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Biomass to bio-ethanol: The evaluation of hybrid *Pennisetum* used as raw material for bio-ethanol production compared with corn stalk by steam explosion joint use of mild chemicals

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A B S T R A C T

The second generation biomass to bio-ethanol production is of growing interest. Energy crop were becoming important for second generation biomass to bio-ethanol production for their growth advantages. Hybrid *Pennisetum* as a new hybrid energy crop was selected as a model to compare with corn stalk. As pre-treatment methods, steam explosion and its combined action with dilute sulfuric acid, bisulfite, and mixed dilute acid and bisulfite were selected. The enzymatic hydrolysis demonstrated that the cellulose conversion is a strong function of the pre-treatment method applied, with corn stalk providing slightly better results. With dilute acid steam explosion (DA-SE), conversions were 67.6% and 54.5% for corn stalk and *pennisetum*, respectively. This can be attributed to the higher Cr. I of *pennisetum* (65.03%) than of corn stalk (54.05%). The cell lumen of pretreated *pennisetum* was smaller than for corn stalk as shown in SEM photos, meaning there was a substantially higher enzyme accessible surface and porosity in *pennisetum*, thus responsible for the higher cellulase adsorption of pretreated *pennisetum*. DA-SE was the most effective pre-treatment method, but the inhibitors' concentration was higher than in other methods. Combined dilute acid and bisulfite can moderately remove hemicelluloses and lignin. Cr. I values, lignin content, accessible surface and porosity were supplied the energy crop evaluation standards for bio-ethanol production.

1. Introduction

As outlined in recent review papers of Baeyens et al. [1] and Kang et al. [2,3] the development of second generation bio-ethanol using lingo-cellulosic biomass as feedstock instead of common sugar-based raw materials is of paramount importance for the future of bio-ethanol. These biomass resources are widely available in different forms [4], including energy crops. China moreover has a lot of marginal or degraded agricultural lands, where energy crops can offer a potential for renewed agricultural activity. There are plenty of energy crops grown in the marginal land of China such as giant reed, Switchgrass, elephant grass and so on. But which can be used as raw materials for bio-ethanol production? In order to screen out the property energy crop for bio-ethanol production, we choose hybrid *pennisetum* as the raw material compared with corn stalk which is an abundant agricultural residue in China [5] and used for bio-enthanol production at the most of time, to evaluation hybrid *Pennisetum* and figure out the screen standard for energy crop chosen to produce bio-ethanol.

Pennisetum (elephant grass) is a target energy crop, with an annual yield of 40–50 ton/acre under optimal growth condition [6]. Hybrid *Pennisetum* can grow on marginal land because it has a strong root system which can be contributed to soil and water conservation, and its salt tolerance is fair since it can grow under conditions up to 0.3% of NaCl. Both corn stalk and hybrid *Pennisetum* are composed of lignin, cellulose and hemicelluloses. Using appropriate pre-treatment, they can be enzymatically hydrolyzed into fermentable compounds [1]. Corn stalk and hybrid *Pennisetum* however differ in their composition content, as shown further in the text, and the comparison of both raw materials can hence provide some insight into the fundamentals of the effects of the pre-treatment and of the subsequent hydrolysis. Within the wide range of pre-treatment techniques described and detailed in literature, steam explosion has been considered as an effective

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pretreated technology with little or no chemical reactants needed [5]. Agriculture residues (herbaceous plant) have a low content of acetyl groups in the hemicellulosic fraction, making steam explosion an effective pre-treatment method [7]. Different chemical reactants can moreover be used, each with different results for the same raw material. Sulfuric acid catalyzed hydrolysis can involve hemicellulose, increase the amorphous fraction of the cellulose and weaken the lignin matrix structure thus increasing the cellulose accessibility [5]. Bisulfite and lignin can form lignosulfonate and dissolve in the broth to free lignin from the substrate and avoid the ineffective adsorption of enzymes by lignin [8]. Combining steam explosion and mild chemicals (dilute acid, bisulfate) can enhance the pre-treatment efficiency.

Numerous researchers examined the effect of steam explosion on the pre-treatment of corn stalk and *Pennisetum*: Sun et al. compared the physicochemical properties of corn stalk pretreated with acid and alkali in steam explosion [9,10]; steam explosion of corn stalk was studied by Sui and Chen [5]; *Pennisetum* was investigated by alkaline or acid pre-treatment, and explosion [6]. There was however no detailed discussion to explain the differences between corn stalk and *Pennisetum* in the same pre-treatment method.

In this research, hybrid *Pennisetum* and corn stalk were compared towards their physicochemical properties, by using different identical pre-treatment methods for both raw materials. Steam explosion as such, and in combination with chemicals, albeit in mild conditions, were assessed. A better understanding of the physicochemical differences between these two biomasses provides an evaluation standard and a clear direction towards how to choose or breeding energy crop for marginal land growing and to produce bio-ethanol for efficiency.

2. Materials and experimental procedures

2.1. Raw materials and chemicals

Corn stalk (C) and hybrid *Pennisetum* (P) were supplied by the Academy of Agriculture and Forestry of China. Corn stalk was grown from June to October at the north of China which belongs to temperate zone. Hybrid *Pennisetum* was harvest at November which had grown 5–6 months. Hybrid *Pennisetum* harvested at the same experimental field with corn stalk. They were naturally dried, milled and screened to below 2 mm.

Analytical grade Sulfuric acid and Sodium bisulfite were purchased from Beijing Chemical Works (China).

2.2. Pretreatment methods

To simplify further references in the text, corn stalk will be indicated as "-C", whereas "-P" refers to hybrid *Pennisetum*. The details of the experiment were shown as Table 1.

In all pre-treatments, 100 g (oven dried) corn stalk or *Pennisetum* particles were soaked in liquors content different chemicals (as shown in the Table 1) overnight (about 10 h). They were thereafter subjected to steam explosion in a 5 L steam explosion equipment (designed by Beijing University of Chemical Technology) at 1.2 MPa for 5 min.

2.3. Solid yield analysis

Solid yield of the pretreated biomass interpreted the hemicellulose and the lignin removal of the raw material. The pretreated samples were washed 10 times with DI water until the spent liquor was removed, then oven-dried at 105 °C overnight. The solid yield was calculated by the weight of the washed dry samples and the raw materials.

2.4. Composition analysis

Oven-dried (105 °C overnight) raw material and pretreated samples were milled and screened below 0.45 mm. The composition of the milled samples such as carbohydrates and Klason lignin was measured by the two step sulfuric acid method [11].

Monosaccharides, furfural, 5-hydroxyl methylfurfural (HMF), and acetic acid (AA) in pretreated spent liquor were detected by High Performance Liquid Chromatography (HPLC) (Thermo Fisher U3000) at a flow rate of 0.6 ml/min 0.5 mM sulfuric acid was used as the inhibitor flow phase with UV detector. DI water was used as flow phase to measure monosaccharides with a RI detector.

2.5. Enzyme hydrolysis

The solids loading of the enzymatic hydrolysis was 2 g per 100 ml liquor. The buffer of the experiment was 0.05 M citrate of pH 5.5 which provided a better glucose conversion than at pH 4.8 [12]. The experiments were carried out at 200 rpm and 50 °C on a shaking bed incubator with 10 FPU/g glucan CTec 2, provided by Novozyme Co. Each sample was put into 40 ppm of antibacterial tetracycline and the results reported were the average of duplicate hydrolysis runs.

2.6. Cellulase adsorption

Substrate accessibility to cellulase was also evaluated by measuring the amount of cellulase adsorption to the substrate. The cellulase adsorption system and chemical loading was the same as for the enzymatic hydrolysis, although adsorption was carried out at room temperature. The detection time was 30 min. After reaction, cellulase adsorption was measured using the Bradford protein assay [13]. The results were the mean of the duplicate experiments and the deviations from the mean were given as error bars.

2.7. X-ray diffractometry (XRD) analysis

The crystallinity index (CrI) is used to characterize the crystalline structure which is a fraction of more ordered structure than amorphous structure in cellulose. XRD scanning used a Bruker D8 Advance diffractometer operated at 40 kV and 40 mA. Scans were collected from $2\theta = 5–50°$ with step size of 0.1 s per step. The scanning is lock-coupled. The amount of cellulose I crystallinity in the untreated biomass samples was calculated from:

Table 1
The experiment operation of different pretreatment.

Samples	Chemicals	Pressure	Time	Solid to liquid ratio
Steam explosion (SE)	No	1.2 MPa	5min	1:3
Steam explosion with dilute sulfuric acid (DA-SE)	0.3 vol% dilute sulfuric acid (DA)			
Steam explosion with bisulfite (B-SE)	3 wt% bisulfite (B)			
Steam explosion with dilute sulfuric acid and bisulfite (DA + B-SE)	0.3 vol% DA and 3 wt% B			