Biomass and Bioenergy 94 (2016) 39–45

Contents lists available at ScienceDirect

Biomass and Bioenergy

journal homepage: http://www.elsevier.com/locate/biombioe

Research paper

Acidified glycerol pretreatment for enhanced ethanol production from rice straw

Ly Thi Phi Trinh [a, b], Jae-Won Lee [c, *], Hong-Joo Lee [a]

[a] Department of Bioenergy Science and Technology, Chonnam National University, 77 Yongbong-ro, Buk-gu, Gwangju, 500-757, Republic of Korea
[b] Research Institute for Biotechnology and Environment, Nong Lam University, Hochiminh City, Viet Nam
[c] Department of Forest Products and Technology, Chonnam National University, 77 Yongbong-ro, Buk-gu, Gwangju, 500-757, Republic of Korea

ARTICLE INFO

Article history:
Received 17 June 2015
Received in revised form
2 August 2016
Accepted 25 August 2016

Keywords:
Ethanol
Glycerol
Optimization
Pretreatment
Rice straw

ABSTRACT

Rice straw was pretreated using an industrial grade glycerol for ethanol production. The pretreatment was conducted at 130–210 °C for 1–24 h with 5% solid loading. The glucan content in the regenerated rice straw increased with increasing pretreatment temperature and time. The production of fermentable sugars initially increased as the pretreatment temperature and reaction time increased, but then decreased somewhat at the higher temperatures and with longer reaction duration. The highest amount of reducing sugar produced by the enzymatic hydrolysis was achieved at 190 °C for 10 h with 5% solid loading, optimal condition for the glycerol pretreatment of rice straw. Furthermore, it was observed that glycerol pretreatment with the addition of HCl improved the digestibility of fermentable sugars by 4–5 times that of untreated samples. Fermentation of hydrolysates resulted in an ethanol yield of 0.44 g/g sugar, corresponding to a theoretical yield of 84.3%. It was concluded that acidified glycerol is one of the good candidates of the organic solvent for the pretreatment of lignocellulosic biomass.

1. Introduction

Ethanol, which can be biologically produced from a variety of biomass, is accepted as an alternative to fossil fuels. Recently, there has been increasing interest over the utilization of non-starch feedstock such as lignocellulosic biomass for the production of ethanol [1,2]. Lignocellulosic biomass is an abundant, inexpensive, and renewable source of sugars, which is considered as a desirable feedstock for the sustainable production of liquid fuels and chemical products through the biorefinery process [3].

Rice straw is one of the largest lignocellulosic biomass sources in the world, and is a common as well as abundant agricultural waste in Asian countries. It contains cellulose (24–34%), hemicellulose (19–29%), lignin (5–11%), and crude ash (10–22%). Rice straw has drawn attention for its potential use in the production of biofuel, since its high content of cellulose and hemicellulose can be readily hydrolyzed into fermentable sugars [4–6].

In general, lignocellulosic biomass is composed mainly of cellulose, hemicellulose, and lignin. The cellulose and hemicellulose are densely packed by layers of lignin, resulting in a crystalline structure that is highly recalcitrant to chemicals or enzymes [7]. This recalcitrance is largely responsible for the high cost of the bioconversion of lignocellulosic biomass. Cellulose can be enzymatically hydrolyzed to the glucose molecules that are then subsequently fermented to ethanol. However, the high crystalline structure of rice straw hinders the bioconversion process in the enzymatic saccharification step. Therefore, rice straw first needs to be pretreated in order to decrease the crystallinity of cellulose, increase the biomass surface area, remove hemicellulose, and break the lignin structures [8]. Through pretreatment, cellulose can be more accessible to enzymes, allowing the conversion of carbohydrate polymers into fermentable sugars to achieve more rapidly and with more yields. Thus, the pretreatment of lignocellulosic biomass is one of the most important processes in ethanol production [9,10].

Many pretreatment technologies have been proposed and developed to overcome the recalcitrance of the lignocellulosic biomass and to enhance the rate and yield of enzymatic saccharification of the lignocellulosic biomass [11–13]. These methods are classified as physical, chemical, biological, and multiple or combinatorial ones. Among the chemical methods, organosolv

* Corresponding author. Department of Forest Products and Technology, College of Agriculture and Life Sciences, Chonnam National University, 77 Yongbong-ro, Buk-gu, Gwang-ju 500-757, Republic of Korea.
E-mail address: ljw43376@chonnam.ac.kr (J.-W. Lee).

http://dx.doi.org/10.1016/j.biombioe.2016.08.017
0961-9534/

pretreatment using organic solvents has gained increased attention because it produces less fermentation inhibitors and is more environmentally friendly than acid pretreatment. In addition, organic solvent pretreatment primarily removes hemicellulose and lignin, thus increasing the rate of enzymatic hydrolysis of cellulose [14]. The organic solvents used include solvents with low boiling point (ethanol, methanol) and high boiling point (ethylene glycol, glycerol, tetrahydrofurfuryl alcohol) solvents, and other organic compounds such as dimethyl sulfonics, ethers, and ketones for solubilizing the lignin [10,15].

Of organic solvents, glycerol, which produces 10% of the products in biodiesel production, is widely available at low cost. Being a high boiling point and non-toxic organic solvent, glycerol increases the pretreatment reaction rate, allowing high temperature processing, and its reaction can occur at atmospheric conditions [16–18]. In addition, organosolv pretreatment is usually carried out using strong inorganic acid catalyst, such as HCl and H_2SO_4, in order to hydrolyze the lignin bonds in the biomass [15]. As far as we know, acidified glycerol pretreatment of rice straw was has not been widely studied, even though it is one of the most abundant agricultural residues.

In the present study, the influence of operation conditions on the bioconversion efficiency of glycerol-pretreated rice straw was investigated. Glycerol pretreatment was examined in the temperature range of 130–210 °C for reaction times over 1–24 h, with 5% solid loading. Furthermore, the addition of HCl under the optimum pretreatment conditions was considered to improve the bioconversion efficiency in the acidified organosolv pretreatment. Using optimal enzymatic hydrolysis condition, rice straw was pretreatment and fermentation of regenerated biomass was carried out.

2. Materials and methods

2.1. Glycerol pretreatment of rice straw

Rice straw was obtained from an agronomy research field of Chonnam National University, Gwangju, South Korea. The rice straw that had been harvested in September 2013, was first air-dried, milled, and screened to a size of 295–833 μm (20–48 meshes) prior to pretreatment.

The milled rice straw was then pretreated with glycerol (industrial grade, 70% purity; Daejung, Incheon, South Korea) in a 100 mL flask immersed in a silicone oil bath (EYELA RCX-1000S, Tokyo Rikakikai Co., Tokyo, Japan), with magnetic stirring at 300 rpm. The pretreatment was carried out at wide temperature range and reaction time, 130–210 °C and 1–24 h, respectively, to investigate the influence of operating condition on the pretreatment performance and bioconversion process at the fixed biomass loading of 5%. Furthermore, enhancement of the pretreatment using HCl (0.1–1%) was considered after the pretreatment condition had been optimized in terms of temperature and time for acidified glycerol pretreatment.

After pretreatment, 50 mL of distilled water was added to the reaction mixture prior to magnetic stirring at 500 rpm for at least 1 h at room temperature. The precipitates were separated by vacuum filtration, collected, and washed with distilled water to remove any residual glycerol and HCl from the surface of the regenerated biomass.

Wet regenerated biomass was directly used for enzymatic saccharification, while the other fraction of the solid residue was dried in an oven at 75 °C for 24 h for characterization. The recoveries of solids and carbohydrates were estimated according to the following equations:

$$\text{Solid recovery}(\%) = \frac{\text{Mass of regnerated biomass}}{\text{Mass of raw biomass}} \times 100 \quad (1)$$

$$\text{Carbohydrate recovery}(\%) = \frac{\text{Carbohydrate in regenerated biomass}(\%) \times \text{solid recovery}(\%)}{\text{Carbohydrate in the nativve biomass }(\%)} \quad (2)$$

2.2. Enzymatic hydrolysis of regenerated biomass

Enzymatic hydrolysis of the pretreated and untreated rice straw was carried out in a conical tube, containing 5% biomass (w/w, dry weight basis) in 0.05 M sodium citrate buffer (pH 4.8), using an enzyme cocktail of cellulase (17.5 FPU/g), β-glucosidase (6.25 CBU/g), and xylanase (25 FXU/g) with 2% sodium azide, at 50 °C for 72 h in a shaking incubator at 150 rpm. The enzymes were provided by Novozyme (Bagsværd, Denmark). After enzyme saccharification, the supernatant was diluted with deionized water prior to high performance liquid chromatography (HPLC) analysis. All the enzymatic hydrolysis experiments were conducted in triplicate. The sugar digestibility of the biomass and the yield of fermentable sugars were calculated using the following equations:

$$\text{Glucan digestibility}(\%) = \frac{\text{Glucose produced via enzymatic hydrolysis} \times 0.9}{\text{Glucan in the native biomass}} \times 100 \quad (3)$$

$$\text{Xylan digestibility}(\%) = \frac{\text{Xylose produced via enzymatic hydrolysis} \times 0.88}{\text{Xylan in the native biomass}} \times 100 \quad (4)$$

$$\text{Fermentable sugar yield}(\%) = \frac{\text{Glucose and xylose produced via enzymatic hydrolysis}}{\text{Glucose and xylose in the native biomass}} \times 100 \quad (5)$$

2.3. Fermentation of hydrolysates

Fermentation of hydrolysates was executed with the separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF) techniques. For SHF, hydrolysates separated from solids by filtration were supplemented with nutrients for cell growth, including 1 g/L KH_2PO_4, 0.5 g/L $MgSO_4$, 5 g/L urea, and 5 g/L yeast extract. Then, the mixture was adjusted to a pH 6.0 using sodium hydroxide and autoclaved at 121 °C for 15 min. After the addition of 2 g/L dry cell weight of *Pichia stipitis* CBS 6054, fermentation was carried out in at a shaking incubator at 30 °C and 150 rpm for 72 h.

For SSF of the regenerated rice straw, a mixture containing rice straw (5% biomass, dry weight basis) and 0.05 M sodium citrate buffer (pH 6) was supplemented with the same nutrients as used for SHF. The mixture was autoclaved at 121 °C for 15 min prior to addition of the enzyme cocktail of cellulase (17.5 FPU/g), β-glucosidase (6.25 CBU/g), xylanase (25 FXU/g), and 2 g/L dry cell weight of *Pichia stipitis* CBS 6054. The SSF was performed at 30 °C and 150 rpm in a shaking incubator for 72 h.