The effect of alkaline-autoclaving delignification on chemical component changes of sugarcane trash

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Abstract. Sugarcane trash (ST) is lignocellulosic biomass that available abundantly during the sugarcane is harvested as a side product of sugarcane plantation. Several research groups have already reported their ST delignification. However, there is still needs to find a suitable delignification technique for improving recovery of cellulose and eliminate lignin and hemicellulose from a complex structure of ST. One of the methods for delignification is used alkaline with a high temperature. Hence, the research aim is to investigate the effect of alkaline-autoclaving delignification method on chemical component changes of ST. The different sample of ST including washing process and microwave-assisted maleic acid pretreatment prior to the delignification process with 2% NaOH combine with autoclaving in 121°C temperature was used in this study compare with the initial sample. The chemical composition changes in pretreated solid fractions and reducing sugar in pretreated liquid were analyzed. The functional properties were also analyzed using Fourier Transform Infrared (FTIR). The results showed that microwave-assisted maleic acid pretreatment before delignification was the effective treatment in this study to eliminate lignin (88.11%) and hemicellulose (44.07%) and cellulose recovery (78.40%) than that other treatment. Besides, the reducing sugar obtained from microwave-assisted maleic acid pretreatment 3.2 times and 3.6 times higher than that initial biomass and washing treatment, respectively. The FTIR spectra showed that washing treatment affects increasing absorbance of hydroxyl group while the maleic acid pretreatment was also increasing absorbance of carbonyl and the aromatic group that is a feature of lignin compound.

1. Introduction
Lots of lignocellulosic biomass are the sustainable alternative resources for renewable and carbon-neutral material in many sectors of production such as energy, electricity, chemicals or others. Sugarcane trash (ST) is one of the lignocellulosic resources that available surplus during the sugarcane is harvested as a side product from sugarcane plantation. ST consist of leaves and upper portion of the sugarcane plant. The production of ST trash approximately 10-15 t/ha of dry matter, mostly they only burning or left in the plantation after harvesting. The effect of burning ST in the field provide the primary source of air pollution in sugarcane producing countries. Restriction on ST burning will be affected to increasing the availability of ST until now the surplus of ST production still limiting utilization for produce value-added products. Therefore it seems promising to utilize ST for raw material in some further conversion into valuable co-products [1-5].
The difficulties of complex lignocellulosic biomass are the main problem to utilize them well. The critical step to solve this problem is by using the most suitable effective pretreatment to eliminate lignin and hemicellulose but also improving recovery of cellulose [5, 6]. This pretreatment is usually known as delignification process that makes lignocellulosic biomass can be converted into cellulose fibres. Delignification process can be affected the chemical bonds among cellulose, hemicellulose, and lignin were broken, and the cellulose pulp was formed. In the other hand, both hemicellulose and lignin were eliminated or dissolved in the solvent [7]. Moodley and Kana [8] explained that leaves of sugarcane have primarily consisted of cellulose (35-50%), hemicellulose (20-35%) and lignin (15-20%). The highest cellulose contained in the ST is seemed potential to utilize them in developing cellulose-based biomaterials products.

Several research groups have already studied regarding ST delignification for separate among cellulose, lignin and hemicellulose. Alkaline pretreatment used NaOH 3% coupled autoclaving with 121°C temperature have been reported by [2, 4] and also a combination between waste glycerol assisted transition [3]. Mutepe et al. [9] explained that the effectiveness for removing lignin on delignification process depends on the type on lignocellulosic biomass used in the research. Pramasari et al. [6] stated that alkaline pretreatment was considered as one of the suitable pretreated lignocellulosic methods. Moreover, acid pretreatment such as microwave-assisted various acid pretreatment [1] and oxalic-acid catalyzed glycerol-based organosolv followed by mild solvent [5] have been reported. To date, there is still a need to find a suitable delignification technique for improving recovery of cellulose and eliminate lignin and hemicellulose from the complex structure of ST.

Therefore, the aims in this study has investigated the effect of alkaline-autoclaving delignification method on some various treated ST, including washing process and microwave-assisted maleic acid pretreatment before the delignification process. In the present study, the chemical component changed especially the amount of cellulose after deliginfication was the main factor to confirm the potential delignification method. Also, this research is expected to get the higher cellulose retained in the sample treated as well as to increase the diversity of utilization ST in developing cellulose-based biomaterials products.

2. Material and Methods
   2.1. Material
   ST was obtained from PT PG Rajawali II, Subang, West Java. The ST was drying and cutting into small pieces. After that, the ST was grinding and fractionated using sieve shaker to make the fraction with particle size between 40 and 60 mesh. The fraction of ST was washed with boiled water with solid ration 1:10 three times and drying at 105°C overnight. The washed- dried sample was a store in sealed plastic bags at room temperature before the delignification process. As a comparison, the microwave-assisted maleic acid pretreatment sample from the previous research conducted by Hermiati et al. [1] used in this study. The preparation of microwave assisted maleic acid pretreatment referred to Hermiati et al. [1], and then they were washed treatment as the first fractioned ST

   2.2 Methods
   The method for this research divided to some of part as below.
   2.2.1. Alkaline-Autoclaving Delignification
   The washed and the microwave-assisted maleic acid pretreatment-washed sample was delignified using alkaline-autoclaving based on Pramasari et al. [6]. 10 g of dried-washed ST slurry in 100 ml of 2% (w/v) NaOH and then stirred for 5 minutes. The sample was autoclaving at 121°C for 1 hour. The reaction was stopped by dipping in an ice bath and then filtration process to separate solid and filtrate fractions. After that washing the solid fraction with distilled water until neutral for chemical component analysis, whilst the filtrate fractions was put in the centrifuge tube for reducing-sugar analysis. The weight and moisture content of solid fraction was calculated to the pulp recovery as
explained by Hermiati et al. [1]. The reducing sugars contained in the filtrate fraction was analyzed by dinitrosalicylic acid (DNS) method [10].

2.2.2. Analysis of Chemical Component
The chemical component was analyzed on ST before and after delignification process. The chemical component analysis consist of moisture and extractives [11], acid insoluble lignin (AIL) [12] holocellulose and α-cellulose [13][14]. The lost of chemical component changes were measured and calculated as explained by Hermiati et al [1].

2.2.3. Functional Groups Analysis
Functional groups of samples (initial and pretreated ST) were determined using attenuated total reflectance (ATR)-Fourier transform infrared (FTIR) spectrometer (Perkin Elmer FTIR Spectrometer-Spectrum Two, C 106456, Perkin Elmer Inc., Waltham, MA, USA). The analysis was conducted at wave number 400-4,000 cm	extsuperscript{-1} with 16 scans.

2.2.4. Statistical Analysis
The experimental design used in this study was a Single-Factor Completely Randomized Design, for the study of comparison between alkaline-autoclaving pretreatment in washing sample and others pretreatment. Data were analyzed for statistical significance by one-way analysis of variance (ANOVA) to determine individual and interactive effects. Duncan's Multiple Range Test (DMRT) was performed to test differences among treatments using STAR (Statistical Tool for Agricultural Research) software. The significance and insignificance effect of pretreatment on the chemical composition changes and loses from the ST, pulp recovery and the reducing sugars in the soluble fraction was analyzed.

3. Results and Discussion
One of the preparation sample processes is washing process both of the initial ST, and the microwave-assisted maleic acid pretreatment ST sample before alkaline-autoclaving delignification. Actually, the washing treatment is physical pretreatment for eliminating residual soluble sugars, or other extractive components might be present in the ST. The visual changes after washing process showed that the microwave-assisted maleic acid pretreatment ST sample is more lightness of brown (Figure 1). These phenomena might be the pretreatment was conducted twice in the sample (microwave-assisted maleic acid pretreatment and washing treatment), so that its caused more extractive compound was soluble in hot water that used in the washing process.

![Figure 1. The powder of a various ST (a: initial sample /untreated; b: the washed sample; c: the microwave assisted maleic acid pretreatment-washed)](image)

Moreover, the colour and visual changes after alkaline-autoclaving delignification in pulp (solid fraction) of the washed and the microwave-assisted maleic acid pretreatment-washed (Figure 2) showed that the delignification affected to a brightness of pulp and the filtrate fraction is dark brown
than that before pretreatment (Figure 1). It might be due to the alkaline-autoclaving delignification conducted the bleaching process, so that the delignified-sample was brighter (white colour) compare with the washed sample or before pretreatment (brown colour). Besides, the browning colour insoluble fraction of delignified-sample (Figure 2) indicated the loss of chemical component especially the easy soluble in alkaline slurry and high temperatures such as lignin component; it can be named with black liquor [6,15].

Figure 2. The pulp of a various alkaline-autoclaving delignification ST (a: initial sample; b: the washed sample; c: the microwave assisted maleic acid pretreatment-washed)

3.1. Effect of Alkaline-Autoclaving Delignification on Chemical Components Changes

The chemical composition (cellulose, hemicellulose and lignin) content shown in Table 1, actually the chemical composition of initial ST (native sample) and microwave assisted maleic acid pretreatment sample used in this study have been reported by Hermiati et al. [1].

Table 1. Chemical composition of delignified and undelignified ST

| Treatment                                      | AIL     | Cellulose     | Hemicellulose | Pulp Recovery |
|-----------------------------------------------|---------|---------------|---------------|---------------|
| Undelignified                                 |         |               |               |               |
| Initial/native ST 17.44 ±3.69<sup>1</sup>      | 35.00 ±1.10<sup>1</sup> | 35.55 ±0.74<sup>1</sup> | -             |
| Washed ST 27.03 ±0.03<sup>2a</sup>            | 36.14 ±0.03<sup>2c</sup> | 25.66 ±0.97<sup>2b</sup> | 79.18 ±0.00<sup>2a</sup> |
| Microwave assisted maleic acid ST 21.18 ±0.52<sup>1</sup> | 47.24 ±1.29<sup>1</sup> | 15.04 ±0.30<sup>1</sup> | 67.50 ±3.52<sup>1</sup> |
| Delignified (alkaline-autoclaving delignification) |         |               |               |               |
| From initial/native ST 3.73 ± 2.17<sup>2b</sup> | 60.08 ±2.67<sup>2b</sup> | 31.09 ±2.82<sup>2a</sup> | 36.59 ±1.77<sup>2c</sup> |
| From washed ST 4.82 ± 1.32<sup>2b</sup>       | 63.24 ±2.45<sup>2b</sup> | 29.52 ±1.13<sup>2ab</sup> | 42.78 ±0.08<sup>2b</sup> |
| From microwave assisted maleic acid-washed ST 5.11 ± 2.41<sup>2b</sup> | 78.40 ±0.72<sup>2a</sup> | 19.52 ±1.09<sup>2c</sup> | 40.30 ±1.17<sup>2b</sup> |

Information: 1 The data from previous research [1].
2 Values in the same column having the same letter were not significantly different at DMRT test (P<0.05).

The component changed consist of retained cellulose, removal of hemicellulose and lignin, and dry matter loss are the important indicator for the effectiveness of delignification methods [6][9][15]. Table 1 show that the lignin content delignified by alkaline-autoclaving was decrease compare with undelignified sample. It is confirmed that the losses of the lignin component were a higher value than other chemical components (Figure 3). The analysis of variance showed that there was a significant difference in lignin content between undelignified and delignified sample. However, there was no
significant difference in all sample delignified in this study. It is clear that alkaline agents (NaOH) combine with high-temperature work on effective pretreatment for delignification, due to NaOH breaks the linkages of hydroxyl ion (OH-) between lignin and hemicellulose in lignin-carbohydrate complexes and dissolves the lignin polymer to its aromatic derivatives. Also, the high temperature can make exploded rapidly in the sample to produce reactive fibre [2, 16].

The lignin content in this study was a similar result with that reported by Shankarappa and Geeta [2]. They used combination (2.5-3%) NaOH coupled autoclaving (121-125°) with various particle size (0.5-10 mm) in sugarcane trash, which was about 4-8% of lignin content. The concentration of NaOH in this study was lower than Shankarappa and Geeta [2], but the lignin content is similar. So, its good for applying a small quantity of NaOH will do the same work in degrading lignin and hemicellulose [15].

The loss of the lignin component of undelignified from washed ST was minus values (Figure 3), its mean the lignin content is higher than initial or native ST. It might be that the washing process mostly impact to the extractive components was easily soluble in hot water except for the lignin. Due to the lignin has been condensed and difficult to remove. Although, the percentage of extractive was decreasing, the lignin was increasing. The lignin loss of microwave-assisted maleic acid is the lowest one both of initial sample (undelignified) and delignified sample. Dilute acid pretreatment did not cause significant delignification, while cause the lignin combined into larger bodies that moving around in the cell wells and develop as droplets on the cell wall surfaces [1].

Figure 3. The loss of chemical component of delignified and undelignified ST

The cellulose content after delignification was increased compared with the undelignified sample, its confirm that alkaline-autoclaving will be suitable for retaining cellulose. The analysis of variance showed that there was a significant difference in cellulose content between undelignified and delignified sample, but only delignified of microwave-assisted maleic acid-washed ST has significantly different than another delignification sample. On the other hand, the hemicellulose content of delignified sample tendency increasing than the undelignified sample. The analysis of variance for hemicellulose content confirms there were significantly different between undelignified and delignified sample. The increase value content of cellulose and hemicellulose in the sample composition due to the marked loss of lignin from the sample, that was ranged 88-90% of the initial lignin in the native ST.

Hemicellulose in undelignified and delignified from microwave-assisted maleic acid-washed ST was the lowest one. Despite that, the loss of hemicellulose from delignified of microwave-assisted
maleic acid-washed ST was the highest one than other delignification sample. It deduced that adding dilute acid make remove hemicellulose insoluble fraction easily, and the pulp will be small amount of hemicellulose [1]. Pulp recovery between undelignified and delignified sample were significant differences based on analysis of variance. The losses of some component of the sample, especially lignin was expected contribution to the value of pulp recovery in delignified sample was lower than undelignified sample. Its means the alkaline-autoclaving delignification affected the pulp recovery of all sample. This confirms with other researchers, Cao et al. [15] and Pramasari et al. [6] stated that alkaline-autoclaving delignification with high temperature and pressure during alkaline condition would be beneficial for retaining cellulose, eliminating lignin and hemicellulose, but not useful for retaining for pulp recovery.

3.2. Effect of Alkaline-Autoclaving Delignification on Reducing Sugars

The highest of reducing sugars concentration in the soluble fraction after alkaline-autoclaving delignification of ST was the sample from microwave-assisted maleic acid-washed ST (Table 2). Analysis of variance showed that alkaline-autoclaving delignification of ST had a significant effect on reducing sugars concentration, and sample from microwave assisted maleic acid-washed ST had significantly different compare the other samples. These data had in accordance with lost of hemicellulose from alkaline-autoclaving delignification of ST was the sample from microwave-assisted maleic acid-washed ST (Figure 3) among 97%. It might be the sample has been conducted twice pretreatment (acid pretreatment and alkaline-autoclaving pretreatment), so that lots of chemical components were soluble in the filtrate fraction than others.

| Soluble Fraction                                  | Reducing Sugars Concentration (g/L) |          |
|--------------------------------------------------|------------------------------------|----------|
| Alkaline-autoclaving from initial/native ST       | 0.39 ±0.00^b                       | 0.39 ±0.00^b |
| Alkaline-autoclaving from washed ST               | 0.34 ±0.03^b                       | 0.34 ±0.03^b |
| Alkaline-autoclaving from microwave assisted maleic acid-washed ST | 1.26 ±0.03^a                       | 1.26 ±0.03^a |

Information: ^Values in the same column having the same letter were not significantly different at DMRT test (P<0.05).

3.3. Effect of Alkaline-Autoclaving Delignification on Functional Groups

There were no new peaks in the spectra delignified and undelignified ST, but there were some differences in parts of absorbance values (Figure 4). The peaks at band around 2850-3300 cm\(^{-1}\) were assosiated with cellulose, especially in OH stretching of intermolecular hydrogen bonds [1].
Information : A: initial sample (1) ; B: the washed sample; C: alkaline-autoclaving from the washed sample; D : alkaline-autoclaving from initial sample; E: the microwave assisted maleic acid pretreatment(1); F: alkaline-autoclaving from the microwave assisted maleic acid pretreatment-washed)

**Figure 4.** FTIR spectra of delignified and undelignified ST

The washing treatment prior to delignified has affected on increasing absorbance of hydroxyl group -OH. Dilute acid pretreatment prior to alkaline-autoclaving delignification (Sample E and F in Figure 4) has affected on increasing absorbance of carbonyl (-CO) and aromatic (-C=C-) functional group. Carbonyl and aromatic functional group are indicated the feature of lignin component [17]. The absorbance near 1605 cm\(^{-1}\) represents the aromatic skeletal vibrations in the lignin. The disappearance of these peaks in the spectra is directly related to delignification [18]. In addition, the methoxyl groups (-COCH\(_3\)) was indicated the feature of cellulose component [19]. Accordance with the Figure 4, the peaks of the methoxyl groups in Sample F (alkaline-autoclaving from the microwave assisted maleic acid pretreatment-washed) showed sharper than others, its means the cellulose was more affected by the delignification process in this study.

4. Conclusion

The best condition of delignification worked in severity condition was washing treatment and combination with microwave, acid, autoclave and alkaline. In this study, the alkaline-autoclaving from washing microwave-assisted maleic can remove lignin and hemicellulose, 88.11% and 44.07%, respectively. In addition, cellulose retained is 78.50% and reducing sugar 1.26 g/L, its more effective delignification than other delignification in this study. The FTIR confirmed that combination alkaline-autoclaving from washing microwave-assisted maleic was increasing absorbance of methoxyl groups, its means the cellulose have been disturbed. These chemical changes in alkaline-autoclaving from washing microwave-assisted maleic suggested the other suitable delignification for utilization sugarcane trash.

5. References

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Acknowledgements
The authors are grateful for the funding research through National Research Program grant funding in 2020 from The Ministry of Research and Technology/ National Research and Innovation Agency of The Republic of Indonesia and LPDP. Integrated Laboratory of Bioproducts (iLaB) of Research Center for Biomaterials – Indonesian Institute of Science for its equipment research support. Prof. Euis Hermiati for assistance and accommodate for sugarcane trash research.