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Kinetics for enzymatic hydrolysis of rice hulls by the ultrasonic pretreatment with a bio-based basic ionic liquid

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ABSTRACT

The enzymatic hydrolysis of rice hulls by the ultrasonic pretreatment using a bio-based basic ionic liquid was investigated. The morphological and characteristic structures of rice hulls before and after the pretreatment were analyzed by field emission scanning electron microscope (FE-SEM), X-ray energy dispersive spectrometer (EDS), focal plane array-Fourier transform infrared spectroscopy (FPA-FTIR), high resolution X-ray diffractometer (HR-XRD), and elemental analyzer (EA). The network structure of rice hulls after the physicochemical pretreatment was seriously corroded and punched to expose the accessible organic region by removing silica, lignin and ash to enhance the hydrolysis reaction. In ultrasound-assisted enzymatic hydrolysis by cellulase, the total reducing sugar yield from rice hulls after the pretreatment was 43.5% at only 3 h of hydrolysis time, and was greater than that from untreated rice hulls (24.0%). A simplified kinetic model was proposed to describe the enzymatic hydrolysis of rice hulls in a heterogeneous system using an impeded reaction of enzyme, with a time-dependent decay coefficient, the initial observed rate constants, and ineffective coefficients were obtained to successfully describe the enzymatic hydrolysis of rice hulls.

1. Introduction

Rice hulls, one of the major types of residues from rice cultivation, are abundant lignocellulosic waste in Taiwan, containing cellulose, hemicellulose, lignin, and crude ash. From the point of view of biomass utilization, rice hulls have the potential to serve as a raw material to produce bioenergy. However, rice hulls possess a more rugged structure with cuticle silica distributed over the outer layer [1], and this silica impedes the enzymes and microorganisms transporting into the network structure of rice hulls to utilize the lignocellulose [2–4]. This difficultly might be overcome by the use of an effective pretreatment for the lignocellulosic biomass to alter their original structure. The pretreatment method including physical, chemical, and biological approaches has been employed for this purpose [2,5], while the combination of such methods might be more promising to strengthen the efficiency of pretreatments.

Recently, ultrasound and ionic liquids (ILs) have attracted increasing attention for the pretreatment of biomass. Ultrasound, referring to high frequency waves (above 20 kHz), can produce cavities in the liquid phase to promote the progress of chemical reactions by violent collapse of bubbles and the generation of hot-spots, and it was reported to be used in the pretreatment and enzymatic hydrolysis of lignocellulosic biomass for modifying the surface structure of lignocellulosic materials, thereby enhancing the bioconversion process [2,6,7]. ILs are considered to be 'green' solvents, and they have been applied extensively in many fields [8,9]; for example, imidazolium-based ILs were used in the treatment of kenaf powder to produce changes of their surface structures for enzymatic saccharification [6]. Choline-based IL is regarded as 'bio-ILs' because of their biocompatibility, low toxicity, and environmental friendliness [10], with the property of higher hydrogen bonding ability than other ILs [11]. In recent studies, choline acetate was reported to treat bamboo powder, bagasse, and southern yellow pine [12,13]. Other choline-based ILs, the cholinium amino acids ILs, were used as effective solvents for the pretreatment of rice straw [14]. Among the choline-based ILs, choline hydroxide (denoted as [Ch][OH]) is one of the choline-based ILs with a basic anion and is termed as 'bio-based basic IL' [15,16]. In our previous study, we found that the pretreatment of rice straw with [Ch][OH] resulted in good enhancement for the enzymatic hydrolysis of the straw [17].

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For the optimization of a bioconversion process, a kinetic model is important to describe the phenomena of the reaction system. For enzyme kinetics, the classical Michaelis–Menten model is practically suitable in a homogeneous system. In fact, many bio-catalytic reactions occur in a heterogeneous system, within which the phenomena of mass-transfer and reaction of enzymes are involved, including the action of soluble enzymes on insoluble substrates, product inhibition, enzyme deactivation, lignin inhibition, accessibility and reactivity of enzyme to substrate, and so on [18,19]; therefore, the Michaelis–Menten model should be further modified when it is used to describe the enzymatic hydrolysis in a heterogeneous system. In the past, various alternative models were proposed to simulate the enzymatic reactions in a heterogeneous system, for example, Michaelis–Menten based models, empirical models, the Langmuir adsorption isotherm based models, the jammed Michaelis kinetics model, the fractal Michaelis kinetics model, and the kinetic model based on shrinking particle theory and the Langmuir isotherm concept [18–20]. However, the application of those models has some restrictions, because of: (i) the models consist of several complicated ordinary differential equations (ODEs) that should be solved, (ii) the models contain many parameters that could not be uniquely determined, and (iii) some parameters are arbitrarily chosen rather than from a fitting process based on experiments [21].

For the utilization of rice hulls and using environmentally benign compound in the pretreatment, a biocompatible type of ILs, like choline-based IL, would be more preferred. The aim of the study was to use an effective physicochemical method using the combination of [Ch][OH] and ultrasound in the mild conditions for the pretreatment of rice hulls, and to construct a simplified kinetic model for the enzymatic hydrolysis of rice hulls by cellulase in a batch reactor, so as to describe the experimental results and to avoid the above restrictions of previous models. The structural changes before and after the [Ch][OH] pretreatment were analyzed by using FE-SEM, EDS, FPA-FTIR, HR-XRD, and EA. The kinetic model was set up by modifying the Michaelis–Menten model with the time-dependent decay coefficient of the impeded reaction of the enzyme in the study, such that it could directly describe the relationship between hydrolysis time and product yield in the lignocellulosic bioconversion.

2. Material and methods

2.1. Materials and ultrasonic irradiation system

Rice hulls were obtained from the Wu-Feng District Farmers' Association, Taichung City, Taiwan, and they were washed thoroughly with reverse osmosis (RO) water until they were clean; then, they were dried, pulverized, and screened through 60-mesh before the experiments. The bio-based basic IL was choline hydroxide solution (46 wt% solution of [Ch][OH] in H_2O, Sigma–Aldrich Co., LLC.). Commercial cellulase aqueous solution from *Trichoderma reesei* ATCC 26921 (EC Number 232-734-4, ≥700 endoglucanase units (EGU)/g, Sigma–Aldrich Co., LLC.) was used in the process of enzymatic hydrolysis. The processes of rice hull powder pretreatment and enzymatic hydrolysis by cellulase were conducted in a thermostatically ultrasonic bath system (LEO-600, Ko Hsieh Instruments Co., Ltd., Taiwan) with 40 kHz and variable electric power (maximum of 300 W) [17].

2.2. Rice hulls pretreated with [Ch][OH]and ultrasound

In the past, for the IL pretreatment in rice hulls, a large amount of IL was used to partially dissolve rice hulls, and subsequently, the cellulose was regenerated [22,23]. However, the dosage of IL in such a pretreatment method could be reduced to reach the high efficiency of rice hulls utilization. In the present study, a less quantity of bio-based basic IL and ultrasound was employed to get the effective pretreatment for rice hulls. The IL solution was prepared with 5 g of choline hydroxide solution and 45 g of deionized (DI) water in a 150-mL, two-neck, round-bottom flask, in which two grams of rice hull powder were added and treated at 60 °C for 180 min under ultrasound. Then, the supernatant was removed by centrifugation at 2690 × *g* (5000 rpm) for 10 min. After that, the precipitate was washed with DI water and centrifuged at least ten times to remove [Ch][OH] and further dried at 80 °C for 48 h to obtain the treated rice hull, which was denoted as CHRH; the untreated rice hull was designated as RH.

2.3. Enzymatic hydrolysis and analysis

Enzymatic hydrolysis of the substrates (RH and CHRH) was conducted in a glass tube at 40, 50, and 60 °C with and without ultrasonic irradiation. The cellulase solution (1.0 g/mL, ≥70 EGU/g) was composed of commercial cellulase and acetate buffer (pH 4.9) at a weight ratio of 1:9 and was used in the enzymatic hydrolysis. The reactor contained 10 mg of substrate and 7 mL of cellulase solution, giving the enzyme dosage to be 49 EGU/mg of substrate (RH or CHRH). For each reaction time, the hydrolysis reaction was stopped by inactivating the enzyme with boiling water. Then, the solution was centrifuged to get the supernatant for analysis. The concentration of total reducing sugar (TRS) in the supernatant was analyzed by the 3,5-dinitrosalicylic acid (DNS) method [17] with a spectrophotometer (BioMate 3S UV–vis spectrophotometer, Thermo Fisher Scientific, Inc.). The equations that were used to calculate biomass recovery, the yield of TRS, and the enhancement ratio (ER, %) were as follows:

$$\text{Biomass recovery}(\%) = \frac{\text{Weight of dry biomass after pretreatment}}{\text{Weight of raw materials}} \times 100$$

$$\text{TRS yield}(\%) = \frac{\text{Weight of total reducing sugars}}{\text{Weight of total dry substrate}} \times 100$$

$$\text{ER}(\%) = \frac{\text{TRS yield with ultrasound} - \text{TRS yield without ultrasound}}{\text{TRS yield without ultrasound}} \times 100$$

2.4. Morphological analysis and characterization

The morphological structures of RH and CHRH were verified by FE-SEM (JEOL JSM-6700F and JSM-7401F), and the functional groups were analyzed by FPA-FTIR (Bruker Vertex 70V, Hyperion 3000, 64 × 64 MCT Focal Plane Array) within the wave number range of 600–4000 cm^{-1}. Elemental compositions (C, H, O, and N) of RH and CHRH were performed with EA (Elementar vario EL III CHNOS Rapid); ash contents of RH and CHRH were determined after they were placed in the muffle furnace (CHANNEL MF-20) at 600 °C for at least 6 h, and the Si content in the ash was analyzed by EDS. The crystallinities of RH and CHRH were examined by HR-XRD (Bruker D8 SSS), for which the condition was set at 40 kV/35 mA, and the samples were scanned in the range of 5–40° (2θ). The crystallinity