

Chemical Characterization of *Imperata cylindrica* ('Lalang') and *Pennisetum purpureum* (Napier grass) for Bioethanol Production in Malaysia

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ABSTRACT

Grass is a candidate biomass producer because it is fibrous and it thrives well on poor soils. The chemical properties of two grass species growing naturally and abundantly on idle lands in Malaysia were investigated in this study. The grasses selected were *Imperata cylindrical* ('Lalang') and *Pennisetum purpureum* (Napier grass). For the purpose of analysis, Napier grass was further divided into male and female plants, and stem and leaves. Lignin, hemicellulose and cellulose contents were determined using the TAPPI standard methods. 'Lalang' was found not to be an attractive biomass producer because of its high lignin content (22%) ($P < 0.05$). On the contrary, Napier grass, particularly the female stem, had low lignin content (13%) ($P < 0.05$) and a favourably high level of cellulose (46%) ($P < 0.05$). In the female leaf, lignin content was higher (20.7%), while the cellulose content (30.4%) was lower compared to the stem. Although the cellulose content in the male stem (51%) was slightly higher ($P < 0.05$) than the female, its lignin was two-fold above that of the female stem, making it a less desirable biomass producer. Hence, it was concluded that female Napier grass has a good potential of becoming a biomass producer in bioethanol production in Malaysia.

Keywords: Bioethanol, biomass, cellulose, chemical properties, grass, lignin

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INTRODUCTION

Lignocellulose from grasses is one of the major resources for bioethanol as it has short rotation coppices and it is not suitable for human consumption. The characteristics of being high-yield, low nutrient requirement,

together with them being easily available throughout the world, have turned grasses into an attractive feedstock for bioethanol production (Hamelinck *et al.*, 2005; Balat *et al.*, 2008). Bioethanol can be produced from several different biomass feedstocks such as sucrose-containing feedstocks (e.g., sugar cane, sweet sorghum), starchy material (e.g., corn, wheat, rice) and lignocellulosic biomass (e.g., wood, rice husk, and oil palm empty fruit bunch) (Balat *et al.*, 2008). However, the production of bioethanol from starch and sugar has been seriously debated over its sustainability as starch and sugar are important food sources for human (Alvira, 2010). Thus, bioethanol from lignocellulosic material may be a better feedstock as there will be no competition against food crops. This form of energy has been produced and used in many developed and developing countries (Demirbas *et al.*, 2009). For example, bioethanol was introduced into the transportation fuel supply in 1970s by the Brazilian government (Manuel *et al.*, 2007). However, the potential bioconversion in lignocellulosic biomass is often limited by the associated aromatic constituents within the grass fibre, including lignins and phenolic acids, which act as barriers to fibre degradation (Anderson & Akin, 2008). In order to confirm the potential of lignocellulosic biomass of a plant, the chemical composition of the materials must first be determined as it greatly affects on the efficiency of bioconversion (Lee *et al.*, 2007). Lignocellulosic biomass consists of three polymers: lignin, hemicelluloses and cellulose. Lignin is a complex of

phenylpropanoid group, which is a common constituent of plant cell walls (Carpita, 1996). Lignins are highly branched, substituted, mononuclear aromatic polymers in the cell walls of certain biomass, especially woody species, and are often bound to adjacent cellulose to form a complex and recalcitrant structure. The lignin contents in both softwood and hardwood (dry basis) ranged from 20% to 40% and from 10% to 40% in various herbaceous species such as bagasse, corncobs, peanut shells, rice hulls and straws (Yaman, 2004). Hemicellulose and cellulose are bonded together by lignin in the microfibril structure. The basic repeating unit of cellulose consists of two glucose anhydride units called a cellobiose unit while hemicellulose is a mixture of various polymerized monosaccharides (Mohan *et al.*, 2006), which also contains some important C₆ sugar (Mohagheghi, 2006). Some of these C₆ sugar can be hydrolyzed into glucose for bioethanol conversion use. Alkaline treatment has been used as a pretreatment method to break down the linkage and release the cellulose. In bioethanol process, cellulose is the most important component as it hydrolyzes into glucose and used in microbial fermentation to produce ethanol.

In this study, the chemical contents of two local grass species available in Malaysia were analyzed. Potential grass candidate can be further explored and used for conversion into bioethanol.

MATERIALS AND METHODS

Plant Materials

Two perennial local grass species, namely, *Imperata cylindrica* (L.) P. Beauv. ('Lalang') and *Pennisetum purpureum* Schumach (Napier grass) were collected from idle lands at Universiti Putra Malaysia, Serdang campus, and from a housing area in Selangor, respectively. The plants selected were in fresh green condition. Napier grasses were collected during their flowering stage at an average height of 2 m. The whole plant was cut using a cutter, about 0.15 m from the base. 'Lalang' was also collected during the flowering stage at an average height of 1 to 2 m. The napier grass was further divided into stems and leaves. The plants were cut into approximately 3 cm and then oven dried at 50°C until constant weight and later ground into powder form using Wiley's mill. The grass powder was sieved to the size of MESH 40-60 as required by the TAPPI Standard.

Chemical Composition

Plant samples were subjected to different extraction methods according to the TAPPI Standard Method (Anon, 1978), with slight modifications. The adaptation of the TAPPI Standard in this experiment was based on the soft fibre-type of material, which was more similar to the materials found in the pulp and paper industry. Besides, the TAPPI Standard Method is more suitable for the preliminary step to determine the amount of chemical properties in the grass material and it does not require any specific equipment to

carry out the experiments, unlike NREL and ASTM. The chemical properties experiment consisted of alcohol-acetone solubility test (TAPPI T6), lignin content test (TAPPI T222), holocellulose content test (Wise *et al.*, 1946) and cellulose content test (TAPPI T203).

Data Analysis

Results were analyzed using SAS program version 9.1.3. (SAS Institute). Procedure Univariate was used in order to determine data normality by conducting Shapiro-Wilk W test and Kolmogorov-Smirnov (K-S) D test. Data were transformed into square root. Procedure General Linear Model (GLM) and Least Square Means (LSM), with probability differences, were used to compare the level of significance between the species, and stems and leaves.

RESULTS AND DISCUSSION

Lignin, holocellulose and cellulose contents of two grass species in Malaysia, 'lalang' and Napier grass were analyzed using the standard methods. Some differences were detected between the species and organ parts. 'Lalang' showed moderate results for the lignin (22.07%), holocellulose (77.69%) and cellulose (51.61%) contents (Figure 1). From the comparison made between 'Lalang' and the male and female Napier grasses, the later had the highest cellulose content ($P < 0.05$) (Fig.1). When comparing the stems and leaves of Napier grass, the cellulose content in the male stem was 2.5-folds above that of the leaves (Table 1). Meanwhile, when comparing

TABLE 1
A comparison of the chemical properties between Malaysia local grass species and other crops

Species	Extractives	Lignin	Holocellulose	Cellulose
'Lalang'	18.26	22.07 ^c	77.69 ^a	51.61 ^a
Napier grass - male (Stem)	3.20	29.67 ^a	76.73 ^b	51.44 ^a
Napier grass - male (Leaves)	6.51	28.09 ^b	72.62 ^d	19.60 ^d
Napier grass - female(Stem)	2.68	12.93 ^c	77.64 ^a	46.01 ^b
Napier grass - female (Leaves)	14.89	20.74 ^d	75.04 ^c	30.39 ^c
Wheat straw ¹	n/a	17.00	27.60*	40.70
Corn stalks ²	3.27	19.00	68.18	42.43
Switch grass ³	17.54	18.13	25.19*	31.98
Softwood ³	2.88	27.67	21.90*	44.55

*Hemicellulose; n/a: not available

1: Reference from Tomás-Pejó *et al.* (2009)

2: https://unit.aist.go.jp/btrc/research_result/database/corn-s.htm

3: Reference from Hamelinck *et al.* (2005)

^a means with the same letters were not significant

The percentages of lignin, holocellulose and cellulose were calculated by taking into account the material's moisture content.

the female leaves and stems, the lignin and cellulose contents were both higher in the stem ($P < 0.05$) (Figures 1a & 1b). Between the female and male Napier grass, the male leaves were found to have less than half of the cellulose in the female leaves ($P < 0.05$) (Fig. 1a), but the female had less than half of the lignin in the male stem ($P < 0.05$) (Fig. 1b). Male leaves, on the other hand, had approximately 10% more lignin when compared to the female leaves (Figure 1b). There were not many differences in the cellulose and holocellulose contents of the female stem when compared to the male stem (Fig. 1a & Fig. 1c). These results indicated that the female Napier grass has a good potential of becoming a biomass producer.

Among the four subsets of Napier grass, the female Napier stem was the best for use in bioconversion into bioethanol as

it contained the lowest amount of lignin. Lignin is the biggest barrier in the hydrolysis process because it forms a cross-link linkage with hemicelluloses, which prevents the hydrolysis of cellulose into glucose. In general, stems always contain more fibre than leaves. In Rye-grass, the stem (39.5%) has more fibre than the leaf (7.9%) (Smole *et al.*, 2005). In addition, stem-to-leaf ratio increases and cell walls which undergo secondary thicken and lignification during maturation resulting in increased content of structural polysaccharides (mainly cellulose and hemicellulose) and lignin (Lindgren *et al.*, 1980). This explains the higher content of holocellulose and cellulose in the Napier female stem compared to the leaf. In the experiments, 'Lalang' was tested with the whole plant (mixture of stem and leaves); therefore, the Napier grass must be combined to reach a fair comparison.

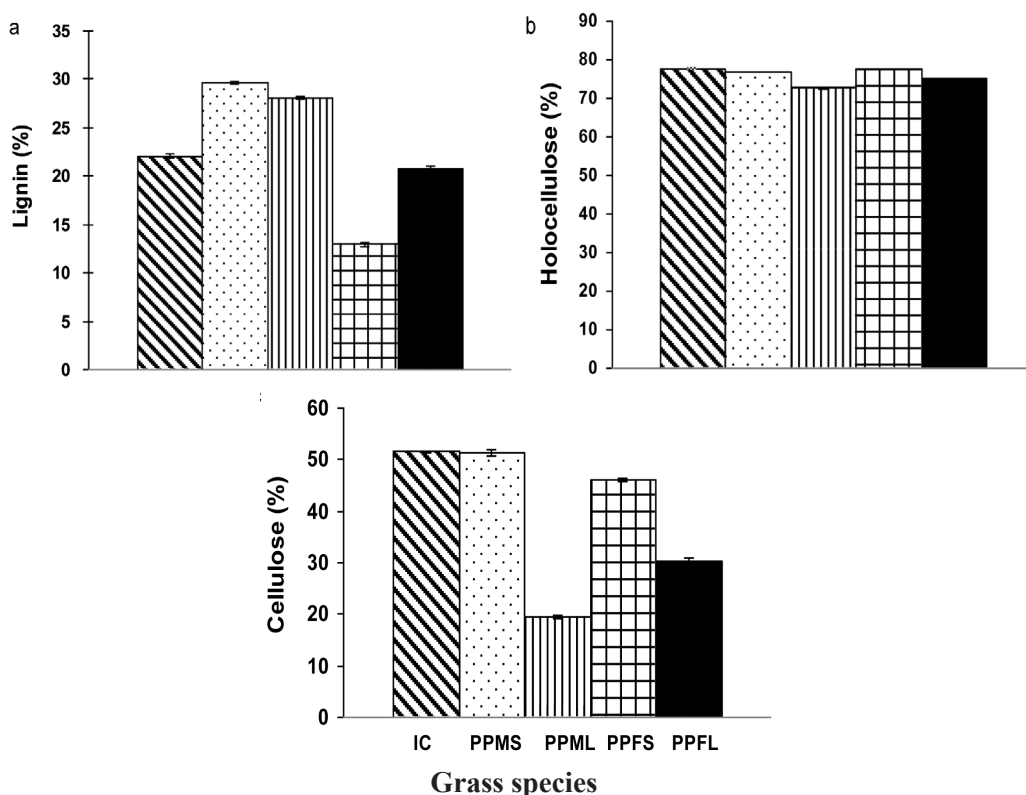


Fig.1: Chemical contents of *Imperata cylindrica* (IC) and *Pennisetum purpureum* (PP) found in Malaysia. Data were the means from six replicates and error bars indicate standard deviations. a) lignin, b) holocellulose, and c) cellulose. MS/ML, male stem or leaves; FS/FL female stem or leaves.

Hence, it was proposed to combine the female Napier stem and leaves together so as to recover more holocellulose and cellulose, which in turn will contribute to higher cellulose content.

Hardwood (24%), softwood (27.67%) and other crops such as wheat straw (17.0%), corn stalks (19.0%) and switchgrass (18.13%) (Table 1) showed similar lignin levels to the Napier grass tested in this study. In addition, Napier grass also had higher cellulose content compared to the other crops (30-42%). One interesting comparison between switchgrass and

Malaysian Napier grass was that the latter had a similar or a higher content of lignin and cellulose to switchgrass (18.13% of lignin and 31.98% of cellulose). When we compared the cellulose content found in the Napier grass with that of softwood (44.55%), wheat straw (30%) and others (Table 1), the Napier grass appeared to have a great potential as a biomass producer. Napier grass, especially the female grass, had higher cellulose content compared to other crops, while the lignin content was about the same. In more specific, the local Napier grass had higher or about similar

lignin and cellulose contents to switchgrass, but lower lignin content than softwood. Lignin can be a problem when it comes to assessing potential biomass as lignin is a major barrier during hydrolysis due to its recalcitrant structure that protects cellulose from being hydrolyzed into simple sugar. The vessel elements of softwood contained mainly guaiacyl units, which restrict fibre swelling and restrict the disruption of lignin structure (Gibbs, 1958; Ramos *et al.*, 1992; Li *et al.*, 2004). Previous research has shown that softwood vessel elements contain a higher ratio of guaiacyl lignins than syringyl lignins (Ramos *et al.*, 1992; Gibbs, 1958). The guaiacyl lignin, which consists largely of coniferyl alcohol, will restrict fibre swelling more than syringyl lignin (Ramos *et al.*, 1992; Henriksson, 2009). Although the HGS-lignin (Hydroxyl phenol, Guaiacyl, Syringyl) in grass contains all three monolignols (*p*-coumaryl alcohol, conigeryl and sinapyl alcohols), the ratio of *p*-coumaryl alcohol is a lot higher, i.e. approximately 33% (Henriksson, 2009). Therefore, the restriction of fibre swelling is not as high as compared to softwood and hardwood. These altered structures of the material will help in hydrolyzing cellulose into glucose during hydrolysis. This increases the efficiency of ethanol conversion as the 6-ring sugar (C₆) hemicellulose can be easily converted into simple sugar using enzyme hydrolysis or acid hydrolysis method.

The chemical properties determination of lignocellulosic biomass is the first step in bioethanol conversion. The content of chemical properties such as lignin,

hemicellulose or holocellulose and cellulose are the main keys to identify the potential of lignocellulosic material in becoming a source of biofuel. Hence, the chemical properties of grasses were determined in this experiment in order to identify the potential of grasses for bioethanol production. We found that the female Napier grass has a potential as a biomass producer.

CONCLUSION

Even though many trees and plants such as *Acacia*, rubber and bamboo produce high cellulose and hemicelluloses contents, they are also rich in lignin. This study has shown that Malaysia's grasses contain less or similar lignin compared to wood and straw. It could be one candidate source of biomass for bioethanol production due to the desirable chemical properties of having less lignin and high or similar content of cellulose compared to woody and herbaceous plants. Among the samples tested, the female stem of Napier grass is the best candidate to serve as a biomass for bioethanol production. Although there are some difficulties in glucose extraction due to the lignin-hemicellulose recalcitrant structures in the fibre, grass material can be pretreated with chemical pretreatment such as diluted alkaline. The significant low content of lignin allows easy extraction of hemicellulose and cellulose after being pretreated. Therefore, in considering the need for lignocellulosic biomass, Malaysia's local grass seems to be a potential candidate due to its abundant availability and its attractive chemical composition.

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