The Utilization of Soybean Straw. II. Dissolution & Regeneration of Soybean Straw in LiCl/DMSO

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Various soybean straw (stem and pod) samples were dissolved in 8% lithium chloride/dimethyl sulfoxide (LiCl/DMSO) following 4 h of planetary ball-milling. The solubility, extractable lignin yield, and crystal structure of the ball-milled soybean straw were greatly affected by the ball-milling pretreatment. The dissolved soybean straw could be regenerated by being poured into excess distilled water under rapid stirring. The total regenerated fraction yield decreased with the increase in the duration of ball-milling. Approximately 10% to 25% of the straw mass was lost in the dissolution-regeneration procedure. For comparison, ethylenediamine (EDA)-pretreated soybean straw was also completely dissolved in 8% LiCl/DMSO to form a homogeneous solution containing 1% straw after 24 h of continuous stirring. The dissolution-regeneration performance of soybean straw submitted to the EDA pretreatment was quite different due to the lack of vigorous ball-milling.

Keywords: Soybean straw; LiCl/DMSO; Ball-milling; Dissolution; Regeneration

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INTRODUCTION

Renewable biomass resources are generally viewed as an important contributor to the development of a sustainable industrial society and the effective management of greenhouse gas emissions. Soybean (*Glycine max*) straw, as an abundant and renewable agricultural residue, would be a low-cost and sustainable source of energy and chemicals in the future (Ashori *et al.* 2014). Soybean is a species of annual legume native to East Asia and widely grown for its edible bean, which has numerous uses (Michel 1995). The mass ratio of soybean straw to soybean fruit is 1.6 (Liu *et al.* 2006), which means that there is a large amount of soybean straw that is grown every year throughout the world.

Currently, soybean straw is mainly used as animal feedstock or a rural energy source or is returned to the field or even arbitrarily discarded (Lucia 2008; Zhu *et al.* 2008; Terashima *et al.* 2009). However, such disposal inevitably results in resource loss and environmental pollution. Some researchers have used soybean straw to remove hazardous dyes from aqueous solutions, such as Cu^{2+} (Zhu *et al.* 2008), Black B, and Acid Orange 7 (Ashori *et al.* 2014), or to produce natural cellulose technical fibers with a structure and properties similar to those of cellulose fibers (Wang and Sain 2007; Reddy and Yang 2009). Therefore, exploiting and utilizing soybean straw in a more efficient manner will certainly provide distinct economic and social benefits.

One promising method for the structural study and efficient utilization of lignocellulose containing soybean straw is through dissolution. However, the complex structure of this substance makes it difficult to dissolve under gentle conditions. Lu and Ralph demonstrated that after extensive ball-milling, wood becomes soluble in dimethyl sulfoxide-tetrabutyl ammonium fluoride (DMSO/TBAF) and dimethyl sulfoxide-imidazole binary (DMSO/NMI) solvent systems (Lu and Ralph 2003). Moreover, both of these two systems require a rather long duration of ball-milling, which could cause structural changes in lignin and cause the degradation of cellulose. Kilpeläinen *et al*. reported that wood could be dissolved in various imidazolium-based ionic liquids (ILs) under moderate conditions (Kilpelainen *et al.* 2007). However, complete wood dissolution was achieved only at a high temperature that also caused the degradation of both cellulose and lignin (Kubo *et al.* 2008). Recently, Wang *et al.* observed that LiCl/DMSO could be used as a solvent system for the dissolution of wood ball-milled for a short period as well as EDA-pretreated pulp with high lignin content (Wang *et al.* 2009, 2010). These results have prompted further examination of the dissolution and regeneration properties of lignocellulose in this solvent system and the utilization of this homogeneous solution in analyzing the structures of certain lignocellulose fractions as well as in converting abundant biomass to valuable chemicals and novel functional composite biomaterials, such as cellulose aerogels (Wang *et al.* 2012). Therefore, in the present study, the LiCl/DMSO was applied to dissolve ball-milled and EDA-pretreated soybean straw. The dissolution and regeneration of soybean straw in the LiCl/DMSO solvent system using different pretreating methods were investigated. Such investigation is crucial for future studies on the isolation and structural characterization of soybean straw components, as well as those on converting this abundant agricultural waste to valuable chemicals and novel functional composite biomaterials.

EXPERIMENTAL

Materials

Soybean straw was collected from the North of Jiangsu, China. Air-dried soybean straw was first manually fractionated into its stem, pod and root components, then ground and passed through the 40 and 80 mesh screens with a Genetic Electric Wiley mill, and extracted with benzene-ethanol (2:1, v/v) for 8 h. These ground extractive-free fractions were vacuum-dried and stored in sealed plastic bags for future use.

Dried ground straw (8 g) was milled in a planetary ball mill (Fristch GMBH, Pulverisette 7 premium line, Idar-Oberstein, Germany) for 0.5, 1, 2, 4, and 6 h to yield milled straw of different ball-milling degrees. Two zirconium dioxide bowls (80 mL) containing 25 zirconium dioxide balls (1 cm diameter) were used in the milling procedure. The milling frequency was 600 rpm. The ball-milling was conducted at room temperature and paused every 10 min between every 5 min of milling to prevent overheating.

Methods

Chemical characterization

The ash and silica contents were analyzed according to TAPPI test methods T211 om-02 and T244cm-99, respectively. The lignin and sugar contents were determined according to NREL/TP-510-42618 (Sluiter *et al.* 2011).

Dissolution and regeneration of ball-milled soybean straw

The dissolution and regeneration scheme is shown in Fig. 1. Soybean straw (stem and pod) samples submitted to ball-milling for different durations were suspended in 8% LiCl/DMSO and stirred continuously at room temperature for 24 h (Wang *et al.* 2009). After being dissolved, the ball-milled stems or pods were regenerated with 5 volumes of distilled water, then centrifuged and washed with distilled water thoroughly until no Cl^- was detected in the supernatant. The samples were then vacuum-dried at 40 ºC for 24 h.

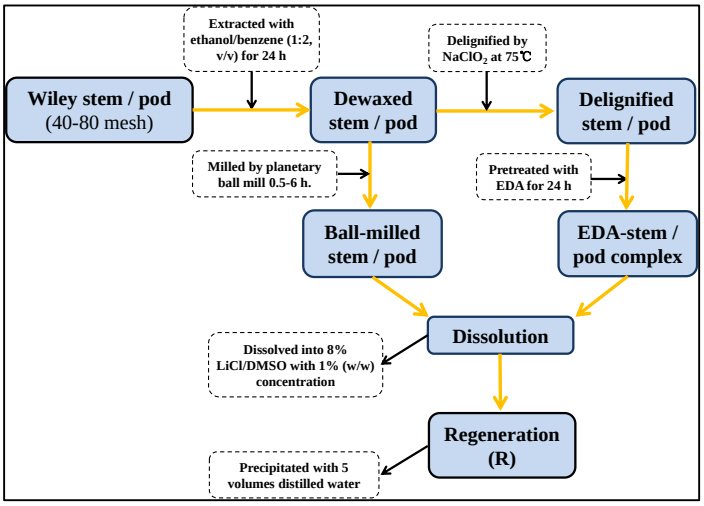

Fig. 1. Scheme for dissolution and regeneration of soybean straw

Extractable lignin yield of ball-milled soybean straw

The yield of extractable lignin from the ball-milled soybean straw was determined according to the method described by Fujimoto *et al.* (2005). The yield was calculated as the weight percentage based on the total lignin content in the corresponding ball-milled soybean straw.

Dissolution and regeneration of EDA-pretreated soybean straw

As shown in Fig. 1, the raw stems and pods were delignified by adding $NaClO_2$ and acetic acid at 75 ºC for 3 h and were then vacuum-dried. The samples (10 g) were soaked and stirred continually in 200 mL of EDA for 24 h at room temperature, then freeze-dried and referred to as "stem-EDA complex" or "pod-EDA complex".

These stem-EDA complex or pod-EDA complex samples were suspended in 8% LiCl/DMSO and stirred continuously at room temperature for 24 h. The samples were then heated to 60 ºC with stirring for 1 h, at which point a homogeneous solution was obtained, and regenerated in distilled water in the same manner in which the ball-milled stem were regenerated.

X-Ray diffraction and FT-IR analysis

The original and ball-milled soybean stems and pods were all fabricated into pellets using a disc apparatus for infrared (IR) measurement and subjected to X-ray diffraction analysis using the reflection method over diffraction angles 2θ ranging from 5º to 40º on a Rigaku RINT 2000 with a Ni-filtered Cu Kα radiation source (λ 0.15418 nm) at 40 kV and 40 mA.

FT-IR spectra of these regenerations were gathered using a Nicolet 750 spectrophotometer within the frequency range of 400 to 4000 cm^{-1} in the transmission mode. The 1% finely ground and dried regenerated samples were mixed with KBr to create pressed plates for measurement.

RESULTS AND DISCUSSION

Dissolution of Ball-milled Soybean Straw in LiCl/DMSO

Dried ground soybean straw (stem and pod) samples were finely ball-milled by planetary ball-milling for various durations to produce different degrees of milling. All of these ball-milled stems and pods were dissolved in 8% LiCl/DMSO separately. Photographs of the straw solutions or suspensions are shown in Fig. 2. The ball-milled soybean stems or pods formed hazy suspensions in 8% LiCl/DMSO when the applied ball-milling time was 0.5 h, even clouding the black line on the rear side of the board. This appearance suggested that the solubility of the ball-milled soybean straw was low when the ball-milling time was short. As the ball-milling time was prolonged to 2 h, the solubility of the stems and pods was increased remarkably. Both the stem and pod samples submitted to 4 h of ball-milling could be dissolved completely in 8% LiCl/DMSO, yielding translucent, amber-colored homogeneous solutions.

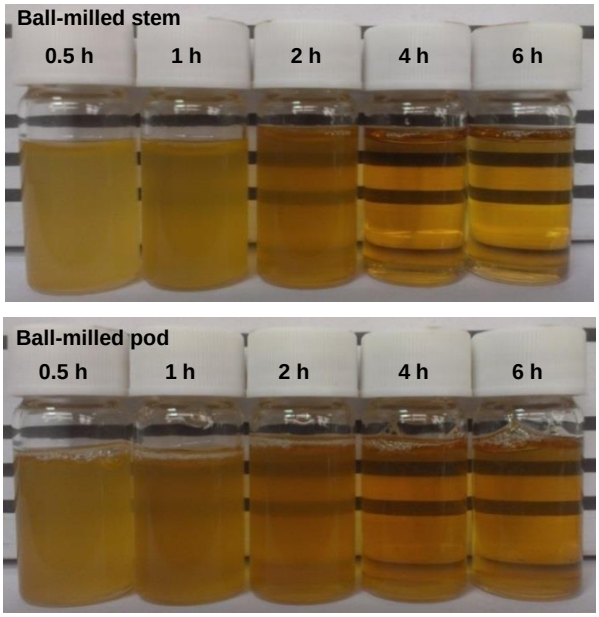

Fig. 2. Photographs of ball-milled soybean stem and pod solutions or suspensions in 8% LiCl/DMSO with a concentration of 1%.
[a] The ball-milling times were 0.5 h, 1 h, 2 h, 4 h, and 6 h.

Under the same conditions, the solubilities of the ball-milled stem or pod powders were observed to follow the order 6 h / 4 h > 2 h > 1 h > 0.5 h with respect to ball-milling time. These results demonstrate that increasing the ball-milling degree could gradually promote the dissolution of lignocellulose by reducing the polymerization degree of the samples. Under the applied ball-milling conditions, 4 h was the critical ball-milling time for the stem and pod materials examined. It was observed that changes in the particle size

and components of lignocellulose could effectively alter the dissolution rate. The complex and compact structure of the lignocellulose cell wall, in which lignin fills the spaces between the cellulose and hemicellulose components, essentially inhibits the diffusion of the solvent into its interior, resulting in only partial dissolution (Kilpelainen *et al.* 2007).

Effect of Ball-milling on The Characteristics of Soybean Straw

Extractable lignin yield of ball-milled soybean straw

When the ball-milling conditions, apparatus, and intensities, *etc.* vary between experiments, milling time cannot be used as the sole criterion of ball-milling degree to compare the effects of milling on lignin structures. However, the yield of extractable lignin from ball-milled lignocellulose can be considered a general criterion of milling degree. Fujimoto *et al*. (2005) showed that when the yields of extractable lignin from ball-milled woods are the same, the structural changes in lignin caused by ball-milling are similar regardless of the differences in ball-milling conditions and apparatus. In the present study, the extractable lignin yield increased with the prolonging of the ball-milling time, as shown in Fig. 3. When the ball-milling time was 4 h, both the stems and pods could dissolve in 8% LiCl/DMSO, and the extractable lignin yields of the materials were 11.4% and 14.3%, respectively. These yields were much lower than those obtained for beech, which could be dissolved completely in LiCl/DMSO, after 2 h of ball-milling (Fujimoto *et al.* 2005; Wang *et al.* 2009). As a result, it can be concluded that the change in the degree of the aromatic part of lignin in the stems and pods was negligible after 4 h of ball-milling.

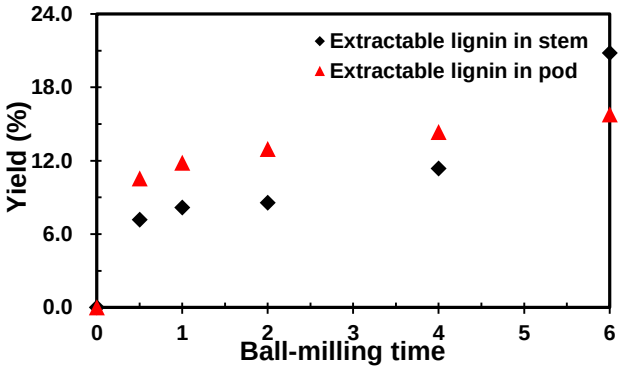

Fig. 3. Extractable lignin yields of soybean stems and pods treated for different ball-milling times

X-ray diffraction pattern of ball-milled soybean straw

The highly crystalline character of cellulose in lignocellulose is attributed to a set of regular intermolecular and intramolecular hydrogen-bonding interactions that, when coupled with the three-dimensional network character of lignin and its potential covalent linkages with carbohydrates, are primarily responsible for the complex and compact structure of wood (Kilpelainen *et al.* 2007). In this study, untreated and ball-milled stems and pods were all converted into pellets and subjected to X-ray diffraction analysis to determine their crystal structure, with the results shown in Fig. 4. The crystal region of cellulose disappeared, even when the ball-milling time was as short as 0.5 h, indicating that the ball-milling process severely affects the crystal structure of cellulose. The destruction of the crystalline region should be one of the decisive factors affecting the dissolution of ball-milled straw in the LiCl/DMSO solvent system (Wang *et al.* 2009). As a result, both the stems and pods submitted to 4 h or 6 h of ball-milling could be completely dissolved in 8% LiCl/DMSO to form homogeneous solutions.

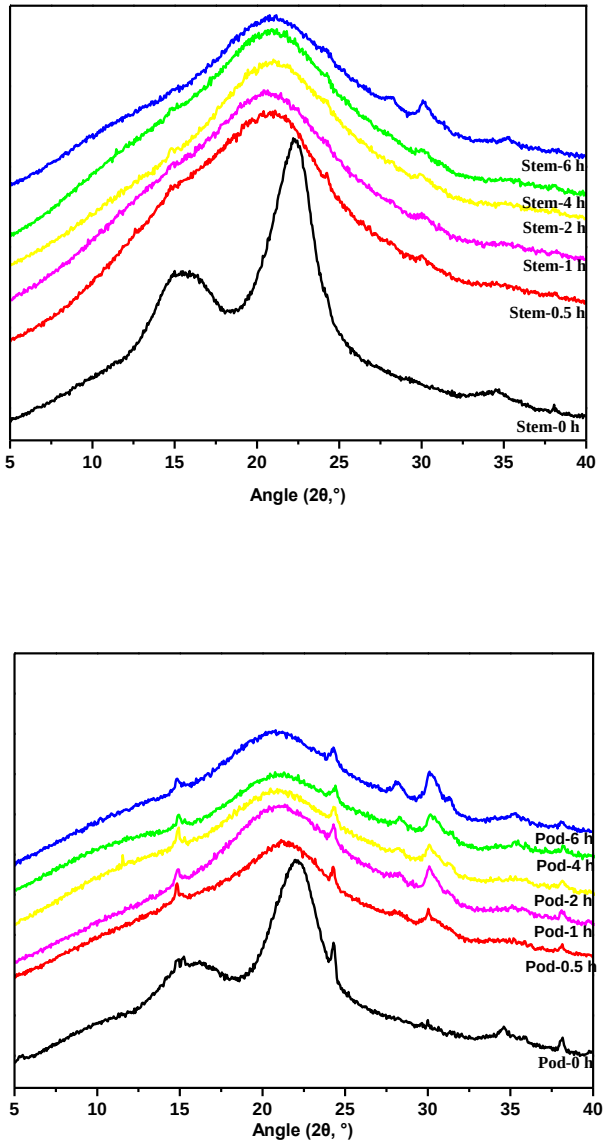

Fig. 4. X-ray diffraction patterns of Wiley and ball-milled soybean stems and pods prepared over different ball-milling times. (The individual diffractograms were shifted on the y-scale to display all data. The y-axis represents intensity).

Regeneration of Dissolved Soybean Stem

After being dissolving in 8% LiCl/DMSO, the lignocellulose could be recovered by simply pouring the solution into an excess of a nonsolvent, such as water. As a result, the dissolved stems were regenerated from these suspensions or solutions with 5 volumes of distilled water under continuous stirring, according to the route shown in Fig. 1. The chemical properties of these various regenerated fractions were also investigated and are listed in Table 1.

The yields of these regenerated fractions clearly decreased with the increase in ball-milling time. The yield of regenerated stems submitted to 0.5 h of ball-milling (R-0.5) was the highest, reaching up to 90.1%. The total lignin content of R-0.5 amounted to 24.8%, which was slightly greater than the lignin contents of R-1 and R-2. Although the yield of

R-4 was only 75.5%, the lowest among the various fractions, the lignin content of R-4 was the highest. R-4, which was regenerated from stems submitted to 4 h of ball-milling, could be dissolved completely in 8% LiCl/DMSO to form a homogeneous and clear solution. All losses of the regenerated fraction during the dissolution-regeneration procedure were caused by the ball-milling step, which could lead to the degradation of both cellulose and lignin and thus the formation of water-soluble products that cannot be precipitated readily with water (Guerra *et al.* 2006; Li *et al.* 2010). However, it could be concluded that although approximately 10.0% to 25.0% of the fractions were lost during the dissolution-regeneration procedure, most of the components could be regenerated from the suspensions or solutions, which could reflect nearly all of the same characteristics of the original stem cell wall components.

Table 1. Characteristics of the Regenerated Fractions (R)

Sample	Total yield (%)	Lignin (%)			Sugar (%)				Ash (%)	Silica (%)
		KL[a]	ASL[a]	Total	Glucan	Xylan	Other sugars[b]	Total		
Raw stem	---	22.5	1.6	24.1	43.6	14.8	10.7	69.1	2.6	0.8
R-0.5[c]	90.1	23.3	1.4	24.8	45.1	15.5	8.7	69.2	0.9	0.3
R-1[c]	86.3	22.7	1.4	24.1	46.3	14.9	8.3	70.5	0.9	0.3
R-2[c]	82.0	22.4	1.4	23.8	42.3	12.2	7.0	61.4	1.0	0.5
R-4[c]	75.5	24.2	1.5	25.6	44.1	9.7	7.0	60.9	1.2	0.8

[a] KL means klason lignin; ASL means acid-soluble lignin.

[b] Other sugars is the sum of rhamnosan, galactan, mannan, and araban contents

[c] Regenerated fractions of the dissolved stems submitted to 0.5 h, 1 h, 2 h, and 4 h of ball-milling pretreatment.

Although the extension of the ball-milling time benefits the extent of dissolution by reducing the polymerization degree of samples, it also produces many low-molecular-weight materials that are easily dissolved in LiCl/DMSO but difficult to regenerate. Compared with the contents of each component in the raw stems, the retention of lignin and all types of sugars in the regenerated fractions all decreased, whereas the retention of ash and silica increased gradually with the extension of the ball-milling time. As shown in Fig. 5, when stems were ball-milled for 0.5 h, the fractions of total lignin, glucan, xylan, and other sugars retained after the dissolution-regeneration procedure were 92.7%, 93.1%, 94.2%, and 73.2%, respectively. The fractions of silica and ash retained were 30.8% and 30.4%, respectively. As the ball-milling time was gradually increased to 2 h, the percentages of these fractions that were retained all decreased significantly, except for those of silica and ash, which increased to 49.6% and 30.7%, respectively. The greatest loss of these fractions occurred between 1 h and 2 h of ball-milling. The retention of xylan decreased from 87.0% (1 h) to 67.6% (2 h), representing a 22.4% reduction rate, which ranked first among the rates observed for all fractions. The second highest reduction rate was that observed for sugars, 20.3%, whose retention decreased from 66.6% (1 h) to 53.1% (2 h). The next highest reduction rate was that of glucan, 13.3%, whose retention decreased from 91.7% (1 h) to 79.5% (2 h). Finally, the minimum reduction rate was that observed for total lignin, 6.2%. The results suggest that lignin is more difficult to lose than carbohydrates during the dissolution-regeneration procedure. However, ash and silica

showed good retention: the retention of silica increased from 30.8% (0.5 h) to 32.9% (1 h) then up to 49.6% (2 h), indicating a 50.6% increase, and the retention of ash increased to 30.7%, despite a slight reduction between 0.5 h and 1 h. This reduction was observed because during the regeneration procedure, the H_2O-DMSO-LiCl neutral solvent system cannot dissolve the ash fraction, which is mainly composed of a large amount of water-insoluble inorganic compounds. When the ball-milling time was further prolonged to 4 h, the fractions of the components retained were 80.4% (total lignin), 76.4% (glucan), 74.5% (silica), 49.6% (xylan), 49.6% (other sugars), and 35.2% (ash). The retention of total lignin and that of total sugar were much greater than the retention of softwood dissolved and then regenerated from an ionic liquid, which demonstrated the retention of 59.0% total lignin and 69.0% carbohydrates (Sun *et al.* 2009).

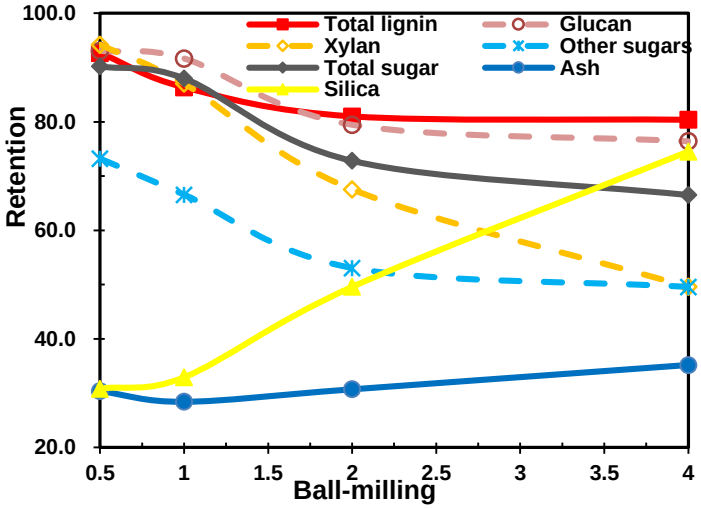

Fig. 5. The retention of each component of the regenerated fraction after the dissolution-regeneration procedure

As a result, during the dissolution-regeneration process, carbohydrates are better retained at short ball-milling times (0.5 h or 1 h), whereas a greater amount of lignin than carbohydrates is retained at longer ball-milling times (2 h or 4 h). This behavior is observed because lignin is a cross-linked polymer, which makes it to more difficult to fragment than cellulose and hemicellulose *via* ball-milling (Assor *et al.* 2013; Ray *et al.* 2014). This characteristic also makes it highly beneficial for the lignin in regenerated soybean stems to be isolated and characterized, which will be studied in future work.

FT-IR of the Regenerated Fractions

Fourier transform infrared (FT-IR) spectroscopy was used to detect the functional groups present in the regenerated biomass. As shown in Fig. 6, the peaks that appeared at 3434 cm^{-1} and 1051 cm^{-1} correspond to O-H stretching and O-H bending frequencies, and the -CH stretching frequencies of CH_2 and CH_3 groups in this case appeared at 2920 cm^{-1} and 2850 cm^{-1}, respectively. The peaks at 1735 cm^{-1} and 1631 cm^{-1} represent carbonyl (C=O) stretching vibrations for acetyl and uronic ester groups in the hemicelluloses and for the aldehydic group present in lignin (Abdul Khalil *et al.* 2010; Peng *et al.* 2012; Zhang *et al.* 2014). The absorption peak at 890 cm^{-1} is associated with the antisymmetric out-of-plane ring stretching of amorphous cellulose (Michell 1990), as well as the presence of β-glycosidic linkages between the sugar units in all of the hemicellulose subfractions. The

aromatic skeleton vibration of the lignin fractions appeared at 1508 cm^{-1} and 1419 cm^{-1}. The absorption peak at 1457 cm^{-1} indicates methoxyl C-H deformation and aromatic ring vibration. The bands at 1338 cm^{-1}, 1245 cm^{-1}, and 1110 cm^{-1} and shoulder at 1160 cm^{-1} indicate a typical lignin structure featuring phydroxy phenylpropane (H), guaiacyl (G), and syringyl (S) units (Faix 1991; Fengel and Shao 1985; Shi *et al.* 2013; Wen *et al.* 2010). It is worth noting that the band at 1383 cm^{-1} represents the nonetherified phenolic OH groups in lignin, which result from the cleavage of β-O-4 and α-O-4 linkages. Additionally, the peaks for the regenerated samples shifted to higher frequencies as the ball-milling time was increased. The blue shift could be due to the different contents of functional groups in the regenerated materials in the form of cellulose, hemicellulose, and lignin caused by damage sustained during milling.

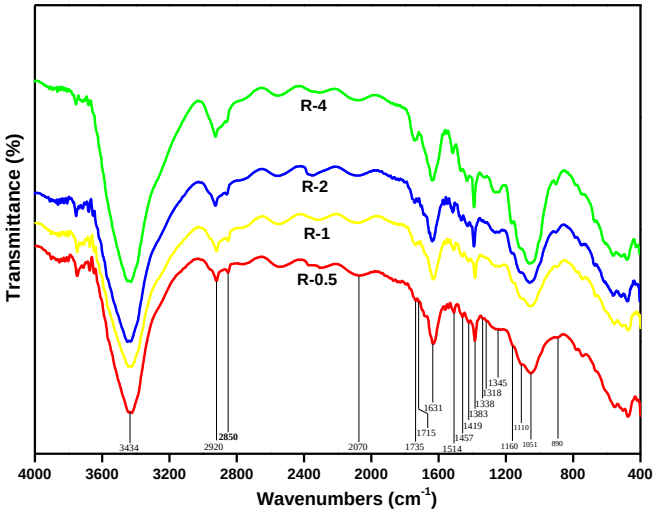

Fig. 6. FT-IR spectra of the regenerated fractions

Dissolution and Regeneration of EDA-pretreated Soybean Straw

EDA treatment was developed as a pretreatment method for dissolving pulp with high lignin content in LiCl/DMSO in our previous study (Wang *et al.* 2010). Because this method does not require ball-milling pretreatment, the structural modification of lignin and the serious degradation of cellulose can be avoided. For comparison, EDA-pretreated soybean straw was also completely dissolved in 8% LiCl/DMSO to form a homogeneous solution containing 1.0% straw after 24 h of continuous stirring. The dissolution-regeneration performance of the soybean straw submitted to the EDA pretreatment was quite different from that of unpretreated straw due to the lack of severe ball-milling.

The raw stems and pods were delignified by $NaClO_2$ and acetic acid at 75 ºC for 3 h. The lignin contents of these materials are shown in Table 2. The raw and delignified ground stems and pods were treated with EDA for 24 h at room temperature to form stem-EDA complex or pod-EDA complex. After being dried, the materials were dissolved in 8% LiCl/DMSO to yield solutions containing 1% of each complex. As shown in Fig. 7, both the stem-EDA complex and pod-EDA complex could be dissolved in 8% LiCl/DMSO to form homogeneous solutions. Due to the presence of lignin, the solutions of stem-EDA complex and pod-EDA complex without delignification were not as clear or as bright as the delignified samples. However, no solid particles were visible to the naked eye.

Additionally, because the EDA pretreatment did not involve ball-milling, which could reduce the polymerization degree of the carbohydrates in the treated materials, the viscosities of the stem and pod solutions were much higher than those of the ball-milled stem and pod solutions. Such homogeneous solutions should be benefit for the chemical modification of lignocellulose to allow for the preparation of different types of chemicals or novel functional biomaterials with special properties.

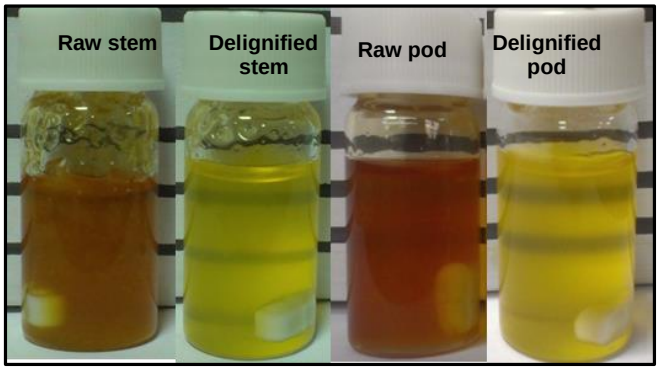

Fig. 7. Photographs of EDA-pretreated soybean stem and pod solutions or suspensions in 8% LiCl/DMSO with a concentration of 1%.

Table 2. Lignin Contents of the Raw and Delignified Soybean Straw

Fractions	KL[a] (%)	ASL[a] (%)	Total lignin (%)
Raw stem	22.5	1.6	24.1
Delignified stem	4.6	6.3	10.9
Raw pod	14.0	2.3	16.3
Delignified pod	3.2	4.3	7.4
[a] KL means klason lignin; ASL means acid-soluble lignin.			

The method applied to regenerate the EDA-pretreated soybean straw was the same as that used to the regenerate the ball-milled straw. Approximately 61.4% of stem-EDA complex could be recovered as a regenerated fraction (R-EDA), as shown in Table 3. The R-EDA fraction was composed of 24.3% lignin, 62.1% carbohydrates, 0.6% ash and 0.04% silica. For this fraction, the retention of total lignin was 62.0%, specifically acid-soluble lignin, which could reach a retention of up to 83.2%. The retention rates of glucan, xylan, and other sugars were 62.7%, 51.6%, and 40.3%, respectively, similar to those observed the ball-milled stems. The regeneration performance of lignin and that of cellulose were better than the regeneration performance of hemicellulose, which has the lowest molecular weight among the three major components of the plant cell wall. In addition, it was interesting that the retention of each component in the R-EDA fraction was much lower than that of the regenerated ball-milled stems. Therefore, considering the excellent dissolution-regeneration performance of the pretreated materials, one may envisage that the methodology described herein could be used in future studies on the chemical conversion of large amounts of lignocellulose to valuable chemicals and novel functional composite biomaterials, as well as in studies on the isolation and characterization of the components of regenerated soybean stems, which will be published in due course.

Table 3. Characteristics of the Regeneration Submitted to EDA Pretreatment (R-EDA)[a]

Fraction	Lignin (%)			Sugar (%)				Ash (%)	Silica (%)
	KL[b]	ASL[b]	Total	Glucan	Xylan	Other sugars[c]	Total		
Content [d]	22.1	2.2	24.3	44.5	12.4	5.1	62.1	0.6	0.04
Retention [e]	60.5	83.2	62.0	62.7	51.6	40.3	55.1	13.3	3.23
[a] The yield of R-EDA was 61.4%.									
[b] KL means klason lignin; ASL means acid-soluble lignin.									
[c] Other sugars is the sum of rhamnosan, galactan, mannan and araban contents.									
[d] The content of each fraction was calculated based on R-EDA.									
[e] The retention of each fraction in R-EDA was calculated based on the content in the raw stems.									

CONCLUSIONS

1. Both soybean stems and pods could be dissolved completely in an 8% LiCl/DMSO solvent system after 4 h of ball-milling.
2. Both the solubility and the extractable lignin yield of soybean straw were greatly improved by the ball-milling pretreatment. The destruction of cellulose's crystalline regions in the milled stems and pods was significant even at a ball-milling time of 0.5 h.
3. After dissolution, most of the stems could be regenerated by being poured into excess water. Greater amounts of carbohydrates in the stems were retained at short ball-milling times, whereas the amount of lignin retained was greater than that of carbohydrates retained at long milling times.
4. Moreover, in this preliminary study, EDA-pretreated stems and pods with different lignin contents could also be dissolved to form homogeneous solutions.

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