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# Effects of LiCl/DMSO dissolution and enzymatic hydrolysis on the chemical composition and lignin structure of rice straw

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ABSTRACT

The exploration of solvent system to completely dissolve lignocellulosic biomass is of great important for the development of biomass based materials and chemicals. Dimethyl sulfoxide containing certain amount of lithium chloride (LiCl/DMSO) was proved an effective solvent system for the dissolution of ball-milled wood. In this paper, four samples of rice straw, internode (stem without node), stem, leaf (leaf and sheath) and whole straw, with and without 1 h of ball milling treatment, were used to investigate the effects of their dissolution in LiCl/DMSO solvent on the chemical composition of regenerated and enzymatically hydrolyzed materials. Because of the structural difference from wood, samples expose d to 1 h of milling were almost completely dissolved. The chemical nature of the regenerated material and of residual after enzymatic hydrolysis of rice straw was analyzed and the susceptibility of this rice straw to the enzymatic hydrolysis was evaluated.



## 1. Introduction

The main components of lignocellulose are lignin, cellulose, and hemicellulose, in which the lignin, derived primarily from *p*-hydroxycinnamyl alcohols, consists of an extensive three-dimensional network which results from radical-coupling polymerization reactions after the polysaccharides have been accumulated [1,2]. The nature of lignin is rather different from that of polysaccharides, i.e., cellulose and hemicellulose in lignocellulose. Lignin binds with the carbohydrates to form a tight compact structure. Consequently, molecular associations and covalent bonds are possibly produced between lignin and carbohydrates [3,4]. Therefore, it is nearly impossible to dissolve lignocellulose in conventional solvents in its native state. In recent years, the dissolution of lignocellulosic biomass in ionic liquids has received a lot of attention since it was first reported by Swatloski et al. [5]. Although dissolving wood is challenging, recent discoveries have demonstrated that suitable media for the dissolution of this lignocellulosic materials are available. It has been reported that pretreatment by dissolution in the ionic

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liquid improved the efficiency of enzymatic hydrolysis of fiber materials [6,7]. For example, more glucose was released from the regenerated materials, than from the starting natural product, during the enzymatic hydrolysis [8,9].

Complete dissolution of wood using an appropriate solvent offers a good opportunity for lignin separation. Recently, a new and facile method for lignin separation from wood, based on complete dissolution, was established by Fasching et al. [1]. They found that the structure of lignin separated by their method was quite similar to the milled wood lignin obtained by the classical Björkman method. Dimethyl sulfoxide and lithium chloride (LiCl/DMSO) solvent system, was reported to be a good solvent for both softwood and hardwood, by Wang et al. [10]. These authors found that wood samples milled for 2 h using a planetary ball mill could be completely dissolved in this system, and the concentration of ball-milled woods could be as high as 10%. Furthermore, lignin structure did not change significantly by milling.

Rice straw offers great potential as a raw material for biorefinery. According to FAO (Food and Agriculture Organization of the United Nations) statistics, the production of rice straw is about 650–975 million tonnes per year globally, calculated according to the ratio of straw to grain [11]. China is the largest agricultural country in the world, and until now, the rice straw has not been used reasonably or just burned in field resulting air pollution and greenhouse gas emission. Efficient utilization of rice straw resource is the best choice for both sides of providing feedstock for biorefinery and releasing risk of environmental pollution [12]. In this paper, different parts of rice straw, with or without ball milling, were dissolved in LiCl/DMSO, then regenerated in water and hydrolyzed by the mixture of cellulase and β-glucosidase. The effects of these processes on the chemical composition and lignin structure were investigated.

## 2. Materials and methods

### 2.1. Materials

Rice straw (*Oryza sativa* L. cv. Kinuhikari) was collected from Oi-machi, Kanagawa, Japan in October, 2010. Air-dried rice straw was stored in a refrigerator at 4 °C before use. The straw was separated in 4 parts to be the starting materials. These 4 parts were internode (stem without node, S-in), stem (S-st), leaf (sheath included, S-ls) and whole straw (S-ws). The weight proportion of leaf to whole straw was around 70%. All samples were milled with a Wiley mill and the fractions retained between 40 and 80 mesh sieves were collected. The straw meals were extracted with a mixture of benzene-ethanol (2:1, v/v) for 8 h to remove solvent extractives. The extractives-free straw meal was dried under air and subsequently under vacuum for further treatment. The main chemical components of the four samples are listed in Table 1.

All chemicals were purchased from Wako Chemicals (Takasaki, Japan) and used as received without further purification.

### 2.2. Ball milling

The dried extractives-free rice straw meal (2 g per bowl) was milled in a planetary ball mill (Fritsch GMBH, Idar-Oberstein, Germany) to give milled samples with different milling hours (0.5 h–4 h). Two zirconium dioxide bowls (45 ml for each) with 18 zirconium dioxide balls (1 cm diameter) in each bowl were used in the milling with a frequency of 10 Hz. The milling was conducted in a cold room (−20 °C), and 5 min intervals were provided between every 15 min of milling to prevent overheating. The ball-milled straw samples were dried under vacuum for further treatment.

**Table 1 – The mass fractions of the components of extractives-free straw materials. Data are the mean of two measurements.**

| | RS-in | RS-st | RS-ls | RS-ws |
|---|---|---|---|---|
| Lignin (%) | | | | |
| KL | 10.9 ± 0.2 | 11.0 ± 0.2 | 11.9 ± 0.3 | 12.3 ± 0.2 |
| ASL | 3.6 ± 0.0 | 3.5 ± 0.0 | 4.8 ± 0.1 | 3.7 ± 0.0 |
| Total | 14.5 ± 0.2 | 14.5 ± 0.1 | 16.7 ± 0.3 | 16.0 ± 0.2 |
| Sugar (%) | | | | |
| Glucan | 42.4 ± 0.1 | 40.4 ± 0.3 | 34.5 ± 0.3 | 35.6 ± 0.1 |
| Xylan | 15.2 ± 0.1 | 16.7 ± 0.2 | 14.8 ± 0.3 | 16.3 ± 0.1 |
| Arabinan | 1.8 ± 0.0 | 2.2 ± 0.0 | 2.8 ± 0.1 | 2.8 ± 0.0 |
| Others | 1.7 ± 0.0 | 1.8 ± 0.0 | 2.1 ± 0.1 | 1.9 ± 0.1 |
| Total | 61.1 ± 0.2 | 61.1 ± 0.4 | 54.2 ± 0.5 | 56.5 ± 0.3 |
| Ash (%) | 16.4 ± 0.1 | 16.7 ± 0.2 | 21.0 ± 0.5 | 18.9 ± 0.3 |
| Sum (%) | 92.0 ± 0.7 | 92.2 ± 0.5 | 92.0 ± 1.2 | 91.4 ± 0.8 |

### 2.3. LiCl/DMSO dissolution and regeneration

A DMSO solution with 8% mass fraction of LiCl was used for dissolving straw samples. Four straw samples, with or without ball milling, were suspended into the solvent system of 8% LiCl/DMSO at a mass concentration of 7.5%, and stirred at room temperature for 48 h, and then at 60 °C for 24 h. At the end of these procedures, all rice straw samples with 1 h ball milling were well dissolved with no significant solid residues while rice straw without ball milling was not completely dissolved in 8% LiCl/DMSO solvent system, but it became well swollen. The LiCl/DMSO treated materials were regenerated in deionized water using a regenerated cellulose dialysis tube (UC 36-32-100, EIDIA Co., Ltd., Tokyo, Japan) till no $Cl^-$ in dialysate was detected. The regenerated materials were freeze-dried for enzymatic hydrolysis. The scheme of LiCl/DMSO dissolution and regeneration for rice straw is illustrated in Fig. 1.

### 2.4. Enzymatic hydrolysis of regenerated rice straw samples

The vacuum dried regenerated samples (RS and RMS in Fig. 1) were hydrolyzed by a mixture of commercial cellulase Celluclast 1.5 L and β-glucosidase Novozyme 188 (Novozymes, Demark) at 37 °C in sodium acetate buffer (pH 4.8) for 72 h. The activity loading was based on a cellulase charge 60 Filter Paper Unit (FPU) per each g of substrate. Cellulase activity was determined by the method of Ghose [13] using the filter paper Whatman #1 as the substrate. Excess β-glucosidase was used to prevent cellobiose accumulation [14]. Enzymatic hydrolysis residue and hydrolysate were separated by centrifugation. The residues were three times washed by acetate buffer and three times by deionized water to remove the protein and other dissolved substances. Frozen and vacuum dried