ETHANOL PRODUCTION FROM COTTON GIN WASTE BY SIMULTANEOUS SACCHARIFICATION AND FERMENTATION

Jiacheng Shen
Foster A. Agblevor
Virginia Tech
Blacksburg, VA

Abstract

Cotton gin waste (CGW) and recycled paper sludge (RPS) are two wastes generated by cotton and paper making industries, respectively. We have developed a simultaneous saccharification and fermentation (SSF) process to convert the mixture of two wastes to bio-ethanol, which also simultaneously solves the waste dispose problem in these industries. Compared to the traditional separate hydrolysis and fermentation (SHF) process, the SSF has the following advantages: (1) less inhibition to enzyme; (2) less capital costs; and (3) higher productivity.

In our developed process, the mixture of CGW (75 wt%) and RPS (25 wt%) was first pretreated by steam explosion at the severity factor of 3.83. The steam-exploded mixture was hydrolyzed and fermented simultaneously in a reactor with *Saccharomyces cerevisiae* at pH 4.8 and temperature 36^{o}C. Two enzyme loadings (Novozymes NS50052) of 10 and 20 FPU/g substrate were used. The experiments showed that the maximum yield (g ethanol/g substrate), theoretical yield (%), and productivity (g ethanol/g substrate/hour) were 0.184, 75.4%, and 0.00383, respectively, after 48-hour SSF at enzyme loading 10 FPU/g substrate. When the enzyme loading was increased to 20 FPU/g substrate, the concentrations of glucose, ethanol, and xylose were obviously higher that those at 10 FPU/g substrate in the early period of SSF, but the final ethanol concentrations for the two enzyme loadings were almost the same.

Introduction

In the south areas of USA, cotton industry produces a large mass of cotton gin waste (CGW). It was estimated that about 2.04 million tons of cotton gin waste were produced annually by the US cotton industry [Holt et al. 2000]. These CGW can be used to produce about 383 million-liter ethanol, which is equivalent to about 2.74% of USA ethanol production in 2006. Similarly, about 4 million dry tons of recycled paper sludge (RPS) were produced annually by the papermaking industry [Glenn, 1997]. Particularly in southeastern Virginia, cotton cultivation is of growing importance. Over the past decade, the cotton cultivation has grown from 3,000 acres per year to over 100,000 acres and six cotton gins have been established in the area. The cotton gin waste generated from these factories must be disposed to meet EPA regulations. However, due to the small size of all the six cotton gins, it is becoming difficult for them to meet EPA clean air rules using combustion technology to dispose of the wastes. A simultaneous saccharification and fermentation (SSF) process has been developed in our laboratory to convert the cotton gin waste to bio-ethanol, while simultaneously to solve the dispose of the cotton gin waste. Compared to the traditional separate hydrolysis and fermentation (SHF) process, the SSF has the following advantages: (1) less inhibition to enzyme because the sugar monomers can be directly utilized to form ethanol after they are depolymerized by the enzyme. Hence, SSF can reduce the inhibitory effect of glucose and cellobiose on the enzymatic activities, and enable to hydrolyze substrates more effectively than SHF; (2) lower capital costs in SSF than in SHF because only one process unit is required; and (3) higher productivity (product per time per volume) for SSF than SHF, resulting from SSF shorter operating time. However, the disadvantage of SSF is that it cannot be operated under optimal conditions for both hydrolysis and fermentation simultaneously.

The overall goal of this study was to convert the mixture of CGW and RPS into bioethanol. The specific objectives were

1) To analyze the components of the steam-exploded mixture of 75% CGW and 25% RSP.
2) To investigate effect of enzyme loading on ethanol concentration produced from the mixture of CGW and RPS in the simultaneous saccharification and fermentation.
3) To study the effects of by-products in SSF on ethanol yield.

Materials and Methods

Materials

Cotton gin waste and recycled paper sludge were obtained from the MidAtlantic Cotton Gin, Inc. (Emporia, VA), and International Paper (Frankin, VA), respectively. The Novozyme NS50052 enzyme was supplied by Novozyme North America Inc. (Research Triangle, NC). Its actual activity determined in our laboratory by filter paper method was 97 PFU/g [Ghose, 1987]. The enzyme concentrations used in the experiments were 2-4 g/l (or enzyme loadings 10-20 FPU/g substrate). *Saccharomyces cerevisiae* was used in the experiments. The growth medium for the seed culture for *Saccharomyces cerevisiae* was YM broth, which consists of yeast extract (0.3%), malt extract (0.3%), peptone (0.5%), and glucose (1.0%). The medium for fermentation included yeast extract (0.3%), $(NH4)_2\ HPO_4$ (0.25 g/l), and $MgSO_4 7H_2O$ (0.025 g/l).

Methods

Pretreatment of Feedstock

The mixture of 75 wt% CGW and 25 wt% RPS were pretreated by steam explosion for 2 minutes at 220°C in a 25-l batch reactor located at the Recycling Laboratory, Thomas M. Brooks Forest Products Center, Blacksburg, VA. The severity factor for the retention time and temperature was calculated to be 3.83 according the concept of the reaction ordinate [Overend and Chornet, 1987].

$$\log(R_0) = \log\left[\int_0^{t_s} \exp\left(\frac{T_r - T_b}{14.75}\right) dt\right] = \log\left[t_s \exp\left(\frac{T_r - T_b}{14.75}\right)\right] \qquad (1)$$

where R_0 is the reaction ordinate, t (minute) is the residence time, T_r (°C) is the explosion temperature, and T_b is the basic temperature (100°C). The constant 14.75 is the activation energy assuming that the overall process is hydrolytic and obeys a first order kinetic law. In Eq. (1) it was assumed that the explosion temperature T_r was constant, *i.e.* the time for raising the material temperature to T_r was ignored during the steam explosion period.

Simultaneous Saccharification and Fermentation

Fresh colonies of *S. cerevisiae* from agar plate were inoculated in 500 ml Erlenmeyer flasks containing 200 ml of the growth medium (YM) concentration 21 g/l. The culture was grown in a shaker bath at 35°C and 200 rpm. The aliquot was taken and diluted 10 times after 18-hour inoculation, and the optical density (OD) of the diluted aliquot was measured at 600nm. When OD of the cell was greater than 0.35, the cells were harvested for fermentation. The suspension containing the cells were centrifuged at 6000 rpm for 5 minutes under sterile condition, the cells were separated from the liquid fraction, and washed by sterile deionized water for three times, and finally the cells were re-suspended in 10 ml of sterile deionized water.

2) Simultaneous saccharification and fermentation

The experiments of simultaneous saccharification and fermentation were conducted in a fermenter of 1 liter (Braun Biotech International, DCO3). At an initial time, the fermenter contained 0.5 l of the 0.05 M citric acid buffer, the 20 g (dried basis) of steam-exploded mixture of 75% CGW and 25% RPS, Novozyme enzyme, and *S. cerevisiae* from inoculation. Temperature of 36°C and pH of 4.8 of the slurry were maintained by a heater, and 2M hydrochloric acid and 2M sodium hydroxide. The agitation rate of fermenter was maintained 300 rpm. The aliquots (2 ml) of the broth were taken at a fixed intervals and centrifuged at 6000 rpm for 5 minutes to separate the substrate from the liquid fraction for measurement of component concentrations in the broth.

(3) Analytical Methods

Acid-insoluble lignin, carbohydrate and ash in the steam-exploded mixture were determined by the ASTM methods: E1721-95 and E1755-95, respectively. Ethanol, sugars, and by-products in the broth were measured by HPLC.

Results

Components of the Steam-Exploded Mixture of 75% CGW and 25% RPS

The cellulose, xylan, acid-insoluble lignin, ash, and other components in the steam-exploded mixture of CGW and RPS were about 43%, 5%, 24%, 20%, and 8%, respectively.

Effect of Enzyme Loading on Glucose, Xylose, and Ethanol Concentrations with Time

The highest ethanol concentration was about 6 g/l after 48-hour fermentation for enzyme loading of 10 FPU/g substrate. After that time, the ethanol concentration slightly decreased. When the enzyme loading was increased to 20 FPU/g substrate, the concentrations of glucose, ethanol, and xylose were higher than those for 10 FPU/g substrate in the early period, because of the faster catalytic reaction in the present of more enzymes. However, the final ethanol concentrations for the two enzyme loadings were almost the same.

Effect of Time on Some By-product Concentrations

The by-products produced in the SSF process included acetic acid, lactic acid, and glycerol. The acetic acid and lactic acid concentrations quickly increased after 35-hour fermentation. For example, acetic acid concentration increased about 10 times when fermentation time increased from 35 hours to 70 hours. This was probably because the microorganisms oxidized the ethanol into the organic acids. However, the glycerol concentration changed slightly.

Effect of Enzyme Loading on Maximum Ethanol Yield, Theoretical Yield, and Productivity

The maximum ethanol yield, theoretical yield, and productivity were 0.184 g ethanol/g substrate, 75.4%, 0.00383 g ethanol/g substrate/hour, respectively, after 48-hour SSF at enzyme loading 10 FPU/g substrate. However, the maximum ethanol yield, theoretical yield, and productivity at enzyme loading 20 FPU/g substrate were almost the same as those at 10 FPU/g substrate (Table 1).

Table 1 The maximum ethanol yield, theoretical yield, and productivity at two enzyme loadings

FPU/g subs	Max. yield g eth/g subs	Theor. yield %	Productivity g eth/g subs/h
10	0.184	75.4	0.00382
20	0.183	75.0	0.00380

Conclusions

The experiments showed ethanol could be produced from the carbohydrates in the steam-exploded mixture of 75% CGW and 25% RPS by SSF process. The maximum ethanol yield and theoretical yield were 0.184 g ethanol/g substrate and 75.4%, respectively. There was no significant effect of enzyme loading increase on the final ethanol concentration. A longer fermentation time had an adverse effect on the ethanol concentration, which increased by-product concentrations and decreased the ethanol concentration. This process demonstrated a new way to produce bio-ethanol from industrial wastes as well as solve the waste disposal problem.

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