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Tween-80 is effective for enhancing steam-exploded biomass enzymatic saccharification and ethanol production by specifically lessening cellulase absorption with lignin in common reed

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HIGHLIGHTS

- Steam explosion much reduces cellulose DP and largely extracts polymers in reed.
- Tween-80 is effective for high biomass saccharification in steam-exploded residues.
- Additional CaO pretreatment leads to the highest ethanol yield at 19% of dry matter.
- Tween specifically blocks lignin absorbing with cellulase for high biomass digestion.
- It provides an optimal biomass process approach for high ethanol yield in reed.

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ABSTRACT

In this study, eight physical and chemical pretreatments were compared in terms of their enhancements on biomass enzymatic saccharification in reed. Despite 8% NaOH pretreatment could result in 100% biomass enzymatic digestion while co-supplied with 1% Tween-80, it only produced bioethanol at 10% (% dry matter). By comparison, 10% CaO pretreatment with Tween-80 is a relatively low-cost biomass conversion with ethanol yield at 12%. Notably, the steam-explosion pretreatment with 1% Tween-80 could cause a complete biomass enzymatic hydrolysis with bioethanol yield at 17%. The sequential 5% CaO pretreatment with the steam-exploded residues could lead to the highest ethanol yield at 19% with an almost complete sugar–ethanol conversion rate. Due to much low-DP cellulose and less noncellulosic polymers (lignin, hemicelluloses) that increase biomass surfaces, the steam-exploded residues were specifically effective for Tween-80 either to block lignin absorbing with cellulases or to disassociate hemicelluloses, leading to an efficient lignocellulose enzymatic digestion. Compared with previously reported pretreatments in other C4-grasses (*Miscanthus*, corn, sweet sorghum, switchgrass), to our knowledge, this study has therefore provided three more applicable approaches for high ethanol production with relatively low cost, less contaminate release and efficient biomass conversion rates in reed.

1. Introduction

Lignocellulose has been increasingly considered for bioethanol production, due to large fossil energy consumption and environmental changes [1–3]. Common reed (*Phragmites australis*) is one of the most widespread wetland plants with high biomass yield for biofuel purpose. As a typical C4 grass, reed grows fast in water and lake margins, but also distributes in deserts and dry lands

Abbreviations: CrI, crystalline index; DP, degree of polymerization; Ara, arabinose; Xyl, xylose; H, *p*-coumaryl alcohol; G, coniferyl alcohol; S, sinapyl alcohol; GC–MS, gas chromatography–mass spectrometer.

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around the world. Over the past years, reed has been broadly used as a valuable raw fiber material in industry, agriculture and daily life. In particular, due to a high proportion of short fibers, reed is favor for paper production [4]. Despite physical and chemical pretreatments have been used in reed biomass process [5], it remains unknown about its optimal technology on biofuel production.

Principally, biomass process involves three major steps: physical and chemical pretreatments for wall polymer disassociation, enzymatic hydrolysis toward soluble sugar release, and yeast fermentation leading to ethanol production [6]. However, as lignocellulose recalcitrance could basically determine a costly biomass process, it becomes essential to find out an optimal pretreatment that not only enhances biomass enzymatic saccharification but also causes high ethanol production with less secondary pollution to the environment [7,8].

Acid and alkali such as H_2SO_4 and NaOH are the classical agents applied in chemical pretreatments, but CaO as a relatively low-cost chemical, can be re-used in industry, which has thus been considered as a relatively economical and environment-friendly chemical pretreatment [9–11]. In principle, alkali pretreatment can extract entire wall polymers by disassociation of hydrogen bonds among polymers, whereas acid pretreatment is able to release soluble sugars and lignin monomers [12,13]. By comparison, hot water and steam explosion are regarded as other relatively economical and environment-friendly physical pretreatments, due to less by-products release during biomass process [14,15]. Notably, the steam explosion pretreatments could largely reduce biomass particle size, extract wall polymers and alter lignocellulose features, leading to much enhanced biomass enzymatic digestibility distinctive in different biomass samples [16–18].

Furthermore, despite of a low cost, Tween has been found as a powerful surfactant for enhancing biomass saccharification by either distinctively disassociating wall polymers or largely increasing cellulases enzyme activity [19,20]. Despite Tween effects on biomass saccharification have been reported in different biomass samples [21,22], little is known about its specific roles in steam-exploded residues and other pretreated lignocelluloses, in particular on reed biomass. Hence, it remains to find out optimal technology of biomass pretreatment and sequential enzymatic hydrolysis for efficient biofuel production in reed.

Plant cell walls are composed mainly of cellulose, hemicelluloses and lignin. Cellulose crystallinity and degree of polymerization (DP) have been characterized as the negative factors on biomass digestibility, whereas hemicelluloses could reduce cellulose crystallinity for high biomass saccharification in many plant species examined [23,24]. By comparison, lignin may play dual roles in biomass enzymatic digestions, due to three monolignols proportions distinctive in different plant species [25].

In the present study, we performed various physical and chemical pretreatments with the mature stem materials of reed plants, and compared their distinct effects on biomass enzymatic saccharification and bioethanol production. Then, we found out optimal technology with relatively economical and environment-friendly biomass pretreatments that are capable for high bioethanol production by means of steam explosion or chemical (CaO) pretreatment followed by Tween-80 co-supply with cellulases into biomass enzymatic hydrolysis in reed.

2. Material and methods

2.1. Plant samples

Common reed (*P. australis*) was grown in lake margins of Tarim, Xinjiang, China. The mature stalks of 5–10 plants were harvested, dried at 50–55 °C and ground into powders through 40 mesh screen. The well-mixed powders were stored in a sealed dry container until in use.

2.2. Plant cell wall fractionation

The procedure of plant cell wall fractionation was used to extract wall polymers as described by Peng et al. [26] and Huang et al. [14]. After soluble sugars, lipids, starches and pectin of the biomass samples were successively removed, the remaining pellet was treated with 4 M KOH and 1.0 mg/mL sodium borohydride for 1 h at 25 °C, and the combined supernatant was used as KOH-extractable hemicelluloses. The remaining one parallel non-KOH-extractable residue was sequentially extracted with TFA for monosaccharides. One parallel was extracted with H_2SO_4 (67%, v/v) for 1 h at 25 °C and the supernatants were collected for determination of free hexoses and pentoses as total cellulose and non-KOH-extractable hemicelluloses. One parallel was extracted with acetic–nitric acids–water (8:1:2; v/v/v) for 1 h at 100 °C and the remaining pellet was regarded as crystalline cellulose for DP detection. All experiments were carried out in biological triplicate.

2.3. Colorimetric assay of hexoses and pentoses

The anthrone/H_2SO_4 method [27] and orcinol/HCl method [28] were respectively used for hexoses and pentoses assay. D-glucose and D-xylose were used in drawing the standard curves, and the deduction from pentoses reading at 660 nm was carried out for final hexoses calculation in order to eliminate the interference of pentose on hexose reading at 620 nm. All experiments were conducted in biological triplicate.

2.4. Total lignin and monolignol detection

Total lignin content was measured by the two-step acid hydrolysis method according to the Laboratory Analytical Procedure of the National Renewable Energy Laboratory as described by Wu et al. [6]. Monolignols were detected by HPLC according to the method described by Si et al. [29].

2.5. Hemicellulose monosaccharide determination

Total hemicelluloses were measured by accounting total hexoses and pentoses from KOH-extractable and non-KOH-extractable hemicelluloses. Monosaccharides of hemicelluloses were detected by GC–MS as described by Li et al. [30].

2.6. Cellulose CrI and DP measurement

The X-ray diffraction method was used for cellulose crystalline index (CrI) assay as described by Zhang et al. [24]. Standard error of the CrI method was detected at ±0.05 to approximately 0.15 using five representative samples in triplicate. The viscosity method was applied for cellulose DP detection using crystalline cellulose samples as described by Huang et al. [14].

2.7. Physical and chemical pretreatments

Steam explosion pretreatment: The dried reed stem materials were pretreated under steam explosion using Steam Explosion Reactor (QBS-200, Hebi Zhengdao Machine Factory, Hebi, China). All conditions were described by Huang et al. [14]. The steam-exploded reed residues were dried and ground into powders through 40 mesh screen, and used for further chemical pretreatments as described below.

Liquid hot water (LHW) pretreatment: The well-mixed raw materials or steam-exploded residues were added into well-