



Short communication

Comparison of lignin, cellulose, and hemicellulose contents for biofuels utilization among 4 types of lignocellulosic crops



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ABSTRACT

Lignocellulose crops serve as an excellent feedstock for biofuels because of their reduced costs and net carbon emission, and higher energy efficiency. To estimate more suitable lignocellulosic crops, we compared the contents of lignin, cellulose, and hemicellulose in miscanthus, switchgrass, sorghum, and reed (from 14 accessions according to the collection site) in the leaves and stems and expressed these as % content based on dry weight. This study shows that miscanthus, switchgrass, and sorghum are valuable lignocellulosic crops owing to the significantly lower lignin content than that in reed, among both whole crops as well as specific plant parts. Although switchgrass has been reported to possess the highest polysaccharide content among the crops examined; our results showed no difference at a 5% significance level. Our study also showed that *Miscanthus sacchariflorus* possesses lower lignin and higher polysaccharide content in its leaves and stalks compared to the other *Miscanthus* species. Furthermore, *M. sacchariflorus* also showed lower lignin and higher polysaccharide contents than those in switchgrass. It is possible that *M. sacchariflorus* is a better resource than switchgrass, although these content assays showed no differences at the 5% significance level. *M. sacchariflorus* plants collected in Hacheonri, Jeju-do, Korea (MFJH), contained 14.12% lignin and 64.23% holocellulose, indicating that Korean miscanthus is a competitive bioenergy crop compared to foreign crops such as switchgrass, which is widely used in the United States.

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1. Introduction

Our society has been highly dependent on fossil fuels since the industrial revolution. However, the amount of available fossil fuels worldwide is limited and its extraction leads to environmental problems including greenhouse gas emission. In response to these impending problems on fossil fuel resourcing, European countries, the United States, and Brazil have now focused on identifying alternative energy resources, including the study of biomass as a source of biofuels. The United States and Brazil are currently equipped for bulk production of bioethanol from corn and sugar cane [1]. However, these plant-based biofuel generation processes are not economically competitive with fossil fuels because of the efficiency, price and availability [2]. Lignocellulosic crops such as miscanthus and switchgrass were more suitable for biofuel production because of their capacity to adapt to barren land; these crops are available even in non-agricultural land. The use of

lignocellulosic crops has also been associated with high input/output energy efficiency and competitive handling cost. It has been reported that these crops have high amounts of lignin and cellulose [3–5]. In Europe, perennial C4 grasses such as miscanthus, switchgrass, and sorghum have been investigated for their potential as biofuel resources based on their comparatively high biomass potentials [3]. The *Miscanthus* species currently considered for bioenergy resourcing include *Miscanthus sinensis* and *Miscanthus sacchariflorus*. Of equal interest is *Miscanthus x giganteus*, an interspecific hybrid of tetraploid *M. sacchariflorus* and diploid *M. sinensis*. *M. x giganteus*, which has been studied in Europe and the United States [4].

Plant biomass resources are complex materials that generally consist of 3 major organic fractions: lignin, cellulose, and hemicellulose. Lignin is a complex, high-molecular-weight structure containing cross-linked polymers of phenolic monomers. Lignin in the primary cell wall provides structural support, impermeability, and resistance against microbial attack [7]. Cellulose is the main structural constituent in plant cell walls and is composed of long chains of cellobiose units that are linked to D-glucose subunits

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through β -(1,4)-glycosidic bonds. These linkage bonds are broken by hydrolysis, which are catalyzed by cellulase or acids [6,7]. Hemicellulose consists of branches of short lateral mono-saccharides such as pentose (xylose, rhamnose, and arabinose), hexose (glucose, mannose, and galactose), and uronic acid [8]. Cellulose in cell walls are packed into microfibrils by the long-chain cellulose polymers linked by hydrogen and van der Waals bonds, which are protected by hemicellulose and lignin [7]. The features of these structural components thus increase the cost of processing lignocellulosic crops [9]. Pretreatment of lignocellulosic crops has been designed to break the lignin seal and disrupt the crystalline structure of cellulose [10–12].

Based on the structural features of lignin, the industrial bioconversion of lignocellulosic crops to biofuel has become quite challenging; therefore, materials with low lignin and high cellulose and hemicellulose contents are now considered more valuable for biofuel production [9,13]. This study aimed to compare the lignin, cellulose, and hemicellulose contents among the lignocellulosic crops such as miscanthus, switchgrass, sorghum, and reed, as well as determine the differences in the contents of these substances between plant parts such as leaves and stems. We also examined content levels in 3 *Miscanthus* species, namely, *M. sinensis*, *M. sacchariflorus*, and *M. x giganteus*.

2. Materials and methods

2.1. Raw materials

Fourteen plants were examined in this study, consisting of 8 miscanthus (4 *M. sinensis*, 3 *M. sacchariflorus*, and 1 *M. x giganteus*), 3 switchgrass (*Panicum virgatum* L.), 2 sorghum (*Sorghum bicolor* L.), and 1 reed grass (*Phragmites australis*) plants. The plants were collected from local sites in Korea, America, India, Nigeria, and Sudan, and they were cultivated in a field in Kangwon University, Korea. Each plant was separated into leaves and stalks for experimentation (Table 1).

2.2. Experimental procedures

The schematic diagram for the determination of lignin, cellulose, and hemicellulose content is shown in Fig. 1.

2.2.1. Sample preparations

The raw plant materials were oven-dried at a temperature below 45 °C for at least 3 days until a constant weight was recorded. The dried materials were ground to a particle size of 1–3 mm and strained using a standard testing sieve (aperture size, 1.40 mm).

2.2.2. Preparation of extractive-free samples

Extractive-free samples were prepared using the Soxhlet extraction method, according to the American Society for Testing and Materials (ASTM) standard D-1105-96 [13–15]. The extraction solvents consisted of ethanol-toluene (1:2) solution, ethanol, and water. The extraction began with 3 gm of the sample placed in the thimble filter of the Soxhlet apparatus and incubated in the extraction solvent mixed with toluene and ethanol (2:1) for 4 h. The sample was then transferred to a Büchner funnel for filtration, and the excess solvent was removed by aspiration. The thimble filter and sample were washed with alcohol to remove the toluene. The sample was returned to the extractor, and ethanol extraction was continued for 4 h or longer, if necessary, until the ethanol in the siphon was colorless. The sample was removed from the thimble, spread out as a thin layer, and air-dried until it was free of alcohol. The extraction procedure was repeated using distilled water. The sample was filtered on a Buchner Funnel, washed with 500 mL of boiling distilled water, and oven-dried at 100 °C.

2.2.3. Determination of lignin content

AIL was estimated according to the modified Klason lignin determination procedure by using the extractive-free sample [13,14,16]. Approximately 3 mL of 72% sulfuric acid was added to 150 mg of the extractive-free sample in an Erlenmeyer flask. The sample was incubated for 1 h in a 30 °C water bath and stirred every 14 min to complete wetting and mixing. Subsequently, 84 mL of deionized water was added to the sample and autoclaved for 45 min at 123 °C. The autoclaved sample was rapidly cooled and filtered using No. 1 Whatman filter paper. The residue in the filter, which was the AIL, was washed with distilled water and oven-dried at 105 °C until a constant weight was recorded. The dried AIL was then subjected to gravimetric analysis. Calculations were performed as follows:

Table 1

Contents of structural components of 4 lignocellulosic plants.

| Plant type | Cultivar | Collection site | Abbreviation [†] | Extractives | Lignin | Holocellulose | | |
|-----------------------|------------------------------------|-----------------------------------|---------------------------|-------------|--------|---------------|---------------|--------------------|
| | | | | | | Cellulose | Hemicellulose | Total |
| Miscanthus | <i>M. sinensis</i> | Bangdongri, Injeun, Korea | MSIB ^a | 20.9 | 14.7 | 30.5 | 29.9 | 60.4 ^{††} |
| | | Nammyeon, Injeun, Korea | MSJN ^a | 19.2 | 24.7 | 31.8 | 24.6 | 56.4 |
| | | Geumbyeongsan, Chuncheonsi, Korea | MSCG ^a | 21.6 | 16.5 | 33.4 | 25.5 | 58.9 |
| | | Malli, West-Bengal, India | MSI ^a | 20.4 | 19.0 | 32.4 | 21.3 | 53.8 |
| | <i>M. sacchariflorus</i> | Yangpyeong, Gyeonggi-do, Korea | MFGY ^b | 25.5 | 18.1 | 31.7 | 22.3 | 54.1 |
| | | Andeokmyeon, Jejudo, Korea | MFJA ^b | 17.6 | 17.6 | 34.6 | 28.9 | 63.5 |
| | | Hacheonri, Jejudo, Korea | MFJH ^b | 18.2 | 14.1 | 36.1 | 28.2 | 64.2 |
| | <i>M. x giganteus</i> | Illinois, USA | MGIN ^c | 24.2 | 17.2 | 33.2 | 23.3 | 56.5 |
| Switchgrass | <i>Panicum virgatum</i> L. | Illinois, USA | SGINA ^d | 16.9 | 21.1 | 35.8 | 21.5 | 57.3 |
| | | Illinois, USA | SGINB ^d | 17.0 | 19.6 | 37.8 | 25.2 | 63.0 |
| | | Illinois, USA | SGINC ^d | 24.8 | 13.9 | 29.5 | 27.4 | 56.9 |
| Sorghum | <i>Sorghum bicolor</i> (L.) Moench | Nigeria | SBNG ^e | 23.7 | 17.9 | 31.4 | 23.4 | 54.8 |
| | | Sudan | SBSD ^e | 20.2 | 20.7 | 35.3 | 23.6 | 58.9 |
| Reed | <i>Phragmites australis</i> | Deokduwonri, Chuncheonsi, Korea | RGCD ^f | 20.3 | 26.2 | 30.0 | 23.8 | 53.9 |
| CV(%) | | | | 3.2 | 3.8 | 2.2 | 2.2 | 1.6 |
| LSD _(0.05) | | | | 1.1 | 1.2 | 1.6 | 1.2 | 1.5 |

a, *Miscanthus sinensis*; b, *Miscanthus sacchariflorus*; c, *Miscanthus x giganteus*; d, switchgrass; e, sorghum; and f, reed.

[†]Abbreviations are derived from the plant type and the site from which it was collected.

^{††}The data in the table show the mean values (n = 3, except cellulose and hemicellulose n = 2; standard deviation < 3) and appear as % based on dry weight. Statistical significance was analyzed by the least significant difference (LSD).

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