Bioresource Technology 235 (2017) 12–17

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Evaluation of the solvent water effect on high solids saccharification of alkali-pretreated sugarcane bagasse

Yunyun Liu [a,b], Bin Zhang [a], Wen Wang [b], Minchao He [b], Jingliang Xu [b], Zhenhong Yuan [b,*]

[a] College of Mechanical and Electrical Engineering, Shaanxi University of Science & Technology, Xi'an 710021, China
[b] Key Laboratory of Renewable Energy, Guangzhou Institute of Energy Conversion, Chinese Academy of Sciences, Guangzhou 510640, China

HIGHLIGHTS

- Oleyl alcohols were used to partially substitute the water to investigate its effect.
- Insoluble solids was found in batch hydrolysis with diverse ratio of water was replaced.
- Fed-batch hydrolysis was carried out with solvent water/oleyl alcohol ratio of 3:1.
- Glucose production was found to be less than pure water hydrolysis system.
- Solids effect was related to the solvent water content.

ARTICLE INFO

Article history:
Received 14 February 2017
Received in revised form 13 March 2017
Accepted 14 March 2017
Available online 18 March 2017

Keywords:
Alkali-pretreated SCB
Solvent water
Oleyl alcohol
Fed-batch strategy
Enzymatic saccharification
High solids loading

ABSTRACT

Solvent water is an essential factor for high solids enzymatic hydrolysis. To investigate its effect on substrate conversion efficiency in high solids hydrolysis of sugarcane bagasse (SCB), oleyl alcohol was used to partially substitute the solvent water. The results in batch hydrolysis tests in which diverse ratio of solvent water was replaced found that the majority of the substrate was insoluble. Then high solids fed-batch hydrolysis with the reaction solution mixed with solvent water and oleyl alcohol in the ratio of 3:1 (solids concentration correspond to 24% (w/v)) was carried out at the final real solids loading of 18% (w/v). The produced sugars were found to be less than pure water system, which indicated that water played a significant role in high solids hydrolysis process, and solids effect was related to the solvent water content.

1. Introduction

Biofuel derived from the largest renewable lignocellulosic biomass offers the potential to partially substitute the fossil fuels and mitigate the clime change. It has been considered as one of the promising solution to world energy crisis and environmental pollution problems. Due to the complex structure and composition of the cellulosic biomass, breakthrough technologies for biofuel production still need to overcome barriers to reach a cost-effective level (Jørgensen et al., 2007a,b; Van Dyk and Pletschke, 2012; Hendriks and Zeeman, 2009). Enzymatic hydrolysis process has been studied to depolymerize the polysaccharides contained in lignocellulose into monosaccharides and further convert them to biofuels, with a more focus on operating at high solids loading (Caspeta et al., 2014; Wang et al., 2012; Mohagheghi et al., 1992; Liu et al., 2015a,b).

High solids enzymatic hydrolysis with the aim to achieve high concentration of the utilized sugars to increase the final ethanol or biogas yield is crucial for large scale development of biofuel production (Koppram et al., 2014; Roche et al., 2009). Operating at high solids level where initially there is generally no significant amounts of free water present in system can lower the cost of downstream processing and waste water consumption. Its system provides high productivity as results of lower energy and water input, as well as a reduction in the size of the equipment needed, and thus, saves the operating capital costs (Jørgensen et al., 2007a,b; Liu et al., 2015a,b; Du et al., 2014; Luiz et al., 2015). However, processing at high initial solids loading involves technical barriers. Viscosity of the slurry is usually very high, the problems of mass transfer limitation and mixing difficulties become more

* Corresponding author.
E-mail address: yuanzh@ms.giec.ac.cn (Z. Yuan).

http://dx.doi.org/10.1016/j.biortech.2017.03.088
0960-8524/

prominent. Meanwhile, continuous accumulation of the end products and inhibitors causing enzymes and yeast cells to not function optimally, and thus reduce the rate of substrate hydrolysis (Ramachandriya et al., 2013; Zhang et al., 2009; Paulova et al., 2014; Geng et al., 2015; Zhao et al., 2013).

To overcome these technical barriers, fed-batch hydrolysis schemes, as an alternative method of achieving high solids loading has been employed with its various advantages (Hoyer et al., 2013; Hodge et al., 2009). Its feeding regime allows time for the solids to liquefy before loading new substrate. The mixing and diffusion problems can be minimized for its initial viscosity is lower, and thus the substrate conversion efficiency can be substantially improved. Although methods for improving high solids enzymatic hydrolysis process have been exploited (Li et al., 2014; Geng et al., 2015), the decreased substrate conversion efficiency with increasing solids loading still presented a challenge to partially offset the advantages of running at high solids loading. Here, the decrease in yield at high solids condition is referred to as solids effect. The specific mechanisms responsible for the decreasing hydrolytic yield are still uncertain.

The possible mechanisms behind the solids effect have been divided into several categories. Since many of these causes and effects seem to impact other properties, it is difficult to determine the factor that has the largest influence on the decreasing efficiency.

Working with high solids low water system may directly affect enzyme performance. The lack of available water is one of the major challenges. Water is essential to mass transfer and lubricity to effective hydrolysis of substrate. Previous works have reported the mechanisms possible for solids effect including water related factors (Modenbach and Nokes, 2013). Roberts et al. (2011) had investigated the water interaction changes in substrate suspensions on mass transfer resistances affecting hydrolysis rates. The studies were conducted with bacterial cellulose, a substrate that is essentially pure cellulose without maintaining a constant system viscosity. Kristensen et al. (2009) studied four mechanisms that possibly contribute to the so-called solids effect in which water concentration was analyzed with filter paper, which is also essentially a pure cellulose substrate.

Based on the above works, this paper aimed to investigate the role of water content in high solids system and analyze if the linearly decreased substrate conversion efficiency with solids loading increasing was related to the lower water content. By partially substituting solvent water with glycerol and sorbitol, the two polyols chosen were known as compatible solutes therefore they could not influence the activity of enzymes (Gervais et al., 1988; Modenbach and Nokes, 2013), it is possible to alter water-substrate ratio with a more or less constant system viscosity. The substrate used in this study was obtained from alkali-pretreated biomass SCB, and fed-batch hydrolysis was applied to achieve high solids loading.

2. Materials and methods

2.1. Raw materials

SCB was provided by Guangxi Fenghao Sugar Co., Ltd. It was pre-milled and screened, and the fractions between 40 and 60 meshes were collected for alkali-pretreatment. Cellulase complex Cellic CTec2 donated by Novozymes A/S, (Bagsaevrd, Denmark) with the activity of about 200 FPU/mL, measured by the description of IUPAC (International Union of Pure and Applied Chemistry) (Ghose, 1987), was used to hydrolyze the substrate. Chemical reagents such as sorbitol, glycerol, acetic acid and sodium acetate were purchased from Sigma-Aldrich Co. LLC.

2.2. Alkali pretreatment

SCB was mixed with 0.5 M NaOH solution (solid to liquid ratio is 1:20), and subjected to a round-bottom flask at 80 °C heated by water bath for 2 h with stirring, as previously described (Liu et al., 2016). After pretreatment, the solid fraction was separated by filtering and then washed with tap water until a neutral pH. The obtained residual fraction was dried at 60 °C. After delignification, the substrate became fractured and showed a rough structure with obvious cracks, ravines and holes. These changes were conducive to the subsequent enzymatic hydrolysis. All experiments were carried out three times, and the given numbers are the mean values.

2.3. Enzymatic hydrolysis

Enzymatic hydrolysis was carried out with 18% (w/v) initial solids loading. In order to investigated the effect of solvent water content on the substrate conversion efficiencies at high solids loading. Various amounts of water was replaced by oleyl alcohol i.e. glycerol or sorbitol respectively, that do not directly affect the function of the enzymes and maintain the constant system viscosities. By substituting part of the solvent water, the solids content was equivalently increased. Hydrolyses were performed at 50 °C, 150 rpm with cellulase loading of 10 FPU/g substrate in 150 mL Erlenmeyer flasks under orbital agitation for 96 h. The final reaction volume mixed with 9g SCB was always 50 mL consisted of 50 mL acetate buffer (corresponding to 18%(w/v) solids loading), 37.5 mL acetate buffer plus 12.5 mL/g oleyl alcohol (24%(w/v) solids loading), 30 mL acetate buffer plus 20 mL oleyl alcohol (30%(w/v) solids loading). After sampling, sugars concentration was analyzed by HPLC.

Fed-batch hydrolysis was initiated with 12% (w/v) solids loading, the viscosity of the slurry measurement was simultaneously started at specific time points during the hydrolysis. After the viscosity was assumed to reach a steady-state value, i.e., the viscosity was decreased below the maximum measuring range of the viscometer, new substrate was quickly added to avoid contamination. At each desired time, the solution was sampled for sugars assay.

The conditions used for enzymatic hydrolysis are described in Tables 1 and 3. The hydrolysis yields were calculated in relation to the amount of glucan (cellulose) and xylan present in the substrate. Glucan and xylan conversion rates were estimated by the following equations:

$$\text{Glucan conversion rate} = \frac{\text{Glucose produced (g)} \times 0.9}{\text{Glucan amount in enzymatic substrate}} \quad (1)$$

$$\text{Xylan conversion rate} = \frac{\text{Xylose produced (g)} \times 0.88}{\text{Xylan amount in enzymatic substrate}} \quad (2)$$

2.4. Analytical methods

2.4.1. Composition assay

The composition of the SCB before and after pretreatment was determined in duplicate according to the standardized methods of the National Renewable Energy Laboratory (NREL, Golden, CO, USA) (Sluiter et al., 2008).

2.4.2. Sugar assay

Sugars (glucose, xylose, cellobiose, and arabinose) concentrations were analyzed by HPLC (Waters model 2695, Wilford, USA) using a Shodex sugar SH-1011 column coupled with a refractive