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Irradiation pretreatment facilitates the achievement of high total sugars concentration from lignocellulose biomass

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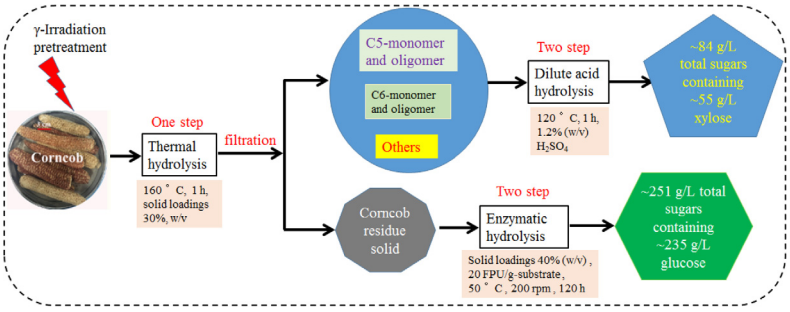
HIGHLIGHTS

- Irradiation pretreatment could reduce particle size and low shear rate of feedstock.
- 251 g/L of high sugars content could be achieved from irradiated biomass.
- 30–40% (w/v) of irradiated biomass loadings was employed for the hydrolysis process.
- 235 g/L glucose content could be obtained in cellulose enzymatic hydrolysate.
- High sugars concentration from lignocellulose opens up a door to biorefinery.

GRAPHICAL ABSTRACT



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ABSTRACT

This study evaluated the two hydrolysis strategies, involving one thermal and one dilute acid/enzymatic hydrolysis, to produce high xylose and glucose concentrations from lignocellulose assisted with irradiation pretreatment. Prior to hydrolysis, lignocellulose was pretreated by γ-irradiation at 800 KGy. The merits of irradiation pretreatment on lignocellulose were contributed to size-reduced particle distributions and low shear rate of material, which allowed high biomass loadings up to 30–40% (w/v, equals to 23–29 wt.%) for the consequent hydrolysis process. Results showed that hemicellulose fraction could achieve ~84 g/L of total sugars containing ~55 g/L xylose and ~21 g/L glucose through this two steps hydrolysis. Cellulose fraction would release ~251 g/L of total sugars consisting of ~235 g/L glucose and ~16 g/L xylose in the ultimate enzymatic hydrolysate. To the best of our knowledge, it was the first report of achieving 235 g/L glucose in cellulose enzymatic hydrolysate derived from lignocellulose.

1. Introduction

High conversion efficiency of hemicellulose and cellulose fractions into C5- and C6- sugars has become an essential prerequisite of next-generation liquid biofuels from lignocellulosic biomass (Anbarasan et al., 2012; Kunkes et al., 2008). To meet this challenge, researches have focused on developing novel lignocellulosic fractionation technologies to separately catalytic conversion cellulose or simultaneously catalytic conversion of holocellulose (including cellulose and hemicellulose) to C5- and C6- sugars in the literatures (Chen et al., 2016a,b; Luterbacher et al., 2014). Chen and co-workers developed DMR (deacetylation and mechanical refining) pretreatment on corn stover, and could achieve 230 g/L of total sugars containing 135.2 g/L glucose and 99.4 g/L xylose during enzymatic hydrolysis at 28 wt.% biomass solids and 20 mg protein enzyme per g biomass loading (Chen et al., 2016a,b). Luterbacher and co-workers (2014) reported the conversion of holocellulose into sugars in a solvent mixture of Gamma-valerolactone

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(GVL) /H_2O (8:2, v/v) with 0.05 wt.% H_2SO_4, and achieved the yield of 127 g/L total soluble sugars under the optimal conditions. Guragain and co-workers (2016) obtained the high sugars concentration of 106 g/L (w/v) in the hydrolysates from alkali-pretreated biomass at solids loading of 17.5% (w/v).

As well known, biomass-derived sugars have been emerged as intermediates for the production of renewable fuels and chemicals (Luterbacher et al., 2014). However, the intrinsic recalcitrant structure of lignocellulose makes it trouble to obtain the high conversion efficiency of hemicellulose and cellulose fractions of lignocellulosic biomass into C5- and C6- sugars. Therefore, chemical, physical and microbiological pretreatment methods were exploited to break down the stubborn lignocellulose structure to solve this enigma (Guragain et al., 2016; Liu et al., 2015a,b; Xie et al., 2015). Rinaldi reviewed the effect of different pretreatment methods on the structure damage of lignocellulose biomass with three catalytic mechanisms: a) deep depolymerization, b) shell peeling, and c) core leaching (Rinaldi, 2014). As a promising pretreatment strategy, more and more articles have demonstrated that γ- irradiation pretreatment can enhance enzymatic hydrolysis efficiency of cellulose for bioethanol production from lignocellulose (Liu et al., 2016; Wang et al., 2012; Yang et al., 2015). In our previous work, approx. 184.3 g/L glucose (total concentrations of soluble C5 and C6 sugars were 231 g/L) was achieved from cellulose fraction of irradiated lignocellulose in GVL/H_2O (40:60, v/v) with no adscititious catalyst (Zhou et al., 2016).

To gain much higher total sugars from biomass, in this present study, a two hydrolysis strategies are developed and evaluated, which converts hemicellulose and cellulose fractions of lignocellulosic biomass to high sugars concentration enrichment in xylose and glucose, respectively. The two hydrolysis strategies are involving one thermal and one acid hydrolysis, as well as one thermal and one enzymatic hydrolysis. The overview scheme of two hydrolysis strategies is depicted in Fig. S1. The final results of the two-step hydrolysis can achieve approx. 86 g/L of total sugars containing *ca.* 60 g/L xylose and *ca.* 26 g/L glucose from hemicellulose fraction, and ~251 g/L of total sugars consisting of ~235 g/L glucose and ~16 g/L xylose from cellulose fraction. To the best of our knowledge, it is the first report of achieving 235 g/L monomeric glucose in cellulose enzymatic hydrolysates derived from lignocellulosic biomass in the literatures.

2. Materials and methods

2.1. Lignocellulose biomass and enzymes materials

Five types of lignocellulose biomass were examined in this work. Corncob and reed stover were gifted from the Hunan Irradiation Center (Hunan, China). Eucalyptus hardwood and pine softwood were obtained from the forest products (Guangxi Gaofeng Forest, China). *Camellia oleifera* shell was gifted from Hunan Dasanxian camellia oil Ltd. Co. (Hengyang, China). The water content of the biomass before irradiation was estimated to be approx. 5.6%. The main components of cellulose, hemicellulose and lignin of the experimental tested biomasses were analyzed and detailed in Fig. S2.

Cellulase (Sigma C9748), β-glucanase (Sigma G4423), and *endo*-1, 4-β-xylanase (Sigma X2629) from *Trichoderma longibrachiatum* were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). The protein content of cellulase, glucanase and xylanase was determined as 139.76 ± 0.99 mg/g powder, 98.62 ± 0.56 mg/g powder, and 153.58 ± 1.25 mg/g powder, respectively. Cellulase and glucanase activity were determined to be 6.70 ± 1.06 FPU/g and 5.60 ± 0.45 FPU/g using whatman #1 filter paper as substrate, respectively. Xylanase activity was determined to be 35.50 ± 1.89 U/mg using beech wood as substrate. β-glucosidase (CAS: 9001-45-0) was bought from Jiangsu Ruiyang Biology Technology Co. Ltd. (Jiangsu, China). The protein concentration of β-glucosidase was determined to be 7.71 ± 1.29 mg/g powder, and its activity was 110 U/g powder.

2.2. γ-Irradiation pretreatment of lignocellulosic biomass

All tested lignocellulose feedstocks were put in a sealed small test casket (10 cm (width) × 10 cm (Length)) and irradiated at room temperature using a ^{60}Co-γ radiation source intensity of 9.99×10^{15} Bq in the Hunan Irradiation Center (Changsha city, Hunan province, China). The dose of γ-irradiation pretreatment on lignocellulose was selected at 800 kGy based on the data in our previous work, in which the procedures of γ-irradiation pretreatment were also described in detail (Liu et al., 2016). Corncob, reed stover, Eucalyptus hardwood and pine softwood were cut into ~5 cm small pieces, while *Camellia oleifera* shell was irradiated without any cut or grinding. The particle sizes of raw biomass subjected to irradiation were 1–5 cm. After irradiation pretreatment, the raw biomass was milled, the effect of irradiation pretreatment on the milling energy consumption, particle size distribution and viscosity of lignocellulose biomass was for the first time evaluated in this work. Furthermore, the influence of irradiation pretreatment on the solid loadings of biomass was examined for consequent hydrolysis, and it was also compared the biomass hydrolysis efficiency with or without addition of dilute acid as catalyst. The ultimate objectives of this work were to achieve the high sugars concentration of xylose and glucose streams from lignocellulose biomass by two steps hydrolysis assisting with irradiation pretreatment. The compositions of cellulose, hemicellulose and lignin of the tested experimental biomasses were analyzed before and after irradiation pretreatment (Seen in Fig. S2). It was observed from Fig. S2 that biomass compositions received slight change but not significant after irradiation pretreatment.

2.3. Hydrolysis process of irradiated lignocellulosic biomass

After irradiation pretreatment, the irradiated raw biomass was grounded and sieved by 60 meshes; the particle sizes of pretreated biomass were 250 μm. It was then successively subjected to thermal hydrolysis following acid hydrolysis for hemicellulose fraction, and enzymatic hydrolysis for cellulose fraction. The schematic overview of hydrolysis process of irradiated lignocellulosic biomass was shown in Fig. S1. In the first thermal step hydrolysis, 90 g irradiated biomass was thermally hydrolyzed in a 500 mL bioreactor with the total volume of 300 mL hot water (solid loadings of 30%, w/v) at 160–170 °C for 1 h. Then, the autoclaving sludge was separated to obtain thermal hydrolysate (278 mL) and the solid residue (54 g) through filtering. The thermal hydrolysate mainly consisted of monomeric and oligomeric xylose derived from hemicellulose fraction, as well as small amount of monomeric and oligomeric glucose derived from cellulose fraction. For the second step acid hydrolysis, the liquid phase was continuously hydrolyzed with 1.2% (w/v) sulfuric acid (H_2SO_4) at 120 °C for 1 h to generate monomer soluble sugars enrichment in xylose. This procedure was so named as xylose stream production from hemicellulose of biomass. The ultimate volumes of hydrolysate in one thermal and one acid step were about 246 mL.

Approx. 54 g of residual solid after thermal hydrolysis of 90 g irradiated biomass was obtained and its compositions were: ~54% glucan, ~3.5% xylan, and 35% lignin. In the case of glucose stream form cellulose fraction of biomass, the solid residue was enzymatically hydrolyzed in 250 mL glass flask filled with solid loadings of 40% (w/v, equals to 29 wt.%), 0.05 M citrate buffer