Biomass and Bioenergy 91 (2016) 204–209

Contents lists available at ScienceDirect

Biomass and Bioenergy

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

Research paper

The addition of hydrolyzed rice straw in xylose fermentation by *Pichia stipitis* to increase bioethanol production at the pilot-scale

Ting-Hsiang Lin [a, b], Gia-Luen Guo [b], Wen-Song Hwang [b], Shir-Ly Huang [a, *]

[a] Department of Life Sciences, National Central University, No. 300, Jhongda Rd., Jhongli City, Taoyuan County 32001, Taiwan, ROC
[b] Chemical Division, Institute of Nuclear Energy Research, No. 1000, Wenhua Rd., Jiaan Village, Longtan Township, Taoyuan County 32546, Taiwan, ROC

ARTICLE INFO

Article history:
Received 19 March 2015
Received in revised form
5 February 2016
Accepted 13 May 2016

Keywords:
Hydrolysis
Lignocellulosic ethanol
Rice straw
Pentose fermentation
Pichia stipitis

ABSTRACT

The pretreatment of agricultural biomass by diluted acid is often employed to facilitate the release of monosaccharide for the subsequent enzyme hydrolysis for lignocellulosic ethanol production. However, furfural and hydroxymethylfurfural are usually generated and markedly decrease the yield of pentose fermentation during this pretreatment. In the present study, the enhancement of lignocellulosic ethanol production was successfully demonstrated at pilot scale with extra addition of hydrolyzed rice straw into pentose fermentation by *Pichia stiptis*. This way has resulted into the increase of *P. stiptis* cell mass was shown to play a positive role. The ethanol yield, 0.45 g_p/g_s, with the addition of hydrolyzed rice straw in hemicellulosic hydrolysate from plywood, bagasse and bamboo were increase 20–51% to demonstrate the applicability of this technology in a variety of lignocellulosic ethanol processes due to the efficient conversion of xylose.

1. Introduction

Fossil fuels with versatile applications are limited in supply and are a non-renewable resource. The worldwide consumption of fossil fuels has resulted in noticeable global warming and environmental problems [1]. The development of alternative fuels is a key to solve the problems derived from the use of fossil fuels. Lignocellulosic ethanol produced from non-food biomass, such as agricultural waste and wood residue, has been regarded as a clean and renewable biofuel among various advanced fuels that were used to replace conventional fossil fuels. Accordingly, bioethanol from lignocellulosic biomass has drawn increasing attention and has been intensively studied recently.

The biochemical processes for lignocellulosic ethanol production usually include pretreatment, enzyme hydrolysis, and fermentation of pentose and hexose sugars. Hydrolysis using diluted sulfuric acid is effective because hemicelluloses are broken down and xylose are released into solution [2]. Pretreatment with diluted acid converts hemicelluloses to xylose, with a yield of 75–90% [3–5]. To increase the mass of glucose released during enzymatic hydrolysis, pretreatment with harsher conditions has been considered a more practical approach. However, several inhibitors, such as weak acids and furanic compounds, are often generated during pretreatment under severe conditions. These inhibitors are regarded as obstacles to the fermentation of hemicellulosic hydrolysate for ethanol production. For example, furfural and hydroxymethyl furfural (HMF) influence the growth of microorganisms [6]. Active charcoal, overliming, and anion exchange resins have been studied for use in eliminating inhibitors to increase the yield of ethanol fermentation [7–9]. However, a common concern of these methods is the increased the cost of bioethanol production. Increasing the fermentative ability of microorganisms by genetic engineering [10] is another way to increase the ethanol production. But genetically engineered microorganisms may cause ecological problems, and their applicability to bioethanol production in commercial scale operations is less studied.

In the present study, the addition of hydrolyzed rice straw (HRS) was tested to understand if this innovative technology is effective for elevating ethanol yield of xylose fermentation. To optimize the standard operation using HRS, different masses of HRS, differently conditioned hemicellulosic hydrolysates were also tested in the present study. In addition, the hemicellulosic hydrolysates from different feedstock and pilot-scale fermentations were further conducted to identify the various applicability and potential for commercial-scale operations with HRS.

* Corresponding author.
E-mail address: slhuang@cc.ncu.edu.tw (S.-L. Huang).

http://dx.doi.org/10.1016/j.biombioe.2016.05.012
0961-9534/

2. Material and methods

2.1. Microorganisms and inoculum preparation

Pichia stipitis was adapted by sequential transfer of culture samples to media containing increasing amounts of NaOH-neutralized hydrolysate [11]. The yeast was grown at 30 °C on YPX agar with 20 g/L xylose, 10 g/L yeast extract, 20 g/L peptone, and 20 g/L agar (Merck, Darmstadt, Germany). A single colony was chosen after 2 days and cultured in YPX medium overnight to prepare a culture with the desired cell concentration.

2.2. Preparation of hemicellulosic hydrolysates from rice straw

Straw from cultivar Taikeng No. 9 was collected from a field near Yunlin County, Taiwan. The straw, on a dry-weight basis, contained 38.8% ± 0.2% glucan, 16.0% ± 0.2% xylan, 3.3% ± 0.1% arabinan, 9.0% ± 0.2% extractive, 13.1% ± 0.1% ash, and 19.5% ± 1.8% lignin. Bamboo, bagasse, and plywood for hydrolysate production were provided by the School of Forestry and Resource of Conservation, National Taiwan University, Taiwan Sugar Corporation and a plywood company in Malaysia, respectively.

The steam-explosion is the most promising pretreatment technology for commercialization of lignocellulosic ethanol and has been exactly employed in numerous pilot plant and demonstration plant [12]. Thus the steam-explosive pretreatment method was conducted under dilute acid conditions in the present study. Before pretreatment, rice straw, chopped to a size of 10 mm, was impregnated with 1% sulfuric acid to produce 20% solid loading at room temperature overnight. The mixture was introduced into a steam explosion reactor, which consisted of a 20-L pressure vessel and a flash tank with a cooling system to collect the slurry. Pretreatments for rice straw, bagasse, and plywood were performed at 200 °C for 1 min. The bamboo pretreatment was performed at 190 °C for 10 min. The hydrolysates were obtained by filtering the slurry through filter press separation as mentioned in a previous study [12].

2.3. Conditioning of hemicellulosic hydrolysates

Overliming was selected as the detoxification procedure to remove inhibitory compounds present in hemicellulosic hydrolysates, such as furfural and HMF. Hemicellulosic hydrolysates were heated to 50 °C, and slaked lime was gradually added, with agitation at 250 rpm. The operation was completed when pH of hydrolysates reached 10. During this process, the temperature of hydrolysates reached 54°C–57 °C because of the formation of gypsum slurry. Agitation of the slurry was maintained for 30 min, and the mixture was filtered through Whatman No. 4 filter paper (Whatman, GE, Taiwan) to separate the gypsum from detoxified hydrolysates. Filtered hydrolysates were re-acidified to pH 6.0 using 72% (w/w) sulfuric acid. Direct conditioning of hydrolysate from diluted acid pretreatment was also performed by the addition of 10 N NaOH or ammonium hydroxide (30% w/w; Sigma-Aldrich, Taiwan), until the pH value reached 6. The conditioned hydrolysates were stored at 4 °C.

2.4. Preparation of hydrolyzed solid from rice straw

HRS was collected and recycled from enzymatic hydrolysis reaction operated in a 5-ton bioreactor. After press separation, the solid part of the rice straw was mixed with water and cellulase (CTec2, Novozyme). The final reaction conditions were 15 FPU/ml and 15% (w/v) solid content in the bioreactor below 50 °C, with initial pH of 5.0. After reaction for 72 h, the hydrolyzed solid from rice straw was separated and collected by press separation. The main composition of HRS is 17.95% cellulose, 3.84% hemicellulose and 51.22% lignin after pretreatment and enzymatic hydrolysis. The pore volume of HRS is estimated to be 0.046–0.05 cc/g. This also indicated the HRS could be thought as the porous material [13].

2.5. Hydrolysate fermentation

For laboratory-scale experiments, 125-ml flasks containing 50 ml of overliming-detoxified or NaOH-neutralized or ammonia solution-neutralized hydrolysates were employed. The fermentation conditions were set at 30 °C and 100 rpm on an orbital shaker. Cell concentration of 0.6–0.8 g/L was used at the beginning of fermentation. The experimental conditions for the addition of HRS were identical to those described above, except for the addition of 2.5 g dry mass of HRS. In addition, experiments with different masses of HRS were conducted under the fermentative conditions described earlier. HRS in masses of 1, 2.5, 5, 10, and 20 g wet weight was added to flasks. The water content of HRS was estimated to be 50%.

For fermentation with individual inhibitors, furfural, HMF, or acetic acid is added to 50 ml YPX solution. The final concentration of these three compounds in solution was 0.44, 0.37, and 2.4 g/L, respectively.

The unit of ethanol yield (g_p/g_s) in this study stands for the ratio of ethanol produced to substrate (including glucose and xylose) consumed. The theoretical ethanol yield from sugar fermentation by yeast is taken as 0.51 g_p/g_s.

2.6. Pilot-scale fermentation

For hydrolysate fermentation performed in a 100-L fermenter, the yeast *P. stipitis* was cultivated in a 30-L reactor overnight with YPX solution containing 10 g/L yeast extract, 20 g/L peptone, and 20 g/L xylose. The initial yeast concentration was 1.0 g/L. The fermentation temperature was controlled at 30 °C, with an agitation rate of 100 rpm. The pH value of the hydrolysate was maintained at 6.0 during each fermentation experiment via automatic additions of NaOH.

2.7. Analytical methods

Each fermentation sample was filtered through a 0.22-μm filter and appropriately diluted using deionized water. Quantitative analysis of xylose, glucose, ethanol, arabinose, acetic acid, and inhibitors, such as furfural and HMF, was performed using an HPLC system (Agilent 1100, Agilent, Palo Alto, CA, USA) equipped with a refractive index detector at 45 °C. Separation was achieved using a Coregel 87-H3 column (Transgenomic Inc., Omaha, NE, USA) maintained at 65 °C, with 4 mM H_2SO_4 as the eluent at a flow rate of 0.8 ml/min.

Cell dry mass was measured in experiments using 125-ml flasks containing 50 ml of overliming-detoxified, NaOH-neutralized, or ammonia-treated hydrolysates. The fermentation conditions were the same as those of hydrolysate fermentation experiments. Samples of 5 ml from each experiment were centrifuged and resuspended in water three times. The resuspended mixtures were heated in a moisture analyzer balance (FD-720, Kett, Japan) for measurement of cell dry mass.