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Comprehensive utilization of glycerol from sugarcane bagasse pretreatment to fermentation

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HIGHLIGHTS

- Glycerol pretreatment was preformed prior to biomass fast pyrolysis.
- After pretreatment the glycerol could be an economic substrate for fermentation.
- Glycerol pretreatment could enhance the yield of levoglucosan in fast pyrolysis.

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ABSTRACT

In this study, the effects of glycerol pretreatment on subsequent glycerol fermentation and biomass fast pyrolysis were investigated. The liquid fraction from the pretreatment process was evaluated to be feasible for fermentation by *Paenibacillus polymyxa* and could be an economic substrate. The pretreated biomass was further utilized to obtain levoglucosan by fast pyrolysis. The pretreated sugarcane bagasse exhibited significantly higher levoglucosan yield (47.70%) than that of un-pretreated sample (11.25%). The promotion could likely be attributed to the effective removal of alkali and alkaline earth metals by glycerol pretreatment. This research developed an economically viable manufacturing paradigm to utilize glycerol comprehensively and enhance the formation of levoglucosan effectively from lignocellulose.

1. Introduction

Currently, due to the forthcoming scarcity of conventional resources the production of fuels from biomass has received a significant interest (Kumagai et al., 2015; Zheng et al., 2015). Sugars are one of the natural intermediates in the biological and chemical conversion of biomass, but access to sugars is hindered by the recalcitrance of plant cell wall. Hydrolysis is a widespread process for breaking down cellulose and hemicellulose into their constituent sugars for fermentation and chemical conversion. The most common approach to recover sugars from biomass is acid or enzymatic hydrolysis. Nonetheless, the hazard of handling acid and the complexity of recycling it are potential obstacles to acid hydrolysis. The low rate of hydrolysis and the costs of pretreatment and enzyme have limited the adoption of enzymatic hydrolysis (Binder and Raines, 2010). Although acid hydrolysis and enzymatic saccharification have received most of attention, fast pyrolysis is a little-explored alternative method for the production of fermentable sugars from biomass (Rover et al., 2014). Fast pyrolysis can depolymerize cellulose to levoglucosan (1,6-anhydro-β-D-glucopyranose) in a very short residence time at 450–550 °C without the utilization of enzymes or catalysts. Levoglucosan yields from crystalline cellulose can be as high as 61% (Jiang et al., 2015a). Pyrolysis has a significant economic advantage and a lower capital cost compared with biochemical pathways (Anex et al., 2010). It has been proven that levoglucosan can be served as fermentation substrate for itaconic acid, citric acid and ethanol production (Nakagawa et al., 1984; Zhuang and Zhang, 2002; Layton et al., 2011). Nevertheless, biomass fast pyrolysis produces a much lower amount of levoglucosan compared to the theoretical yield based on cellulose fraction and pretreatment prior to pyrolysis is essential (Carpenter et al., 2014). The ash of biomass has significant catalytic effects on the pyrolysis process. Some effort has been made for demineralization to achieve a high yield of levoglucosan from lignocellulose such as hot water washing and dilute acid pretreatment (Chang et al., 2013; Dalluge et al., 2014; Jiang et al., 2015a).

Glycerol is the main byproduct of fatty acid methyl ester process, taking up 10% of the total biodiesel output (Yang et al., 2012). It is projected that the world biodiesel market will reach

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37 billion gallons by 2016, which implies that approximately 4 billion gallons of crude glycerol can be produced (Anand and Saxena, 2012). The large-scale production makes glycerol readily available and cheap. Although there is an extensive utilization of high purity glycerol in the food, cosmetic and pharmaceutical industries, it is technically difficult and costly to obtain pure glycerol. One of the promising applications of glycerol is the production of 1,3-propanediol, ethanol, 2,3-butanediol, as well as acetic acid and H_2 by microorganisms fermentation (Wu et al., 2011). Furthermore, glycerol has a boiling point of 290 °C, which makes it suitable for atmospheric reaction. Recently, glycerol pretreatment as a prerequisite for effective enzymatic saccharification to produce fermentable sugars has gained momentum (Zhang et al., 2013; Kurosawa et al., 2015). The pretreatment of sugarcane bagasse with microwave associated to glycerol turned out to be an effective method to improve digestibility of biomass structure and previous research indicated that aqueous glycerol pulping could lead to high lignin removal from lignocellulose (Sun and Chen, 2008; Moretti et al., 2014). Most of the structural ash in biomass was located in the cross-linking structure of lignin. Unsatisfactorily, little research focused on the glycerol pretreatment for overcoming biomass recalcitrance and consequently improving levoglucosan production (Zheng et al., 2015). Meanwhile, most of research ignored the utilization of glycerol after glycerol pretreatment.

Herein, the present work was aim to investigate the influences of glycerol pretreatment on subsequent glycerol fermentation and biomass fast pyrolysis. Glycerol pretreatments of sugarcane bagasse and microcrystalline cellulose were performed. Then the fermentability of liquid fraction from the pretreatment process was tested and the constituent of product from fast pyrolysis of pretreated biomass was analyzed.

2. Methods

2.1. Material

Sugarcane bagasse was obtained from Dehong in Yunnan, China. It was ground and sieved to the particle size range 0.11–0.18 mm and then dried in an oven at 105 °C for 12 h. Microcrystalline cellulose (Fluka, Avicel® PH-101), levoglucosan, 2,3-butanediol, acetoin, acetic acid, ethanol, furfural and 5-hydroxymethylfurfural (5-HMF) were purchased from sigma (Shanghai). Glycerol (99%) was purchased from Tianjin Fuyu Fine Chemical Co. Ltd. Glucose, xylose, mannose, galactose, arabinose and cellobiose (purity > 98%) were purchased from Bomei Biotech Co., Ltd., Heifei, Anhui. The strain *Paenibacillus polymyxa* (CICC 10010), was purchased from China Center of Industrial Culture Collection (Beijing).

2.2. Chemical composition of lignocellulose

Characterization of biomass was carried out following the National Renewable Energy Laboratory (NREL) procedure (Sluiter et al., 2008). Firstly, the extractives in the biomass were removed by water and ethanol extraction. Then 0.3 g of the extractives-free sample was weighted and treated with 72% H_2SO_4 for 1 h at 30 °C. Subsequently, the mixture was diluted with 84 mL deionized water and further hydrolyzed at 121 °C for 1 h in an autoclave. The hydrolysate was filtered to separate the filtrate and solid residue. The sugars in the neutralized filtrate were analyzed by performance liquid chromatography (HPLC, Waters 2695) and Aminex HPX-87P column (Bio-Rad, USA) at 80 °C with deionized water as mobile phase with a flow rate of 0.4 mL/min. The filtrate was also analyzed by an ultraviolet (UV)–Visible spectrophotometer to determine acid soluble lignin. The residue after acid hydrolysis was collected for the measurement of the content of ash and acid insoluble lignin.

2.3. Elemental analysis

Carbon (C), hydrogen (H) and nitrogen (N) contents were measured with an Organic Elemental Analyzer (Vario EL cube, Hanau, Germany). The contents of potassium (K), sodium (Na), calcium (Ca) and magnesium (Mg) were determined by an inductively coupled plasma optical emission spectrometry (ICP-OES) (Optima 8000, PerkinElmer, USA). For ICP-OES analysis, the 0.3 g sample was digested for about 6 h in the 4 mL mixed acids of concentrated HNO_3 and $HClO_4$ (3:1, v/v). Then the digested sample was diluted to 20 mL with deionized water. In ICP-OES analysis, nebulizer flow was 1.5 L/min. The flush time, delay time and wash time were 10 s, 40 s, and 40 s, respectively. Five standard solutions of each metal were prepared and analyzed to generate external calibration curves for quantitative determination.

2.4. Glycerol pretreatment

Previous work on glycerol pretreatment established the optimum experimental conditions as 150 W for 18 min in a microwave reactor (Zheng et al., 2015). Based on this, glycerol pretreatment experiments in this work were performed at 200–240 °C for 1 h. The pretreatment were prepared by adding 10 g sugarcane bagasse or cellulose in a 250 mL flask containing 100 g glycerol. Then, without removing air, the flask was sealed with a cork and placed in oil bath with stirring at 200 rpm. Afterwards, the pretreated biomass was filtered, washed thoroughly with 1000 mL deionized water at 90 °C to remove glycerol and then freeze dried for 24 h (EYELA 1200 freeze dryer, Tokyo Rikakikai Co., Ltd.). The biomass sample was dried in an oven at 105 °C for 6 h to constant weight before subsequent structure characterization and fast pyrolysis. The liquid fraction of the pretreatment process containing high concentration of glycerol was diluted and prepared for fermentation. The filtrate was analyzed by HPLC and Aminex HPX-87H column (Bio-Rad, USA) at 60 °C with 0.005 M H_2SO_4 as mobile phase with a flow rate of 0.6 mL/min. Concentrations of sugars (such as glucose and xylose), organic acids were measured by refractive index detector. Furfural and 5-HMF were analyzed by an UV detector at 280 nm.

2.5. Fermentation of glycerol

The culture was maintained on Luria–Bertani agar slant at 4 °C. The seed medium was composed of glucose (10 g/L), peptone (10 g/L), beef extract (10 g/L) and NaCl (5 g/L) with the *pH* of 6.8–7.0. For seed preparation, a full loop of strain from a fresh slant tube was inoculated in a 250 mL flask containing 50 mL fresh seed medium in a rotary shaker at 200 rpm for 12 h at 30 °C. Seed culture (5%, v/v) was then inoculated into the fermentation medium. A serious of fermentation experiments were carried out in the following medium: glycerol (25 g/L), peptone (5 g/L), beef extract (5 g/L), K_2HPO_4 (12.55 g/L), KH_2PO_4 (3.9 g/L). The substrates were sterilized at 115 °C for 15 min. All the fermentation process occurred in the 250 mL flask loaded with 50 mL sample solutions at 200 rpm and 30 °C. The samples were withdrawn at 12 h, centrifuged at 10,000 rpm for 5 min and the supernatant was analyzed. Concentrations of glycerol, 2,3-butanediol, acetoin, acetic acid and ethanol were measured by HPLC fitted with a refractive index detector and Aminex HPX-87H column (Bio-Rad, Hercules, CA) at 60 °C with 0.005 *M* H_2SO_4 as mobile phase at a flow rate of 0.6 mL/min. Each product was calibrated by its standard solutions with four different concentrations (*e.g.*, 1, 2, 3, 4 g/L). All