Delignification of Cassava Peel by Using Alkaline Hydrogen Peroxide Method: Study of Peroxide Concentration, Solid/Liquid Ratio, and pH

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ABSTRACT

Cassava peel is a natural material with cellulose content reaching 33.33%. In order to utilize cassava peel as a biodegradable polymer and renewable energy alternative, a delignification process is essential to separate cellulose from hemicellulose and lignin, which prevents the penetration of cellulose hydrolyzer. The delignification method chosen in this study was alkaline hydrogen peroxide (AHP). The AHP is based on the autooxidation of lignin using hydrogen peroxide (H₂O₂) in an alkaline environment. This method was chosen because it can damage the lignocellulosic structure with relatively low energy and is more selective for lignin. However, under certain conditions, AHP can trigger carbohydrate depolymerization, which decreases yield. Therefore, it is necessary to study the effect of H₂O₂ concentration, Solid/Liquid ratio (S/L) (%v), and pH to evaluate the effectiveness of lignin removal in cassava peel. The concentration of H₂O₂ was varied into 1.5%, 3%, 4.5%, 6%, and 7.5%. The S/L ratio was varied to 1:3, 1:5, 1:7, 1:9, 1:12. The pH of the solution was varied to 8, 9, 10, 11, and 12. The reaction temperature was maintained at 70-90 °C for 3 hours. The results showed that lignin could be reduced to 84.05% for 3 hours by using 6% H₂O₂, an S/L ratio of 1:5, and a pH of 11. The reaction carried out under these conditions can also increase the amount of cellulose from 33.33% to 49.00%.

Keywords: alkaline hydrogen peroxide, delignification, lignocellulose

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1. INTRODUCTION
Banjarnegara is one of the districts known as the cassava producer in Central Java. Central Bureau of Statistics data in 2019 showed that cassava productivity in Banjarnegara was approximately 90,200 tonnes[1]. The cassava in Banjarnegara is primarily cultivated as the raw material for tapioca starch and MOCAF production. The fact related to the dependency on importing wheat experienced by Indonesia encourages the local communities to increase cassava-derived productivity. On the one hand, cassava processing can be considered as an alternative way to reduce wheat dependency. However, on the other, it will open up opportunities for environmental pollution caused by solid and liquid effluent. Liquid effluent produced during cassava processing consists of organic materials that can be utilized as feedstock for biogas energy [2] or organic fertilizer [3]. The solid waste from cassava processing consists of leaves, stems, and peels. The leaves and stems are not a primary threat to the environment. It is because the leaves can be used as the castle feed, besides the stem can be dried to be used as fuel. Unlike leaves and stems, the small number of peels is only used as a mixture of castle feed after several techniques to reduce the cyanide content [4]. The rest then discharge into the environment and become a pollutant.

The major component of cassava peel is cellulose. The preliminary study in this research showed that cassava peel contains up to 33.33% cellulose, 8.57% hemicellulose, and 19.05% lignin. Besides polysaccharides, cassava peel also contains 4.40% protein, 1.81% ether extract, 8.00% ash, 65.92% nitrogen-free extract, and minerals, such as 0.31% calcium, 0.32% phosphorus, 0.57% potassium, and 0.01% magnesium, as minor components [5].

Based on the components inside the cassava peel, it can be utilized as a promising source of fermentable sugar that can be further processed into cellulose-based products, such as membranes [6], hydrocolloids [7], and industrial intermediate products such as carboxymethyl cellulose [8], cellulose acetate [9], and bioethanol [10], [11].

The first step in the cellulose-based product manufacturing process using agricultural waste is delignification. Delignification refers to the separation process of cellulose and hemicellulose from lignin. This step has become crucial because lignin prevents access to the hydrolytic agents unless the lignin needs to be removed [12], [13]. Several processes used to remove the lignin are dilution using sulfuric acid [14], organosolv and steam explosion [15], alkaline and lime treatments [16]. Several studies stated that using acids enhances the rate of hemicellulose [15,16], but some concentrated acids, such as sulfuric acid and hydrochloric acid, are toxic and corrosive. Steam explosion is another promising method to treat lignocellulosic agricultural waste. This method uses less hazardous chemicals, but the generation of the toxic chemical and the energy requirement in the form of heat and incomplete disruption of lignin are the drawbacks.

Like the acid method, the alkaline usage in the pretreatment process increases the cellulose's internal surface by breaking the ester bond of lignin and hydrocarbon complexes, which causes a decrease in the degree of polymerization crystallinity. The other process is disrupting and solubilizing the lignin [19]. Numerous research proposed alkaline methods due to low energy requirement, simple reactor design, less corrosion, minimal formation of inhibitors, and preserving carbohydrates [20]. Nevertheless, alkaline usage is only remarkable for low lignin-content biomass [12].

In order to improve the alkaline performance, the alkaline method was coupled with autooxidation processes, namely alkaline hydrogen peroxide (AHP). The AHP method uses hydrogen peroxide ($H_2O_2$) in an alkaline environment. This method was chosen because it can damage the lignocellulosic structure using relatively
low energy, more selective for lignin, thus preventing carbohydrate degradation [21]. The delignification using AHP can be done in mild conditions so that only little energy is required to control the process. This condition will be more favorable for large-scale applications.

The principle of delignification using the AHP method is the oxidation of lignin in the form of phenolic bonds, Cα-Cβ lignin ring bonds opening, side chain displacement, or cleavage of alkyl aryl ether bonds by per hydroxyl anions (HOO-) which are activated by hydroxyl radicals (•OH) and superoxide anions (•O2-), an oxidizer of lignin. The cleavage of the lignin chromophore group produces lactic acid, formic acid, succinic acid, and acetic acid, which are readily soluble in water [19].

Several researchers have contributed to the development of AHP pretreatment. Mittal [22] studied the influence of peroxide loading on the delignification of corn stover during AHP pretreatment. The examination was conducted over 30–500 mg H₂O₂/g dry corn stover at 50°C for 3 h. The research result showed that AHP pretreatment at 250 mg H₂O₂/g dry CS resulted in the pretreated solids with more than 80% delignification, enriching the carbohydrate fraction to >90%. Tareen [23] studied the effect of hydrogen peroxide concentration (1–5%), temperature (50–90 °C), and time (30–90 min) in the AHP treatment of oil palm trunks (OPT). The optimum condition to pretreat the OPT using AHP was obtained at 70°C, 30 min, and 3% H₂O₂ g/g of biomass. The process used in this condition increased the cellulose content from 38.67% in raw material to 73.96% and removed lignin and hemicellulose up to 50% and 57.12%, respectively. Damaurai [24] stated that AHP improved the efficiency of enzyme hydrolysis of rice straw to fermentable sugars. The process conducted under the optimal pretreatment conditions (2.5% H₂O₂ (%w), 55 °C, 24 h, and 7.5% biomass loading (%w) produced reducing sugar in the range of 499±6 mg/g native biomass.

Although considered as an effective alternative to reduce lignin content, several factors must be considered when applying the AHP method. The first factor is the possibility of carbohydrate depolymerization, which causes a decrease in yield. The second is the possibility of peroxide decomposition due to metal oxides. Therefore, to develop cassava peel pretreatment using AHP, this research studied the effects of hydrogen peroxide concentration, the ratio of Solid/Liquid (S/L) (%w), and pH.

### 2. RESEARCH METHODS

#### 2.1. MATERIAL

Cassava peel was obtained from the Getuk Goreng Outlet at Sokaraja, Banyumas. This research only used the cassava's inner skin. It was washed and dried at 80°C until the weight reached a constant. Hydrogen peroxide (H₂O₂), sodium hydroxide (NaOH), and sulfuric acid (H₂SO₄) were purchased from Merck (Darmstadt, Germany).

#### 2.2. DELIGNIFICATION USING AHP METHOD

The pH of H₂O₂ was adjusted by adding NaOH. This solution was then poured into the three-necked flask containing cassava fiber. The delignification was carried out at 70-90°C for 3 hours. After reaching the reaction time, the solids obtained were bleached with H₂O₂ 10%, then washed using aquadest to obtain neutral pH. The fiber dried at 80-90°C. The experimental instrument used in the delignification process was showed in Figure 1.
2.3. HEMICELLULOSE, CELLULOSE, AND LIGNIN ANALYSIS
The analysis of hemicellulose, cellulose, and lignin was conducted based on the Chesson Datta [25]. One gram of dry sample (weight a) was diluted by 150 mL H₂O and refluxed at 100°C for an hour. The result was filtered, and the residue was washed with 300 mL of hot water. Then, the residue was dried until it reached the constant weight (weight b). The residue was added with 150 mL H₂SO₄ 1 N, then was refluxed for an hour at a 100°C. The result was filtered and washed to neutral, and the residue was dried to a constant weight (weight c). The dry residue was added by 100 mL H₂SO₄ 72% and soaked at room temperature for 4 hours, then was added by 150 mL H₂SO₄ 1 N and was refluxed at 100°C for an hour. Residues were filtered and washed with H₂O until neutral. The residue was then reheated in an oven at 105°C until constant weight (weight d). Furthermore, the residue was furnace at 750°C and weighed (weight e). The percentage of cellulose, lignin, and hemicellulose was calculated using the Equation (1), (2), (3)

\[
\text{Hemicelullose(\%) = } \frac{b-a}{a} \times 100\% \quad (1)
\]

\[
\text{Celullose(\%) = } \frac{c-b}{a} \times 100\% \quad (2)
\]

\[
\text{Lignin(\%) = } \frac{d-e}{a} \times 100\% \quad (3)
\]

FTIR analysis was conducted by Shimadzu IRTracer-100.

2.4. DATA ANALYSIS
Research data were evaluated by using one-way ANOVA using IBM SPSS version 20. The characteristic of the polysaccharide structure of cassava peel before and after the AHP treatment was evaluated using Fourier Transform Infra-Red

3. RESULTS AND DISCUSSION
3.1. EFFECT OF HYDROGEN PEROXIDE CONCENTRATION
The treatment of agricultural waste under alkaline conditions causes the oxidation of lignin and some hemicellulose. Moreover, chemical treatment with hydrogen peroxide also causes pigment degradation [26]. The H₂O₂-catalyzed oxidation of the lignin structure begins with separating one electron from the donor substrate's aromatic ring and producing an aryl cation radical. These aryl cation radicals then undergo various post-enzymatic reactions to produce veratyl aldehyde and veratyl alcohol [27].

To investigate the effect of H₂O₂, the concentration of H₂O₂ was varied to 1.50%, 3.00%, 4.50%, 6.00%, and 7.50%, while the S/L ratio and pH were kept constant at 1:9 and 11. The reaction time was 3 hours. The concentration of hemicellulose, cellulose, and lignin after pretreatment using AHP is shown in Table 1.
The data (See Table 1) shows that the increases in H$_2$O$_2$ concentration from 1.5% to 4.50% significantly (P-value <0.05) affected the removal percentage of lignin. However, the decrease in lignin content is not as significant as the increase in H$_2$O$_2$ concentration to 6.00%, so the H$_2$O$_2$ concentration range used to treat cassava peel is 4.50%-6.00%.

Increasing the concentration of H$_2$O$_2$ up to 7.50% decreased the removal percentage of lignin. Sari et al. [28] stated that increasing H$_2$O$_2$ concentration improves the number of ·OH that plays a role in the non-phenolic units of lignin breaking bonds. Nevertheless, the number of H$_2$O$_2$ outside the maximum conditions increases the possibility of glycosidic bond breakdown and decreasing cellulose yield. Contrary to the theory, data in Table 1 only shows the decreasing of lignin removal, but the cellulose content increases in accordance with H$_2$O$_2$ concentration. At a higher concentration of H$_2$O$_2$ (7.50% H$_2$O$_2$), the rate of O$_2$ formation (observed from the froth formation) increases rapidly. This condition reduces the oxygen incorporation at lignin sites and decreases delignification efficiency. The decreasing of lignin removal rate has also been reported by Rojith and Singh [29]. The result of their research showed that the addition of H$_2$O$_2$ of more than 2% decreased the delignification efficiency of Coir Pith Black Liquor from 53.10% to 37.89%.

The concentration of H$_2$O$_2$ required in the pretreatment process is influenced by the concentration of lignin, cellulose, and hemicellulose. By means agricultural waste has different optimum conditions. Pudjiastuti [30] studied the effect of H$_2$O$_2$ concentration in the coffee pulp waste pretreatment using AHP. The research result showed that the highest lignin removal was achieved by adding 7.50% H$_2$O$_2$. The treatment using that condition could reduce lignin up to 49.50% (from 16.85% to 8.50%). The treatment of fall leaves using 3 M NaOH and 3 M H$_2$O$_2$ recovered hemicellulose and cellulose up to 99.50% and 81.60%, respectively [31].

### 3.2. THE EFFECT OF SOLID/LIQUID RATIO

The S/L ratio shows the size of the cassava peel mass to the volume of H$_2$O$_2$ used in the delignification. The effect of the solid/liquid ratio was studied by varying the S/L ratio to 1:3, 1:5, 1:7, 1:9, and 1:12. The delignification process was carried out using 6% H$_2$O$_2$ at pH 11 for 3 hours. The research data in Table 2 shows that the maximum S/L ratio that can be used to extract lignin from cassava peel is ratio of 1:5. The total yield of cellulose obtained by using the ratio S/L 1:4 was 49%, while the residual lignin content attained 3%. From these results, the highest lignin separation effectiveness that could be achieved is 84.28%.

<table>
<thead>
<tr>
<th>H$_2$O$_2$ Concentration (%)</th>
<th>Percentage (%)</th>
<th>Removal of lignin (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>H</td>
<td>C</td>
</tr>
<tr>
<td>1.50</td>
<td>23.30 ± 0.14$^b$</td>
<td>33.01 ± 0.29$^c$</td>
</tr>
<tr>
<td>3.00</td>
<td>34.86 ± 0.08$^a$</td>
<td>35.78 ± 0.31$^b$</td>
</tr>
<tr>
<td>4.50</td>
<td>9.90 ± 5.51$^c$</td>
<td>37.62 ± 0.17$^a$</td>
</tr>
<tr>
<td>6.00</td>
<td>31.19 ± 0.27$^a$</td>
<td>35.78 ± 0.39$^b$</td>
</tr>
<tr>
<td>7.50</td>
<td>18.92 ± 0.11$^b$</td>
<td>36.94 ± 0.84$^a$</td>
</tr>
</tbody>
</table>

H: Hemicellulose, C: Cellulose, L: Lignin. The data homogeneity is marked by superscript letters. The letter ‘a’ to ‘d’ symbolize the highest to the smallest value.
Delignification can be analogous to the solid-liquid extraction process. The solute to be separated is lignin, while the solvent that plays a role is \( \text{H}_2\text{O}_2 \). The solid-liquid ratio is vital in solid-liquid extraction. This is because the amount of solvent used affects the penetration of the solvent into the solute. If the amount of solvent used is insufficient, only a small portion of the solute can be dissolved. The data in Table 2 shows that increasing the S/L ratio from 1:3 to 1:5 can be used to eliminate the lignin up to 3%. In other words, the rate of delignification is 84.28%. When the S/L ratio is increased, the amount of lignin content decreases. It is because the addition of solute above optimal conditions allows the dissolution of impurities that cause changes in the properties of the extracted material [32]. Research by Asharudin et al. [33] showed that cassava peels contain metallic minerals from oxides. Cassava peel contains several types of micro minerals such as \( \text{K}_2\text{O} \), \( \text{CaO} \), \( \text{Fe}_2\text{O}_3 \), \( \text{SiO}_2 \), \( \text{SO}_3 \), \( \text{Al}_2\text{O}_3 \), and \( \text{P}_2\text{O}_5 \) are composed of transition and non-transition metals, which catalyzes the decomposition of \( \text{H}_2\text{O}_2 \), thereby reducing the effectiveness of delignification. Jong et al. [34] stated that metals that can decompose \( \text{H}_2\text{O}_2 \) include \( \text{Mn} \), \( \text{Cu} \), \( \text{Fe}^{3+} \), \( \text{Fe}^{2+} \), \( \text{Mg} \), and \( \text{Ca} \). The mechanism of \( \text{H}_2\text{O}_2 \) decomposition catalyzed by transition metals occurs through the ionic reaction mechanism according to Equation (4).

\[
\text{H}_2\text{O}_2 + \text{HO}^- \rightarrow \text{H}_2\text{O} + \text{O}_2 + \text{H}^+ \quad (4)
\]

If reaction (4) occurs, the deprotonation reaction that produces \( \text{HOO}^- \) and \( \cdot \text{OH} \), which plays a role in bleaching and delignification, will be inhibited.

### 3.3. THE EFFECT OF PH

Ingrid et al. [35] stated that delignification with \( \text{H}_2\text{O}_2 \) is highly dependent on pH. Hydrogen peroxide will dissociate at pH 11.5-11.6 by Equation (5).

\[
\text{H}_2\text{O}_2 \leftrightarrow \text{H}^+ + \text{HOO}^- \quad (5)
\]

Hydroperoxy anions (\( \text{HOO}^- \)) can react with undissociated \( \text{H}_2\text{O}_2 \) to form highly active Hydroxyl radicals (\( \cdot \text{OH} \)) and Superoxide (\( \text{O}_2^- \)) according to Equation (6).

\[
\text{H}_2\text{O}_2 + \text{HOO}^- \rightarrow \cdot \text{OH} + \text{O}_2^- + \text{H}_2\text{O} \quad (6)
\]

In the absence of other reactants, hydroxyl and superoxide react to form \( \text{O}_2 \) and water by Equation (7).

\[
\cdot \text{OH} + \text{O}_2^- + \text{H}^+ \rightarrow \text{O}_2 + 2\text{H}_2\text{O} \quad (7)
\]

The general reaction of hydrogen peroxide under alkaline conditions is:

\[
\text{H}_2\text{O}_2 + [\text{HOO}^- + \text{H}^+] \rightarrow \text{O}_2 + 2\text{H}_2\text{O} \quad (8)
\]

The pH effect on the AHP treatment was investigated by varying pH to 8, 9, 10, 11, and 13. The \( \text{H}_2\text{O}_2 \) concentration was set to 6%, while the S/L ratio was set to 1:5. The delignification was done for 3 hours. The effect of pH on the effectiveness of delignification is shown in Table 3.

### Table 2. Effect of alkaline hydrogen peroxide (AHP) pretreatment of cassava peel at different S/L ratio

<table>
<thead>
<tr>
<th>S/L Ratio</th>
<th>H</th>
<th>C</th>
<th>L</th>
<th>Removal of lignin (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:3</td>
<td>32.00 ±0.14^c</td>
<td>37.00 ±1.27^c</td>
<td>15.00 ±0.28^a</td>
<td>21.25 ±1.48^e</td>
</tr>
<tr>
<td>1:5</td>
<td>29.00 ±0.85^d</td>
<td>49.00 ±1.41^a</td>
<td>3.00 ±0.14^e</td>
<td>84.28 ±0.74^a</td>
</tr>
<tr>
<td>1:7</td>
<td>37.00 ±0.14^b</td>
<td>38.00 ±0.28^c</td>
<td>9.00 ±0.28^b</td>
<td>52.75 ±1.48^d</td>
</tr>
<tr>
<td>1:9</td>
<td>37.00 ±0.71^a</td>
<td>41.00 ±1.41^b</td>
<td>7.00 ±0.14^d</td>
<td>63.25 ±0.74^b</td>
</tr>
<tr>
<td>1:12</td>
<td>34.00 ±0.56^a</td>
<td>36.00 ±0.00^e</td>
<td>8.00 ±0.14^c</td>
<td>58.26 ±1.11^c</td>
</tr>
</tbody>
</table>

H: Hemicellulose, C: Cellulose, L: Lignin. The data homogeneity is marked by superscript letters. The letter 'a' to 'c' symbolizes the highest to the smallest value.

Table 3. Effect of alkaline hydrogen peroxide pretreatment of cassava peel at different pH

<table>
<thead>
<tr>
<th>pH</th>
<th>H Percentage (%)</th>
<th>C Percentage (%)</th>
<th>L Percentage (%)</th>
<th>Removal of lignin (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>23.00 ± 0.84d</td>
<td>31.00 ± 0.00a</td>
<td>17.00 ± 0.28a</td>
<td>10.76 ± 1.48e</td>
</tr>
<tr>
<td>9</td>
<td>36.00 ± 1.31a</td>
<td>25.00 ± 0.71c</td>
<td>11.00 ± 0.28c</td>
<td>42.25 ± 1.48e</td>
</tr>
<tr>
<td>10</td>
<td>32.00 ± 0.71b</td>
<td>31.00 ± 0.42b</td>
<td>10.00 ± 0.28d</td>
<td>47.51 ± 1.48b</td>
</tr>
<tr>
<td>11</td>
<td>29.00 ± 0.85d</td>
<td>49.00 ± 1.41a</td>
<td>3.00 ± 0.14e</td>
<td>84.28 ± 0.74a</td>
</tr>
<tr>
<td>12</td>
<td>28.00 ± 0.84c</td>
<td>31.00 ± 0.71b</td>
<td>13.00 ± 0.14b</td>
<td>31.75 ± 0.74d</td>
</tr>
</tbody>
</table>

H: Hemicellulose, C: Cellulose, L: Lignin. The data homogeneity is marked by superscript letters. The letter ‘a’ to ‘d’ symbolizes the highest to the smallest value.

Table 3 shows that the maximum pH required for the cassava peel treatment using AHP is 11. The process under these conditions retained the lignin concentration at 3%. The removal of lignin using 6% H₂O₂, 1:5 S/L ratio, and pH of 11 reaches 84.28%. This treatment also increased the cellulose content from 33.33% to 49.00%. This result is in line with other studies. Anwar [18] studied lignin extraction from rice husks using AHP. The result showed that the optimum conditions for lignin isolation were a solvent/solid ratio of 9:1 ml/gr, hydrogen peroxide concentration of 1.5% v, and pH of the mixture of 11. Gould [36] stated that the isolation of lignin catalyzed by H₂O₂ was a vital function of pH. At a pH of 11.5, the amount of solubilized lignin approached 60%. If the pH of the solution is increased to 12, the amount of solubilized lignin is decreased because of H₂O₂ decomposition through Equation (9).

H₂O₂ → H₂O + O₂  
(9)

3.4. FTIR ANALYSIS

Fourier Transform Infrared (FTIR) spectroscopy was used to analyze the polysaccharide structure of cassava peel before and after the AHP treatment. The polysaccharide structure was evaluated by spectral sorption. Sasmal et al. [16] reported that the range of 1266-1325 cm⁻¹ represents syrinxyl, p-coumaryl, and coniferyl alcohol that build the lignin. The peak in the 1325-1330 cm⁻¹ and 1266-1270 cm⁻¹ represents the wave number of the syringyl ring and guaiacyl ring. The 1620-1670 cm⁻¹ transmittances represent the C=O of lignin aromatic ring vibrations. While the peak of wave numbers ~1725 cm⁻¹ is a specific area of hemicellulose absorption with a distinct C=O functional group [37]. FTIR data trend in Figure 2 shows that transmittance in the 1000-2000 cm⁻¹ was significantly changed because of lignin degradation during pretreatment using AHP.

Figure 2. FTIR spectra of untreated and treated cassava peel using 6% H₂O₂, 1:5 S/L ratio, and pH of 11.

Absorbance in the region between 3800-000 cm⁻¹ indicates the vibration of valence bands of the hydrogen bond of the O-H group and the bands of infra-molecular and intermolecular hydrogen bonds [17]. The peak trend in this area tends to be wider and shifts towards a larger wavenumber due to disturbances in the cellulose crystal structure. This change refers to the disruption of intramolecular hydrogen in cellulose [31]. As a result, the absorption
intensity of the OH group decreased even though cellulose degradation can not be revealed 4.

4. CONCLUSION
The research showed that the H$_2$O$_2$ concentration, ratio S/L, and pH affected the percentage of the amount of lignin that could be removed. The range of the H$_2$O$_2$ concentration between 4.5%-6% gave the best choice to treat the cassava peel. The maximum ratio of S/L and pH used to alter the lignin content was 1.5 and 11, respectively. Using the maximum condition stated in this work (6% H$_2$O$_2$, 1:5 ratio S/L and pH of 11) increased the cellulose content from 33.33% to 49.00% and decreased the lignin from 19.05% to 3%.

From the described data, it can be concluded that AHP is a promising method that can be chosen as an alternative method to treat the cassava peel before further processing to produce cellulose-based material.

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