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Characterization and ethanol potential from giant cassava (*Manihot esculenta*) stem waste biomass

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Abstract. *Manihot esculenta* stem waste biomass is promising material for ethanol production since it is unutilized substance from cassava production. Nowadays, cassava is the most common food in Indonesian society. The aims of this study were to identify availability and characteristic of giant cassava (*M. esculenta*) stem waste biomass for ethanol feedstock. In term of that, four plots with the size of 5m x 5m were made to calculate the total stem biomass obtained after harvesting process. In this study, various concentrations of alkaline were used to degrade lignin from the substrate. The effects of alkaline pretreatment were investigated using TAPPI method and the ethanol yield was estimated using modified NREL protocol. The results showed that the potential dry stem waste biomass from harvesting of *M. esculenta* was approximately 10.5 ton/ha. Further, alkaline pretreatment of stem waste biomass with 2% of NaOH coupled with the enzymatic saccharification process using meicelase was showed the highest production of sugar to reach of 38.49 % of total reduction sugar and estimated potentially converted to 2,62 L/ha of ethanol. We suggested *M. esculenta* stem waste biomass could be used as sustainable feedstock for ethanol production in Indonesia.

1. Introduction

As the human populations and industries increases, energy consumptions in general also increasing gradually, resulting in increased levels of pollution. Nowadays, until 2050 it is estimated that the world's population will increase by more than two billions or more people [1]. Concerning of energy consumption increased as the beginning of the development of bioethanol and biodiesel products resulting from food crops [2,3]. During this time, the production of bioethanol is more directed to the ingredients of starch and sugar, such as sugar cane, cassava root, and corn. Though these materials are basically a potential source of food, so the development of bioethanol from food in the future will be able to cause new problems due to competition for the fulfillment of food demands of the community. Therefore, it is necessary to develop bioethanol from materials that are not a food source of the community, namely lingo-cellulosic materials that allow forwards to the utilization of agricultural waste and industrial waste for bioenergy. One of kind the potential raw material source developed in Indonesia is cassava. Cassava is one of the most important root crops known and used in many countries of Africa, Latin America, and some Asian countries. Though it has its origin in South America and some Asian countries. The crop is widely grown as staple food and animal feed in these regions with a total cultivated area over 18 million ha [4,5]. In Indonesia, especially in East Kalimantan have been cultivating giant cassava (*Manihot esculenta*) that have superior quality than

other cassava. Seeing the promising prospect of *M. esculenta* with the potential of large tubers, of course, many stem wastes that is not utilized. So far, few reported has been done regarding the utilization of *M. esculenta* stem biomass as the raw material for bioethanol manufacture and the characteristic properties and process technology that can be used simply to produce it. In this study, we characterized the ethanol potential of cassava stem waste into renewable energy sources and environmental benefits.

2. Methods

2.1. Giant cassava stem materials

The plant material was wood biomass from Kota Bangun, Kutai Kartanegara, East Kalimantan, Indonesia. Amount of 1% sampling intensity plot was used in this study to estimate total biomass per hectare. The 4 random sampling plots with the size of 5 m x 5 m was made. Giant cassava is commonly harvested at 12 months with the planting distance of 1 m x 1 m.

2.2. Alkaline pretreatment

Alkaline pretreatment of stem biomass was carried out for 60 min at 170ºC using a liquid-to-solid ratio 4: 1 (w/w) and NaOH concentration between 2, 4, and 6%based on dry weight of the woody biomass, respectively. The reactions were carried out using a rotary digester equipped with a controller for pressure, rotary speed, and temperature. After the reaction, the pulp fraction was separated by filtration and washed extensively with tap water until neutral pH.

2.3. Chemical component analysis

The Klason lignin content was determined by the TAPPI standard method [6]. The holocellulose and α-cellulose contents were determined according to Wise's chlorite method [7] and the TAPPI standard method [8], respectively. The reducing sugar content was determined by the Somogyi–Nelson method [9].

2.4. Enzyme activity

Filter paper unit activities were assayed in reaction mixture containing 50 mg (w/v) Whatman filter paper number 1, 50 mM tartrate buffer, pH 4.5 and the enzyme. After incubation at 50ºC for 30 min., the reducing sugars produced were determined by Somogyi-Nelson method [9]. One unit (U) of each enzyme activity is defined as the amount of enzyme, which produce 1 μmol reducing sugar as glucose in the reaction mixture per minute under above specific condition.

2.5. Saccharification of stem M. esculenta

The wet pulp fraction was hydrolyzed with a commercial cellulose preparation, meicelase from *Trichoderma viride* (Meiji Seika Co., Ltd., 224 filter paper units (FPU)/g, β-glucosidase activity 264 IU/g). The cellulase enzyme loading was an 8-FPU/g substrate. Enzymatic hydrolysis was performed at a substrate concentration of 2% in 0.05 M sodium citrate buffer (pH 4.5) containing 0.02% sodium azide at 45ºC on a rotary shaker (NTS-4000C, Rikakikai, Japan) at 140 rpm for 48 h [10]. The saccharification ratio per pulp was calculated according to the NREL LAP-009 procedure [11]. The sugar yield per wood is based on the weight percentage of the reducing sugars to the original wood. The overall yield of sugars per wood is calculated by multiplying the saccharification ratio per pulp and the pulp yield. All enzymatic hydrolysis experiments were performed in triplicate.

2.6. Estimation of ethanol production from stem M. esculenta

Potential ethanol production from Macaranga wood was estimated based on the amount of hexose sugar (HXTEL) in the lignocellulosic material obtained by enzymatic saccharification of the insoluble pulp fraction. Due to the high content of glucose in the pulp fraction, HXTEL was approximated by

the amount of reducing sugars obtained from the pulp fraction (equation 1). The ethanol yields (EtOHBIO) based on the weight of original biomass was calculated from equation (2).

$$HEXTEL = HEX \times a\ (mg/Kg) \quad (1)$$

$$EtOHBIO = HEXTEL \times Ye.\frac{h}{b}\left(\frac{mL}{Kg}\right) \quad (2)$$

Where, HEX is the hexose (D-glucose) yield upon saccharification from hexosan (w/w), of original wood basis), a is the weight of substrate (1000 mg, 1kg), Ye.h is the theoretical ethanol yield from hexose (D-glucose) (0.511), and b is the ethanol density (0.789 kg/L) (modified from Premjet) [12].

3. Results and Discussion

3.1. Measurement of cassava (M. esculenta) biomass

Overall, the number *M. esculenta* every unit produced 81 stems with total population (*M. esculenta*) per hectare obtained as many as 8,100 stems/hectare. The results showed that the percentage of *M. esculenta* in each part are root 59.39%, stem 31.84%, tuber 5.96%, and leaves 2.80%, respectively (Figure 1). Acquisition of this value is based on the post-harvest results of *M. esculenta* conducted on each plot.

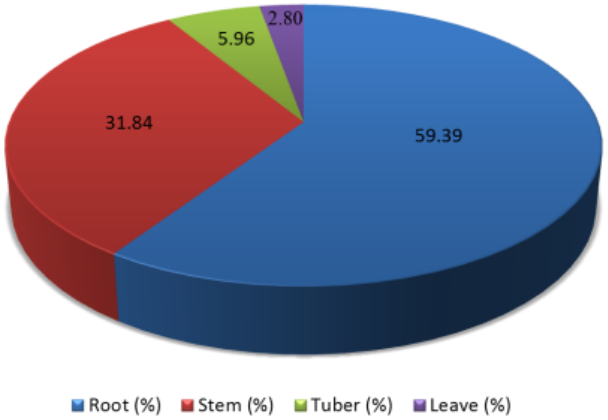

Figure 1. Percentage of *M. esculenta* in each part

Based on percentage results of *M. esculenta*, particularly the stem portion shown potential results when developed into bioethanol feedstock compared to root and other parts. According to Sumada [13], the utilization of *M. esculenta* stem wastes is not yet optimal because only 10% of the stems can be used for replanting and the remaining 90% are untapped waste. The dry biomass value obtained in *M. esculenta* stem was 10,533 kg/ha. Calculation of biomass values related to moisture contents that part of physic characteristic of *M. esculenta* stem.

3.2. Lignocellulosic components of wood biomass

The chemical properties of wood are related to the chemical content in the wood. Chemical timber or chemical components of wood are required for their existence in the chemical industry that processes wood (rayon, pulp, and paper industries) [14] and determine the timber harvest for the purpose of producing certain products. In this research, we evaluated lignin, holocellulose, and cellulose contents of wood biomass to point out their correlations to saccharification and estimated yield of ethanol. We found that the lignin content was 23.34%, holocellulose 67.26%, and cellulose 56.47% (Table 1), respectively.

Table 1. Lignocellulosic compositions (original wood components)

Lignin (%)	Holocelllulose (%)	Cellulose (%)
23.34 ± 0.39	67.26 ± 0.83	56.47 ± 0.58

3.3. Effects alkaline pretreatment on lignocellulosic components of M. esculenta stem

Effects of alkaline pretreatment on the change components were also analyzed. We found the alkaline pretreatment was effectively defibrillated and delignified the woody biomass that marked by the residual pulp fractions and decreased of Klason lignin content (Table 2,3).In general discussion, we found two different types of the residual pulp properties, hard fiber, and soft fiber. The formation of residual pulp fraction was really dependent on the concentration of alkaline and also biomass species used (Table 2, Figure 2).At the remaining 2% and 4% alkali concentrations the pulp properties are fiber with hard fiber, whereas at the remaining 6% alkali concentration the pulp properties is soft fiber. The formation of the remaining properties of the pulp depends on the concentration of alkaline and also the type of biomass used [15].

Figure 2. Pulp properties (a) 2% NaOH (b) 4% NaOH (c) 6% NaOH

Table 2. Pulp yield and pulp properties of *M. esculenta* stem

Alkaline concentration	Pulp yield (%)	Pulp properties
2%	65.81	hard fiber
4%	59.54	hard fiber
6%	40.16	soft fiber

Table 3. Residual lignin, holocellulose, and cellulose of *M. esculenta* stem

Alkaline	Lignin (%)	Holocellulose (%)	Cellulose (%)
2%	11.31 ± 0.12	43.85 ± 0.22	50.44 ± 0.01
4%	12.84 ± 0.14	41.79 ± 0.18	47.44 ± 0.21
6%	12.95 ± 0.13	36.04 ± 0.15	33.98 ± 0.37

The results showed that the pulp yield value at cooking conditions 2% was greater than the cooking conditions of concentration 4 and 6%. Differences in pulp yield produced in various types of raw materials, caused by differences in chemical components, especially holocellulose and lignin and reactions that occur during the pulping process. According to Fengel and Wegener [16] and Sjöström [17], suggesting that high levels of active alkali concentrations will cause the pulping process to

proceed rapidly, but as process speed increases, lignin degradation is unavoidable and cellulosic damage will be even greater.

The highest decrease of lignin content was obtained when 6% NaOH applied at 170˚C to give the value of 12.95%. Based on the values obtained in accordance with the opinion of Dewi [18], which states that the lignin content increases along with the increase of Sodium Hydroxide (NaOH) concentration. This occurs because the depolymerization process of lignin in the pulp material whose polymer turns into monomers, then the monomers react with the polymer still contained in the pulp and produce a new polymer or new lignin.

3.4. Enzymatic saccharification of M. esculenta stem

Lignocellulosic biomass consists of carbohydrates (cellulose and hemicellulose), lignin and other components (proteins, lipids, and inorganic substances) [19]. The presence of lignin greatly inhibits the process of degradation of cellulose and hemicellulose into glucose. Therefore, lignin must be eliminated either chemically or enzymatically which is a delignification process and thereafter can be fermentation process for bioethanol production. This delignification process is needed to break the long polymer chain into shorter polymer chains, increase the amorphous area (decrease the degree of crystallinity) and separate the lignin portion of the holocellulose.

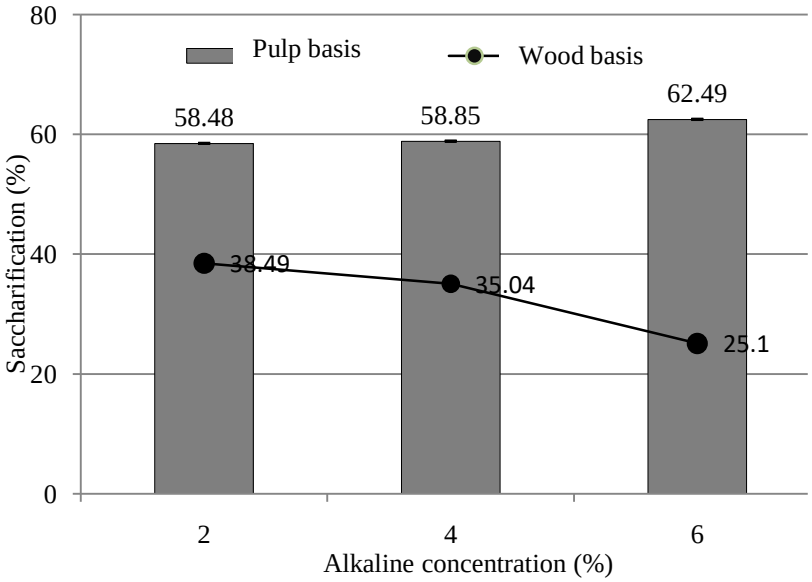

Figure 3. Saccharification yield of *M. esculenta* stem

The figure above reflects the calculation of the saccharification result of *M. esculenta* stem from the alkaline treatment concentration of 2, 4, and 6%, respectively. The higher alkaline concentration given will increase the saccharification result on the wood pulp. At the concentration of 2% alkaline addition, the saccharification result shows a number of 58.48% reducing sugars which can be produced from the pulp fraction. Meanwhile, the addition of 4% alkali concentration was able to produce reduced sugar as much as 58.85% while at 6% concentration able to reduce sugar up to 62.49% from total of pulp fraction. The effect of lignin on the saccharification process can inhibit the performance of the enzyme in breaking the glycoside bond in cellulose to produce simple sugars. According to Ko [20], lignin is a physical barrier that can limit the access of enzymes to cellulose. Lignin is able to bind to the active side of the enzyme thereby decreasing and causing unproductive enzyme performance. This causes a decrease in the activity of enzymes in degrading cellulose resulting in reduced productivity of reducing sugars.

If we look closely together, based on the overall base of the wood actually shows the opposite result. The higher the concentration given causes the decreasing of sugar yield. It also shows that the cassava wooden stem structure that is not too hard only requires preliminary treatment with mild concentration. The result of this research is obtaining optimum point of utilization of *M. esculenta* stem waste as raw material of ethanol with alkaline treatment of 2% NaOH addition with wood saccharification result 38.49%. As reported by Amirta [21], some tropical wood species such as macaranga require concentrations of up to 5% to find their optimum use point as ethanol feedstock. Moreover, this result is interesting because the used of concentrations of chemicals had suppress the production cost of the use of biomass cassava stems in the future.

3.5. Theoretical ethanol production from wood biomass

According to Premjet [12], the basic theory of ethanol calculation described in Figure 4, the highest ethanol production value was obtained from 2%, 4% and 6% alkali concentration of 249 L/ton, 227 L/ton and 163 L/ton, respectively. According to Aveline [22], the properties of biomass and plant species vary to be one of the causes of different ethanol yields at each given alkali concentration.

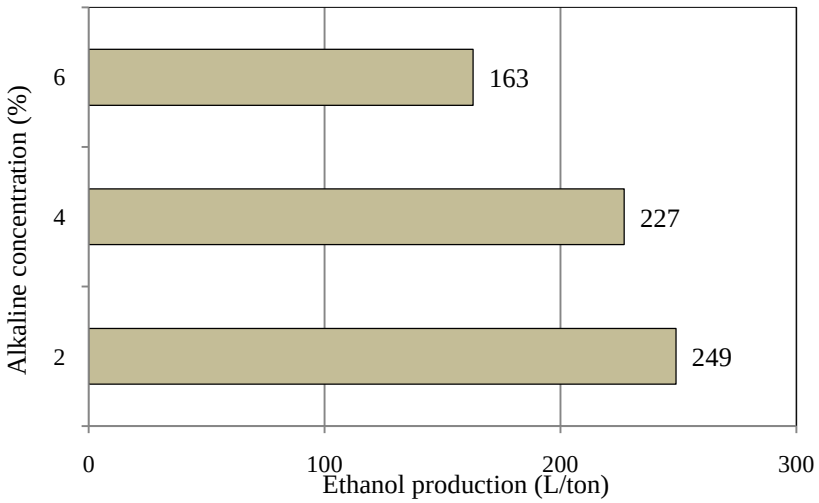

Figure 4. Potential ethanol production from wood biomass

Furthermore, the ethanol production from utilization of *M. esculenta* waste is higher than some tropical timbers from previous studies, such as *A. moluccana* (241 L/ton), *G. arborea* (239 L/ton), *A. cadamba* (233 L/ton), *A. altilis* (222 L/ton) and *P. falcataria* (220 L/ton) [15], respectively. The tropical wood species use too high concentrations of chemicals (NaOH above 3.5%), while *M. esculenta* stem waste use only 2% alkaline concentrations.

4. Conclusion

In conclusions, we found that production of ethanol from *M. esculenta* stem can be promoted as one of the more friendly process for environment, besides also explore the cassava waste stem utilization which has been widely wasted in society.

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