

## Bio Pulping Of Bagasse As The Material For Paper Making Using Different Species Of White Rot Fungi And Incubation Time

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### Abstract

The Biopulping is defined as the biological process of lignin degradation by utilizing microorganisms that produce some enzymes. A microorganism which widely known in the degradation of lignin and cellulose is a group of white-rot fungi. The aims for this research to know the most effective white rot fungi species of *G.lucidum*, *P.tuber-regium*, and *T.versicolor* in the degradation of lignin and cellulose with different incubation time on bagasse substrate. The effectivity of biopulping indicated by the highest degradation of lignin concentration and the lowest degradation of cellulose concentration. This study used an experimental design with Completely Randomized design with a two factorial pattern. The independent variable of this study is white rot fungi species and incubation time while the dependent variable is the concentration of lignin and cellulose. The main parameter was the concentration of lignin and cellulose, supporting parameters were pH, weight loss of substrate and mycelial growth. The result showed the degradation of lignin and cellulose in all treatment. The best degradation of lignin and cellulose showed by species *T.versicolor* and *P.tuber-regium* within 30 days of incubation.

Keyword: Bagasse; Biopulping; White Rot Fung

### INTRODUCTION

Bagasse is a solid waste remnant of sugarcane (*Saccharum officinarum*). In general, bagasse contains lignocellulose compounds such as lignin (13-22%), cellulose (26-43%), hemicellulose (17-23%), and pentose (20-33%) (Shabiri, 2014). The waste of sugarcane mostly used as bioethanol. Bagasse and straw are mostly used for generating heat and electricity, and recently bagasse is used as paper material. The process of papermaking from bagasse can be done through biopulping (Del Rio *et al.*, 2015).

Bio-pulping is an environmentally friendly technology which currently being developed in the pulp and paper industry. The application of biopulping in the pulp and paper industry is before the cooking process using chemicals. The pulping process pretreated with White Rot Fungi (WRF) has proven as a technology that is efficient and economically feasible, energy savings, reducing chlorine consumption and reducing pollutants into

the environment and produce stronger pulp. Selective degradation or modification of native lignin in wood by WRF facilitates removal or softening in subsequent pulping processes. However, the benefits of biopulping are not proportional to the level of biodelignification or biological elimination of certain wood components in biomechanics in organosolv and kraft pulping processes (Purwita & Hendro, 2016).

The objectives of this research were (1) to find out the interaction effect of white-rot fungi species and incubation time in the concentration of lignin and cellulose of bagasse in bio-pulping process, and (2) to find out the best optimum interaction between white-rot fungi and time of incubation in the concentration of lignin and cellulose from bagasse.

### MATERIALS AND METHODS

#### Materials

The materials used were *G. lucidum*, *P. tuber-regium* from Asa Agro Corporation Cianjur

collection, *T. versicolor* from Laboratory of Mycology and phytopathology Biology Faculty, bagasse of Sugarcane (*Saccharum officinarum*) from PT Gendhis Multi Manis, Potato Dextrose Agar (PDA), H<sub>2</sub>SO<sub>4</sub>, alcohol 70 %, distilled water, sorghum, mask, gloves, 25x15x0.8 cm polypropylene plastic bags, 50x60x0.8 cm polypropylene plastic bags.

#### **Location and Time of Research**

The research have been conducted in Mycology and Phytopathology Laboratory of Biology Faculty in University of Jenderal Soedirman, Panti Asuhan Siloam Purwokerto for field work, and Laboratory of Food Technology Science of Agricultural Faculty Universitas Jenderal Soedirman Purwokerto for analysis of lignin-cellulose concentration from January – May, 2019.

#### **Design of Research**

The experimental design used in this research was Completely Randomized Design (CRD) with a Factorial pattern with 3 replication. The independent variables were three species of white rot fungi and incubation time, while the dependent variable was the degradation of lignin and cellulose. The main parameters were concentration of lignin and cellulose before and after treatment. The supporting parameters were pH of substrate and weight loss of bagasse before and after treatment, and mycelial growth.

#### **The procedure of Research**

The laboratory equipment was used in this research such as petri dish, reaction tubes, beaker glass, measuring glass, and other tools were sterilized using autoclave with temperature 121°C, 2 atm, for 15 minutes (Hadioetomo, 1994). Bagasse was prepared by filtering through muslin cloth then it's dried under the sunlight for approximately 5 hours in a day until it's dry. Further, the bagasse collected into sacks. (Salbeti *et al.*, 2018).

The commercially PDA medium powder and distilled water prepared. The distilled water was measured for 1 L and the PDA powder for 39 g, then put the entire ingredient to Erlenmeyer flask. The flask boiled on a hotplate and homogenized it with a magnetic stirrer. The Erlenmeyer was covered with a cotton plug and paper, then tied with a rubber band. It was sterilized in the autoclave for 15 min at 121°C, then poured into sterilized petri dish after the steam released and cooled down in LAF (Tudses, 2016).

The three species fungal isolates (*T. versicolor*, *P. tuber-regium* and *G. lucidum*) were recultured into the PDA medium. The reculturation of the fungal has been done by transferred each of the fungal colonies as much as three plugs with the size of 5 mm using cork drill into a new PDA medium in LAF. The new PDA medium will be incubated for about 5-7 days.

#### **Fungal Spawn Inoculum Preparation (Kusnadi & Yayan, 2003. modified).**

The fungal spawn inoculum prepared using sorghum. Five kg of sorghum and glass bottles in 350 ml size prepared. Sorghum was soaked in water for two hours and the floating dirt are removed. The sorghum drained and steamed for 10 minutes, then cooling down for minutes. The CaCO<sub>3</sub> and dextrose were measured in an analytical scale as much as 25 g and 50 g respectively, then inserted into the sorghum medium and stirred. The sorghum medium was filled into the bottle as much as 2/3 from bottle size. The inoculum bottle covered with the cotton plug. They were then sterilized in the autoclave 121°C in 1 atm for 15 minutes. Furthermore, release the steam and wait for the cooling down.

#### **Fungal Spawn Inoculation (Kusnadi & Yayan, 2003, modified).**

The isolates of *G. lucidum*, *P. tuber-regium*, and *T.versicolor* in PDA medium prepared.

Inoculation done by forming a plug using cork borer, and take as much as five plugs of each fungal and take it into a bottle medium and wrapped the medium aseptically in LAF then incubated it for one month.

#### **Bagasse Substrate Preparation (Suryani & Carolina, 2017, modified).**

Bagasse substrate was taken 1 kg and put into a container, then 1 L distilled water was measured and put it container and homogenized. The pH of the bagasse also measured using the soil tester. The bagasse substrate filled as much 200 g into 25x20x0.008 cm polypropylene plastic bags. The baglogs were carried out with the process of compaction. The paralon ring was given on the top of the baglog followed by putting the cotton and plastic. The bagasse substrate medium was sterilized in the autoclave for 2 hours at 121°C. The media that has been sterilized was cooled for 12 hours before inoculation.

#### **Fungal Inoculation on Bagasse (Suryani & Carolina, 2017, modified).**

The fungi isolated from sorghum medium was taken 10% from the weight of the bagasse substrate medium. Inoculation was done by making a deep hole approximately 2 cm in the middle of the baglog media using the sterilized wooden stick. Then fungal spawn was inserted into the polybag by using spatula aseptically. It's incubated in room temperature with an incubation time of 0, 15, and 30 days.

#### **Measurement pH in Bagasse Substrate**

The measurement of pH in the bagasse substrate was done with soil tester tools. The soil tester was dipped into bagasse medium and the pH measured. The measurement was done before the treatment and after treatment.

#### **Measurement of Weight Loss Media**

The weight loss measurement of bagasse substrate has been done by measuring the bagasse substrate on day 0, 15 days and 30 days before and after treatment using an analytical scale.

#### **Lignin and Cellulose Analysis (Chesson, 1981)**

Determine lignin and cellulose concentration with different incubation time (0, 15, and 30 days) was done by Chesson method.

#### **Data Analysis**

The quantitative data of the lignin and cellulose contents at the beginning and the end of treatments were analyzed using analysis of variance (F test) with the error contents is 5% and 1% followed by Duncan's Multiple Range Test (DMRT).

## **RESULT AND DISCUSSION**

### **1. Lignin Concentration**

The degradation of lignin concentration in bagasse using three species of white-rot fungi were occurred in all treatment (Table 1). The initial lignin concentration of bagasse was 17.23%. Lignin concentration after 15 days and 30 days incubation time has been reduced by *Ganoderma lucidum* to 10.54%-10.88% and 10.54%-10.61%, respectively. *Pleurotus tuber-regium* has reduced lignin concentration to 10.21-11.03% and 9.55%-10.21%, while *Trametes versicolor* has reduced lignin concentration to 11.62%-11.71% and 9.55%-10.21%, respectively.

The interaction between fungal species and incubation time significantly affect the degradation of lignin concentration as well as a factor of incubation time while the fungal species did not significantly affect to degradation of lignin concentration. It was then followed by Duncan Multiple Range Test (DMRT) with level 5%, as it is seen on Table 1.

**Table 1.** Duncan Multiple Range Test on the interaction of WRF species and incubation time on lignin concentration (%)

Treatment	Average	Notation
A1B0	17.38	a
A1B1	10.61	c
A1B2	10.58	c
A2B0	17.38	a
A2B1	10.77	c
A2B2	9.78	d
A3B0	17.38	a
A3B1	11.67	b
A3B2	9.78	d

Note:

A1B0: *G. lucidum* with incubation time 0 day

A1B1: *G. lucidum* with incubation time 15 days

A1B2: *G. lucidum* with incubation time 30 days

A2B0: *P. tuber-regium* with incubation time 0 day

A2B1: *P. tuber-regium* with incubation time 15 days

A2B2: *P. tuber-regium* with incubation time 30 days

A3B0: *T.versicolor* with incubation time 0 day

A3B1: *T.versicolor* with incubation time 15 days

A3B2: *T.versicolor* with incubation time 30 days

The treatment of A2B2 and A3B2 were the most effective fungi (*P. tuber-regium* and *T. versicolor*) and incubation time (30 days) to degrade lignin from bagasse, reducing its concentration from 17.38% to 9.78% (Table 1). The previous studies reported that *T. versicolor* had a high delignification rate after 14 days of wheat straw fermentation, while levels of the analyzed ligninolytic enzyme activities were similar or even lower (Knezevic *et al.* 2013). *Trametes* species are well recognized as good laccase producers, especially in a copper-enriched substrate. Other research reported that *T. versicolor* able to degrade lignin concentration on hemp with 15.4% after 7 days incubation (Hossain & Anantharaman 2006). *T.versicolor* also showed the ability to produce three types of ligninolytic enzymes such as LiP, MnP and laccase with the dominated of enzyme activity by LiP. According to Ćilerdžić *et al.* (2017), the study of degradation of lignin in wheat straw using *G. lucidum* showed 38% degradation within 21 days incubation. Jonathan *et al.* (2008), investigated that *P. tuber-regium* able to degrade the waste wood. The greatest lignin degradation was noticed in the sawdust of *Holoptelia grandis*

within 0, 30, 60 and 90 incubation days (from 83.33% to 24.00 %).

The highest degradation showed by *T. versicolor* and *P.tuber-regium*, both of the fungal reported have the capability in producing LiP, MnP and laccase enzymes. The laccase enzyme activity in *T.versicolor* is higher among the three enzymes while in *P.tuber-regium* the MnP enzyme activity was significantly higher than LiP and laccase activities (Asif *et al.*, 2017; Nwogu *et al.*, 2012). Yanru *et al.* (2016), *G. lucidum* has the ability to produce lignocellulolytic enzymes such as peroxidase or laccase which can degrade cellulose, hemicellulose and lignin. This is in accordance with the statement of Mtui (2012). Lignin peroxidase (LiP) has different immunological and structural characteristics from manganese II-dependent peroxidase (MnP), which has the ability to oxidize important bonding members in lignin. In contrast, the role of MnP in lignin degradation has been suggested as indirect by providing H<sub>2</sub>O<sub>2</sub> for the lignin peroxidase reaction (Hossain & Anantharaman, 2006). The three ligninolytic enzymes, LiP, MnP and laccase work by different mechanisms. LiP was degraded non-phenolic lignin units, while MnP degraded on phenolic and non-

phenolic lignin units through lipid peroxidation reactions, oxidizing  $Mn^{2+}$  to  $Mn^{3+}$  which oxidizes the phenol ring to radicals phenoxy which lead to degradation of lignin. Laccase catalyzes the oxidation of phenolic units in lignin and phenolic compounds. The potential of laccase to degrade lignin is increased by acid phenolic compounds which will act as redox mediators. Without the role of redox mediators, the impact is limited (Saloheimo & Nikku-Paavola, 1991).

The longer the incubation time the more effect to degradation of lignin concentration. Incubation time play a vital role in degradation lignin and cellulose. Each fungal species has optimal enzyme activity in degrade lignin and cellulose concentration, certain incubation time is needed to optimize ligninolytic enzyme activity. According to Krik & Chang (1990), the optimum incubation time is a factor that allows good bio delignification in sengon wood sawdust. The best incubation time is the incubation time which results in the optimum decrease in lignin, resulting better degradation process.

## 2. Cellulose Concentration

The degradation of cellulose concentration in bagasse using three species of WRF and different incubation time also showed the degradation in cellulose concentration. The initial cellulose concentration of bagasse was 23.64%. The degradation by *G. lucidum* during 15 and 30 days has reduced cellulose concentration of bagasse to 16.66-18.06% and 18.83-20.17%, respectively. Species *P. tuber-regium* has reduced cellulose concentration to 18.92-22.08% and 19.93-22.75 % while species *T.versicolor* has reduced cellulose concentration to 19.53-22.16% and 22.16-22.99% respectively. The interaction of the three species of white-rot fungi and incubation time affected degradation of cellulose concentration, as well as

the factor of incubation time and the factor of WRF species (Table 3).

The interaction between incubation time and WRF showed a significant effect on the amount of degraded lignin and cellulose. The degradation of cellulose concentration in bagasse indicated that WRF is an organism which not only capable of completely degrading lignin into  $CO_2$  and  $H_2O$ . Its uniqueness in degrading lignin is very selective so it does not damage cellulose fibers relatively and fungal groups that produce ligninolytic enzymes (Risdiyanto *et al.*, 2007). The result of the analysis of variance in showed that the interaction between fungal species and incubation time significantly affects the degradation of cellulose concentration as well as a factor of incubation time and fungal species (Table 2). It was then followed by Duncan Multiple Range Test (DMRT) with level 5%, as it is seen on Table 3.

**Table 3.** Duncan Multiple Range Test on the interaction of WRF species and incubation time on cellulose concentration (%)

Treatment	Average/Standart Deviation	Notation
A1B0	23.64/0.000	a
A1B1	17.59/0.4973	e
A1B2	19.75/ 0.2409	cd
A2B0	23.64/0.000	a
A2B1	19.176/ 0.3120	d
A2B2	19.753/0.2468	b
A2B0	23.64/0.000	a
A3B1	19.946/ 0.493	c
A3B2	22.47/0.2502	b

Note:

A1B0: *G. lucidum* with incubation time 0 day  
A1B1: *G. lucidum* with incubation time 15 days  
A1B2: *G. lucidum* with incubation time 30 days  
A2B0: *P. tuber-regium* with incubation time 0 day  
A2B1: *P. tuber-regium* with incubation time 15 days  
A2B2: *P. tuber-regium* with incubation time 30 days  
A3B0: *T.versicolor* with incubation time 0 day  
A3B1: *T.versicolor* with incubation time 15 days  
A3B2: *T.versicolor* with incubation time 30 days

The effectivity of biopulping showed by the highest reduction of lignin and the lowest reduction of cellulose concentration. The DMRT of cellulose concentration in bagasse (Table 3.) showed that the interaction between WRF species and incubation time was significantly different in

the degradation of cellulose content. The most significant treatments were showed by A2B2 (*P. tuber-regium*, incubation time 30 days) that lowered cellulose concentration from 23.64% to 19.753%; and A2B3 (*T. versicolor*, incubation time 30 days) that reduced cellulose concentration from 23.64% to 22.47%. The degradation of cellulose concentration may happen because of the ability of WRF in producing enzymes and the utilization of cellulose as their carbon source (Rouches *et al.*, 2016). Cellulose contains about 50-90% of the crystallized part and amorphous (Aziz *et al.*, 2002).

The fungi could degrade cellulose by producing a group of cellulolytic enzymes that work synergistically. The rate of degradation of polysaccharides and lignin from the substrate can differ between species of white-rot fungi. According to Solar *et al.* (2006) & Aguiar *et al.* (2014), *T. versicolor* had ability to degrade lignin and cellulose loss resulting from bio-treatment. The Degradation of cellulose using *T.versicolor* and beech wood as the substrate showed the degradation of cellulose in 15 days is 53.57% while in 30 days of incubation time is 54.26%. Degradation of cellulose, similarly as in the case of lignin, proceeded since the early stages. The cellulose degradation caused fungal used the cellulose and lignin as a source of carbon.

According to Kuforiji & Fasidi (2009), the degradation of *P.tuber-regium* using different waste showed degradation of cellulose. The cellulose degradation of Rice straw showed the initial concentration from 38.6% to 31.5% while in cocoyam waste the degradation of cellulose concentration showed from 50% to 42.5% after 3 weeks incubation time. Adinipekun *et al.* (2012) reported that hemicelluloses and celluloses reduced when *Pleurotus tuber-regium* was used during biodegradation of agro industrial wastes. The degradation of cellulose concentration from

8.90% to 6.40% in rattan wood. The degradation of cellulose concentration based on incubation time. Fadilah (2008) reported that degradation of cellulose reaches 22.3% within 30 days of incubation time on leaf stem as a substrate. The longer incubation time the greatest reduction of cellulose. The incubation time play important role in degradation of cellulose concentration.

The incubation time has large influence in the degree of milling and strength of pulp. The previous study showed that incubation of 12-14 days produced a better quality of pulp compared to 10 days of incubation (Septiningrum & Ikhwan, 2017). The utilization of fungi in biopulping process can reduce the use of chemical substances, reduce the cooking time on chemical pulping, modified the specific fibers and improve the physical properties of pulp sheets such as tensile resistance and bursting resistance. Increased physical properties of these paper can occur because fungi are able to increase external fibrillation and internal fiber (Yang *et al.*, 2007). The efficiency of fungi in the bio pulping process depends on several factors, such as the type of fungal species, incubation time, pH and temperature.

**Table 4.** The Average pH and Weight of Bagasse Substrate

Isolate code	pH			Weight of Bagasse (g)		
	Incubation Time			Incubation Time		
	0	15	30	0	15	30
GL	6.8	6.5	5.43	200	195.3	190.3
PT	6.8	6.5	5.26	200	196.3	193.5
TV	6.8	6.6	5.7	200	194.3	192.3

Note:

GL: *G.lucidum*

PT: *P.tuber-regium*

TV: *T.versicolor*

The initial pH was 6.8. After 15 days of incubation the average pH of *G. lucidum*, *P.tuber-regium* and *T.versicolor* ranged between 6.5 -6.6 while the treatment of 30 days incubation showed that the average pH was 5.43 - 5.7(Table 4). These pH values are suitable for the growth of the fungi.

Generally, the value of pH for fungal growth ranges between 5-6. According to Away *et al.* (2000), to utilize hemicellulose, cellulose and lignin as a source carbon, the fungi should secrete enzymes. The action of enzymes influenced by several factors such as substrate, pH, cofactor, and inhibitor. Each enzyme have different optimum pH and temperature. Enzymes are proteins, which can change shape if the temperature and acidity change. Beyond the appropriate temperature or pH value, the enzyme cannot work optimally or the structure will be damaged. Besides pH, temperature also plays an important role in the production of the enzyme lignin peroxidase. Each fungi has a different optimum, minimum and maximum temperature for growth. Growth at temperatures below the optimum temperature can reduce the average cell metabolism. Temperature above the optimum, causing growth to decline and the possibility of death if it exceeds the maximum temperature. Table 4 showed weight loss of bagasse before and after treatment. The initial weight of bagasse substrate was 200 g, then after 15 days incubation time, the weight of bagasse decreased. Species *T.versicolor* showed the highest weight loss of bagasse with an average of 194.3 g. After 30 days of incubation, the species of *G.lucidum* showed the best treatment with an average of bagasse weight is 190.3 g. The weight loss of bagasse substrate caused by the fungal that utilize the component of bagasse such as lignin, cellulose and hemicellulose as their carbon source. According Septiningrum & Ikhwan (2017), the fungal degradation of lignin and cellulose affected to pulp yield as much 12.3%.

## CONCLUSION

Based on research result and discussion, it can be taken conclusions are the following that the interaction of white rot fungi species and incubation time affected in lignin and cellulose

degradation of bagasse in bio-pulping process. the best interaction between white rot fungi and time of incubation to degradation of lignin concentration from bagasse was fungal species of *P.tuber-regium* and *T.versicolor* within 30 days of incubation time, while the best interaction between white rot fungi and time of incubation to degradation of cellulose concentration from bagasse was fungal species of *P.tuber-regium* and *T.versicolor* within 30 days of incubation time.

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