PROCEEDINGS

2nd International Conference on Adaptive and Intelligent Agroindustry (ICAIA)

September 16 - 17, 2013

IPB International Convention Center
Bogor - Indonesia

Organized by:

Department of Agroindustrial Technology

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Indonesian Agroindustry Association

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Organized by :

Departement of Agroindustrial Technology, Faculty of Agricultural Engineering and Technology Bogor Agricultural University

George Mason University, Fairfax, Virginia, USA

Indonesian Agroindustry Association (AGRIN)

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WELCOMING ADDRESS

Prof. Dr. Ir. Nastiti Siswi Indrasti
Head of Agroindustrial Technology Department
Faculty of Agricultural Engineering and Technology
Bogor Agricultural University
On
Second International Conference on Adaptive and Intelligence Agroindustry (2nd ICAIA)
Bogor, September, 16 – 17, 2013

Assalamu'alaikum Warohmatullahi Wabarokatuh
In the name of Allah, the beneficent and the merciful,

Distinguish Guest, Ladies and Gentlemen
Let me first thank you all for accepting the invitation to participate in this 2nd International Conference on Adaptive and Intelligence Agroindustry (ICAIA). In particular I would like to thank Rector of IPB (Institut Pertanian Bogor/Bogor Agricultural University) Prof. Herry Suhardiyanto for supporting this event as part of the series academic event in celebrating the 50th Anniversary of Bogor Agricultural University.

In fact, the idea of organizing this conference was the continuation of the International Workshop on Computational Intelligence and Supercomputing Technology for Adaptive Agroindustry held by the Department of Agroindustrial Technology, Bogor Agricultural University last year.

Professor Kenneth A De Jong from George Mason University, US has successfully conducted joint international research with some staff from the Department of Agroindustrial Technology and Department of Computer Science, Bogor Agricultural University. The research aims to develop an integrated and intelligent system (namely SMART-TIN©) for the design of adaptive agroindustrial system in order to achieve a sustainable agroindustry that can mitigate global climate change and at the same time secure food, water, energy and natural medicine supply.

We are certainly proud to have been able to assemble this event in IPB, Bogor. The range of participants and audience at this conference is precisely something I would like to stress. The main goal of the conference is to provide an effective forum for distinguished speakers, academicians, professional and practitioners coming from universities, research institutions, government agencies and industries to share or exchange their ideas, experience and recent progress in Adaptive and Intelligent Agroindustry.

Distinguish Guest, Ladies and Gentlement,
Global climate change is the most challenging problems for us today and in the near future. This global change in our climate can lead to the shortage of the food, water, bioenergy and natural medicine that will affect the quality of human life. Many studies indicate that the threat of food, water, bioenergy and natural medicine crisis due to global climate change still worries our society. This problem can be solved by the development of agroindustry, i.e. an interrelated value chain entities from farming, to agro-processing industry and then to the end-customers. In fact, the design of agroindustry is complex and involves many factors and large data bases and more importantly, needs a good intelligence to process data and information to good decisions. Therefore, the way to design and manage agroindustry should be improved in order to meet the design objectives.

Agroindustries consume quite significant amount of energy on one side, on the other side they generate sizable amount of industrial wastes and its utilization as a captive energy resource is a kind of potential. Based on our study, a plywood industry with the production capacity of 200.000 m^3/year could generate 32 percentage of solid waste. If this amount of waste used as an energy alternative, it may result on the saving of 131.037.768.597 rupiah per month. Similar to plywood industry, sugarcane industry with the production capacity of 480 ton per hour could generate 154 ton per hour of waste (bagasse) and this amount of waste contribute to the saving of energy consuming by 19.250 Kwh. Recent study we conducted, indicated that cassava starch industry may contribute to a significant amount of waste. It has also potential usage as an energy resource. Based on our study the conversion of its waste into energy will contribute to the saving of energy usage of 4100 liter biogas per ton material.

The three industries mentioned is only examples of how potential the role of agroindustrial waste as an alternative resource in replacing the conventional energy resource as its presence will be significantly

reduced. The new, incremental energy contributions that can be obtained from waste biomass will depend on future government policies, on the rates of fossils fuel depletion, and on extrinsic and intrinsic economic factors, as well as the availability of specific residues in areas where they can be collected and utilized. All of these factors should be in detail examined to evaluate the development of the industrial waste contribution. Hope this conference will also discuss this issue in more detail as it is an important matter for all of us. We should no more think just how to produce high value product but it is also necessarily important how to keep our live in good quality by understanding following old saying…" only when the last tree has been cut, only when the last fish has been angled, and only when the last river has been polluted, then we realized that we could not eat money".

I do not to take up any more of your time with these opening remarks. Let me simply thank you once again for sharing your thoughts with us. Here's wishing every success for the conference. May Allah bless all of us.

Thank you for your kind attention,
Wassalamu'alaikum Warohmatullahi Wabarokatuh

AGENDA of
2nd International Conference on Adaptive and Intelligent Agroindustry (ICAIA)

Time	Activities	Room
Day 1 (16 September 2013)		
08.00 – 09.00 (60')	Registration	
09.00 – 10.00 (60')	Opening Ceremony • Welcoming Address: Prof. NastitiSiswiIndrasti (Head of Dept TIN, Fateta, IPB) • Conference Opening: Prof. HerrySuhardiyanto(Rector of IPB) o ABET Certification announcement and short ceremony o Launching International Double Degree Master Program in Innovation and Technopreneurship in Cooperation with University of Adelaide, Australia o Soft-launching Master in *Logistik Agroindustri* (Agroindustrial Logistics)	Ballroom
10.00 – 10.45 (45')	Opening Speeches: Prof. IrawadiJamaran (Agroindustry Guru, IPB: 25') Prof. Eriyatno (Industrial and System Engineering, IPB: 20')	Ballroom
Session 1		
10.45 – 11.15 (30')	Keynote Speech Dr. YandraArkeman (IPB)	Ballroom
11.15 – 12.00 (45')	Keynote Speech Prof. Kenneth De Jong (George Mason University, USA)	Ballroom
12.00 – 13.30 (90')	Lunch Break	
Session 2		
13.30 – 15.15 (105')	Moderator: Prof. EndangGumbiraSa'id (IPB) Invited Speakers (1-4) (4 x 20 minutes) Discussion (25 minutes) Tentative Schedule: Prof. Kim Bryceson (Australia), Prof. SyamsulMa'arif (IPB), Prof. KudangBoro Seminar (IPB), Prof. HaruhiroFujita (Japan)	Ballroom
15.15 – 15.45 (30')	Break	
15.45 – 17.30 (105')	Moderator: Prof. Marimin (IPB) Invited Speakers (5-8) (4 x 20 minutes) Discussion (25 minutes) Tentative Schedule: Dr. Gajendran (UK), Prof. Noel Lindsay (University of Adelaide), Dr. KuncoroHartoWidodo (UGM), Prof. UtomoSarjonoPutro (ITB)	Ballroom
Day 2 (17 September 2013)		
08.00 – 08.30 (30')	Registration	
08.30 – 10.15 (105')	Moderator: Prof. KudangBoro Seminar (IPB) Invited Speakers (9-12) (4 x 20 minutes) Discussion (25 minutes) Prof. Egum (IPB), Prof. Marimin (IPB), Dr. AgusBuono (IPB), Dr. HeruSukoco (IPB)	
10.15 – 10.30 (15')	Coffee Break	
10.30 – 12.30 (120')	Parallel Session 1 Moderator: Prof. Fujita (7 paper @ 15 minutes) Discussion (15 minutes)	Parallel Session 2 Moderator: Prof. Ono Suparno (7 paper @ 15 minutes) Discussion (15 minutes)
		Parallel Session Moderator: Prof. Suprihatin (7 paper @ 15 minutes) Discussion (15 minutes)

12.30 – 13.30 (60’)	Lunch Break	
13.30 – 15.00 (90’)	Open Discussion (Open Forum) with Prof. Kenneth De Jong Topic: Foundations and Applications of Genetic/Evolutionary Algorithms	Ballroom
15.00 – 15.30 (30’)	Conference Closing	Ballroom
15.30 – 17.00 (90’)	Indonesian Agroindustry Association (AGRIN) National Congress (PIC: Prof. Suprihatin)	Ballroom
17.00 – 17.45 (45’)	Refreshment and Closing of AGRIN National Congress	Ballroom

CONTENT LIST

Corncob Biodelignification Process Using White Rot Fungi

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ABSTRACT

Corncob has a big potency to be converted become simple sugars which can be used as raw materials for various products such asbioethanol and xylitol. Biodelignification is a process to remove lignin from cellulosic materials by using microorganism like fungi, bacteria or enzyme. Delignification need to be done because lignin is main barrier at lignocellulose hydrolysis process. Some types of white rot fungi often applied for delignification are *Schizophyllum commune*, *Phanerochaete chrysosporium* and *Pleurotus* EB9. The aims of this research is to obtain the best type of white rot fungi,to get optimum concentration of mycelia, the bestconcentration of added glucose and the most optimum incubation time for the chosen fungi. The results of preliminary researchshowed that corncob delignification by fungi *Phanerochaete chrysosporium*was the best one by decreasing of lignin content up to 26.07% andby giving degradation of dry weight up to 28.22%. Therefore, *Phanerochaete chrysosporium* was chosen to be used in main research. Results of the main research showed that optimum condition for delignification of corncob by *Phanerochaete chrysosporium* wasas follows: incubation time of 24.25 days, concentration of mycelia 1.89 ml/10 g substrate, and concentration of glucose added was 0.23 g/10 g substrate. From these conditions,the obtained value of lignin rate ratio to holocellulose was 0.210689 g/g.
Keywords: Corncob biodelignification, *Schizophyllum commune, Phanerochaete chrysosporium* and isolate *Pleurotus* EB9.

A. INTRODUCTION

Corncob is a byproduct of agricultural industry activity which is also the source of lignocellulose. Indonesian dry shelled corn production reached 19.38 million tons in 2012 (BPS, 2013). Corn contains approximately 30% of corn cobs and the rest are seeds and skins (Koswara, 1992).

According to Iswanto (2009), a corncob contains 15% of lignin, 45% of cellulose and 35% of hemicellulose. With this abundant amount of availability and the high content of cellulose and hemicellulose in it, corncob has a great potency to be processed into high economic products and also improved animal feeds. Corncob hydrolysis will produce simple sugars such as glucose, xylose, mannose and arabinose. These kind of sugars can be used as raw materials for huge variety of refined products such as carboxylic acids, bioethanol, xylitol, amino acids or other complex products.

Prior to corncob hydrolysis process, a delignification pretreatment is required. Delignification needs to be done because lignin is a major obstacle in the process of cellulose hydrolysis. Lignin which protects cellulose is resistant to hydrolysis because of the bond and the alkyl aryl ether linkage (Perez *et al*., 2002). There are several common delignificationmethods, such as mechanical, chemical and semi chemical process. However, those methods have some weaknesses such as high needs of chemicals and energy. In

addition, the liquid waste of delignification processwhichcontain chemicals are classified as hazardous waste. It is toxic and tends to pollute the environment (Martina, 2002).

Several studies have been done to solve those problems by implementing biological delignification method that known as biodelignification. White rot fungi is one of microbes that can break down lignin selectively but just breaking down cellulose and hemicellulose in very small amounts (Eaton and Hale, 1993). There are several types of white rot fungi that are often be used for delignification process such as *Schizophyllum commune, Phanerochaete chrysosporium* and *Pleurotus* EB9.

Those three types of fungi are able to do biodelignification process in wood. Realizing that characteristic, this study observed the ability of those fungi in corncob delignification process. In addition, this study also observe the influence of incubation time, the amount of mycelia and the amount of additional glucose that was added to the lignin content ratio to holocellulose. To measure biodelignification effectiveness process, the ratio of holoselulosa to lignin content was calculated. Lowest ratio shows that these conditions are optimum conditions for the growth of fungi.

B. METHODOLOGY

1. Preliminary Research

Preliminary research was conducted to know the characteristic of corncob and to select the best fungi that can degrade the lignin content from corncob. There some steps that were being taken.

a. Corncob Characterization

At this stage, corncob were being diminished up to ± 20 mesh using Hammer Mill and followed by disc milling cutter up to ± 40 mesh. The downsizing result was dried using oven at temperature of 50 °C (up to 10% final moisture). At this stage, proximate tests were performed to measure moisture, ash, fat, protein, and fiber content. In addition, fiber component analysis was also conducted, i.e. extractives, holocellulose, cellulose, hemicellulose and lignin

b. The Best Fungi Selection

This stage aims to obtain the best white rot fungi among the three types of fungi which are *Schizophyllum commune, Phanerochaete chrysosporium* and *Pleurotus* EB9 isolate. As its phases are:

a. Isolate Refreshment

Fungi were refreshed on *Potato-Dextrose Broth* (PDB) which already been sterilized at temperature of 121^{o}C for 15 minutes using autoclave then followed by incubation in room temperature for 5-10 days.

b. Culture Preparation

Materials that were used in this stage are shown at Table 1.

Table 1. Materials of Culture

Materials	Amount
KH_2PO_4	7.2 g
$MgSO_4.7H_2O$	1.5 g
$CaCl_2.H_2O$	0.3 g
$FeCl_3.6H_2O$	0.045 g
$ZnSO_4.7H_2O$	0.023 g
$CuSO_4.5H_2O$	0.015 g
$MnSO_4.H_2O$	0.03 g
Aquades	150 ml

Source: Fadilah (2009)

c. Fungi Suspension Production

Fungi suspension was made by separating mycelia from its liquid media using sterilized filtering paper. Then fungi suspension was mixed with 20 ml Tween solution 80 0.01% in a volumetric flask 50 ml and diluted with sterilized water up to 50 ml.

d. Media Preparation and Incubation

10 grams of 40 mesh corncob powder in a 6 cm diameter glass bottles, added with 0.01gram of glucose and been stirred until homogeneous, followed by 15 ml culture medium addition. The media were sterilized in an autoclave at a temperature of 121^{o}C for 15 minutes, cooled and inoculated by adding 2.5 ml of fungi suspension. This media were incubated in an incubator for 30 days at a temperature of 30^{o} C. The best fungi was selected based on its ability to degrade lignin.

2.Main Research

Activities undertaken in the main research include refreshment of isolates, culture preparation, fungi suspension production, media preparation, incubation of white rot fungi and observation of lignin degradation result.

a. Isolate Refreshment

Fungi are refreshed on *Potato-Dextrose Broth* (PDB) which already been sterilized at temperature of 121^{o}C for 15 minutes using autoclave and followed by shuffling using shaker during incubation in room temperature for ± 5-10 days. The aim of shuffling was to downsize the size of mycelia so that easier to be extracted.

b. Media Preparation and Incubation of White Rot Fungi

This stage aims to provide optimum concentrations of fungi, glucose concentration and optimum incubation time for fungi that obtained from the preliminary research. An amount of 10 grams of 40 mesh corncob powder in a 6 cm diameter glass bottles, was added with 0.01gram of glucose and stirred until homogeneous. Then 15 ml culture medium was added. The media were sterilized in an autoclave at a temperature of 121^{o}C for 15 minutes, cooled and inoculated by adding fungi suspension. Then the media were incubated in an incubator for at a temperature of 30^{o} C

e. Observation to Lignin Degradation Result

Parameters measured in this study were the reduction of dry weight percentage, levels of extractive substances (TAPPI T 6m-59), levels of lignin (TAPPI T 13 os-54), levels holosclullose (TAPPI T 9 m-54), cellulose (TAPPI T 17 m-55) and hemicelluloses. Moreover, this study also measure the dry weight of mycelia /ml suspension of fungi and microscopic observation to corncob structure before and after delignification.

3.Determination of Influence Factor Against Lignin/Holocellulosa Ratio

This stage determines the influence of the amount of glucose, the amount of mycelia that were added and the incubation time to the ratio of lignin / holocellulose. The experimental design used in this research was factorial design 2^3*Full Factorial Central Composide* (CCD). This experimental design has a response equation as follows:

$$LH_N^* = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{33} x_3^2 + \beta_{12} x_{12} + \beta_{13} x_{13} \ldots + \beta_{23} x_{23}$$

LH_N^* is the ratio of lignin content to holocellulosa (g/g), x_1 is the incubation time (days), x_2 is the number of mycelia that were added (ml), and x_3 is the amount of glucose added (g), while β is the coefficient. Value of these factors are shown in Table 2.

Table 2. Value of Research Treatment Factor.

Factor	Extreem Value	Minimum Value	Average Value	Maximum Value	Extreem Value
Incubation Time (day)	3.18	10	20	30	37.8
Micelial (ml)	1	2.5	6.25	10	12.56
Glucose (g)	0.032	0.1	0.2	0.3	0.368
Code	+α-1.68	1	0	+1	+α = +1.68

3. Data Analysis

Lignin content and holocellulose data that were obtained from chemical analysis been analyzed statistically to determine the incubation time, the addition of glucose and mycelia to the ratio of lignin/holocellulose. Analysis and determination of the best conditions in the delignification process was done by using *Statistic Analysis Software* (SAS) version 9.1. MINITAB 14 was used to provide three dimensional images of the surface response.

C. RESULT AND DISCUSSION

1. Characterization of Corncob

a) Proximate Analysis

Proximate analysis was conducted to determine initial conditions of corncob. The result is shown in Table 3. Initial corncob water content was 10.71%. This data indicates the corn cob have low moisture content that cause the material more resistant to microbial damage during storage.

Table 3. Corncob Proximate Analysis Result

Component	% (w/w)	%(d.w)
Water	10.71	-
Ash	1.69	1.89
Protein	0.60	0.67
Fat	2.34	2.62
Fiber	79.15	88.64
Carbohydrate (*by difference*)	5.51	6.18

Note: % w.w = wet weight percentage; % d.w = dry weight percentage

The ash content in corncob was about 1.69%. Ash content shows the amount of inorganic and mineral content in a particular substance.

Protein content in corncob was very low, only 0.60 %. This shows that the corncob in this study has not enough nutrition to be applied directly as an animal feed. This corncob has 2.34 % of fat. Fat is an extractive substance that can be dissolved in organic solvent such as eter, aceton, etc. (Fengel and Wegener, 1995).

The high content of fiber in the corncob (79.15%) showed that this substance is a good carbon source which support microbial growth, especially microbe that can degrade fiber component (ligninolyticand celullolytic).

b) Lignocellulose Content Analysis

Lignocellulose is the main component of a crop that represents the amount of organic matter that can be renewed (Sjostrom, 1995). Lignocellulose consists of cellulose, hemicellulose, lignin and some other extractive materials. All components of lignocelluloses can be found in cell walls. The result of lignocelluloses analysis is shown in Table 4.

Tabel 4. Composition of Corncob's Lignocellulosesbefore Delignification

Component	% d.w
Extractive material	4.92
Lignin	19.74
Cellulose	41.45
Hemicellulose	33.91

Note: % d.w = dry weight percentage

The initial corncob lignocellulose components analysis showed that corncob has 19.74% lignin content, 41.45%cellulose content and 33.91% hemicellulose. While the corncob that were used by Iswanto in his research (2009) has 15%lignin, 45% cellulose and 35% hemicellulose content. It shows that each corncob has different lignocelluloses compositions. Differences in the chemical composition of the corncob is influenced by several factors such as differences in varieties, place of growth, weather and humidity while harvesting (Shofiyanto, 2008).

2. The Best Fungi Selection

The main parameters to select the best fungi is the ability of each fungi which are, *Schizophyllum commune,Phanerochaete chrysosporium* and isolat *Pleurotus* EB9 to degrade lignin during 30 days of incubation. The reduction percentage of lignin content by each fungi is shown in Picture 1 as follows.

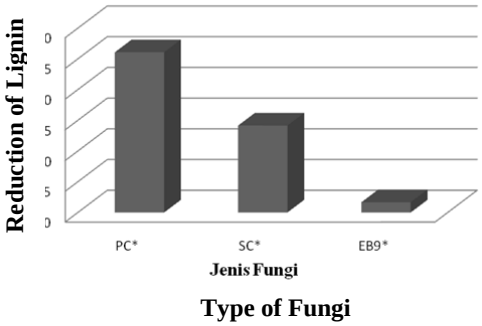


Figure 1 : Percentage of Reduction Corncob Lignin Content

After 30 days of incubation, corncob which was incubated with *P. chrysosporium* has the highest reduction of lignin content level among the other two types of fungi (26.07 %). This fungi has the ability to degrade lignin component of most large corncob among others. Therefore, *P. chrysosporium* was selected to be used in the main research.

Decreasing lignin content by *Pleurotus* EB9 isolate after 30 days of incubation was very low which was 1.66%. Different from *P. chrysosporium* which can produce enzyme that can degrade components of phenolic (MnP) and non-phenolic components (lip) at once, *Pleurotus* EB9 only produces one type of lignin-degrading enzymes which is *laccase* enzyme. According to Kimura *et al.* (1990), attempts to detect the activity of the enzyme in lignin peroxidase *P.ostreatus* under some culture conditions did not show positive results. Sannia *et al*. (1991) found that there is *laccase* enzyme activity in white rot fungi *Pleurotus*. *Laccase* is a copper-containing phenol oxidase that does not require H_2O_2 but oxygen (Thurston, 1994). *Laccase* can oxidize non-phenolic components if there is a mediator such as (2,2- azinobis (3-etilbenzthiazolin-6-sulfonat) or HBT (hydroxybenzotriazol) (Bourbonnais and Paice, 1990). In fact, approximately 90% lignin structure composed of non phenolic unit (Srebotnik *et al*., 1994). This caused the small decreasing lignin percentage in substrate which incubated with *Pleurotus* than *P. chrysosporium*

Likewise *Pleurotus*, *S. commune* is known as fungi that can degrade lignin by only producing *laccase* enzyme. The lower end of the weight lignin experienced by the substrate after incubation with *S. commune* is mainly because the greater dry weight reduction of the substrate.

Moreover, corncob also experienced decreasing in its dry weight. Likewise lignin reduction content, corncob that was incubated with *P. chrysosporium* has the highest reduction dry weight among the othe fungi at 28.22% (Picture 2).

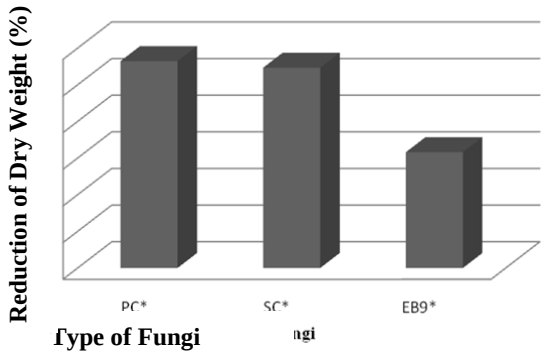


Figure 2 : Reduction of Corncob Dry Weight Percentage

Reduction of corncob dry weight percentage is one indicator of the existence of biodegradation by fungi. Refer Picture 2, it can be inferred that *P. chrysosporium* has the highest ability to decrease corncob's dry weight than the other fungi in this study. Reduction of corncob's dry weight during incubation happened because there was enzyme produced by the fungi that degrade corncob. According to Hatakka (2001), *white-rot fungi* produce some kind of enzyme that involved in lignin degradation process, and also produce cellulase, xilanase dan hemicellulase.

3. MAIN RESEARCH

1. Corncob Microscopic Structure

Initial fiber corncob microscopic structure and fiber corncob microscopic structure after incubation with *P. chrysosporium* is shown in Picture 3. In that picture looks thread-like structures called fibrils. Fibrils is a combination molecules of cellulose and contains regular and less regular part. Initial corncob structure Picture 3 (a) shows fibrils structure which still straight arrayed. However, after 30 days of incubation (Picture3 (b)) it can be seen that fiber structure seems more tenuous. This happened because during the incubation there was degradation activity by enzymes released by fungi, thus causing the cell walls are increasingly porous and produce a honeycomb structure. Cellulose fibrils lysis zones are unprotected and loose (Fengel and Wegener, 1995).

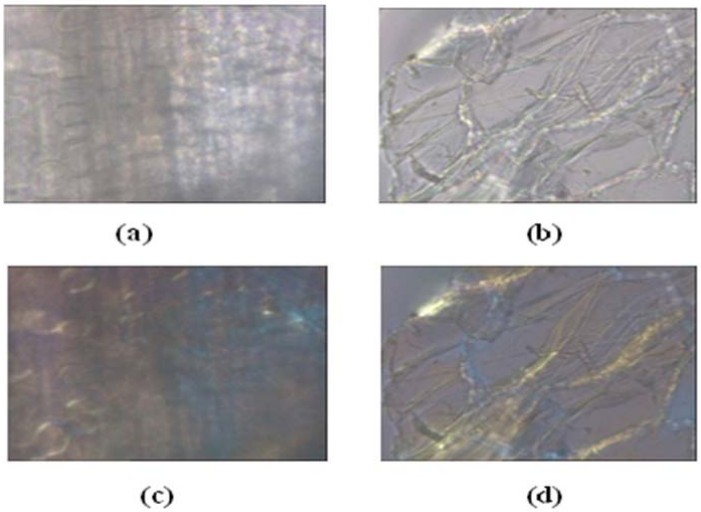


Figure 3 : Corncob Fiber Microscopic Structure (400x of zooing): **without polarized light** (a) before lignification, (b) after 30 days incubation; **with polarized light**; (c) before delignification, (d) after 30 days incubation

Corncob appearance with polarized light (Picture 3 (c), (d)), there is a bluish color that shows the structure of crystalline cellulose. On the initial corncob structure (before delignification) appereance there is a structure of crystalline cellulose which characterized by a bluish color. In the 30 days of incubation bluish color in polarized light become less then before. This shows that the lignin content directly proportional with the length of incubation time. According to Knauf and Moniruzzaman (2004), changes in the structure of lignocellulose that has already been pretreated with separation between the cellulose and material that protect it (hemicellulose and lignin). Changes in the structure of cellulose which was originally shaped crystals become amorphous, makes it easy to be hydrolyzed.

2. Biodeliginification by *P. chrysosporium*

a. Dry Weight After Incubation

Incubation treatment *P. chrysosporium* on corncob has caused decreasing and increasing its dry weight. The result of dry weight measurement of initial corncob and post delignification corncob is shown in Picture 4.

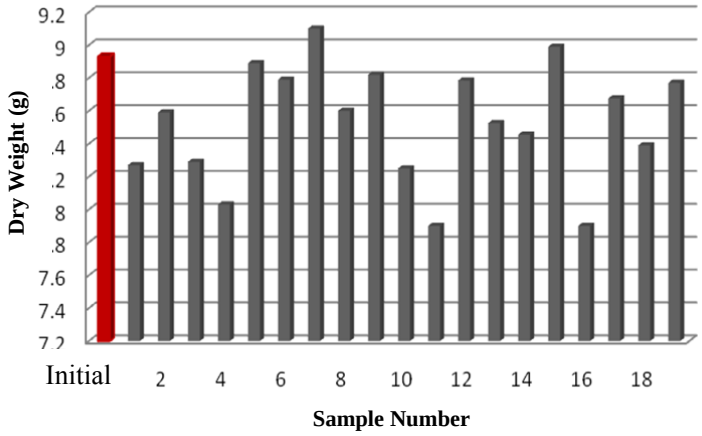


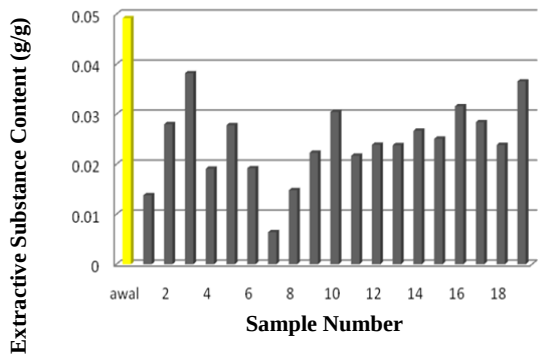
Figure 4 : Dry weight of Corncob before (initial) and after Delignification

Degradation of lignocelluloses component is marked by decreasing of dry weight substance. Enzyme that produced by fungi catalyzes the biochemical reactions in lignocellulose media, so that the holocellulose and lignin can be changed into simpler compounds. Then fungi can absorbed and metabolize these compounds (Herliyana, 1997).

However, the results obtained from this study showed an increasing dry weight in both treatments. This increasing dry weight happened due to the fast growth of mycelia. According to Fadilah (2009), the addition of nutrients such as glucose in the medium has two advantages; one of them is the rapid growth of fungi on media. Because of the addition of mycelia, there was an increase in the dry weight of the substrate after delignification.

b. Extractive Substance Content

Extractive substance consists of several organic compounds such as acids, resins, fatty acids, terpenes and alkaloids (Sjostrom, 1995). Extractive substances have varies molecular weights. The substances can be found in lignocellulose contained materials but not construct the cell wall. The ability of *P. chrysosporium* degrades extractive component is influenced by the character of its extractive substance. There are some extractive substance in corncob which are 0.7% of fat and uronat acids up to 3.36% (Parajo *et al.,* 2003) (Figure 5).



Figu Initial ractive substance content (g/g) which dissolved in etanol:benzene (1:2) before (initial) and after delignification

According to Rayner and Boddy (1995) the presence of wood extractive substances can affect the growth of fungi, which acts as a carbon source, as a growth stimulant and as an impediment to growth. Decreasing number of extractive substances happened because it is estimated that the extractive substances contained in the corncob are used as a carbon source by fungi.

c. Lignin Content

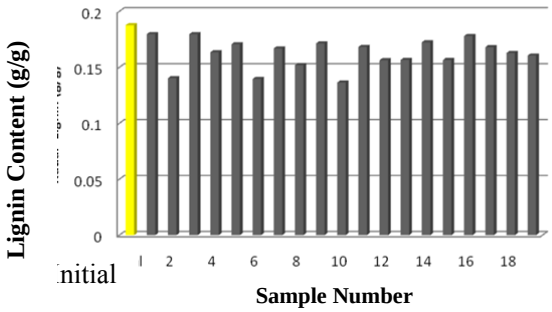


Figure 6 : Lignin Content (g/g) before (initial) and after Delignification by *Phanerochaete chrysosporium*

The results (Figure 6) showed that the incubation time of 37.8 days (Experiment No. 10) causes the higest decrease in lignin content from 0.187 (g/g) to 0.136 (g/g) 32.82%. From the data also found that the longer incubation time, the bigger decrease in corncob lignin content. This shows that the fungi *P. chrysosporium* is an efficient ligninolytic fungi.

d. Cellulose Content

Beside degrading lignin components, *P. chrysosporium* also capable to degrade cellulose. Cellulose degradation is a work of a group of cellulolytic enzymes that work synergistically. Fungi *P. chrysosporium* produces cellulase enzymes which has similar activity with endogluconases (EGs) and exocellobiohydrolases (CBHs) depends on the carbon source that available (Broda *et al*., 1996).

Cellulose content was decreasing during incubated with *P. Chrysosporium* (Figure 7). This decreasing shows that during the incubation *P. chrysosporium* has broken down cellulose component by using cellulolytic enzyme.

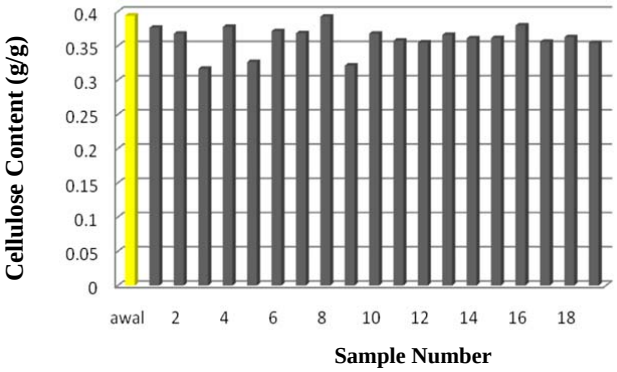


Figure 7 : Cellulose content (g/g) before (initial) and after Incubation Treatment with *Phanerochaete chrysosporium*

Cellulose will be broken down into simpler compounds by fungi which then used as nutrition for its growth. Those compounds then will be used in its metabolism cycles. Additional sugar as nutrition is a factor that decreases cellulose degradation. The more glucose that is added, the less rate of cellulose degradation will happen. This is because the fungi will consume glucose first before degrade cellulose into simpler sugars

e. Hemicellulose Content

Hemicellulose is biodegraded into sugar monomer and acetic acids with the help hemicellulase e Initial Hemicelulase as like as many other enzyme which can hydrolize cell plant. Xylan is the main carbohydrates that develop hemicellulose. Xylanase is also the main hemicellulase which hydrolyze β-1,4 bonds. Fungi *P. chrysosporium* produced endoxylanase that plays a role in the breakdown of xylan into oligosaccharides (Perez *et al*., 2002).

In Picture 8 it can be seen that some samples experienced increasing number of hemicellulose content. Increasing in the hemicellulose content of the substrate can be caused by miscounting of mycelia as part of the hemicellulose. This is probably happened because in some samples mycelial growth rate higher than the rate of degradation of lignocellulosic components. According to Chang and Miles (1989), the average mycelial fungi have fiber content ranging at 7.4%-24.6%.

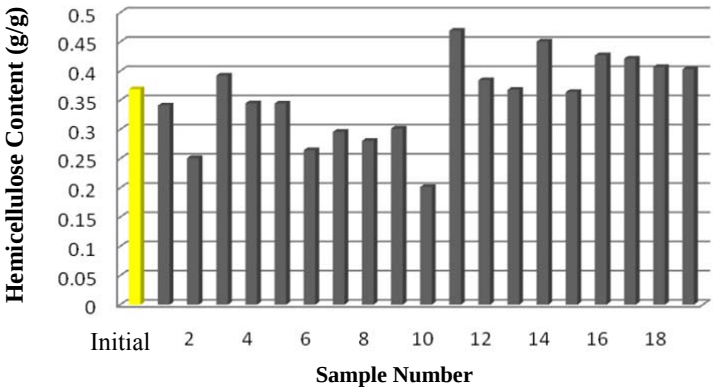


Figure 8 : Hemicellulose content (g/g) before (initial) and after incubation treatment by *Phanerochaete chrysosporium*

3. Influence of Factor against Ratio Lignin/Holocellulose Content

Biodelignification process is influenced by some factors which are duration of incubation, amount of nutrition and amount of additional mycelia that is added into media. Those factors can be optimized so that can increase delignification efectivity. Increasing delignification efectivity can be seen from degradation lignin levels by *P. chrysosporium*.

Ideal delignification process is happened when lignin was decomposed in large numbers but holocellulosa components broken down in small amounts. Holocellulose which is a part of the fiber that is free from extractive substances and lignin, consist of all the cellulose and hemicellulose components (Chang and Alan, 1971). Therefore, the main parameter to assess the effectiveness of delignification is decreasing lignin and holocellulose content level.

This research measures the influence of incubation time, the amount of mycelia that were added and the amount of glucose that is added to the ratio of lignin/holocellulosa as well as the interaction of these three factors to the ratio of lignin/holocelulose. Relation between factor reaction with response can be determined through a systematic series of experiments that tested through statistical analysis.

Table 5. Influence of Linear and Quadratic Factors Against the Ratio of Lignin/Holocellulose

Source	Probability p > F	Effect
X_1	< 0001	Significant
X_1*X_2	< 0001	Significant
X_2	0.8335	Not significant
X_2*X_2	0.7999	Not significant
X_3	0.6172	Not significant
X_3*X_3	0.4023	Not significant

Results of statistical analysis (Table 5) showed that both the linear and quadratic effect of incubation time gave a significant effect on the ratio of lignin / holoselulosa. Yet, in this study the addition of mycelia (x_2) in delignification process did not decrease lignin content ratio against holocellulose. Though it appears from the graph that the lignin content against holocellulosa ratio slightly decreased with increasing the amount of glucose that is added, but from the linear and quadratic effects analysis it can be inferred that the amount of glucose was not significantly affected the response (Figure 9).

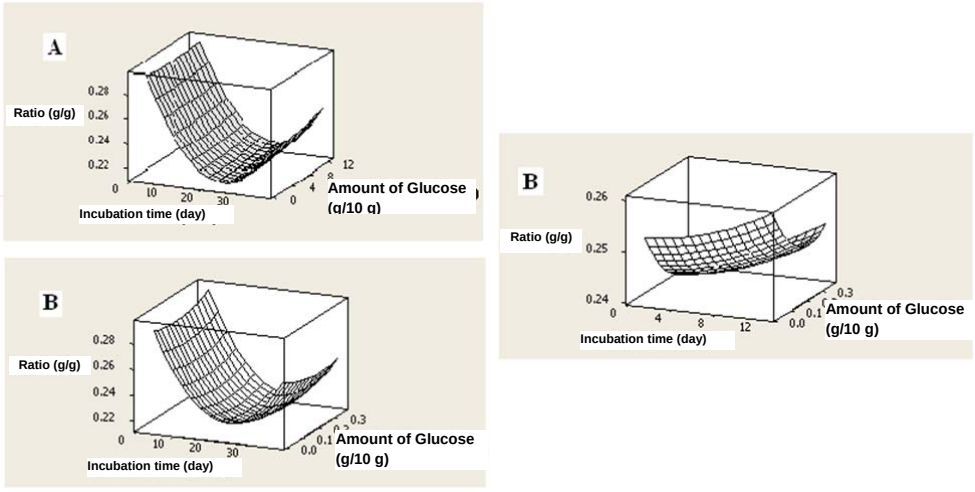


Figure 9 : Influence Combination of Length of Incubation Time (day), Amount of mycelia (ml/10 g substrate), Amount of glucose (g/10 g substrate) that is added against ratio lignin/holocellulose

Interaction effect between the number of mycelia, length of incubation time and the amount of glucose that is added to the process showed no significant effect to the value ratio lignin/ holoselulosa. It can be seen from the shape of response surface that which not tend to change in various combinations with other factors.It is reinforced by opportunities value p> F that showed interaction effect was not significant (Table 6).

Table6. Interaction InfluencebetweenLength of Incubation Time, Amount of Mycelia and Glucosethat is Added againstLignin/Holocellulose Ratio

Source*	Probability p > F	Effect
X_1- X_2	0.8117	Not Significant
X_1- X_3	0.6916	Not Significant
X_2-X_3	0.9754	Not Significant

4. THE BEST CONDITION FOR BIODELIGNIFICATION PROCESS

The result of data analysis ratio lignin/holocelluloseat various treatment producedoptimization equation ratio lignin/holocellulose for delignification process as follows.

$$LH_N^* = 0.314541 - 0.007922\ x_1 - 0.000709\ x_2 - 0.061692\ x_3 + 0.000168\ x_1^2 + 0.000046\ x_2^2 + 0.194656\ x_3^2 + 0.000020\ x_{12} - 0.001181\ x_{13} + 0.000246\ x_{23} \quad \text{..... (2)}$$

$LH_N^*$is symbol for ratio lignin/holocellulose (g/g), $x_1$is length of incubationtime(day), $x_2$is additional mycelia (ml), and $x_3$is additional glucose (g).

The results of the canonical analysis to the response surface showed that the response surface model is minimum. This causes the optimum value can be determined from the response surface. Determination of the best conditions of biodelignifikasi process using *Phanerochaete chrysosporium* is shown in Table 7.

Tabel 7. The Best Condition of Biodelignification Process Using *Phanerochaete chrysosporium*

Factor*	Code	Value
X_1	0.22	24.25
X_2	-0.85	1.89
X_3	0.18	0.23

***** x_1 : Length of Incubation time (day); x_2 : Amount of mycelia (ml/10 g substrate) ; x_3 : amount of glucose(g/10 g substrate).

That condition is estimated giving ratio of lignin content against holoselulosaup to0.210689 g/g. It means there is 0.210689 g of lignin in each 1g of holocellulose. This ratio is obtained in 24.25 days length time of incubation using *Phanerochaete chrysosporium*, the amount of additional mycelia is 1.89 ml/10 g substrate and the additional glucose is 0.23 g/10 g substrate.

5. CONCLUSION

Based on its ability to degrade lignin, *Phanerochaete chrysosporium* isolate has better capability than *Schizophyllum commune* and *Pleurotus* EB9 isolate. This is proven by the decreasing value of lignin content from corncob that was incubated with *P. chrysosporium* for 30 days at room temperature 26.07^{o}C.

Corncob biodelignification process effectiveness using *P. chrysosporium* can be seen from decreasing ratio lignin/holocellulose level. The result of statistical analysis showed that length of incubation time significantly affected the decreasing of lignin/holocellulose ratio level. However, various additional mycelia and amount of glucose did not significantly affect the decreasing lignin/ holocellulose ratio.

From the result of surface respon analysis, it can be inferred that the optimum condition for corncob biodelignification process is determined by response equation model, as follows.

$LH_N^* = 0.314541 - 0.007922\ x_1 - 0.000709\ x_2 - 0.061692\ x_3 + 0.000168\ x_1^2 + 0.000046\ x_2^2 + 0.194656\ x_3^2 + 0.000020\ x_{12} - 0.001181\ x_{13} + 0.000246\ x_{23}$

This equation provided decreasing value of ratio lignin/holoselulosa up to 0.210689 g/g. This ratio derived from 24.25 days of incubation using *Phanerochaete chrysosporium* on the substrate of corncob with additional mycelia up to 1.89 ml/10 g substrate and the amount of glucose is 0.23 g/10 g substrat.

6. ACKNOWLEDGEMENT

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