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Full Length Article

Application of hydrogen peroxide presoaking prior to ammonia fiber expansion pretreatment of energy crops

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HIGHLIGHTS

- The H-AFEX pretreatment was an effective pretreatment method for *Miscantus* species.
- The lignification of energy crop was between that of agricultural residues and wood.
- A strong negative correlation between solid recovery and H_2O_2 loading.
- The addition of hydrogen peroxide cause competition of glucose gain and xylose lose.
- SEM images showed that the porous structure was formed during H-AFEX pretreatment.

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ABSTRACT

This work presents the application of ammonia fiber expansion (AFEX) pretreatment both with (H-AFEX) and without hydrogen peroxide presoaking of energy crops moso bamboo, giant reed, and *Miscanthus*. The influences of AFEX and H-AFEX pretreatments on chemical composition and enzymatic hydrolysis were assessed. The results address the solid recovery, delignification, hydrolysis sugar yields, and micro-structural morphology of varying H-AFEX pretreatment conditions of energy crops. A strong negative correlation between solid recovery and H_2O_2 loading was found for all pretreated samples with H_2O_2 loading <2.0. In comparison with AFEX process, the addition of hydrogen peroxide in H-AFEX pretreatment could result in increases in glucose yields at the expense of xylose. The maximum sugar yields of moso bamboo, giant reed, and *Miscanthus* were 269.0, 424.6, and 485.0 g per kg dry biomass respectively, which were obtained under their optimal pretreatment conditions following enzymatic hydrolysis. The micrograph of H-AFEX-treated giant reed demonstrated that the modified porous structure resulted in increased enzyme accessible surface area, and facilitated the subsequent enzymatic hydrolysis.

1. Introduction

With the exhaustion of available oil reserves, the development of renewable energy and materials is an inevitable choice for human beings [1,2]. The lawmakers of USA have envisioned cars burning up to 36 billion gallons of biofuel per year by 2022 [3]. The European council has endorsed a mandatory target that 20% energy consumption is generated from renewable sources by 2020 by all member states [4]. Lignocellulosic biomass is the most abundant and renewable resource on the earth. The global annual production of biomass through photosynthesis could reach 10 trillion tons, which is about 20–27 times more than the total annual petroleum production in the world [5,6].

In general, lignocellulosic biomass can be divided into agricultural residues, wood and forestry residues, energy crops, urban wastes, human and livestock manure and so on [7]. Energy crops are abundant and highly productive, but most of them are still in wild or semi-wild state. Researchers are studying the application of genetic improvement, artificial cultivation and advanced technology to produce a variety of bio-based products [8]. Rapid growth, high yield, and abundant sources make energy crops have a competitive advantage in cost [9,10]. The burning of biofuel produced from lignocellulosic biomass could reduce carbon emissions compared with fossil fuels [11]. Lastly, the direct source of biomass energy is the photosynthesis of green plants which is renewable and sustainable [12]. At present, more than 20 kinds of energy

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crops have been selected by researchers [13]. Among them, switchgrass (*Panicum virgatum*), giant reed (*Arundo donax*) and *Miscanthus* have the most promising potential. The annual biomass productivity of bamboo (*Lingnania wenchouensis wen*) is up to about 60 tons of dry matter per hectare, which is far higher than that of *Miscanthus* (about 10–30 tons of dry matter per hectare) [9] [14]. The carbon sequestration and biomass productivity of bamboo are even more than those of tropical rain forest, which has resulted in great attention from scientists in biomass energy field [15,16]. Therefore, bamboo can also be utilized as a preferred species of energy crops.

Pretreatment is the first and one of the key steps in the bioconversion of energy crops [17,18]. The physicochemical properties of the plant cell wall are modified by pretreatments to decrease biomass recalcitrance, hence improving the subsequent enzymatic hydrolysis [19]. For bioconversion route, bioethanol and biobutanol could be derived through glucose or other sugars, and various kinds of bio-based chemical or materials could be converted through 5-hydroxymethyl furfural [20]. A variety of pretreatment methods have been developed in recent years, such as acid, alkali, ammoniation, steam explosion, etc. Ammoniation is effective and has been widely studied. In general, ammoniation includes aqueous ammonia, ammonia fiber expansion, ammonia recycle percolation, low-moisture anhydrous ammonia and recycled aqueous ammonia expansion, etc [21–23]. Garlock et al. [21] reported that the AFEX is very suitable for agricultural residues and herbaceous crops. AFEX undergoes physical (high temperature and high pressure) and chemical (ammonia) processes. The toxicity of inhibitors which are generated from AFEX pretreatment is very low during enzymatic hydrolysis and fermentation [24,25]. Therefore, there is usually no water-washing of AFEX-treated substrate, and the process is simplified [26,27]. However, AFEX pretreatment mainly shows the structural modification of cell wall rather than lignin or hemicellulose removal [25]. The inexpensive and environment-friendly strong oxidant, hydrogen peroxide is introduced to AFEX pretreatment in our laboratory. A novel pretreatment method, named hydrogen peroxide presoaking prior to ammonia fiber expansion (H-AFEX) is proposed. Alkaline peroxide pretreatment often leads to improving lignin removal and enzymatic hydrolysis [28–30]. To our best known, this was the first time to apply the H-AFEX pretreatment to energy crops. We also focused on the changes in chemical composition and enzymatic hydrolysis by adding hydrogen peroxide presoaking in comparison with AFEX pretreatment only. The results would be helpful for the future economic analysis using the new H-AFEX pretreatment.

In this paper, moso bamboo, giant reed and *Miscanthus* were chosen as representative materials of energy crops. The AFEX and H-AFEX pretreatments of different energy crops were studied. The effects of AFEX and H-AFEX pretreatments on chemical composition and enzymatic hydrolysis were assessed. We were also interested in the composition characteristics of different energy crops and their relationship to enzymatic hydrolysis yields. In addition, the micro-structural morphology changes in pretreated substrate were observed.

2. Materials and methods

2.1. Materials and chemicals

Moso bamboo, giant reed and *Miscanthus* (the growth period about 1 year) were harvested in December 2013 from Linan coutry (30.10° N; 119.23° E), Zhejiang Province, China. The whole plant was used in the experiment. The raw materials were cut into strip less than 1 cm using a broken branch machine (ES-S4002, Esen Power Garden Tools Co. Ltd, Ningbo, China) after air-dried. Then the samples were further dried using an oven until the moisture contents were less than 15%. Afterwards, the materials were ground through 50 mesh screen sieve using a grinder (Cosuai CS-700, Haina Electric Appliance Co. Ltd., Zhejing, China), and then put into freezer at −20 °C in self-sealing plastic bags.

All chemicals were purchased from Sigma-Aldrich Chemical Co. Ltd (Shanghai, China) unless otherwise noted. The cellulase from *Trichoderma reesei* and β-glucosidase from *Aspergillus niger* were bought from Novozymes (China) Investment Co. Ltd. Xylanase was bought from Shandong Zesheng Bio-engineering Technology Co. Ltd. (China). The cellulase activity was assayed by the IUPAC (International Union of Pure and Applied Chemistry) standard procedure [31]. The β-glucosidase and xylanase activities were determined using the procedures described by Saha and Cotta [32]. The average activity of cellulase, β-glucosidase and xylanase were 75 FPU/mL, 250 CBU/mL and 8000 IU/mL, respectively.

2.2. AFEX and H-AFEX pretreatment

The H-AFEX process was similar to AFEX process just adding a presoaking of hydrogen peroxide solution. 20 g of dry materials (moso bamboo, giant reed and *Miscanthus*) were mixed with 30% hydrogen peroxide solution at room temperature. The mixture was treated in a high-pressure reactor under the conditions of 2.0 ammonia loading (the mass ratio of ammonia to dry biomass), 0.4 water loading (the mass ratio of water to dry biomass), 130 °C for 10 min. The AFEX process was detailed by Zhao et al. [30]. The pretreatment variable was H_2O_2 loading (the mass ratio of 30% hydrogen peroxide solution to dry biomass) and set at 0.0, 0.5, 1.0, 2.0 respectively. When the H_2O_2 loading was 0.0, it meant this was an AFEX process. The pretreated samples were cooled to room temperature in hood and put into freezer at −20 °C in self-sealing plastic bags for composition analysis and enzymatic hydrolysis. The solid recovery was defined as follows:

$$S_r(\%) = m_1/m_2 \times 100\% \tag{1}$$

where S_r is solid recovery (%), m_1 is the total mass of pretreated biomass (g, dry basis), m_2 is the total mass of feedstock (g, dry basis).

2.3. Analytical methods

2.3.1. Composition analysis and enzymatic hydrolysis

The composition analysis of raw materials and pretreated substrates (without water-washing) was determined by the national renewable energy laboratory technical report NREL/TP-510-

Table 1
Compositional analysis of different raw materials (dry basis %).

Feedstock	Components	Glucan	Xylan	Lignin	Reference
Energy crops	Moso bamboo	32.9 ± 0.8	21.6 ± 1.4	25.4 ± 0.5	–
	Giant reed	33.2 ± 1.1	19.2 ± 0.1	23.2 ± 0.3	–
	Miscanthus	39.6 ± 0.2	21.3 ± 0.3	22.9 ± 1.3	–
Agricultural residues	Corn stover	34.7–38.7	21.0–23.3	17.1–20.6	[23,35,36]
Wood	Poplar	39.8–44.3	18.4–19.7	26.9–29.1	[37–39]
	Eucalyptus	40.2	19.0	35.9	[7]