CHARACTERIZATION OF PHYSICOCHEMICAL PROPERTIES OF *MISCANTHUS FLORIDULUS* STEMS AND STUDY OF THEIR OIL ABSORPTION ABILITY USING GOLD NANOPARTICLES

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Miscanthus floridulus, which originated from a high elevation mountain area in Taiwan, is a newly cultivated species of *Miscanthus*. Instead of *Miscanthus* × *giganteus*, *M. floridulus* can be used as an alternative fuel for energy production as well. Except for leaves, stems of *M. floridulus* count for a major portion of the biomass. In this study, the lignin and cellulose contents of *M. floridulus* stems were determined to be 22.33 ± 2.21% and 43.13 ± 2.79%, respectively. In addition, a new application of *M. floridulus* stems was proposed. Oil absorption ability represented by the amount of soybean and motor oils absorbed by one gram of pulverized *M. floridulus* stems was estimated to be 2.25 ± 0.25 and 2.33 ± 0.18 g, respectively. Gold nanoparticles were used to investigate the absorption ability of *M. floridulus* stems. The absorption of gold nanoparticles by *M. floridulus* stems was visualized using SEM and TEM. In addition, the IR spectrum of *M. floridulus* stems was recorded for comparison with other studies.

Keywords: Miscanthus floridulus; Lignin; Cellulose; Oil absorption; Gold nanoparticles; IR spectroscopy

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INTRODUCTION

Miscanthus is an important C_4 energy plant that grows easily and free of diseases. *Miscanthus* can grow 2 to 3 m in height, producing 10 to 40 tons of dry biomass per hectare annually (Lewandowski *et al.* 2000). In the European Union, *Miscanthus* has been burned with charcoal for electricity production (Lewandowski and Kicherer 1997; Clifton-Brown *et al.* 2004; Bauen *et al.* 2010). The heat capacity provided by 20 tons of *Miscanthus* biomass is approximately equal to that of 12 tons of hard coal. The heat generated by one ton of *Miscanthus* is less than that by fossil fuel, but the environmental impact is much less. Comparing the provision and combustion of *Miscanthus* with hard coal, burning *Miscanthus* emits 90% less CO_2 to produce the same amount of energy than burning hard coal (Lewandowski *et al.* 1995). Since *Miscanthus* has a low content of nitrogen, phosphorus, and potassium, combustion of hard coal along with *Miscanthus* reduces the emission of harmful substances including NO_x and SO_2 (Lewandowski and Kicherer 1997; Lewandowski and Heinz 2003) as well. As a result, using *Miscanthus* biomass in combustion not only reduces the consumption of fossil fuel, but also reduces the problems associated with greenhouse gases. Thus, *Miscanthus* has become a highly valuable bio-fuel crop which provides clean and renewable energy.

The *Miscanthus* genus, which is widely populated in Asia, has several species. However, *Miscanthus* $\times$ *giganteus*, a hybrid variety with high biomass, is almost the exclusive species used in energy production and research. In the European Union, growth of *M. giganteus* is greatly influenced by weather. Frost damage of *M. giganteus* rhizomes greatly reduces its first winter survival rate. Biomass production is usually high in summer, but much lower in winter (Clifton-Brown *et al.* 2000; Lewandowski *et al.* 2000; Clifton-Brown and Lewandowski 2002; Tuck *et al.* 2006). Thus, introduction of a new *Miscanthus* crop that can survive extremely cold conditions of northern Europe is required. Stable and continuous supply of large quantities of biomass helps scientists to perform research on *Miscanthus* and also increases the use of *Miscanthus* for energy production.

Miscanthus species are easily found in Taiwan, even on mountains with high elevation where the temperature in winter is low. Thus, the Chia-Yi Agricultural Experiment Station has been collecting different *Miscanthus* species from the mountain area of Taiwan during winter and studying their agronomic traits. Among the Taiwanese native species collected, *M. floridulus* produces the largest amount of biomass. Agronomic traits of four Taiwanese native *M. floridulus* lines have been studied (Hung *et al.* 2011). These lines were collected in the mountain area from low to high elevation. Among these four lines of *M. floridulus*, the line collected at the altitude of 1,000 m, which sustains the cold weather and grows at low temperature, has emerged as a new potential source of bio-fuel.

Since *M. floridulus* became a new source of bio-fuel, physiochemical properties of *M. floridulus* need to be studied. In addition to the leaves, stems of *Miscanthus* count as a major portion of the biomass. The lignin and cellulose of *Miscanthus* stems collected at elevation of 1,000 m were determined in this study. In addition, in this study a new application of *Miscanthus* stem is proposed. *M. floridulus* stems can be used as natural absorber to clean up an oil spill. When oil spills occur, sorbents can be used to remove oil. Sorbents used include synthetic polymer and natural materials. Synthetic polymers have high sorption capacity, but they are not biodegradable. Thus natural materials such as agricultural products and wastes emerge as potential sorbents in oil spills (Suni *et al.* 2004; Annunciado *et al.* 2005; Lim and Huang, 2007; Cojocaru *et al.* 2011). Using *M. floridulus* stems to absorb spilled oil will solve problems of environmental pollution. More importantly, *M. floridulus* stems with oil absorbed may provide extra energy after burning.

In this study, the oil absorption ability of *M. floridulus* stems was determined using soybean oil or motor oil. Since gold nanoparticles can be easily observed by tunneling electron microscope (TEM) and scanning electron microscope (SEM), the absorption ability of *M. floridulus* stems were directly visualized by electron microscopy in the presence of gold nanoparticles. In addition, the infra-red spectra of *M. floridulus* stems were recorded with or without gold nanoparticles. The IR spectrum of *M. floridulus* stems can be used as a comparative reference for future studies. In the presence of gold nanoparticles, IR spectra may be helpful to investigate the compositions of *M. floridulus* stems that are important in absorption.

EXPERIMENTAL

Preparation of *M. floridulus* Stems

M. floridulus plants were provided by the Chia-Yi Agricultural Experiment Station. This species originated in the mountain area of Taiwan at an altitude of 1,000 m, where they were planted in the area with the longitude, latitude, and altitude of E120.468049, N23.484503, and 79 m, respectively. After harvesting, *Miscanthus* stems were dried in an oven at 50 °C for 24 hours and pulverized. Pulverized *Miscanthus* stems were sieved with 20- and 60-mesh screens and stored in a cool place for further use. For IR and electronic micrograph analysis, pulverized *Miscanthus* stems were further sieved with 200-mesh screen.

Lignin Contents of *M. floridulus* Stems

The acid-insoluble lignin of *M. floridulus* stems was determined as the ash free residues by gravimetric analysis from the two stage sulfuric acid hydrolysis (Jung *et al.* 1999; Raiskila *et al.* 2007; Yao *et al.* 2010). Acid-soluble lignin was determined spectroscopically (Raiskila *et al.* 2007; Yao *et al.* 2010). For each analysis, 3 mL of 72% sulfuric acid was added to 0.3 g of pulverized stems. The mixture was then sonicated at room temperature for 1 hour followed by adding 82 mL of deionized water. The whole mixture was then autoclaved at 121 °C for 1 hour. After cooling, the acid-insoluble lignin and ash were collected by vacuum filtration and dried at 103 °C until constant weight.

Dry acid-insoluble lignin was then ignited at 575 °C for a minimum of 3 hours to determine the contents of lignin and ash. Acid insoluble lignin was calculated based on the difference in weight before and after igniting. The filtrate collected was used to determine the acid soluble content. Exactly 0.2 mL of filtrate was diluted with 1.0 mL of diluted sulfuric acid. The absorbance at 205 nm was recorded to calculate the acid soluble lignin.

Cellulose Content of *M. floridulus* Stems

The content of α-cellulose was determined by the anthrone method with modification (Updegraff, 1969; Ververis *et al.* 2004). Briefly, 0.3 mL of acetic/nitric acid was added to 5.0 mg of pulverized *Miscanthus* stems. The mixture was heated up to 100 °C. After 30 minutes, the mixture was centrifuged, and the supernatant solution was discarded. The residue was washed with 1 mL of deionized water. After centrifugation to discard the supernatant, 0.5 mL of 67% H_2SO_4 was added to dissolve cellulose. Exactly 0.01 mL of this solution was further diluted with deionized water to a final volume of 1.0 mL.

To measure the cellulose content, 0.1 mL of this diluted solution was mixed with 0.4 mL of deionized water followed by adding 1 mL of cold anthrone reagent. The mixture was then heated to 100 °C for 16 minutes.

After cooling in an ice bath, the mixture was placed at room temperature for 10 minutes. The absorbance at 620 nm was recorded using an ELISA reader (TECAN^R, Austria). A calibration curve of α-cellulose standard was constructed to determine the cellulose content.

Oil Absorption by *M. floridulus* Stems

The pure soybean oil and motor oil (10W40) were purchased from a local store to study the oil absorption ability of *Miscanthus* stems. The density and viscosity of soybean oil were 0.920 g/mL and 54.5 mPa·s, at 26 °C. The density and viscosity of motor oil are 0.861 g/mL and 137.5 mPa·s, at 26 °C, respectively. For each absorption experiment, 5 grams of pulverized *Miscanthus* stems were mixed with 100 mL of either the soybean oil or motor oil at 26 °C. After 12 hrs of soaking, the mixture was then filtrated with filtering paper and weighed. The oil absorption ability (S) of *Miscanthus* stems was calculated as:

$$S = \frac{W_{oil}}{W_{stem}} \tag{1}$$

In equation (1), W_{oil} is the weight (g) of oil absorbed and W_{stem} is the weight (g) of dry pulverized *Miscanthus* stems.

Absorption of Gold Nanoparticles by *M. floridulus*

Gold nanoparticles were used to visualize the absorption ability of *Miscanthus* stems. Gold nanoparticles synthesized using the standard protocol described by Graber *et al*. (1995) were purified by centrifugation to remove residual chemicals before use. Purified gold nanoparticles were resuspended in deionized water. The size distribution of gold nanoparticles was determined using a dynamic laser scattering particle size distribution analyzer (Horiba LB-550, Kyoto, Japan), which showed an average size of 24 nm. In a typical absorption experiment, approximate 3 mg of pulverized stems was soaked in 200 μL of gold nanoparticle solution for 24 hrs at room temperature. *Miscanthus* stems having gold nanoparticles bound to them were then collected by centrifugation. After washing with deionized water, *Miscanthus* stems with gold nanoparticles were resuspended in deionized water for TEM analysis. Small pieces of *Miscanthus* stems were soaked in gold nanoparticles for SEM analysis. For IR analysis, stem samples soaked in deionized water for 24 hrs were used as reference samples. Samples used for IR analysis were dried thoroughly in an oven at 50 °C.

Electronic Micrographs of *M. floridulus* Stems

A Hitachi TM-1000 tabletop scanning electron microscope (Hitachi Corporation, Tokyo, Japan) was used to examine the morphology of *Miscanthus* stems at a magnification of 1,000 and 1,500X. For TEM analysis, *Miscanthus* stems with gold nanoparticles were re-suspended in 200 μL of deionized water and loaded onto copper grids. Absorption of gold nanoparticles onto *M. floridulus* stems was examined using a transmission electron microscope (JEM-2010, JEOL Co. Ltd., Tokyo, Japan) equipped with a field-emission gun. The acceleration voltage was set to 100 kV.

FT-IR Spectroscopic Analysis of *M. floridulus* Stems

FT-IR spectroscopy was used to examine the absorption of gold nanoparticles on *M. floridulus* stems. Approximately 3.0 mg of *M. floridulus* stems with or without gold nanoparticles were mixed with KBr and pressed into a disc of 1 mm thickness. The spectra of the stem fibers were obtained using a Perkin-Elmer FT-IR spectrometer

(Spectrum One, Waltharm, Massachusetts, USA). Spectra of samples were recorded in the range of 400 to 4,000 cm^{-1} with a resolution of 4 cm^{-1}.

RESULTS AND DISCUSSION

Lignin, cellulose, and ash contents of *M. floridulus* stems are shown in Fig. 1. The acid-insoluble lignin content was determined gravimetrically, whereas the acid-soluble lignin was determined spectroscopically. As shown in the figure, the acid-insoluble lignin was 21.02 ± 2.12%, and the acid-soluble lignin was only 1.31 ± 0.86%. The total lignin, which is the summation of acid-soluble and acid-insoluble lignin, was 22.33 ± 2.19%. In comparison to previous studies (de Vrije *et al.* 2002; Ververis *et al.* 2004; Brosse *et al.* 2009; Hage *et al.* 2010), the acid-soluble lignin was comparable to that of *M. giganteus*. On the other hand, the acid-insoluble lignin was slightly lower than reported values for *M. giganteus*. The average total lignin content of *M. giganteus* in literature was about 26%. The lignin content of *M. floridulus* was about the same as that of *M. sinensis* whose lignin content was 24.4%. These differences in lignin content were probably due to different plant species. In addition to lignin, the cellulose content was determined for *M. floridulus* stems. As shown in Fig. 1, the cellulose content of *M. floridulus* stems was determined to be 43.13 ± 2.79%, which was comparable to the literature value reported for *M. giganteus* (Ververis *et al.* 2004).

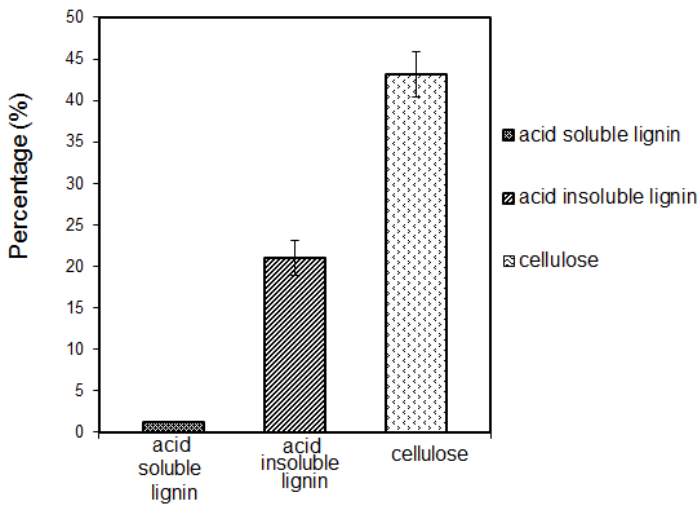

Fig. 1. Lignin, cellulose, and ash contents of *M. floridulus* stems

The oil absorption abilities of *M. floridulus* stems were evaluated using soybean and motor oils. Results are shown in Fig. 2. Since stems of fresh *Miscanthus* are too tough to process, only dry stems were used in oil absorbing experiments. Dry stems were pulverized to increase the surface area which enhances their oil absorbing ability. The amounts of motor and soybean oils absorbed by one gram of pulverized *Miscanthus* stems were 2.25 ± 0.25 and 2.33 ± 0.18 g, respectively. The compositions between these two kinds of oils are different, but the difference in motor oil or soybean oil absorbed by *Miscanthus* stems was minimal. The main fatty acids of triglycerides in soybean oil are palmitate, stearate, oleate, linolenate, and linoleate, while the major hydrocarbons in motor oil include polyalphaolefins and esters. Thus, similarity in absorbing soybean oil or motor oil indicates that the hydrophobic parts of hydrocarbon may be an important factor to govern the oil absorbing ability of *Miscanthus* stems.

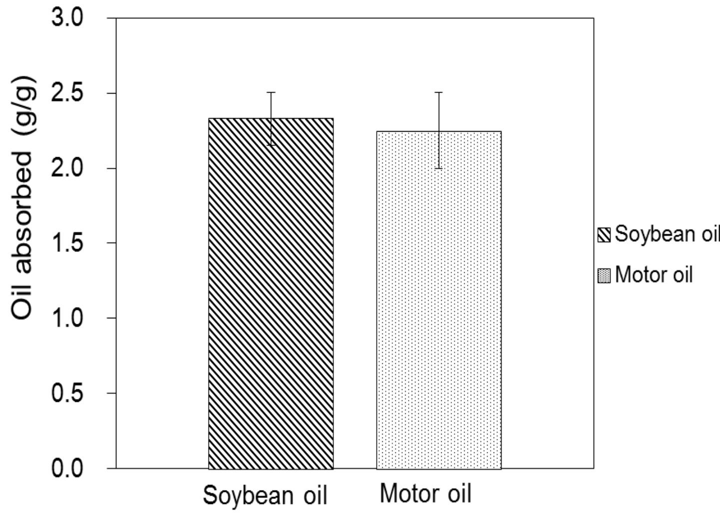

Fig. 2. Oil absorption ability of *M. floridulus* stems

The absorbing ability of *Miscanthus* stems was directly visualized by an electron microscope in the presence of gold nanoparticles. Figure 3 shows representative TEM images of *Miscanthus* stem tissues with gold nanoparticles absorbed. In all TEM images, gold nanoparticles indicated by arrows appeared as black dots. Figures 3A and 3B demonstrated that gold nanoparticles clustered on the edge and surface of stem tissue. Since stem tissues were repeatedly washed with deionized water to remove excess free gold nanoparticles, gold nanoparticles observed in TEM images were tightly bound to the stem. As shown in the images, some gold nanoparticles were scattered on the surface of the *Miscanthus* stem, while some were concentrated on a specific area. The areas with dense coverage of gold nanoparticles were likely cracks or rough areas on the surface.

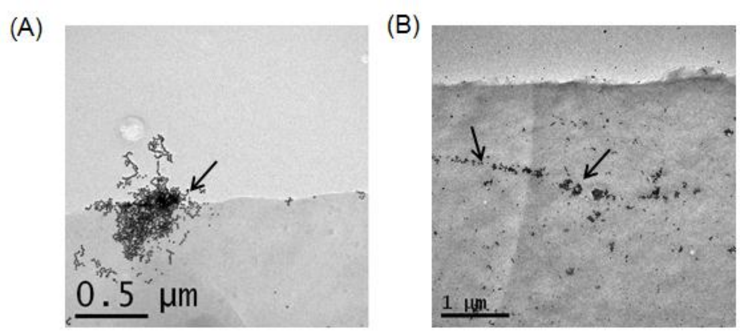

Fig. 3. Representative TEM micrographs of *M. floridulus* stem tissues with absorbed gold nanoparticles at a magnification of 10,000× (A) and 6,000× (B), respectively. Gold nanoparticles shown as black dots are indicated by arrows.

In addition to TEM, absorption of gold nanoparticles on *Miscanthus* stem was also visualized by SEM. Figure 4 shows representative SEM images. Figure 4A shows the longitudinal section of *Miscanthus* stem at a magnification of 1,000×, while Figs. 4B and 4C show the longitudinal surface in the presence of gold nanoparticles at a magnification of 1,500×. As shown in Fig. 4A, the stem surface of *M. floridulus* was not smooth. Protuberances and valleys appeared alternately. Similar protuberances and valleys were also observed in Figs. 4B and 4C. In SEM images, gold nanoparticles marked by arrows appeared as bright dots. Figure 4B showed that some gold nanoparticles clustered on the stem surface, and some even clung on the side of protuberances. Similarly, Figure 4C demonstrated absorption of gold nanoparticles on the edge of chipped stem.

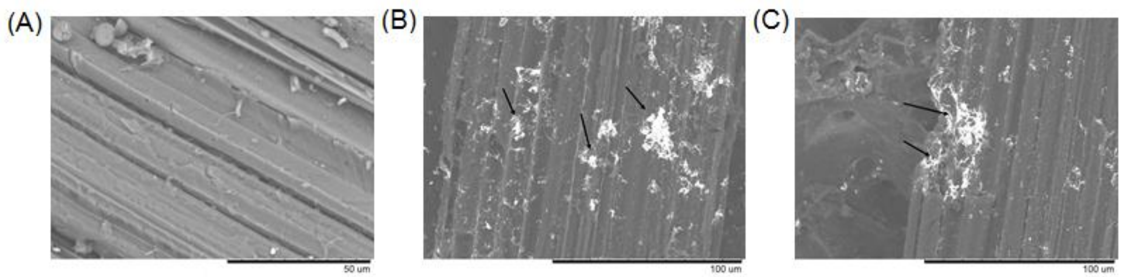

Fig. 4. Representative SEM images of *M. floridulus* stems with or without gold nanoparticles at a magnification of (A) 1,000×; (B) 1,500×; (C) 1,500×. Gold nanoparticles appeared as white spots were indicated by arrows.

IR spectroscopy was used to investigate the possible interaction between absorbents and molecules involved in absorption. If IR signals of functional groups change upon absorption, then the types of molecules that are affected by absorption may be speculated. Thus, gold nanoparticles were used to investigate the compositions that may be important in absorption in this study. In addition, the IR spectrum of *M. floridulus* stem can be a reference for comparison in future studies. The IR spectra of *M. floridulus* stems recorded with or without gold nanoparticles are shown in Fig. 5. The lower and upper panels in Fig. 5 were the IR spectra of stems soaked in either water or gold nanoparticles for 24 hours prior to drying, respectively. The IR spectrum of *M. floridulus* stems was similar to that of *M. giganteus* reported in the literature (Wang *et al.* 2010). The major absorption frequencies were at 3459, 2961, 1741, 1519, 1386, 1262, and 1061 cm^{-1}, respectively. These absorptions are mainly due to chemical bond vibration or stretching of lignin and polysaccharides, such as cellulose and hemicellulose. For example, the OH stretching of lignin, cellulose, or hemicellulose was around 3436 or 3422 cm^{-1} (Sun *et al.* 1995; Sun *et al.* 2004; Sain and Panthapulakkal 2006). The characteristic absorption at 2961 cm^{-1} was from CH_2 stretching (Wiśniewska *et al.* 2003). Both 1519 and 1430 cm^{-1} originated from the aromatic carbon-carbon double bond stretching of lignin. Details of assignments are summarized in Table 1.

When gold nanoparticles were present, the IR signals did not change except for the absorption at 1386 cm^{-1} (Fig. 5). A small shoulder appeared at around 1378 cm^{-1} in the presence of gold nanoparticles compared with the IR spectrum of the stem alone.

Since the signal around this frequency was correlated to aliphatic C-H vibration, which was one of the most abundant motions among chemical bonds of molecules, this change indicated possible interaction between gold nanoparticles and stems. Even the interaction between gold nanoparticles and *M. floridulus* stem was not strong enough to produce apparent changes of IR signals in this study, using gold nanoparticles to investigate the absorption interaction is still possible. Because gold nanoparticles are easily fabricated by chemical reactions to make their surfaces either hydrophobic, hydrophilic, or having distinct recognition moieties, specific absorption between gold nanoparticles and plant tissues may be established to produce spectroscopic changes in the future.

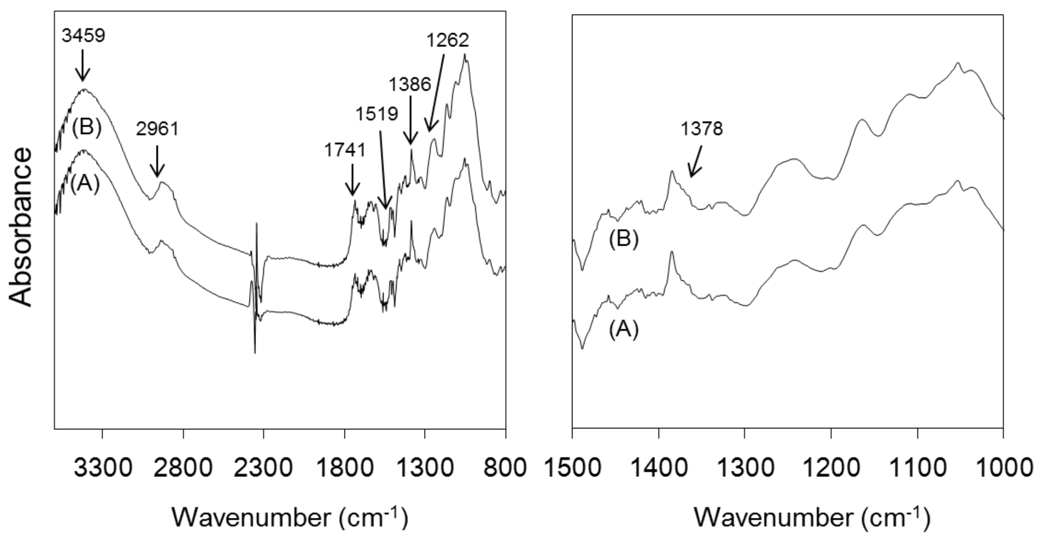

Fig. 5. Representative FT-IR spectra of *M. floridulus* stems with or without gold nanoparticles. The frequency region between 1000 and 1500 cm^{-1} was expanded in the right-hand plot. (A) *M. floridulus* stems; (B) M. floridulus stems in the presence of gold nanoparticles.

Table 1. Characteristic IR absorption of *M. floridulus* Stems

Absorption frequency (cm^{-1})	Origination of IR absorption
3459	-OH stretching of cellulose, hemicellulose, and lignin [a, b, c]
2961	-CH stretching vibration of methyl or methylene group [d, e]
1741	Carbonyl stretching of ketone, ester or other carboxyl groups [b, f, g]
1519	Aromatic C=C stretching of lignin [e,h]
1465	-C-H vibration of aromatic ring [e]
1430	Aromatic C=C stretching of lignin [e, h]
1386	Vibrations of aliphatic $–CH_2$, or $–CH_3$ [e, g, h]
1262	Aromatic C-O stretching of lignin [e, g]
1170	Stretching of C-O-C at β-glucosidic bonds in cellulose or hemicellulosec [e, g, i]
1061	Stretching of C-O and C-C bonds, or C-OH bending in cellulose or hemicellulose [e, g, i, j]

[a]Sun *et al.* 2004. [b]Sain and Panthapulakkal, 2006. [c]Sun *et al.* 1995. [d]Wiśniewska el al., 2003. [e]Wang *et al.* 2010. [f]Sun *et al.* 2002. [g] Xu *et al.* 2006. [h]Sun *et al.* 2005. [i]Mascarenhas *et al.* 2000. [j]Xiao *et al.* 2001.

CONCLUSIONS

1. The lignin and cellulose contents of *M. floridulus* stems cultivated in the area with the longitude, latitude, and altitude of E120.468049, N23.484503, and 79 m were determined to be 22.33 ± 2.21% and 43.13 ± 2.79%, respectively. Both cellulose and lignin contents were comparable to the literature values reported for *M. giganteus*.
2. One gram of pulverized *M. floridulus* stems was able to absorb approximately 2.3 grams of either soybean or motor oil.
3. Considering the role of *Miscanthus* as an alternative fuel and its ability to absorb oils, application of *M. floridulus* stems as an absorber may be an effective, economic, and environment-friendly strategy to clean up oil spills.
4. SEM and TEM micrographs showed that gold nanoparticles absorbed on the *M. floridulus* stem surface.
5. The IR spectra of *M. floridulus* stems were recorded with or without gold nanoparticles. The IR spectrum of *M. floridulus* stems can be a comparing reference for future studies.

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