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Research article

Reinforced alkali-pretreatment for enhancing enzymatic hydrolysis of sugarcane bagasse

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ABSTRACT

Microwave irradiation (MWI) and ionic liquid (IL) assisted methods were chosen to strengthen the alkali pretreatment process to improve saccharification efficiency of sugarcane bagasse (SCB). Four different pretreatment approaches namely alkali, IL, MWI-alkali and IL-alkali pretreatments were compared. The chemical composition, morphology and crystal performance of the substrates were analyzed by the standardized methods of the National Renewable Energy Laboratory, field emission scanning electron microscope (SEM) and X-ray diffraction (XRD), respectively. IL assisted treatment brought the highest lignin removal (78.51%), and the glucan and xylan conversion achieved 99.51% and 92.76%, respectively, after 72 h of enzymatic hydrolysis. The similar effect occurred for MWI assisted alkali-pretreatment approach. SEM and XRD analyses showed that the strengthened process obviously changed the structure and crystallinity of the substrates. Moreover, as a persuasive tool, the fractal-like theory was used to study the enzymatic hydrolysis kinetics.

1. Introduction

Lignocellulosic biomass mainly consists of hexose, pentose and lignin. Crystalline cellulose is partially surrounded by the amorphous hemicelluloses, which is aligned with cellulose fibers and densely packed by the layers of lignin. Lignin constructed of phenylpropane units is the most obstinate component of the plant cell wall, which is generally resistant to enzymatic hydrolysis [1]. The degree of polymerization, available surface area, lignin content and distribution are important factors that hinder the enzymes attack. Due to the inhomogeneous structure of lignocellulosic materials, efficient pretreatment technologies are required to disrupt the recalcitrance of lignocellulosic biomass for further downstream processing [2,3].

Among different pretreatment methods, alkali pretreatment has the advantage of simple device, convenient operation with high efficiency, which induces the swelling reactions and the removing of lignin, uronic acids and acetyl groups from polysaccharides, therefore, making the substrates more susceptible to enzymatic reaction [4,5]. Although alkali pretreatment can remove the majority of lignin, the residual lignin and hemicelluloses in the pretreated substrate can still hinder enzymatic hydrolysis. The digestibility improvement depended on the removal of lignin and parts of hemicelluloses, the reduction of acetyl content, and the disruption of lignin–carbohydrate matrix [6,7]. In order to achieve efficient hydrolysis, using the synergistic benefits of the combined methods may be an integrated novel pretreatment approach.

Microwave irradiation (MWI) with the advantages of high heating efficiency and easy operation has been widely used in many areas. It has been shown in some studies that MWI can change the ultrastructure of cellulose and degrade lignin and hemicelluloses. The pretreated particles were characterized by swelling and fragmentation, which was favorable for enzyme accessibility. This technique can be easily combined with chemical reaction and accelerate the reaction rate [8–10]. The recent discovery of ionic liquid (IL) can effectively dissolve cellulose and biomass and is suggested to be a potential alternative to established pretreatment techniques [11]. IL pretreatment can reduce the cellulose crystallinity and partially remove lignin and hemicelluloses, thus enhance the biomass digestibility. It has high degree of flexibility in process design with less energy costing, and is easier to handle than other pretreatment methods [12,13].

The enzymatic hydrolysis of cellulose can be thought as a heterogeneous reaction with less than three dimensions [14] along a cellulosic fiber chain. Before the reaction, enzyme must be absorbed on the substrate surface and then diffuse to a reactive site. These complex reactions and mechanisms can be understood using a mathematical model [15]. The fractal-like kinetic model has been applied to analyze the effect of pretreatment on the enzymatic saccharification kinetics. With two parameters (fractal exponent and rate coefficient), it fitted very well to the experimental data in the literatures [16–18].

In the present study, alkali pretreatment was enhanced by MWI or IL considering the synergistic benefits of them for improving the hydrolysis

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Table 1
Chemical compositions of untreated and pretreated SCBs.

Pretreatment method	Composition (%)		
	Glucan	Xylan	Klason lignin
Untreated	38.51	20.65	25.03
Alkali	63.43	26.11	8.26
Alkali + MWI	65.62	26.54	7.13
IL	44.82	21.49	27.16
Alkali + IL	69.24	21.68	5.47

efficiency. The comparison of single alkali and IL pretreatments was also applied on sugarcane bagasse (SCB). 1-Allyl-3-methylimidazolium chloride (AMIC) was used as a representative IL because of its enhanced hydrogen bond basicity and lower viscosity [19]. The structural characterization of SCB substrates was investigated by field emission scanning electron microscope (SEM) and X-ray diffraction (XRD), and the fractal-like model was used to theoretically analyze the effect of various pretreatments on enzymatic hydrolysis.

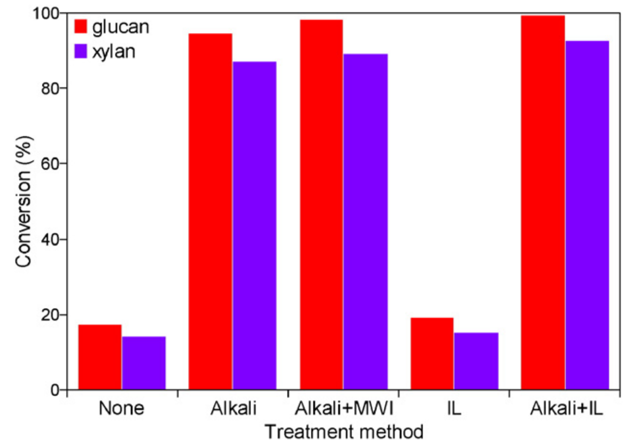

Fig. 1. Effect of pretreatment method on enzymatic conversion of the substrates.

2. Materials and methods

2.1. Materials

SCB was obtained from Guangxi Fenghao Sugar Co., Ltd. (Chongzuo, China). It was pre-milled and screened, and the fraction between 18 and 40 meshes was used for alkali-pretreatment. AMIC was purchased from Shanghai Cheng Jie Chemical Co. Ltd. A cellulase mixture, CTec2, was kindly provided by Novozymes A/S (Bagsaevrd, Denmark), with an enzyme activity of 200 FPU/mL measured by the description of International Union of Pure and Applied Chemistry [20]. All other chemicals used were of analytical or reagent grade and directly used as purchased without further purification.

2.2. Pretreatment procedure

2.2.1. Alkali pretreatment

SCB was pretreated by 0.5 M NaOH in a round-bottom flask, at 80 °C for 2 h with agitation (solid–liquid ratio is 1:20). After the pretreatment, the solid fraction was separated by filtering and then washed with tap water until a neutral pH. The obtained residual fraction was dried at 65 °C and then used as substrates for the subsequent enzymatic hydrolysis. All experiments were carried out three times, and the given numbers were the mean values.

2.2.2. IL pretreatment

Details of IL pretreatment were described elsewhere [21]. 5 g of SCB was quickly mixed with 95 g of AMIC solution at a solid/liquid ratio of 1:20, in a 250 mL dried flask. The flask was sealed with a cork and placed in oil bath at 80 °C for 2 h with stirring. After the reaction, the obtained substrate was precipitated using deionized water (90 °C) with vigorous shaking for 10 s. The regenerated substrate was then transferred into a beaker and washed with 50 mL deionized water (75 °C) thoroughly, and freeze-dried before subsequent enzymatic hydrolysis. The filtrate, containing water, IL, and some degraded carbohydrates as well as lignin, was separated under the reduced pressure.

2.2.3. MWI assisted alkali pretreatment

MWI was carried out in a MCR-3 microwave chemistry reactor. After the alkali pretreatment, the bottle with substrate mixtures was placed into the microwave reactor and the treatment program with the desired time of 5 min and a power of 600 W was started [22]. After the run, the bottle was removed from the reactor. The obtained solid was separated and dried according to Section 2.2.1.

2.2.4. IL assisted alkali pretreatment

The alkali-pretreated substrate was successively treated with IL, and the same pretreatment procedure was conducted according to Section 2.2.2. After the reaction, the wet regenerated SCB was freeze-dried for 24 h prior to enzymatic hydrolysis and further analysis.

2.3. Enzymatic hydrolysis

Enzymatic hydrolysis was carried out with 2% (w/v) substrate at pH 5.0, a temperature of 50 °C and a rotation speed of 150 rpm for 72 h in 250 mL Erlenmeyer flasks. The enzyme-loading was 9.6 FPU/g of biomass. Samples for sugar assay were taken at time points (specified in the text) and analyzed by high performance liquid chromatography (HPLC). Glucan and xylan conversion rates were estimated by the following equations:

$$\text{Glucan conversion rate} = \frac{\text{Glucose produced (g)} \times 0.9}{\text{Glucan amount in enzymatic substrate}} \quad (1)$$

$$\text{Xylan conversion rate} = \frac{\text{Xylose produced (g)} \times 0.88}{\text{Xylan amount in enzymatic substrate}}. \quad (2)$$

Table 2
Glucose and xylose concentrations (g L^{-1}) produced from enzymatic hydrolysis of different substrates.

Time (h)	Raw		Alkali-treated		Alkali + MWI		IL-treated		Alkali + IL	
	Glucose	Xylose	Glucose	Xylose	Glucose	Xylose	Glucose	Xylose	Glucose	Xylose
6	0.96	0.44	8.79	3.79	10.09	3.91	1.27	0.49	12.06	3.69
12	1.12	0.48	10.12	4.19	11.16	4.20	1.38	0.54	12.72	3.88
24	1.25	0.55	11.33	4.59	12.04	4.54	1.69	0.64	13.39	4.04
48	1.28	0.60	11.95	4.61	12.47	4.62	1.69	0.66	13.61	4.00
72	1.35	0.62	12.02	4.55	12.90	4.73	1.73	0.66	13.78	4.02