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Practicability of Lignocellulosic Waste Composite in Controlling Air Pollution from Leaves Litter through Bioethanol Production

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Abstract. Environmental degradation through greenhouse emission have spurred nation’s interest on feedstock-based fuel. Yet, development of this clean biofuel is obstructed by the expensive feedstock which takes up most of the production cost. Therefore, as an alternative, utilization of widely available lignocellulosic residues with relatively no commercial significance has been considered. This present work emphasizes on mango (*Mangifera indica*) leaves one of the most abundant lignocellulosic waste in Malaysia. Through implementation of this biomass for bioethanol production, continuous allowance of air pollution with a deleterious impact to the country’s environment could be reduced. The high concentration of sugar (16-18%w/v) in the form of cellulose and hemicellulose is ultimately the reason behind the selection of these leaves as a substrate for bioethanol production. Hence, in this study, a comparison of biomass composition in Harum Manis, Sunshine and Chokanan mango leaves were conducted to detect the most suitable substrate source to produce biofuel. At the end of the biomass evaluation, Harum Manis mango leaves turned out to be the most competitive bioethanol crop as these leaves reported to be made up of 34.71% cellulose and 44.02% hemicellulose which summed up to give highest fermentable sugar source with a lignin content of 19.45%.

1. Introduction

Bioethanol has received much attention in recent years due to its potential as a clean sustainable petroleum based fuel that offers vital economic values [1]. Besides its contribution as an alternative fuels source, bioethanol have also become an ultimate priority of chemical industry in terms of solvent, disinfectant and sterilizing agent [2]. Initially, bioethanol is obtained from conversion of reducing sugar stored in sugarcane, corn and maize through fermentation process [3]. However, as the demand for bioethanol increased tremendously, utilization of human feedstock for reducing sugar supply has been less practicable [4]. Hence, to ensure a long sustainability of bioethanol, biotechnologists considered non edible lignocellulose material (LCM) as a promising option to produce bioethanol [5].

There has been extensive research regarding the use of LCM as a source of fermentable sugars for bioethanol production because these substrates are inexpensive and highly available. Thus, in terms of raw material, there will be a great reduction in cost [6]. Yet, there is not much recognition given to agricultural leafy residues, specifically leaves of mango (*Mangifera indica*), a LCM source with a potential to produce bioethanol. *Mangifera indica* of Anacardiaceae family is well distributed in tropical regions, especially in Malaysia, India, Thailand, Philippines, China and Srilanka [7]. In Malaysia, the equatorial climate facilitates a vastly growth of mangoes [8]. Harum manis, Chokanan and Sunshine are known to be among the popular mango varieties in Malaysia [9].

Although this tropical fruit have recorded an overall production of 42 million tons worldwide for its juice and rich taste, the remaining part of the plant remains as waste [10]. The leaves are generally the most widely available crop residue apart of the flowers, stems and barks. These mango leaves usually overcrowds the mango plantations during tree pruning and harvesting season. This scenario leads to an alarming disposal issue in various countries [11]. In some countries, the open burning of collected leaves waste is practiced as a step to manage the leaves waste. However, this action led to air pollution whereby 85% particle of the leaf smoke could cause severe chemical effect to the lung when inhaled [12]. Eventually, these airborne particles will aggravate to chronic respiratory illness and shorten life span of humans. Furthermore, when the burning goes out of control, the nation will be economically challenged to overcome the cost of property damages, personal injuries and fire station services [13].

Therefore, using the mango leaves as LCM for producing bioethanol could be a strategic goal to reduce air pollution. According to Das and co-workers [14], cellulose (26.3%) and hemicellulose (54.4%) content of leafy biomass could be promptly hydrolysed into fermentable sugar that subsequently is bio transformed into ethanol. However, there is no any clear data on the variety of mango leaves that could aid in bioethanol production. Hence, this paper presents a comparison between mango leaves varieties with regards to biomass composition through gravimetric analysis, as this is a crucial step for determination of the most viable substrate in bioethanol production.

2. Materials and Methods

2.1 Chemicals

The extraction solvent, acetone with a Molecular Weight (MW)= 58.08 g/mol was purchased from Fisher Scientific. Sodium hydroxide (MW = 40 g/mol) was supplied by MacRon. Sulphuric acid (Purity= 96%: MW=98.079 g/mol) was received from Fisher Scientific. Distilled water was used as a diluting agent and for washing the biomass.

2.2 Substrate Preparation

The dried leaves of Harum manis, Chokanan and Sunshine mango (*Mangifera indica*) were collected from Institute of Sustainable Agrotechnology (INSAT), Perlis, Malaysia. The collection was carried out to accumulate 500 grams of leaf sample for each mango variety. The leaves were washed with running tap water thrice to ensure removal of dirt and dust particles and then cut into small pieces. Next, the leaf samples were dried overnight in oven (Memmert, Germany) at 80 ℃. For an efficient fermentation process, the leaves were then ground using laboratory blender (GM 200, Retsch, Germany) and allowed to pass through via 1 mm mesh sized sieve shaker (Retsch, Germany). Lastly, the sample of powdered mango leaves as illustrated in Figure 1 (A) was stored in an air tight container at -20 ℃ chest freezer (REMI, Malaysia).

2.3 Moisture Content Analysis

The moisture content of mango leaves before grinding process was determined through oven drying method. Initially, the weight of the mango leaves was recorded using an analytical balance (Sartorius, Germany). Next, the leaves were well distributed on the aluminium tray and inserted into the oven (Memmert, Germany). Then, the sample was dried for an hour at 105℃ to ensure complete removal of moisture. The dried leaves sample were measured for the moisture content analysis by using the formula as in e.q (1):

$$\text{Moisture content (\%)} = \frac{W_2 - W_3}{W_2 - W_1} \times 100 \quad (1)$$

Where,
W_1= weight of aluminium tray, g
W_2= weight of aluminium tray + sample, g
W_3= weight of aluminium tray + oven dried sample, g

2.4 Extractives
For soxhlet, 2.5 grams of dried leaves samples was first loaded into thimble followed by filling up the round bottom flask with 150 mL of acetone. The soxhlet apparatus (DXY, China) was then assembled. Subsequently, the temperature was set up at 70℃ for 4 hours. After extraction, the sample was air dried at room temperature for 10 minutes followed by conventional oven drying with a temperature of 105℃ until a constant final weight was achieved. The sample free of extractive in demonstrated in Figure 1 (B). Lastly, the % (w/w) of extractive was calculated based on differences between initial (2.5 grams) and final weight.

2.5 Hemicellulose
1 gram of extract free dried biomass was transferred into 250 mL conical flask and added with 150 mL of 500 mol/m^3 sodium hydroxide (NaOH). The mixture was heated at 95 ℃ in water bath shaker (WiseBath, Korea) in static mode for 3.5 hours. After that, the mixture was neutralized by washing with distilled water. Subsequently, the mixture was separated into supernatant and pellet via centrifuge (Thermo Scientific, U.S.A) at 4000 rpm for 20 minutes. Finally, the residue (pellet) was dried at 105℃ until a constant weight was attained. Figure 1 (C) shows the mango leaves powder with absence of hemicellulose content. The hemicellulose content % (w/w) was equalled to changes in weight of sample before and after sodium hydroxide treatment.

2.6 Lignin
0.3 grams of dried extract raw material was weighed and inserted into a test tube. Subsequently, 3 mL of 72 % of H_2SO was pipetted into the test tube. The sample was kept in room temperature for 2 hours. Throughout the incubation period, the test tube was vortexed by (Ika® Genius 3 Vortex, Sigma-Aldrich, U.S.A) every 30 minutes to complete the hydrolysis. Next, 87 mL of distilled water was poured into the test tube for an autoclave (Hirayama, Japan) session of 1 hour at 121℃. The slurry was cooled down at room temperature and then centrifuged at 4000 rpm for 30 minutes. Acid insoluble lignin was equivalent to the pellet dried at 105℃.The drying process was carried out until a constant weight of lignin was achieved. The remaining mango leaves powder with lignin content are shown in Figure 1 (D).

2.7 Ash
The sample hydrolysed to quantify the lignin content was further used for ash determination. The sample was incinerated at 575℃ in a muffle furnace (Cole Parmer, U.S.A) with a heating rate of 20℃/min. Then, the sample was removed from furnace and placed in a desiccator until the sample cooled down. At last, the ash was determined by weighing the sample in the crucible. Figure 1 (E) demonstrates the left-over sample of mango leaves which represents the ash composition of the biomass.

2.8 Cellulose
The cellulose content was determined by elimination of amount of the hemicellulose, lignin, ash and extractives in term of percentage from 100% (total amount of biomass).

A.
B.
C.
D.
E.

Figure 1: Biomass composition of mango leaves; A. Initial powder, B. Extractive free, C. Hemicellulose free, D. Acid insoluble lignin and E. Ash.

3. Result and Discussion

Figure 2 is a bar chart that illustrates moisture content and three main organic fractions (cellulose, hemicellulose and lignin) along with minor constituents (extractives and ash) of Harum Manis, Chokanan as well as Sunshine mango leaves. Each set of experiments was replicated thrice to obtain a more accurate and precise results. With this, the values reported is in the mean of 3 replicates ± standard deviation.

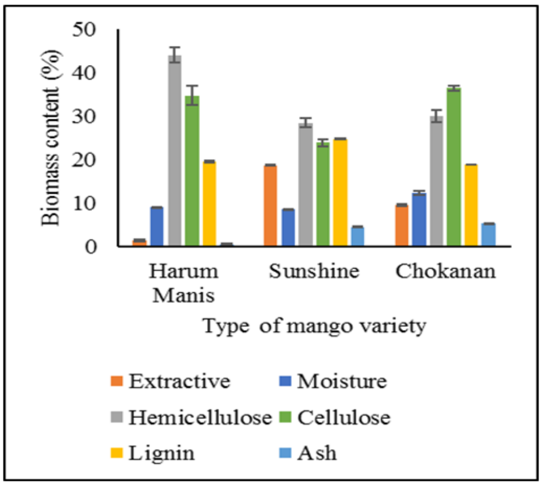

Figure 2: Biomass content of mango varieties

In term of bioethanol application, feedstock with minimal moisture content or hydrophilic nature is given priority as this is a promising feature for a substrate. With this, there is commonly an urge to determine hydrophilic traits of the leaves. Since, the moisture content of the biomass correlates to the hydrophilicity of the biomass, an analysis of the water content is crucial in the selection of an appropriate substrate for fuel biotransformation [2]. Hence, a moisture analysis was carried out whereby leaves of Chokanan mango have recorded maximum reading of 12.38±0.48% followed by Harum Manis (8.94±0.17%) and Sunshine (8.50±0.16%). Yet, Chokanan leaves with highest moisture adsorption are not preferred because the biomass will exhibit low density [15]. According to previous findings, biomass fuel with high density is more economical to be transported, stored and utilized compared to low density biomass. Thus, based on data presented above, both Harum Manis and Sunshine with relatively lower moisture content would satisfy the role as substrate to produce bioenergy in comparison to Chokanan.

Cellulose is known to be a linear sequence of homopolysaccharides made out of glucose units linked consecutively by β-(1,4) glyosidic bonds through condensation reaction [16]. According to Saifuddin and his co-researchers [5], the effectiveness of lignocellulosic crop to bioethanol majorly depends on the carbohydrate source which includes both cellulose and hemicellulose. These constituents of LCM are prominent extent of polymeric sugar that could be hydrolysed into monomers of pentose and hexose sugar to be promptly aided in biofuel production. Among three varieties of mango leaves investigated, Chokanan mango leaves resulted the greatest cellulose composition of 36.42±0.55%. However, the content of cellulose in Harum Manis mango leaves were only 1.72% lower compared to Chokanan. Hence both Chokanan and Harum Manis mango leaves would make a reliable candidate for bioethanol production.

Subsequent to cellulose, hemicellulose is the most inexhaustible (20-50%) sugar source in lignocellulosic biomass [17]. Hemicellulose is a configuration of branched heterogeneous polymer structured by hexoses (galactose, glucose and mannose), pentose's (eg: arabinose and xylose), as well as uronic acids (4.0- methyl galacturonic acids and galacturonic acid) [18]. Hemicellulose could be found in plant cell wall in the form of xylans, glucomannans, xyloglucans and mannans [19]. Through the experimental procedure for hemicellulose determination, the respective content for all three mango varieties were found to be in the range of 28-45%. Furthermore, this part of the study has also recommended that Harum Manis mango leave with the greatest hemicellulose content of 44.02±1.81% would aid the bioethanol production in a most significant manner. This is because apart of cellulose, biofuel orientated biomass is also highly dependent on its hemicellulose content for a worthy conversion process [4].

Lignin is comprised of coumaryl alcohol, sinapyl alcohol and coniferyl alcohol which are a part of phenyl propionic alcohol monomer units. These monomers are bonded with each other through aryl-aryl, alkyl-aryl and alkyl-alkyl linkage [20]. Besides that, lignin is an unstructured heteropolymer that contributes to the resistance, structural pillar and recalcitrance of lignocellulosic biomass [21]. Furthermore, owning to the fact that lignin's presence is the rationality to recalcitrance of lignocellulosic crops towards enzymatic attack for the release of reducing sugar. Thus, LCM with abundant of lignin content is not preferable [22]. In lignin analysis, Sunshine mango leaves have shown the highest composition of lignin (24.73±0.11%), this mango variety is assumed to be least favourable in production of bioethanol as compared with that of Harum Manis (19.45±0.23%) and Chokanan (18.83±0.09%).

Nitrate, chlorophyll, protein, waxes, steroids, fats, terpenes as well as phenolic compounds are known be the components of biomass extractive [17]. Extractives are non-structural constituents which are not linked chemically to the biomass. Furthermore, extractive actually is the most redundant composition of a biomass which interferes with the downstream analysis of a LCM sample [23]. The percentage of extractive in Sunshine, Chokanan and Harum Manis mango leaves were found to be 18.67±0.14%, 9.47±0.22% and 1.32±0.17%, respectively. Thus, through the finding obtained, Harum Manis could be the most competent LCM stock as these leaves have least percentage of extractive.

The final composition investigated in this research work was the ash content of the mango leaves. Ash could be defined as the non-flammable solid mineral element of a biomass which consist of Magnesium (MgO), Aluminium (AlO), Iron (FeO), Silica (SIO) and Calcium (CaO) [24]. In common, ash represents the most minimal percentage of a biomass constitution. This study has demonstrated that ash content was recorded in a range of 0.5-6%. Apart of that, the lowest amount of ash was recorded by Chokanan (5.28±0.13%). However, this criterion would not be supportive to account these leaves to be suitable for bioethanol production [2]. This is due to ash could not be converted into energy. Apart of it, dispersion of ash into the media could result the ash to exist as cations. This form of the ash would hinder the enzymatic saccharification of the LCM to fermentable sugars [24]. overall, it could be summarized that Harum manis with the smallest percentage of ash (0.5±0.21%) would be the most desired choice of substrate.

4. Conclusion

A reduction in lignin content of the mango leaves enhances a more proficient hydrolysis of cellulose and hemicellulose to its monomer that results a comprehensive yield of fermentable sugar. Consequently, the biotransformation of lignocellulosic crops to biofuel could become more productive. Hence, LCM with high cellulose and hemicellulose composition followed by low lignin content are currently taken into option to be more valuable for bioethanol production. Thus, through this study, Harum Manis mango leaves with the highest polysaccharide content of 78.73% (inclusive cellulose and hemicellulose) in comparison to Chokanan (66.42%) and (52.28%) has been suggested to be the most competitive biofuel crop. Besides that, Harum Manis have also recorded the least content of undesired substrate composition at 21.27% which comprises lignin, ash, moisture and extractive. Thus, by using Harum Manis leaves for bioethanol production, a higher yield of biofuel could be obtained. With this, a future research related production of bioethanol of Harum Manis leaves litters could developed to cut down air pollution caused by open burning. Finally, this study could be an upper hand to the nation because besides reducing the dependence of fossil fuel, bioethanol could also decrease the emissions carbon monoxide, particulate matter, toxic chemicals, and greenhouse gasses, creating a cleaner, safer motor fuel to improve the air quality.

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