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Combination of ultrasonic irradiation with ionic liquid pretreatment for enzymatic hydrolysis of rice straw

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HIGHLIGHTS

- Ultrasound promotes the pretreatment and enzymatic hydrolysis of rice straw.
- Ionic liquids were used to pretreat rice straw under ultrasonic irradiation.
- Choline hydroxide pretreatment with ultrasound can erode the structure of rice straw.
- High total reducing sugar yield in enzymatic hydrolysis was obtained with ultrasound.
- The efficient process for treating rice straw with ultrasound and ILs was developed.

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ABSTRACT

The application of ultrasonic irradiation and ionic liquids (ILs) in the degradation of rice straw under different processes of pretreatment and enzymatic hydrolysis was investigated. Various substrates for enzymatic hydrolysis by cellulase with and without ultrasound were as follows: untreated rice-straw powder (RS); RS treated by ILs of 1-ethyl-3-methylimidazolium ethylsulfate and trihexyl (tetradecyl) phosphonium decanoate with ultrasound at 300 W/(40 kHz, 28 kHz); RS treated by IL of choline hydroxide ([Ch][OH]) with ultrasound at 300 W/40 kHz (CHRS). In ultrasound-mediated enzymatic hydrolysis, the yield of total reducing sugar (TRS) converted from CHRS was up to 96.22% at 240 min and was greater than that from the other substrates; the TRS yield for CHRS with ultrasound was 19.5% greater than that without irradiation. Lignocellulosic biomass pretreated with [Ch][OH] showed the highest efficiency among the tested ILs, and ultrasound can be applied effectively in rice-straw pretreatment and enzymatic hydrolysis.

1. Introduction

Rice straw (RS), the major residue of rice production, is a common and abundant agricultural waste in Taiwan. It contains an abundant lignocellulosic biomass, including cellulose (24–34%), hemicellulose (19–29%), lignin (5–11%), and crude ash (10.4–21.8%) (Juliano, 1985); therefore, RS has the potential to serve as a raw material for the production of biofuel. However, RS contains around 11–15% of cuticle silica (Juliano, 1985), which makes it difficult for the lignocellulose to be utilized by enzymes and microorganisms. In Taiwan, the silica content of rice straw is low, generally less than 6% (Wang et al., 2007; Korndörfer et al., 2001). In recent studies, various methods have been reported that can destroy the morphological structure of RS so that the lignocellulose can be used and converted to fermentable sugars more effectively; these methods include physical pretreatment (e.g., steaming, electron beam irradiation, grinding, and milling), chemical pretreatment (e.g., alkali, acid, ammonia, oxidizing agent, and organo-solvent), biological pretreatment, and enzymatic hydrolysis (Ranjan and Moholkar, 2013; Binod et al., 2010; Bak et al., 2009; Kim and Han, 2012; Sindhu et al., 2012; Nguyen et al., 2010).

Ultrasound, the high frequency waves that are generally over 20 kHz, has been applied to assist in the pretreatment of lignocellulosic biomass with different reaction solutions (Subhedar and Gogate, 2013). For example, we have investigated the potential of ultrasound for treating rice hull as the fermentation substrate for the production of xylooligosaccharides (Yang et al., 2012). In addition, ultrasound pretreatment of sugarcane bagasse (Liu et al., 2006), buckwheat hulls (Hromádková and Ebringerová, 2003),

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wheat straw (Sun and Tomkinson, 2002), kenaf powder (Ninomiya et al., 2012), and rice hulls (Yu et al., 2009) also has been reported. Ultrasonic irradiation on liquid–solid interfaces showed some surface erosion or particle size reduction (Luche, 1998; Sasson and Neumann, 1997). The effect of ultrasonic irradiation is to produce cavitation in the liquid to assist the progress of chemical reactions from bubble creation and hot-spot generation. Cavitation, the property occurred by ultrasound, has the potential to destroy the surface structure of lignocellulosic biomass.

Ionic liquids (ILs), regarded as a type of green solvent, are organic salts that are comprised entirely of cations (usually organic) and anions (usually inorganic). Ionic liquids have been used extensively in many fields due to their properties of negligible vapor pressure, high thermal stability, and non-flammability (Vancov et al., 2012; Quijano et al., 2010). The most common types of ILs used in biotechnological processes are imidazolium, pyridinium, pyrrolidinium, tetrafluoroborate, methylsulfate, quaternary ammonium, quaternary phosphonium, hexafluorophosphate, and bis[(trifluoromethyl) sulfonyl] amide, among which the ILs of imidazolium-based salts have been the most investigated in biotechnology (Quijano et al., 2010).

Recently, ILs have been applied in cellulose dissolution or biomass pretreatment. Swatloski et al. (2002) found that cellulose could be dissolved in the IL, 1-butyl-3-methylimidazolium chloride ([Bmim]Cl), and many studies have focused on investigating the efficiencies of various ILs in treating lignocellulosic biomass, and diversifying their recovery, structures, enzymatic hydrolysis, and yields of fermentable sugar. In addition, ILs were found to have the potential for removing lignin, reducing the crystallinity of cellulose, and enhancing the activities and stabilities of several enzymes (Nguyen et al., 2010; Fu and Mazza, 2011; Lynam et al., 2012).

The aim of this study was to investigate the application of ultrasound and ionic liquids in the degradation of rice straw under different processes of pretreatment and enzymatic hydrolysis. Three different types of ionic liquid including [EMIM][$EtSO_4$], THTDPD, and [Ch][OH] were used to pretreat rice straw under ultrasound, the structural and elemental composition changes were verified by field emission scanning electron microscope (FE-SEM) and X-ray energy dispersive spectrometer (EDS). The enzymatic hydrolysis of substrates was conducted by cellulase from *Trichoderma reesei* ATCC 26921 with and without ultrasonic irradiation.

Specifically, the new method of pretreatment with [Ch][OH] and ultrasound for the utilization of rice straw was developed in this study, due to the basic ionic liquid [Ch][OH] being able to replace the acid and alkali pretreatments for rice straw and effectively promoting the enzymatic hydrolysis under ultrasound. The pretreatment strategy by combing basic ionic liquid [Ch][OH] and ultrasound not only generates high availability of lignocellulosic biomass in high efficiency of bioconversion within limited processing time but also makes possible reduction of pretreatment cost by using [Ch][OH], which is cheaper than other types of ILs, demonstrating a rather preferred potential of commercial feasibility.

2. Methods

2.1. Biomass and chemical reagent

The biomass of rice straw was Taikeng 9, and it was obtained from Ershui Town, Changhua County, Taiwan. Prior to the experiments, the rice straw first was cut into short lengths and washed thoroughly with reverse osmosis (RO) water until it was clean. Then, it was dried, pulverized, and screened through 60-mesh sieves. The ILs used in the study were 1-ethyl-3-methylimidazolium ethylsulfate ([EMIM][$EtSO_4$], 98%, from Strem Chemicals, Inc.), trihexyl(tetradecyl)phosphonium decanoate (THTDPD, 95%, from Strem Chemicals, Inc.), and choline hydroxide solution ([Ch][OH], 46 wt% in H_2O, from Sigma–Aldrich). The commercial cellulase from *T. reesei* ATCC 26921 (aqueous solution, 1.2 g/mL of density at 25 °C, ⩾700 endoglucanase units (EGU)/g, CAS Number 9012-54-8, EC Number 232-734-4, Celluclast® 1.5 L, C2730, Sigma–Aldrich Co., LLC.) was used in enzymatic hydrolysis.

2.2. Pretreatment of rice straw under ultrasonic irradiation

The pretreatment of rice straw powder was conducted with treated solutions in a 150-mL, two-neck, round-bottom flask at 60 °C for 180 min in an ultrasonic bath (LEO-600, Ko Hsieh Instruments Co., Ltd., Taiwan). The ultrasonic bath was equipped with dual frequencies (28/40 kHz) and variable electric power (maximum = 300 W), and the power density was 0.0126 W/cm^3 for 300 W (Yang et al., 2012; Yang and Chu, 2014). FE-SEM was used to observe the structural change of samples treated by the ILs and ultrasonic irradiation.

2.2.1. RS pretreated with the ionic liquids [EMIM][$EtSO_4$] and THTDPD

The solution of ILs was prepared with 10 g of [EMIM][$EtSO_4$], 1 g of THTDPD, and 10 mL of RO water in a 150-mL, two-neck, round-bottom flask under ultrasound (600 W/40 kHz) for 10 min. Then, 1 g of RS was put into the solution of ILs to be pretreated by the ultrasonic system (300 W/40 kHz) at 60 °C for 180 min. The supernatant was removed by centrifugation at 2690×g (5000 rpm) for 30 min at room temperature. The precipitate from the pretreatment was washed with deionized (DI) water and centrifuged at least five times. Finally, the precipitate was dried at 80 °C for 48 h, and the dried powder was called ILRS-A. The substrate of ILRS-B was prepared in the same pretreatment process, but with a different ultrasonic frequency of 28 kHz (300 W). In addition, the polysaccharide was extracted from the supernatant by precipitation with ethanol (Yang et al., 2012). The extracted polysaccharide was dried and milled to avoid aggregation. The extraction yield of polysaccharide from RS was calculated and total soluble sugar (TSS) was analyzed by phenol–sulfuric acid assay.

2.2.2. RS pretreated with ionic liquid [Ch][OH]

The pretreatment solution was prepared with 5 g of [Ch][OH] and 45 g of DI water in a 150-mL, two-neck, round-bottom flask. The 2 g of RS were put into the prescribed solution to be pretreated by the ultrasonic system (300 W/40 kHz) at 60 °C for 180 min. The supernatant was removed by centrifugation at 2690×g (5000 rpm) for 10 min. The precipitate was washed with DI water and centrifuged at least ten times to remove the [Ch][OH]. The precipitate was dried at 80 °C for 48 h and designated as CHRS. The procedure of polysaccharide extraction for the supernatant was the same with that described in Section 2.2.1.

2.3. Enzymatic hydrolysis and analysis

The enzymatic hydrolysis reactions of untreated rice-straw powders (RS) and treated rice-straw powders of ILRS-A, ILRS-B, and CHRS were conducted in a glass tube at 50 °C under ultrasound (300 W/40 kHz) in the ultrasonic bath or without sonication. The reaction mixture contained 10 or 20 mg of RS, ILRS-A, ILRS-B, or CHRS using 7 mL of cellulase solution. The cellulase solution was prepared by using cellulase aqueous solution (10%, w/w) and acetate buffer (90%, w/w, pH 4.9 at room temperature). For each reaction time (0, 30, 60, 120, 180, and 240 min), the hydrolysis reaction was stopped by heating the sample in boiling water for 10 min, after which the sample was taken for subsequent analysis. After centrifuging the heated sample, the concentration of total reducing