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# Isolation and Enzymatic Degradation of Hemicellulose from Corncobs Waste

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**ABSTRACT**
Corncobs are rich in hemicellulose, which has very important applications in the food industry and biofuels. Hemicellulose is a heteropolysacharide which contains hexosan such as glucan, mannan, galactan and pentosan such as xylan and arabinan. The aims of this research are determining the optimum condition of hemicellulose isolation and identifying enzymatic degradation products of hemicellulose. Hemicellulose is extracted from corn cobs using various NaOH concentrations and extraction times. Acetic acid was added to the mixture after hemicellulose A reflux process, whereas hemicellulose B was precipitated with ethanol 96%. Enzymatic hydrolysis is carried out using xylanolytic enzyme from a recombinant of *E. coli* DH5α. The yield of hemicellulose is nearly 64.74% (w/w) using NaOH 4 M for 2 h of extraction time. Based on High Performance Liquid Chromatography data indicating that the enzymatic hydrolysis products of hemicellulose A are xylose and arabinose. While xylose, arabinose, and xylooligosaccharide are hemicellulose B and unextracted hemicellulose hydrolysis products.



## 1. Introduction

Indonesia is an agricultural country that has a lot of agricultural land, one of them is corn field. According BPS data in 2018, annual corn yields are around 30.05 million tons per year. An increase in corn production causes an increase in the waste produced [[1]]. Agricultural industrial waste is lignocellulosic biomass. Corncobs are the largest component of corn products, reaching 40–50%. Corncobs lignocellulose contains 45% cellulose, 35% hemicellulose, and 15% lignin [[2]]. Hemicellulose is the second most abundant renewable component of lignocellulosic biomass after cellulose [[3]]. Hemicellulose is a long-chain polysaccharide formed from several kinds of monosaccharides such as arabinose, xylose, mannose, or galactose. Most hemicelluloses are soluble in alkaline solutions, with the solubility dependent on the concentration of the alkali [[4]]. So far, the extraction process of hemicellulose uses alkaline solutions. Alkali is used to reduce the amount of lignin in hemicellulose components. Alkali solubilization of hemicellulose is the more mature and widely used extraction strategy for obtaining high molar mass hemicellulose [[5]]. The common alkali types used for extraction are KOH (potassium hydroxide) and NaOH (sodium hydroxide) [[6]]. The corncob powder was subjected to pretreatments with 2% $H_2SO_4$, 2% NaOH, 2% $H_2SO_4$, 2% NaOH, and 15% $NH_4OH$, making the hydrolysis process easier than hydrolysis without extraction [[7]]. The utilization of hemicellulosic sugars is essential for the efficient conversion of lignocellulosic materials to ethanol fuel

and other value-added fermentation products [[2]]. The hydrolysis products of hemicellulose have high usability, so the process of enzymatic degradation of corn cobs will be very useful.

Xylan is the principal type of hemicellulose. It is a linear polymer of β-D-xylopyranosyl units linked by (1,4) glycosidic bonds. In nature, the polysaccharide backbone may be added to 4-O-methyl-α-D-glucuronopyranosyl units, acetyl groups, α-L-arabinofuranosyl, and others in variable proportions. An enzymatic complex is responsible for the hydrolysis of xylan, but the main enzymes involved are endo-1,4- β-xylanase and β-xylosidase. Xylanases, the xylan hydrolyzing enzymes, are ubiquitous and diverse in nature. A number of different sources have been found to produce these enzymes, which include marine and terrestrial bacteria, rumen bacteria, fungi, marine algae, protozoa, snails, crustaceans, insects, terrestrial plants, and their seeds [[8]]. One of the enzymes that can be used for the degradation of agricultural by-products is the xylanolytic enzyme group from bacteria. Xylanolytic enzymes can hydrolyze hemicellulose because xylan is a constituent of hemicellulose. This xylanolytic enzyme hydrolyzes glycoside bonds at -1,4-D-xylopiranose and -1,3-L-arabinofuranose to produce xylose and arabinose monomers. Xylooligosaccharides are produced due to the activity of the enzyme endo-xylanase which breaks the glycoside bonds in hemicelluloses randomly. Thus, in this research, the process of hemicellulose isolation from corncobs waste and the enzymatic degradation of hemicellulose products will be described.

## 2. Materials and methods

### *2.1. Isolation of hemicellulose*

Hemicellulose was obtained from corn cobs of pioneer varieties from Mojodhuwur Village, Kediri. Corncobs are cut into pieces and dried in the sun, then ground into powder. Five grams of corn cob powder added with 100 mL of NaOH (2.0 M, 2.5 M, 3.0 M, 3.5 M and 4.0 M) in a three-necked round-bottom flask then stirred for 2, 4, and 6 h time variations. The round-bottom flask is equipped with a thermometer and reflux. After the process was completed, the mixture was cooled and filtered using a Buchner funnel. The filtrate was added with 4 N Acetic acid until pH 5.5-6.0 then centrifuged (10,000 rpm, 20 min) to separate the precipitate. The remaining filtrate was added to 96% ethanol. The ratio of filtrate to 96% ethanol was 1:2 and allowed to stand for 24 h. The mixture was centrifuged (10,000 rpm, 20 min) to separate the precipitate. The precipitate was washed with 96% ethanol. Both Hemi A and Hemi B were freeze dried to remove the water [[9]].

### *2.2. Enzyme productions*

The recombinant *E. coli* DH5α bacteria that were used in this study are a collection of the Proteomics Laboratory, University Centre of Excellence Research Center for Bio-Molecule Engineering. A 1% inoculum of *E. coli* DH5 recombinant bacteria was inoculated in Luria Bertani medium containing 100 mg/mL ampicillin. The medium containing the bacteria was shaker at 37 °C at 150 rpm for ± 18 hours. Enzymes were harvested using 6,000 rpm centrifuge for 10 min. The precipitate was separated from the supernatant and then added with 50 mM phosphate citrate 7 buffer. Precipitate was lysed using sonication at 20 Hz. Xylanase was obtained after centrifugation at 10000 rpm for 10 min.

### *2.3. Activity assay of xylanase*

The enzyme activity was determined by measuring the release of reducing sugar using 3,5-dinitrosalicylic acid (DNS) method [[10]].100 µL oat-spelled xylan substrate, and 100 µL of enzyme were incubated at 70 °C for 1 h. The mixture was then treated with 600 L of DNS (3.5-dinitrosalicylic acid) reagent and heated in a boiling water bath for 15 minutes before being immediately cooled in ice water for 20 minutes. The absorbance was read at 550 nm. The control used 100 µL enzyme, 100 µL

substrate and 600 µL of DNS reagent were treated in the same way as above but without incubation. The absorbance was converted to determine the amount of reducing sugar produced.

### *2.4. Enzymatic hydrolysis of hemicellulose*

Hemicellulose, which was extracted and without extraction, was added with xylanase enzyme using a 1:3 ratio of substrate to enzyme. The hydrolysis was carried out at 70 °C for 24 hours.

### *2.5. Quantitative analysis of hydrolysis product*

The hydrolysis products of the enzyme were analyzed using High Performance Liquid Chromatography (HPLC). HPLC analysis used a carbohydrate column (µbondapak, waters 2487), an index refractory detector, 80% methanol solvent in water, a 1 µL flow rate, and the column temperature was set at room temperature.

## 3. Results and discussion

### *3.1. Hemicellulose from corncob waste*

Hemicellulose A (Hemi A) and hemicellulose B (Hemi B) were obtained from the isolation of corn cobs using a variation of NaOH concentration. Hemi A is the main hemicellulose product, while Hemi B is the residual hemicellulose product. The hemicellulose form is a powder with a light brown to dark brown color. Hemi A powder is darker than Hemi B. Basically, Hemi A and Hemi B are the same hemicellulose that only differ in molecular weight. Hemi B has a lower molecular weight, so it precipitates after Hemi A.

Optimization of hemicellulose extraction conditions aims to obtain a high yield and good quality of hemicellulose. The concentrations of NaOH were 2.0 M, 2.5 M, 3.0 M, 3.5 M, and 4.0 M, with extraction times varying between 2 hours, 4 hours, and 6 hours. Based on the optimization results, the highest total yield of hemicellulose (Hemi A and Hemi B) was obtained at 4.0 M NaOH at 2 h. The results of hemicellulose yield are shown in Table 1. The total hemicellulose yields in corncobs were nearly 68.74% of the total available hemicellulosic polysaccharides.

Table 1. Yield of Corn Cob Hemicellulose Extraction

| Time (h) | NaOH Concentration (M) | Sample Weight (g) | Hemicellulose (g) | | Total Hemi Weight (g) | Average Total (%) |
|---|---|---|---|---|---|---|
| | | | A | B | | |
| 2 | 2.0 | 10.1050 | 3.0270 | 1.1536 | 4.1806 | 41.77 |
| | | 10.0370 | 2.8567 | 1.3760 | 4.2327 | |
| | 2.5 | 10.0935 | 3.2928 | 2.3749 | 5.6677 | 51.96 |
| | | 10.0014 | 2.7633 | 2.0132 | 4.7765 | |
| | 3.0 | 10.0157 | 4.1376 | 1.9490 | 6.0866 | 66.34 |
| | | 10.0011 | 4.0144 | 3.1781 | 7.1925 | |
| | 3.5 | 10.0031 | 2.1950 | 2.5894 | 4.7844 | 52.58 |
| | | 10.0797 | 2.9910 | 2.7881 | 5.7791 | |
| | 4.0 | 10.0655 | 3.8114 | 3.3732 | 7.1846 | 68.74 |
| | | 10.0428 | 3.5650 | 3.0729 | 6.6379 | |

| | | | | | | |
|---|---|---|---|---|---|---|
| 4 | 2.0 | 10.0024 | 1.4062 | 1.8772 | 3.2834 | 33.77 |
| | | 10.0100 | 2.0496 | 1.4255 | 3.4751 | |
| | 2.5 | 10.0217 | 2.4687 | 1.7738 | 4.2425 | 41.08 |
| | | 10.0104 | 1.9741 | 2.0123 | 3.9864 | |
| | 3.0 | 10.0061 | 2.4693 | 2.2076 | 4.6769 | 45.66 |
| | | 10.0234 | 2.3658 | 2.1034 | 4.4692 | |
| | 3.5 | 10.0015 | 3.6380 | 2.6273 | 6.2653 | 57.46 |
| | | 10.0017 | 3.6523 | 1.5763 | 5.2286 | |
| | 4.0 | 10.0105 | 2.0728 | 2.5900 | 4.6628 | 53.23 |
| | | 10.0108 | 1.9649 | 4.0306 | 5.9955 | |
| 6 | 2.0 | 10.0073 | 2.6169 | 2.1752 | 4.7921 | 41.86 |
| | | 10.0014 | 2.0280 | 1.5563 | 3.5843 | |
| | 2.5 | 10.0012 | 1.8878 | 1.8036 | 3.6914 | 38.50 |
| | | 10.0035 | 2.3815 | 1.6288 | 4.0103 | |
| | 3.0 | 10.0236 | 5.0196 | 2.4897 | 7.5093 | 60.74 |
| | | 10.0138 | 2.5517 | 2.1120 | 4.6637 | |
| | 3.5 | 10.0054 | 4.2850 | 1.7215 | 6.0065 | 52.71 |
| | | 10.0046 | 1.9170 | 2.6248 | 4.5418 | |
| | 4.0 | 10.0036 | 1.6674 | 2.5243 | 4.1917 | 53.56 |
| | | 10.0015 | 1.6174 | 4.9054 | 6.5228 | |

### *3.2. Production of xylanase*

Colonies of recombinant *E. coli* DH5α were grown in LB medium containing ampicillin, IPTG, and X-gal. The addition of antibiotics aims to maintain the growth of recombinant *E. coli* cells free from other bacterial contamination. The addition of IPTG and X-gal resulted in *E. coli* DH5α in white color, while the recombinant *E. coli* DH5α (pBKS+) was blue. This color change was due to genetic engineering of the lacZ gene. In the pTP510 plasmid, there was inactivation of the lacZ gene due to the insertion of a xylanolytic encoding gene, while in the pBKS+ plasmid it did not occur, so that the lacZ gene remained active [[11]]. Enzyme production was carried out at 37 °C at 150 rpm for ± 18 h using 1% inoculum. Xylanase was obtained by precipitating the product using centrifugation at 6,000 rpm for 10 min. The precipitate was dissolved in PC pH 7 buffer and then sonicated at 20 Hz for 2 min. The supernatant after centrifugation was freeze-dried to obtain water-free xylanase. The crude xylanase obtained was 2.8 grams.

### *3.3. Activity assay of xylanase*

Incubation of xylanase and substrate was carried out at 70 °C for 1 h. This is the optimum temperature for xylanolytic enzymes. 600 µL DNS reagent was added and put in a boiling water bath for 15 minutes to complete the reaction, then immediately cooled in ice water for 20 minutes to stop the reaction. Xylose from the hydrolysis of oat-spelled xylan can be identified by the color change from yellow to brown because DNS is reduced to 3-amino-5-nitrosalicylic acid. The reducing sugars produced

from the hydrolysis of oat-spelt xylan with xylanase are xylose, arabinose, xylooligosaccharides, and glucuronic acid. The main constituent monosaccharide of xylan is xylose, so in this activity test, xylose is measured. The concentration of reducing sugar was determined by measuring the absorbance against a xylose standard at 550 nm. The activity of xylanase was obtained at 0.1156 U $mL^{-1}$. This shows the unit of xylanase activity was 0.1156 mol xylose per minute for every 1 mL of xylanase.

### *3.4. Enzymatic hydrolysis of hemicellulose*

Hemicellulose hydrolysis was carried out at 70 °C for 24 hours. The products from the hydrolysis were analyzed using HPLC. The hydrolyzed hemicelluloses were hemicellulose A, hemicellulose B, and hemicellulose without being extracted (corncob). The products of hydrolysis are shown in **Table 2**.

**Table 2.** Retention Time for Hydrolysis Product

| Hydrolysis Product | Retention Time (minutes) | | |
|---|---|---|---|
| | Hemi A | Hemi B | Non-extracted Hemi |
| Xylose | 2.100 | 2.065 | 2.037 |
| Arabinose | 2.595 | 2.516 | 2.556 |
| Xylooligosaccharide | - | 3.008 | 3.092 |

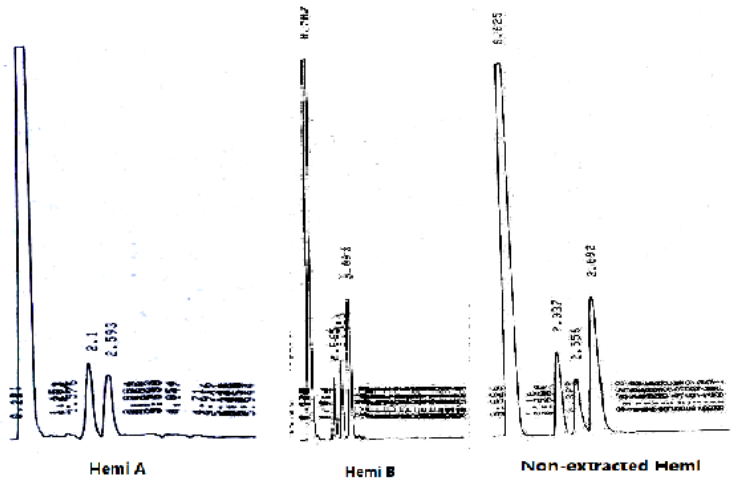


**Figure 1.** Chromatogram of hemicellulose hydrolysis product

**Figure 1.** shows the chromatogram of the hemicellulose hydrolysis product. Xylanolytic enzymes can hydrolyze hemicellulose because xylan is a constituent of hemicellulose. This xylanolytic enzyme hydrolyzes glycoside bonds at -1,4-D-xylopyranose and -1,3-L-arabinofuranose to produce xylose and arabinose monomers. Xylooligosaccharides are produced due to the activity of the endo-xylanase enzyme which breaks the glycoside bonds in hemicelluloses randomly (z). Based on the results of the hydrolysis, the resulting concentration of hydrolysis products is as follows (**Table 3**).

**Table 3.** Concentration of Hydrolysis Product

| Sample | Concentration of hydolysis Product (ppm) | | |
|---|---|---|---|
| | **Xylose** | **Arabinose** | **Xylooligosaccharide** |
| Hemi A | 25.126 | 44.318 | - |
| Hemi B | 18.458 | 37.163 | 268.509 |
| Non-extracted Hemi | 36.372 | 36.905 | 356.367 |

### *3.5. Discussion*

Environmental pollution caused by agricultural by-products is increasingly being found in Indonesia. This is due to the increasing demand for community food needs along with the increasing population in Indonesia, while innovation in the use of agricultural biomass waste is still very lacking. Corn cobs are one of the most abundant agricultural wastes in Indonesia. Corn cobs contain lignocellulosic crude fiber which is composed of lignin, hemicellulose, and cellulose, and each is a compound that has the potential to be converted into other compounds biologically. Lignocellulosic biomass waste can undergo bioconversion using lignocellulose enzymes which are a collection of cellulolytic, hemicellulolytic, and lignolytic enzymes. Hemicellulose enzymes can produce various monosaccharides and oligosaccharides that can be utilized further. The most explored hemicellulolytic enzymes are xylanolytic enzymes that can degrade xylan into its constituent monomers. Xylan as a major component of plant hemicelluloses; xylan's presence and structure and its interaction with plant cell walls; properties, production, purification, and immobilization of enzymes and their industrial application; and multiple forms of xylanases and the synergism between the enzymes of the xylanolytic complex [[8]].

Hemicellulose A (Hemi A) and hemicellulose B (Hemi B) were obtained from the isolation of corncobs using a variation of NaOH concentration. Xylan extract is completely soluble in alkali, soluble in hot water, slightly soluble in cold water, and insoluble in acids. Therefore, the purpose of using 4N NaOH as a solvent was to dissolve the xylan extract in corncob powder. The alkaline method is more effective at damaging ester bonds between lignin, hemicellulose, and cellulose and prevents fragmentation of hemicellulose polymers. So, alkaline solutions dissolved not only xylan extract but also cellulose which has high solubility in alkaline solvents after the ester bond was broken. Lignin cannot be dissolved in an alkaline solvent because 4N NaOH can degrade lignin which will result in lysis of the lignin structure, then cause some of the molecules to condense and settle [[12]]. From these methods, the yields of the hemicellulose obtained were nearly 68.74% of the total available hemicellulosic polysaccharides in corncobs. This apparent yield indicated that most of the hemicellulose from corncobs was easily solubilized by the alkali solution, which was consistent with previous research by Ma et al. [[13]]. This is because the $OH^-$ charge on the alkali fills the microfibers in the cell wall so that the hydrogen bonds between cellulose and hemicellulose are broken, and also hydrolyzes ester bonds connected to other polymers in the cell wall.

Production of xylanase was obtained from colonies of recombinant *E. coli* DH5α, which will be carried out by enzymatic hydrolysis of hemicellulose. The results of this xylanase production were tested for activity using the DNS method and were measured its activity of 0.1156 U $mL^{-1}$. This shows the unit of xylanase activity was 0.1156 mol xylose per minute for every 1 mL of xylanase. The dinitrosalicylic acid (DNS) method gives a rapid and simple estimation of the extent of saccharification by measuring

the total amount of reducing sugars in the hydrolysate [[10]]. The reducing sugars produced from the hydrolysis of oat-spelt xylan with xylanase are xylose, arabinose, xylooligosaccharides, and glucuronic acid, so that the xylose standards are used to determine xylanase activity.

The products from the hydrolysis were analyzed using HPLC. The hydrolyzed hemicelluloses were hemicellulose A, hemicellulose B, and hemicellulose without being extracted (corncobs). The hydrolysis process was carried out at 70 °C for 24 hours. Hydrolysis of Hemi A did not produce xylooligosaccharides, in contrast to Hemi B which produced xylooligosaccharides. The result of the hydrolysis of hemicellulose with extraction is greater than that without extraction. This is due to the hemicellulose without extraction being still mixed with cellulose and lignin. Besides, the hemicellulose that is extracted with NaOH has lost lignin, which makes it more easily hydrolyzed by xylanase. Exo-xylanase activity was detected from the release of xylooligosaccharide. This result is consistent with previous research [[11]]. The product similarity between hydrolysis of oat-spelt xylan and corncob xylan provides a new alternative in the use of substrates for xylanolytic activity. Xylanolytic enzymes can hydrolyze hemicellulose because xylan is a constituent of hemicellulose. This xylanolytic enzyme hydrolyzes glycoside bonds at -1,4-D-xylopiranose and -1,3-L-arabinofuranose to produce xylose and arabinose monomers. Xylooligosaccharides are produced due to the activity of the enzyme of endo-xylanase which breaks the glycoside bonds in hemicelluloses randomly.

## 4. Conclusions

In summary, the xylanolitic enzyme in this study exhibited a great potential to hydrolyse hemicellulose from corncob. The hidrolysis product of hemicellulose using an extraction process showed a higher product (xylose and arabinose) than hemicellulose without extraction. This xylanolitic enzyme has great potential to hydrolyse corncob without the extraction process. Therefore, this enzyme is potent to reduce the use of alkaline solutions in the hydrolysis process.

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