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Short communication

Kinetics of enzymatic hydrolysis of rice straw by the pretreatment with a bio-based basic ionic liquid under ultrasound

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ABSTRACT

The kinetics of enzymatic hydrolysis of rice straw pretreated by a bio-based basic ionic liquid (choline hydroxide) under ultrasound was investigated. The structural characterization of rice straw before and after the pretreatment was performed with EA, HR-XRD, AFM, and FPA-FTIR, by which the increase of crystallinity, the destruction of surface structure, and the changes in functional groups and compositions were observed after the pretreatment on rice straw with choline hydroxide under ultrasound, thus significantly enhancing the enzymatic hydrolysis by cellulase. A simplified kinetic model with the impeded reaction of enzyme was developed to successfully describe the enzymatic hydrolysis of rice straw in a heterogeneous system. Based on the experimental observation, a time-dependent decay coefficient for the impeded reaction was proposed. The initial observed rate constants as well as ineffective coefficients were obtained to elucidate the interaction of enzyme and substrate, and the applicability of the simplified model was demonstrated.

1. Introduction

Rice straw, a type of agricultural waste, contains rich lignocelluloses that can be utilized as a raw material for the production of bioenergy. In general, rice straw has 11–15% cuticle silica [1], which could be one of the obstacles for enzymes to hydrolyze the lignocellulose and should be reduced as much as possible before enzymatic hydrolysis. This requirement might be attained by an effective pretreatment to alter the original structure of lignocellulosic material [2]. Ultrasound can produce cavities in a liquid phase to generate liquid jets and hot-spots, which would be useful in the pretreatment and enzymatic hydrolysis of biomass [3–6]. Ionic liquids (ILs) with properties of negligible vapor pressure, high thermal stability, etc. [7,8] are employed in many fields, such as electrochemistry and catalysis [9,10]. Various imidazolium-based ILs were reported in the pretreatment of biomass at a rather high temperature (typically, >110 °C) [11–13], but a mild condition for pretreatment would be more favorable. Choline-based ILs as 'bio-IL' are biocompatible and low toxic, and are environmentally friendly solvents [14–17], and some of them have been used in the treatment of bamboo, southern yellow pine, and rice straw [18–21].

For a bioconversion process, a kinetic model that can appropriately describe the enzymatic reaction is essential in the reactor design. The Michaelis–Menten model for enzyme kinetics is suitably applied in a homogeneous system. However, many biocatalytic reactions occur in a heterogeneous system with effects of mass-transfer and reaction involved, including accessibility and reactivity of soluble enzymes on insoluble substrates, enzyme deactivation, etc. [22,23]. In the past, alternative models were proposed to simulate enzymatic reactions in a heterogeneous system, for example, the jammed Michaelis model, the fractal Michaelis model, and the model by shrinking particle theory [22–24]. But, the application of those models has some disadvantages because of (i) the models consisting of complicated ordinary differential equations (ODEs) that should be solved, (ii) the models containing many parameters that could not be uniquely determined, and (iii) some parameters being arbitrarily chosen rather than coming from a fitting process by experiments [25].

Choline hydroxide ([Ch][OH]) is a choline-based IL (as bio-IL) with a basic anion, and is called as a 'bio-based basic IL' [26]. In our previous study, the pretreatment of rice straw with different ILs was investigated, and the enzymatic hydrolysis of pretreated rice straw was significantly enhanced by using [Ch][OH] [6]. The aim of this study was to further explore the structural changes of rice straw after pretreatment with [Ch][OH] and ultrasound at a mild

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condition, including crystallinity, compositions, and functional groups, by using elemental analyses (EA), atomic force microscope (AFM), high resolution X-ray diffractometer (HR-XRD), and focal plane array-FTIR (FPA-FTIR), and to develop a simplified kinetic model that could show the efficiency of the pretreatment, describe the catalytic behaviors of enzyme in the solid substrate, and avoid the disadvantages of solving complicated ODEs and using many uniquely determined parameters. The kinetic model was set up by modifying the Michaelis–Menten model with the time-dependent decay coefficient for the impeded reaction of the enzyme, such that the relationship between the yield of product and hydrolysis time was directly described.

2. Materials and methods

2.1. Materials and ultrasonic system

The rice straw used in this study was Taikeng 9 (*Oryza sativa*) from Ershui, Changhua County, Taiwan. The rice straw was first cut into small pieces with 3–5 cm of length and washed thoroughly with reverse osmosis water until it was clean. Then, it was dried, pulverized, and screened through 60-mesh sieves for the pretreatment and enzymatic hydrolysis. The bio-based basic IL was choline hydroxide solution (46 wt% solution of [Ch][OH] in H_2O, from Sigma–Aldrich). Commercial cellulase from *Trichoderma reesei* ATCC 26921 (EC number 232-734-4, ≥700 endoglucanase units (EGU)/g, from Sigma–Aldrich) was used in the enzymatic hydrolysis. The ultrasonic system [6] used in the pretreatment and enzymatic hydrolysis of rice straw was set at 40 kHz and 300 W.

2.2. Pretreatment of rice straw with [Ch][OH] and ultrasound

The pretreatment conditions in this study were the same as that described in the work of Yang and Fang [6] and were summarized as follows. The pretreatment solution containing 5 g choline hydroxide solution and 45 g deionized water was used to pretreat 2 g rice straw under ultrasound at 60 °C for 180 min. After pretreatment, the reaction solution was centrifuged at 5000 rpm to remove the supernatant, and the precipitate was washed and centrifuged with deionized water at least 10 times to remove [Ch][OH]. Then, the precipitate was dried at 80 °C for 48 h to obtain the treated rice straw, which was designated as CHRS. The untreated rice straw was denoted as RS.

2.3. Structural characterization

For RS and CHRS, the elemental compositions (C, H, and O) were determined using EA (Elementar vario EL III CHN-OS Rapid), the morphological structures were observed by AFM, and the functional groups were verified by FPA-FTIR within the wave number range of 600–4000 cm^{-1}. The crystallinities of RS and CHRS were examined by HR-XRD, 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 index (CrI) was calculated with Eq. (1) by the method of Segal et al. [27],

$$\mathrm{CrI}\,(\%) = \frac{I_{\mathrm{crystalline}} - I_{\mathrm{amorphous}}}{I_{\mathrm{crystalline}}} \times 100, \tag{1}$$

where $I_{\mathrm{crystalline}}$ means the intensity data of crystalline regions at about $2\theta = 22°$, and $I_{\mathrm{amorphous}}$ is the intensity of the amorphous portion at $2\theta = 18°$ [28,29].

2.4. Enzymatic hydrolysis and analysis

As described in the work of Yang and Fang [6], the process of enzymatic hydrolysis is summarized as follows. The cellulase solution (1.0 g/mL, ≥70 EGU/g) that consisted of commercial cellulase and acetate buffer (pH 4.9) at a weight ratio of 1:9 was used in the enzymatic hydrolysis. The substrate (10 mg or 20 mg) was hydrolyzed with 7 mL cellulase solution at 50 °C with and without ultrasound. For each time period, the reaction was stopped by the inactivation of enzyme in boiling water. The reaction solution was centrifuged to obtain the supernatant for the analysis of the total reducing sugar (TRS) via the 3,5-dinitrosalicylic acid (DNS) method. Eq. (2) was used to calculate the yield of TRS (Y_{TRS}) as follows:

$$Y_{TRS}\,(\%) = \frac{\text{Weight of total reducing sugars}}{\text{Weight of total dry substrate}} \times 100. \tag{2}$$

2.5. Development of the kinetic model (the "impeded" Michaelis model)

The enzymatic hydrolysis of rice straw is conducted in a heterogeneous system, for which many factors affect the reaction kinetics, including mass-transfer resistance, interactions of enzyme and lignocellulosic biomass, and enzyme inhibition [30]. Based on the Michaelis–Menten model with a modification, the kinetic model (the "impeded" Michaelis model) in this study is constructed to describe the enzymatic hydrolysis in a heterogeneous system. The Michaelis–Menten model is illustrated by the scheme, $E + S \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} ES \xrightarrow{k_2} E + P$, where S, P, E, and ES represent the substrate, the product, the enzyme, and the enzyme–substrate complex, respectively. The rate constants k_1 and k_2 refer to the forward reactions and k_{-1} refers to the reverse reaction. Several assumptions in the model are made: (i) the adsorption of enzymes on the solid substrate is very fast in comparison with the enzymatic reactions, (ii) the actions of enzymes on the inert and non-reactive materials, the product inhibition, and the mass-transfer resistance for enzymes are combined as the impeded reaction of enzymes with a time-dependent decay coefficient, (iii) the enzymatic reaction is considered as the combined effect of the enzyme system (E) on the substrate, and (iv) the effect of the pretreatment for changing the structure of solid substrate is reflected in the rates of reaction for the corresponding k_1, k_{-1}, and k_2. With the assumption in the Michaelis–Menten kinetics, [ES] is at a quasi-steady-state [31], and the reaction can be formulated by the equations,

$$\frac{d[\mathrm{ES}]}{dt} = k_1[\mathrm{E}][\mathrm{S}] - k_{-1}[\mathrm{ES}] - k_2[\mathrm{ES}] \cong 0, \tag{3}$$

$$[\mathrm{ES}] = \frac{k_1[\mathrm{E}][\mathrm{S}]}{k_{-1} + k_2} = \frac{[\mathrm{E}][\mathrm{S}]}{k_{\mathrm{M}}} \quad \text{with } k_{\mathrm{M}} = \frac{k_{-1} + k_2}{k_1}, \tag{4}$$

where k_M is the half-saturation constant [32]. In addition, the rate of change of the product can be represented by Eq. (5),

$$\frac{d[\mathrm{P}]}{dt} = k_2[\mathrm{ES}] = k_2\left(\frac{[\mathrm{E}][\mathrm{S}]}{k_{\mathrm{M}}}\right) = \left(k_2\frac{[\mathrm{E}]}{k_{\mathrm{M}}}\right)[\mathrm{S}]. \tag{5}$$

Taking the material balance for the substrate and the product gives,

$$[\mathrm{S_0}] = [\mathrm{S}] + [\mathrm{ES}] + [\mathrm{P}], \tag{6}$$

where $[S_0]$ is the initial concentration of the substrate. Because $[ES] \ll [P] + [S]$, [ES] can be negligible, and Eq. (6) can be simplified and rearranged as

$$[\mathrm{S}] = [\mathrm{S_0}] - [\mathrm{P}] = [\mathrm{S_0}]\left(1 - \frac{[\mathrm{P}]}{[\mathrm{S_0}]}\right). \tag{7}$$

Combining Eqs. (5) and (7) gives

$$\frac{d[\mathrm{P}]}{dt} = \left(k_2\frac{[\mathrm{E}]}{k_{\mathrm{M}}}\right)[\mathrm{S_0}]\left(1 - \frac{[\mathrm{P}]}{[\mathrm{S_0}]}\right) \tag{8}$$

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