INTERNATIONAL JOURNAL OF HYDROGEN ENERGY XXX (2016) 1–8

Available online at www.sciencedirect.com

ScienceDirect

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

Pretreatment conditions of rice straw for simultaneous hydrogen and ethanol fermentation by mixed culture

Biswarup Sen [a,*], *Yen-Ping Chou* [b], *Shu-Yii Wu* [b,**], *Chun-Min Liu* [b]

[a] *Amity Institute of Biotechnology, Amity University Haryana, Gurgaon 122413, India*
[b] *Department of Chemical Engineering, Feng Chia University, Taichung 40724, Taiwan*

ARTICLE INFO

Article history:
Received 30 April 2015
Received in revised form
25 October 2015
Accepted 26 October 2015
Available online xxx

Keywords:
Mixed culture
Fermentation
Bioenergy
Fermentable sugars
Hydrolyzate

ABSTRACT

The major hurdle to produce bioenergy from rice straw is the low yield of fermentable sugars. This study was carried out to evaluate the effect of pretreatment conditions (rice straw concentration, particle size, hydrolysis time, acid concentration, $FeCl_3$ and enzyme additions) that can yield the maximum sugar monomers for simultaneous production of hydrogen and ethanol by mixed culture fermentation. The results of the evaluation showed that 0.8–1.0 M hydrochloric acid could give the maximum total sugar yield of 52.9%, 2.8 g/L glucose, 14.5 g/L xylose, and 38.6 g/L total reducing sugar from 100 g/L rice straw with particle size 0.15 mm, hydrolysis time 20 min. $FeCl_3$ addition did not enhance the sugar yield but enzymatic hydrolysis increased the reducing sugar yield to 49.8 g/L. High volumetric hydrogen production of 771 mL/L and ethanol production of 1776 mg COD/L was obtained from the pretreated rice straw hydrolyzate. And the total energy from simultaneous hydrogen and ethanol production was highest at 355 J/g polysaccharides.

Introduction

In recent years, the energy crisis, climate change and greenhouse gas emissions have raised serious concern to our society and its sustenance. For energy crisis and global warming issues, alternative fuels are considered as one of the best solutions. Sustainable alternative fuels for e.g., biohydrogen are in high demand and are of interest for the next generation [1]. Lignocellulosic based biorefinery seems to be most promising concept which can provide fuels and other value-added products [2–7]. In fact, large-scale production of biofuels from lignocellulosic biomass (LCB) has proven successful and feasible [8–11]. Various biofuels can be produced from LCB, such as hydrogen, alcohols and hydrocarbon either by thermochemical or fermentative method [1,3,4]. Fermentative route of bioenergy production generally produces a major product either as hydrogen or butanol and a minor product ethanol [12]. This simultaneous production of two fuels improves the overall energy

* *Corresponding author*. Tel.: +91 124 2334015; fax: +91 124 2337637.
** *Corresponding author*.
E-mail addresses: bsen@ggn.amity.edu, bisen0102@gmail.com (B. Sen), sywu@fcu.edu.tw (S.-Y. Wu).

http://dx.doi.org/10.1016/j.ijhydene.2015.10.147

Please cite this article in press as: Sen B, et al., Pretreatment conditions of rice straw for simultaneous hydrogen and ethanol fermentation by mixed culture, International Journal of Hydrogen Energy (2016), http://dx.doi.org/10.1016/j.ijhydene.2015.10.147

production of the bioprocess [13]. Hydrogen and ethanol production from LCB has drawn attention as alternative fuels due to their production potential from widespread, low cost, and other fuel properties [14].

Among various LCB, rice straw is one of the most abundant in the world, especially in Asia. In terms of total agricultural production, rice is the third most important main crop in the world. In Asia, rice is a widely grown crop in many agriculture-economic countries such as China, India, Indonesia, Thailand, Vietnam, and Taiwan, and accounts for 87% of world's total rice production. Rice straw makes up about half of the yield of rice and is traditionally disposed through open-field burning, a common practice in Asian countries. This practice is soon being protested for environmental reasons; hence, using rice straw as a resource for fermentable sugars for hydrogen and ethanol production is the best alternate. Rice straw has several characteristics that make it a potential feedstock for hydrogen [15–17] and ethanol [18–22] production. It has high cellulose and hemicellulose content that can be readily hydrolyzed into fermentable sugars [19,23,24].

Like other LCB [25–31], the major hurdle which needs to be overcome before rice straw can be amenable to fermentation is delignification and deconstruction of its rigid structure. Many pretreatment methods have been tested on rice straw for production of hydrogen or ethanol [19,23,32–36,17,37,38,24], but none of the studies reported hydrogen and ethanol production in a single fermentation of pretreated rice straw. Among various pretreatment methods for LCB, dilute acid hydrolysis is widely used for the depolymerization of hemicellulosic fraction into xylose and other sugar monomers [39]. Nevertheless, the choice of the optimal hydrolysis conditions is very important for maximum yield of sugars, minimal formation of toxic compounds, and high productive fermentation [39,30]. The objective of this study was to examine the effect of dilute hydrochloric acid pretreatment conditions on the rice straw conversion into fermentable sugars, and use the rice straw hydrolyzate to produce hydrogen and ethanol in single-step batch fermentation using mixed culture enriched from anaerobic sludge. We followed one-factor-at-a-time design to identify the optimal conditions for dilute hydrochloric acid pretreatment method for rice straw.

Materials and methods

Feedstock and pretreatment

Rice straw was collected from Wurih East garden, Taichung City, Taiwan. The rice straw was crushed prior to hydrolysis with dilute hydrochloric acid. The hydrolysis was conducted in a 100 mL capped bottle by autoclaving at 121 °C. After autoclaving the hydrolyzate was cooled to room temperature and its pH was adjusted to 6.0 by 18 N NaOH. The range of rice straw concentration, particle size, hydrolysis reaction time, concentrations of hydrochloric acid, and $FeCl_3$ concentration for batch hydrolysis test were 75–250 g/L, 0.15–0.5 mm, 20–60 min, 1.0–3.0 M, and 10–50 respectively. Cellulase (1% v/v) from *Trichoderma reesei* ATCC 26921 (Sigma–Aldrich) was used for enzymatic hydrolysis.

Inoculum and fermentation medium

The seed inoculum used in this study was sludge collected from a local bamboo park at Miaoli city, Taiwan. The sludge was pretreated by heating around 95–100 °C for 1 h to remove the hydrogen consuming microorganisms. Mixed culture from the sludge was enriched under anaerobic conditions in reinforced clostridia medium (Himedia) at 37 °C for 24 h prior to inoculation. The SEM image of the mixed culture indicating rod shaped bacteria (generally *Clostridium* sp.) is shown in Fig. 1. The fermentation medium was composed of the following components (per liter): $(NH_4)_2SO_4$, 10 g; KH_2PO_4, 4 g: $MgSO_4.7H_2O$, 0.5 g; $CaCl_2.2H_2O$, 0.662 g and the initial pH of fermentation was adjusted to 5.5 with 2 M NaOH or 2 M HCl. Fermentation was done at 37 °C.

Batch fermentation

The batch experiment was conducted in serum vials (250 mL) with a working volume of 100 mL filled with 15% mixed culture (15 mL), rice straw hydrolyzate as the carbon source, and fermentation medium. The serum vials were flushed with argon to maintain anaerobic environment after adjusting the initial pH 5.5, and then sealed with aluminum cap. The serum vials were placed in an incubator and kept on shaking at 200 rpm with the temperature controlled at 37 °C. Gas and liquid samples were collected at regular intervals.

Analytical methods

The total sugar concentrations were measured by the DNS method. The components of biogas in the headspace, including hydrogen, nitrogen and carbon dioxide were determined using gas chromatography (SHIMADZU GC-14B with TCD). The fermentation was filtered through a 0.22 mm filter and analyzed by liquid chromatography (SHIMADZU LC-10AT with FID) for sugar composition and soluble metabolites. Total solids and pH were measured as per standard methods [40].

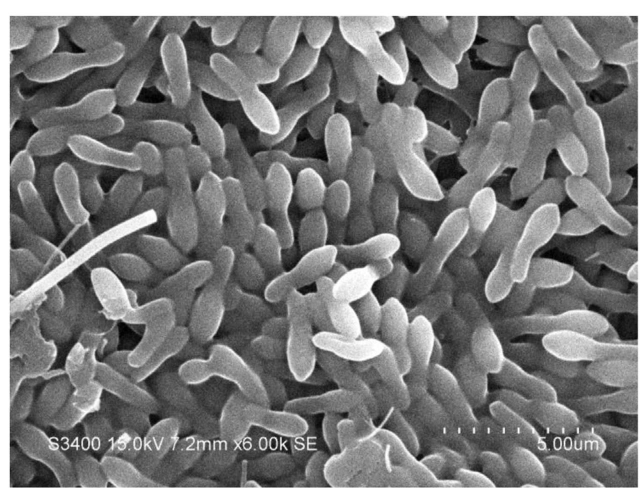

Fig. 1 – Scanning electron microscope image of the enriched mixed culture used for rice straw hydrolyzate fermentation.

Please cite this article in press as: Sen B, et al., Pretreatment conditions of rice straw for simultaneous hydrogen and ethanol fermentation by mixed culture, International Journal of Hydrogen Energy (2016), http://dx.doi.org/10.1016/j.ijhydene.2015.10.147