Preliminary study to determine the glucose levels in cassava peel waste (Manihot esculenta Crantz) as a result of enzymatic activities of fungi Aspergillus fumigatus

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Abstract. The aim of this study was to utilize cassava peel waste, with a lignin content of 19% as a raw material for bioethanol. The process of obtaining bioethanol from this material was conducted in 4 stages, and these include pretreatment/delignification, hydrolysis, fermentation, and distillation. The delignification was conducted using fungi Aspergillus fumigatus in Potato Dextrose Broth media and 40 mesh was prepared. Moreover, the pretreatment process was conducted by varying the ratio of cassava peel powder as a substrats to fungi at 1:1, 2:1, and 1:2 and at contact times of 1, 3, and 5 days and analyzed through the use of Chesson method. Moreover, the hydrolysis stage used variations of sulfuric acid (H2SO4) at 1, 3, 5% (v/v) heated at 100°C with contact time varied at 30, 60, and 90 minutes. The process was further analyzed by using the DNS method to obtain sugar levels. The result therefore showed the best lignin content after pretreatment to be 8% and the delignification of cassava peel was obtained from the enzymatic activity of A. fumigatus. It was also discovered that 10 g/L of sugar was produced from the total sample used. However, further research is needed to increase the sugar content of cassava peel raw material.

1. Introduction
Increasing human needs in the agricultural sector cause the production of cassava (Manihot esculenta crantz) to be high, such that in 2014, the amount of cassava peel waste reached 9,752,345 tons/year [1]. The percentage of outer peel ranges between 0.5-2% of the total weight of fresh cassava while the inner part reaches 8-15% [2]. The peel is often used as animal feed or discarded as garbage, however, it contains cellulose, hemicellulose, and lignin at 43.626%, 10.384%, and 7.646% respectively [3].

Bioethanol (C2H5OH) or ethyl alcohol is an alcohol conformation that has recently emerged as an environmentally friendly bio-renewable energy, used as an alternative fuel in automotive engines and a potential substitute for gasoline in vehicles [4]. This compound is synthesized from the fermentation of sucrose or simple sugar, from various types of biomass either raw materials or non-raw sources [5]. However, cassava peel has a high carbohydrate content, making it useful as a raw material for bioethanol. Currently, the production of this compound using cellulose and lignocellulose materials, especially waste, provides alternative solutions to overcome environmental, economic and energy problems being faced throughout the world [6]. Fortunately, lignocellulose material from cassava peel can be used as a raw material to produce this bioethanol, which has recently been seen as a promising alternative to overcome the increasingly scarce availability of fossil fuels [7].
Bioethanol production from lignocellulosic biomass includes several processes such as biomass pretreatment, enzymatic hydrolysis, fermentation, and distillation processes to separate water and ethanol [8]. During the last two decades, research has been focused on the optimization and incorporation of each process using various types of biomass and biocatalysts [9]. Moreover, ethanol production from lignocellulose biomass has been reported to require higher costs than sugar-based raw materials [10].

Cassava peel contains a lot of lignocellulose, therefore, it has the potential to produce bioethanol. However, pretreatment and hydrolysis processes are needed to increase sugar levels as the main raw material to be fermented into ethanol [11]. Samples of this waste at the pretreatment stage were delignified by enzymatic processes to remove lignin. This is necessary due to the presence of sturdy-walled polymer contained in the lignin which has been found to be inhibiting the hydrolysis and microbial growth in the fermentation process [9]. The next process is the chemical hydrolysis, a process of degrading hemicellulose and cellulose into glucose through the use of strong acids [12].

Several studies reported that the enzymatic lignin removal process can be conducted through the use of several fungi, one of which is A. fumigatus [13]. As a biocatalyst, A. fumigatus isolated from cellulose waste contaminated soil is reported to have cellulase enzymes needed to degrade cellulose [14]. Besides this, filamentous fungi such as Trichoderma spp. is an efficient and well-known cellulase producer [15]. Although this cellulase is not commercially available, the enzyme does not have a significant level of β-glucosidase activity, therefore, it faces feedback barriers. However, Aspergillus species are reported to have cellulase and β-glucosidase activity which are preferred as alternatives [16].

Based on the description above, there is a need to study bioethanol production from plant-based biomass waste in order to solve environmental and energy problems. This research, therefore, serves as a preliminary study to explore the potential of A. fumigatus fungi with its lignolytic properties in the delignification process and also to determine the initial glucose content before the fermentation process is conducted. This research is also expected to provide scientific information about the use of cassava peel waste into bioethanol through an environmentally friendly and effective method.

2. Material and methods
The laboratory scale research was conducted in stages as shown in the flow diagram in Figure 1.

![Figure 1. Research flow diagram.](image-url)
The first stage was the pretreatment, which comprises of the mechanical preparation of cassava peel and delignification while the second stage was the hydrolysis process. Moreover, the lignin content was analyzed using the gravimetric method while glucose content made use of DNS (3,5-dinitrosalicylic) method [17].

2.1. Growth of A. fumigatus in Potato Dextrose Broth (PDB) as growth media
Aspergillus fumigatus was obtained from the collection of the Laboratory of Environmental Biology/Microbiology, Department of Environmental Engineering, Universitas Trisakti, Jakarta, Indonesia. It was grown in the growth medium of Potato Dextrose Broth (PDB) and observed for 7 days, up to the exponential phase on the 5th day when it was harvested. Furthermore, in order to obtain A. fumigatus concentration of 1% (w/v) in the form of a spore suspension, a total of 1 mg of dry biomass was put into Erlenmeyer containing 100 ml of PDB.

2.2. Mechanical preparation of cassava peel
Cassava peel was obtained from Pasar Minggu, Jakarta and the fresh peel was soaked for 3 days and later cut into smaller parts. These were dried for 5 days, mashed, sifted using a 40-mesh sieve, and dried again in the oven at ±100°C for 2 hours to form cassava powder or substrate.

2.3. Determination of the ratio of A. fumigatus to the substrate in the delignification process
This study aimed to determine the amount of A. fumigatus in the delignification process at a specific contact time. The process started with the mixture of cassava powder and A. fumigatus at a ratio of 1:1; 2:1; 1:2 into Erlenmeyer containing GDP at a pH of 7 and room temperature. The delignification process was observed for a contact time (Td, hours) of 24, 72, and 120. Furthermore, the levels of lignin were analyzed by using the Gravimetric method.

2.4. Determination of the Sulfuric Acid (H$_2$SO$_4$) concentration in the hydrolysis process
After the determination of the number of A. fumigatus which produced the highest percentage of lignin removal, the concentration of sulfuric acid (H$_2$SO$_4$) in the hydrolysis process was also determined. This compound at a varying concentration of 1, 3 and 5% was added to Erlenmeyer containing GDP, cassava powder (substrate) and A. fumigatus with a certain ratio, and heated at a temperature of 100°C. The hydrolysis process was observed during the contact time (Td, minutes) of 30, 60, and 90 while the glucose levels were analyzed by using the DNS (3,5-dinitrosalicylic) method [17].

2.5. Lignin removal calculation
The removal efficiency of lignin (%) was obtained through the following equation:

\[
\text{Removal efficiency (\%)} = \frac{W_{(a)} - W_{(b)}}{W_{(a)}} \times 100\% 
\]

$W_{(a)}$: Initial lignin percentage (%)
$W_{(b)}$: Final lignin percentage (%)

3. Results and discussion

3.1. Lignin removed by A. fumigatus
Table 1 shows the percentage of lignin before pretreatment to be 17.90% and after to be (the value should be inputted here) and the efficiency is as shown in figure 2. In table 1, A. fumigatus is also observed to have the ability to delignify at all treatment ratios. However, the best delignification was found at 1:1 for all contact times with the lowest at 2:1 for all contact times. This shows that the enzymatic delignification process requires at least the presence of minimal A. fumigatus in the same amount as the substrate to be degraded.
Table 1. Lignin removal (%) with various substrate ratios : A. fumigatus at different contact times.

| Substrat : A. fumigatus | Contact time (hours) | % Lignin (Before) Average | Std | % Lignin (After) Average | Std | % Lignin Removal |
|-------------------------|---------------------|---------------------------|-----|--------------------------|-----|------------------|
| 1 : 1                   | 24                  | 13.75                     | 0.0252 | 23.52                   |
|                         | 72                  | 13.19                     | 0.0604 | 26.61                   |
|                         | 120                 | 10.94                     | 0.0186 | 39.36                   |
| 2 : 1                   | 24                  | 15.60                     | 0.0241 | 13.02                   |
|                         | 72                  | 17.90                     | 0.5727 | 16.95                   |
|                         | 120                 | 15.41                     | 0.0428 | 14.12                   |
| 1 : 2                   | 24                  | 13.77                     | 0.0515 | 23.38                   |
|                         | 72                  | 12.76                     | 0.0190 | 29.09                   |
|                         | 120                 | 12.48                     | 0.0338 | 30.67                   |

The highest lignin removal efficiency of 39.36% occurs after the pretreatment process has run for 120 hours at 1:1 and the same trend was observed for the other contact times where 1:1 showed high efficiency than other ratios. As stated above, cassava peel powder needs to be dignified because of its ability to inhibit the hydrolysis and microbial growth in the fermentation process [18]. Furthermore, previous research obtained the efficiency of lignin removal by utilizing H\(_2\)SO\(_4\) and NaOH to be up to 33.17% [19].

Lignin-forming monomers are arranged irregularly to make it difficult for microorganisms to degrade them. They consist of three types of phenylpropane compounds, which are p-kumaril alcohol, coniferil alcohol, and synapyl alcohol with the main difference in their chemical structure being the substitution of the methoxyl group (-OCH\(_3\)) in positions 3 and 5 of the aromatic ring [18].

Figure 2. Efficiency removal of Lignin with various ratios of cassava powder : A. fumigatus at different contact times.

This irregularity in the structure of lignin causes the degradation process to be complex by making the enzymes playing a significant role in the degradation of lignin work in a non-specific manner. This process takes place through the formation of free radicals with the ability to attack large numbers of organic molecules. This causes the lignin-degrading fungi to get great attention in the biodegradation of various types of organic pollutants.

3.2. Glucose levels as a result of the hydrolysis process
The hydrolysis process with the addition of sulfuric acid (H\(_2\)SO\(_4\)) at various concentrations has the capacity to work effectively in producing glucose levels for all treatments as shown in Table 2. However, enough time is needed to produce higher glucose levels.
Data observed in table 2 are presented in a linear graph to show the relationship between variations in sulfuric acid concentration and contact time as shown in Figure 3. The highest glucose level of 10.89 g/L was obtained when 5% sulfuric acid (H$_2$SO$_4$) was included in the hydrolysis process for 90 minutes as shown in figure 3. A research conducted by Hikmiyati and Yanie reported that the hydrolysis process using HCL and H$_2$SO$_4$ produced glucose between 0.6 - 9.9% [2].

| Table 2. Glucose levels (g/L). |
| Pretreatment | Time (minutes) | Glucose Levels (g/L) | X | STD |
|--------------|----------------|----------------------|---|-----|
| 1%           | 30             | 8.027                | 0.115 |
|              | 60             | 9.457                | 0.557 |
|              | 90             | 9.337                | 0.112 |
| 3%           | 30             | 9.473                | 0.240 |
|              | 60             | 9.580                | 0.056 |
|              | 90             | 10.740               | 0.148 |
| 5%           | 30             | 9.587                | 0.074 |
|              | 60             | 10.380               | 0.062 |
|              | 90             | 10.893               | 0.032 |

Hydrolysis causes discoloration because cellulose has been converted to glucose with the difference in colour change caused by differences in the hydrolysis strength of the acid concentration and contact time. Moreover, high acid concentration has been reported to affect this strength and causes further degradation of hemicellulose and cellulose to carbon [20].

The aim of the hydrolysis process is to obtain glucose. This happens when the H + group of HCl converts fibers from cassava peel into a free radical group which later binds to the OH-group of water molecules to produce glucose. However, the amount produced depends on the concentration of the hydrolysis solution used. Such that a high concentration of acid solution decreases glucose production due to the formation of a fiber-free radical group. Moreover, the addition of these concentrations causes fewer water molecules in the hydrolysis solution which consequently leads to a reduction in the OH-groups needed for the binding process which further causes low production of glucose [21].

4. Conclusion
The enzymatic activity of A. fumigatus has been shown to play a fairly good role in the delignifying cassava powder. The best delignification process was observed when A. fumigatus is in the same amount as the substrate. Furthermore, the highest lignin removal efficiency of 39.36% occurred after the pretreatment process ran for 120 hours while the highest glucose level of 10.89 g/L was observed at the inclusion of 5% sulfuric acid (H$_2$SO$_4$) in the hydrolysis process for 90 minutes. Moreover, the delignification and hydrolysis processes were found to be important initial stages before fermentation is conducted in a series of bioethanol formation from cassava peel.

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