waste delignification by Phanerochaete chrysosporus

Delignification of research results was significantly decreased, the waste weight of 47.8% of 500 grams of palm sugar solid waste into 261 grams. However, the decomposed lignin was reduced by 55% from 14.04% to 6.32%. This may be due to the presence of lignin and its toxic derivatives. The scope of the research was only focusing on bioprocess, so the lignin separation was not done.

Delignification results were used as inserts on the saccharification process by using *Trichoderma viride* fungus. Common bioprocess biomass research into ethanol tends using cellulase enzymes (Maki et al., 2013). But economically the use of off-site cellulase enzymes (cellulase enzymes produced in different places) is not feasible because of the high price of pure cellulase enzymes.

In this study, saccharification was done with an on-site cellulase enzyme where the enzyme was produced by *Trichoderma viride* with an output substrate of the delignification process. It was expected that *Trichoderma viride* can produce sufficient cellulase enzymes to break down cellulose. Saccharification with the on-site cellulase enzyme was carried out at room temperature, making it more energy-efficient than saccharification with an off-site cellulase enzyme that requires about 48°C warming.

Glucose testing result at the stage of saccharification with *Trichoderma viride* showed that the highest glucose content was saccharification with 1 tube *Trichoderma viride* at 3 days curing at 1.95% / 0,5kg of delignification powder so that for subsequent experiments using 2 tubes Trichoderma / 1kg powder delignification. See **Table 3**).

Fermentation is divided into liquid and solid fermentation. In this research, liquid fermentation was carried out. Liquid fermentation is divided into two types: subsurface fermentation (batch process, fed-batch, and continuous process) and surface fermentation. The batch process is a fermentation once downloaded, the media and the inoculum are inserted simultaneously into a fermentation device and the product is taken at the end of the fermentation. Fed-batch is a combination of batch and continuous systems, a constant concentration of nutrients is fed into a fermentation device with a certain volume until the product is obtained close to the maximum. While continuous process, drainage of substrate and product taking were done continuously. Fermentation conducted in this research was a batch process. Before fermentation, all tools, media, and materials were sterilized beforehand.

Glassware sterilized oven at 160°C for 2 hours (Dion & Parker, 2013). While the media and hydrolyzate were sterilized using autoclave at 121°C and 2 atm for 15 minutes (Jin, 2015).

Fermentation was done by using *Saccharomyces Cerevisiae* microorganisms. *Saccharomyces Cerevisiae* is purified by scratch graft method and then incubated in the media to tilt as a stock culture. It can then be used by activating it in the media for italics as shown in the figure in the documentation attachment of research activities. The agar medium used was YMA (yeast malt agar) consisting of: yeast extract (3 g / L), malt extract (3 g / L), peptone (5 g / L), glucose (10 g / L), and agar (15 g / L).

The fermentation raw materials used were derived from saccharification products. The fermentation process was stopped by heating the tube containing the fermented samples in a water bath 60 ° C for 10 minutes (Mussatto et al., 2010). This heating aimed to stop the activity of *Saccharomyces Cerevisiae* without destroying existing ethanol. After that the sample was distilled at 80 ° C for 3 hours and the alcohol content was then analyzed (Ko et al., 2009). **Table 5** shows some data displaying of changing period of solid waste.

The first experiment the addition of mushrooms and yeasts was done gradually in accordance with the stages of each process. In the process of saccharification of 3-7 days, time shows the results were still fluctuating. Highest glucose concentration was obtained at 1.95% after 3 days. These results form the basis of the further fermentation process. The fermentation process was carried out with time variables of 3, 5 and 7 days at fermentation temperature of 30-35°C. The initial pH of the fermentation process of 4-5 showed relatively more stable results until the fermentation time is over. The purification process is then carried out by one level distillation at the boiling point of alcohol (78°C). Test results showed the obtained alcohol content was about 8.92%.

The second experiment of saccharification and fermentation were done in integratively (addition of *Trichoderma viride* fungus and yeast *Saccharomyces Cerevisiae* done simultaneously) (Rame et al., 2017). The operating conditions were all done similar to the first experiment with the weight of processed the raw material was about 6 kg. After the purification process, ethanol content obtained was about 4% (distillation).

The ethanol content itself represents the enzymatic efficiency of *Trichoderma viride* and Saccharomyces cerevisiae. In the presence of *Trichoderma viride* and Saccharomyces cerevisiae, glucose obtained was higher and will be used in the growth of *Trichoderma viride* and Saccharomyces cerevisiae. So at the end of the saccharification process, the resulting glucose was smaller and will reduce the efficiency of the saccharification process.

Trichoderma viride's optimal growth at the saccharification stage will increase the cellulase enzyme during saccharification (Posts et al., 2016). *Trichoderma viride*, however, tends to use glucose in its growth compared to the existing cellulose in solid waste of sago palm fiber. Though glucose is a product of the process of saccharification will then be used as a substrate in the fermentation process to become bioethanol, so it is necessary to develop *Trichoderma viride* optimally without reducing the resulting glucose product.

Table 5. The content of Material Analysis Result at Process stages

No	Data	Solid waste	Delignification	Saccharification	Fermentation
1	Weight	6 kgs	3,5 kgs	3 kgs	1,8 kgs
2	Cellulosa	31,61 %	30,49 %	-	-
3	Lignin	12,63 %	6,32 %	-	-
4	Glucose	-	-	1,95 %	-
5	Ethanol	-	-	-	8,92%

Judging from the results of ethanol content obtained, the gradual process of saccharification and fermentation is a better process than an integrated process. The availability of abundant waste of palm sugar, makes it possible for the production of pure cellulase enzyme with a solid waste substrate of sago palm fiber so that the saccharification efficiency will increase to convert cellulose into glucose.

4. CONCLUSION

The pretreatment and bioprocess of the solid waste fiber of pulp mill industries has the potential as a raw material for bioethanol production that is sustainable and renewable. The addition of *Trichoderma viride* and yeast Saccharomyces cereviceae gradually obtained alcohol content ± 8.92%. While the integrated process of saccharification and fermentation (addition of *Trichoderma viride* fungus and yeast Saccharomyces cereviceae done simultaneously) obtains alcohol content of about 4%. Judging from the results of ethanol content obtained, the process of saccharification and fermentation in stages is a better process than the integrated process. The operating conditions of bioprocess did not reach optimum, because of the presence of inhibitors that inhibit enzyme activity in each bioprocess stages. A simple distillation process (one level) is also one of the factors causing low levels of bioethanol obtained.

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