Coupled Pretreatment with Liquid Nitrogen and Ball Milling for Enhanced Cellulose Hydrolysis in Water

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ABSTRACT: A key problem in the conversion of cellulose into chemicals and fuels is the low product yield from cellulose due to its robust structure. In this work, for the first time, cellulose was pretreated with coupling of liquid nitrogen and ball milling (LN−BM) for cellulose hydrolysis. After the LN−BM treatment, the glucose yield from cellulose by HCl in water increased by almost 2 times and yield of formic acid catalyzed by H$_2$SO$_4$−NaVO$_3$ was more than 3-fold that obtained from untreated cellulose. The yields were also much higher than that from the individually ball-milled cellulose. The structure variation of cellulose indicated that reduction of both crystallinity index and molecular weight contributed to improving the conversion efficiency, but the former was the dominant factor. The combination of liquid nitrogen and ball milling developed in this work is an effective and environment-friendly approach for cellulose pretreatment.

INTRODUCTION

Cellulose is the most abundant and renewable biomass resource in nature, with an estimated production of 1.5 × 10$^{12}$ tons per year. Cellulose is composed of glucose units, which can be converted to a variety of high-value-added chemicals and fuels. Thus, lignocellulosic biomass has been regarded as one of the most promising alternative to fossil resources. Unfortunately, the conversion efficiency of native cellulose is usually very poor. This is because cellulose is a polymer consisting of hundreds to thousands of β(1→4) linked D-glucose units. The glucose chains are linked by a network of inter- and intramolecular hydrogen bonds, endowing cellulose with a robust crystal structure with high stability. Cellulose is insoluble in water and most of the organic solvents. Therefore, cellulose is very difficult to be hydrolyzed especially in water. During the past years, scientists all over the world have concentrated on the technologies of improving the conversion efficiency of cellulose.

Pretreatment is one of the most effective ways to relieve the recalcitrance of cellulose, thereby enhancing its utilization efficiency. Chemicals such as acid and base have been used to pretreat cellulose. However, these chemicals are quite corrosive and they need to be neutralized and removed after pretreatment. Explosion process such as steam explosion and carbon dioxide explosion is another widely used technique to pretreat cellulose. However, the explosion process is usually conducted under harsh conditions (high temperature/high pressure), followed by the sudden reduction of the pressure leading to the mechanical destruction of cellulose. Thus, the process needs high energy and high temperaturepressure, which will cause some of the formed sugars to decompose during the pretreatment. Biological treatment of cellulose is a promising method with several advantages such as mild operation condition and no waste production. Unfortunately, biological treatments suffer from problems such as high cost and long treatment time. Ionic liquid is a novel solvent that can dissolve cellulose by destroying the hydrogen bonds in cellulose. Therefore, ionic liquid has been used for the pretreatment of cellulose under mild conditions and showed excellent performance. However, most ionic liquids have side effects on enzymatic hydrolysis process and their cost is still very high.

Recent studies have indicated that ball milling (BM) is a powerful physical technique to change the structure of biomass. With the mechanical milling treatment, the crystallinity of cellulose could be reduced, which greatly improved the accessibility of catalysts to the reactive sites of cellulose. Unlike most of the other cellulose treatment methods, no chemicals are needed in the ball-milling process. The ball-milling-pretreated cellulose can be directly used for the following conversion reactions. To further improve the efficiency of ball milling, in this work, coupling application of liquid nitrogen (LN) and ball milling was developed for the pretreatment of cellulose. Liquid nitrogen was widely used for the frozen treatment of biological samples such as plant tissue to extract intracellular compounds. Besides, liquid nitrogen has been applied in the mechanical production of cellulose microfibers. Castoldi et al. reported that liquid nitrogen pretreatment was an effective way to improve the enzymatic digestibility of plant biomass by decreasing lignin content.
The reduction of CI could be the main factor in determining the conversion of cellulose. Hydrolysis of cellulose to glucose or oligosaccharides is the first and also the most difficult step for cellulose utilization. Herein, the LN–BM pretreatment of cellulose on its hydrolysis to glucose in water was investigated. Figure 2 shows that ball-milling time had great influence on the hydrolysis efficiency of cellulose. The yield of glucose increased rapidly when the ball-milling time increased from 0 to 2 h. The maximum yield of glucose from LN–BM-2 was 41.4%, which was slightly lower than that from LN–BM-3 (42.2%). From the viewpoint of energy consumption, the LN–BM treatment of cellulose for ball-milling time of 2 h is appropriate, and the sample of LN–BM-2 was used in the following studies.

To prove the function of liquid nitrogen on the ball-milling pretreatment of cellulose, untreated cellulose and cellulose treated by sole ball milling were used as control groups to produce glucose. The results of comparison are shown in Figure 3. The maximum yield of glucose from untreated cellulose was only 24.4%. After single BM treatment, the yield of glucose from cellulose was significantly increased, and a maximum glucose yield of 34.4% was obtained in 2 h reaction time. With the assistant of liquid nitrogen, the maximum yield of glucose from LN–BM was further improved to 41.4%.

Table 1. CI and M_w of Cellulose Pretreated with Different Methods

|          | BM    | LN–BM |
|----------|-------|-------|
| no treatment |       |       |
| CI (%)   | 63.2  | 52.4  |
| M_w     | 41 845 | 39 482|

(Mean ± 5% for samples)
Moreover, the reaction time to reach the maximum yield was also shortened to 1.5 h. Therefore, liquid nitrogen freezing treatment of cellulose before ball milling is an effective way to improve the pretreatment efficiency and thus enhance the cellulose hydrolysis performance.

Conversion of Untreated and Pretreated Cellulose to Formic Acid. The LN−BM-pretreated cellulose was also applied in the production of formic acid in water. Formic acid is a commodity chemical that can be applied in many fields such as organic synthesis and energy storage. Generally, the conversion of cellulose to formic acid involves two steps: hydrolysis of cellulose to monosaccharide and the subsequent oxidation of the monosaccharide to formic acid.28 Herein, \( \text{H}_2\text{SO}_4−\text{NaVO}_3 \) catalytic system was used to produce formic acid. They act as acid catalyst and oxidation catalyst, respectively. As shown in Figure 4, the yield of formic acid from untreated cellulose was low and only 10.1% of formic acid was formed within 2 h reaction time. However, the maximum yield of formic acid increased significantly to 36.0% after LN−BM pretreatment. This value was also much higher than that obtained from the single BM-treated cellulose (26.4%). Figure 4 also shows that the reaction time to reach maximum formic acid yield was shortened by the LN−BM treatment. The above results proved that LN−BM is an effective way to improve the cellulose conversion efficiency not only for the hydrolysis of cellulose but also for the multistep process of cellulose utilization.

## CONCLUSIONS

This study demonstrated that liquid-nitrogen-assisted ball milling (LN−BM) is an effective way to pretreat cellulose for increasing its conversion to value-added chemicals such as glucose and formic acid. Compared with cellulose treated with traditional single ball-milling treatment (BM), LN−BM-pretreated cellulose was more easily converted. This can be attributed to the fact that crystallinity and molecular weight of cellulose could be reduced more readily with the assistance of liquid nitrogen. The developed LN−BM process is an effective and green way for cellulose pretreatment that has advantages such as no corrosion and no need of chemicals and separation step.

## MATERIALS AND METHODS

**Materials.** Microcrystalline cellulose was purchased from Yuanye Bio-Technique Co., Ltd. (Shanghai, China). Glucose, formic acid, and sodium metavanadate (NaVO3) were obtained from Aladdin Biochemical Technology Co., Ltd (Shanghai, China). Hydrochloric acid (HCl) and sulfuric acid (H2SO4) were provided by Sinopharm Chemical Reagent Co., Ltd (Beijing, China).

**Pretreatment of Cellulose.** Microcrystalline cellulose was pretreated by liquid-nitrogen-assisted ball milling (LN−BM). Typically, 2 g of cellulose was added to a stainless steel pot (50 mL) along with 15 zirconium oxide balls (diameter of 8.50 mm). Then, 30 mL of liquid nitrogen was poured into the pot. After the evaporation of liquid nitrogen, the pot was immediately placed in a planetary ball miller (Retsch PM100, Germany). The speed was set as 300 rpm. The freezing cellulose was ball-milled for 1, 2, and 3 h. The obtained cellulose samples were denoted as LN−BM-1, LN−BM-2, and LN−BM-3, respectively. For comparison, cellulose was pretreated with sole ball milling (BM), and the process was the same as that for LN−BM but without the addition of liquid nitrogen.

**Characterization of Cellulose Samples.** The structures of cellulose before and after treatments were characterized with X-ray diffraction (XRD, D8 ADVANCE, Germany) and gel permeation chromatography (GPC, Waters 1525). XRD analysis was conducted on the X-ray diffraction equipment. The crystallinity indexes (CI) of all the samples were calculated according to formula 1

\[
\text{CI} (%) = \frac{I_{\text{crystalline}} - I_{\text{amorphous}}}{I_{\text{crystalline}}} \times 100\%
\]

where \( I_{\text{crystalline}} \) is the intensity of the crystalline peak (\( \sim 22^\circ \)) and \( I_{\text{amorphous}} \) is the intensity of the amorphous peak (\( \sim 17.5^\circ \)).

GPC was performed on Waters 1525 chromatography system equipped with a refractive index (RI) detector (Waters 2414). The column was Agilent PLGEL 5 μm MIXED-C. The mobile phase was dimethylformamide with a flow rate of 1 mL/min. The injection volume was 10 μL. Polystyrene was used as the standard.

**Hydrolysis of Pretreated Cellulose to Glucose and Formic Acid.** Hydrolysis of the pretreated cellulose to glucose was performed in a 30 mL stainless steel autoclave. Typically, 0.05 g of pretreated cellulose with 5 mL of dilute HCl aqueous solution (0.025 M) was added into the steel autoclave. The reaction was conducted in the closed autoclave at 180 °C for different times (0.5−3 h). After the reaction, the mixture was filtered and the glucose concentration in the filtrate was quantified by high-performance liquid chromatography (HPLC). Conversion of the pretreated cellulose to formic acid was carried out in a 30 mL stainless steel autoclave with gas inlet and outlet. Typically, 100 mg of cellulose sample and 6 mL of NaVO3−H2SO4 aqueous solution (NaVO3: 0.35 wt %, H2SO4: 2 wt %) was added into the autoclave, which was sealed and purged with O2 for three times. After the introduction of O2 at a pressure of 3 MPa, the reaction was conducted at 180 °C for a given time (0.5−2 h). After the reaction, the mixture was filtered and liquid products such as formic acid were quantified by high-performance liquid chromatography (HPLC). Both formic acid and glucose were quantified by HPLC with the same conditions as follows. HPLC analyses were performed with Waters ACQUITY UPLC H-CLASS liquid chromatography system equipped with an RI detector. The column was SHODEX SH1011. A mobile phase of 5 mM H2SO4 was used at a flow rate of 0.5 mL/min. The
temperatures of column and RI detectors were 50 and 35 °C, respectively. The injection volume was 10 μL. All of the experiments were conducted in triplicate, and the provided data are the mean values of the repeated experiments that had relative deviations below 3%.

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**Notes**
The authors declare no competing financial interest.

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