Bioresource Technology xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Bioresource Technology

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

Development of a combined pretreatment and hydrolysis strategy of rice straw for the production of bioethanol and biopolymer

Raveendran Sindhu *, Mathiyazhakan Kuttiraja, Thunoli Payyanvalappil Prabisha, Parameswaran Binod, Rajeev K. Sukumaran, Ashok Pandey

Biotechnology Division, National Institute for Interdisciplinary Science and Technology, CSIR, Trivandrum 695 019, India

HIGHLIGHTS

- First report on combined pretreatment and hydrolysis of rice straw.
- Fermentation of the hydrolyzate yielded bioethanol and biopolymer.
- Maximum reducing sugar yield was 0.374 g/g of dry biomass.
- Hydrolyzate is devoid of fermentation inhibitors like organic acids and furfurals.
- Residue contains hemicellulose and lignin as the major component.

ARTICLE INFO

Article history:
Received 27 January 2016
Received in revised form 17 February 2016
Accepted 19 February 2016
Available online xxxx

Keywords:
Pretreatment
Hydrolysis
Combined
Lignocellulose
Rice straw

ABSTRACT

The present study highlights the development of a combined pretreatment and hydrolysis strategy of rice straw for the production of bioethanol and biopolymer (poly-3-hydroxybutyrate). Maximum reducing sugar yield was 0.374 g/g. The hydrolyzate is devoid of major fermentation inhibitors like furfural and organic acids and can be used for fermentation without any detoxification. Fermentation of the non-detoxified hydrolyzate with *Saccharomyces cerevisiae* yielded 1.48% of ethanol with a fermentation efficiency of 61.25% and with *Comamonas* sp. yielded 35.86% of poly-3-hydroxybutyrate without any nutrient supplementation. Characterization of native, control as well as the residue left out after combined pretreatment and hydrolysis of RS by scanning electron microscopy and X-ray diffraction showed difference. Compositional analysis revealed that the residue contains lignin and hemicellulose as the major component indicating that major portion of cellulose were hydrolyzed in this strategy.

1. Introduction

Increase in dependence as well as depletion of fossil fuels and the need to reduce green house gas emissions because of its influence on climatic change leads to search for alternative sources of energy from renewable source. Lignocellulosic biomass is the main potential raw material for the production of biofuels because of its availability, high sugar content and low price (Travaini et al., 2016). Lignocellulosic biomass has a complex structure composed mainly of cellulose, hemicelluloses and lignin. Several agricultural by products like sugarcane bagasse, sugarcane tops, rice straw, cotton stalks, sorghum stover, chili post harvest residue etc. serve as potential sources for the production of bioethanol.

Conversion of lignocellulosic biomass to bioethanol involves three major steps- pretreatment, enzymatic saccharification and fermentation. Pretreatment alter the structural composition of the lignocellulosic biomass by removing hemicelluloses and lignin. It is one of the most energy intensive processes in lignocellulosics biorefinery. Most of the conventional pretreatment were carried out at higher temperature leads to generation of inhibitory compounds which have a negative impact on subsequent fermentation.

The advantages of ultrasound pretreatment have been reported by Nikolic et al. (2010). Ultrasound helps in swelling and fragmentation of the biomass due to cavitational effect of ultrasound. Beneficial effects of ultrasound on saccharification have been reported by Rolz (1986). Sonication has been reported to reduce cellulase requirements by 1/3 to 1/2 (Ingram and Wood, 1998). Sonication cause homolysis of lignin-carbohydrate bonds to release lignin and hemicelluloses (Sulman et al., 2011; Li et al., 2012a,b). The aromatic rings in the lignin are opened up at the α-position by

* Corresponding author. Tel.: +91 471 2515426; fax: +91 471 2491712.
E-mail addresses: sindhurgcb@gmail.com, sindhufax@yahoo.co.in (R. Sindhu).

http://dx.doi.org/10.1016/j.biortech.2016.02.080
0960-8524/

cleavage of the C—C bonds (Goncalves and Schuchardt, 2002). Disruption of the C—C and C—H bonds leads to formation of macroradicals. These macroradicals together with ·H and ·OH radicals produced by cavitation stimulate the depolymerization of the lignocellulosic biomass. Cavitation associated with sonication leads to disruption of bonds within polymers. Unlike other pretreatment methods sonication does not alter the chemical composition of the lignocellulosic biomass. Sonication induced structural changes depends on the sonication power and duration (Rehman et al., 2013).

Few reports are available on exploiting the ultrasound potential in biomass saccharification (Velmurugan and Muthukumar, 2012). The potential of ultrasound without any inhibitor generation in pretreatment and reducing the incubation time for enzymatic saccharification is exploited in this study.

The objective of the present study was to develop a combined pretreatment and hydrolysis strategy of rice straw for the production of biopolymer and bioethanol. Optimizations of various process parameters as well as characterization of the native and combined pretreated and hydrolyzed residue were carried out.

2. Methods

2.1. Feed stock

Rice straw received from Hyderabad, Andhra Pradesh, India was used in this study. The samples were dried and milled using a knife mill. Compositional analysis of native and pretreated samples was carried out by adopting NREL protocol (Sluiter et al., 2008).

2.2. Combined pretreatment and hydrolysis

Sonics (Vibra cell) ultrasonic cell disrupter (USA) was used in this study. The samples (biomass, commercial cellulase (Zytex India Pvt. Ltd., Mumbai, India), surfactant (Tween 80), 0.01 M citrate phosphate buffer pH 4.5) were taken in 50 ml falcon tubes and sonicated using a Sonics Vibra cell ultrasonic cell disrupter (USA) for different time points (4, 6 and 8 min). For sonication, the samples were kept in a beaker containing ice flakes to prevent heating as well as denaturation of the enzyme. After sonication the samples were incubated at 50 °C, 200 rpm in a shaking water bath (Julabo, Switzerland) for different time points (6, 9 and 12 h). After incubation the samples were centrifuged to remove the unhydrolyzed residue and reducing sugar analysis were carried out by 2,5-dinitrosalicylic acid method (Miller, 1959).

2.3. Optimization of various process parameters affecting combined pretreatment and hydrolysis of RS

Optimization of various process parameters affecting combined pretreatment and hydrolysis of RS was carried out by adopting a Box–Behnken design. The experiment consists of a total of 33 runs. The parameters selected were biomass (solid) loading, enzyme loading, surfactant concentration, sonication time and incubation time. Five parameters were selected at three levels- lower, middle and higher levels. Biomass loading was selected at three levels (6%, 9% and 12% w/w), enzyme loading at three levels (10, 20 and 30 FPU/gds), surfactant concentration at three levels (0.20%, 0.25% and 0.30% w/w), sonication time at three levels (4, 6 and 8 min) and incubation time at three levels (6, 9 and 12 h). The software Minitab 15 (Minitab Inc., USA) was used for experimental design, data analysis and quadratic model building. The response surface graphs were obtained using the software to understand the effect of variables individually and in combination, and to determine their optimum levels. The experimental set up is presented in Table 1.

2.4. Validations for optimized conditions of combined pretreatment and hydrolysis of RS

For the validation of the model, four confirmation experiments were carried out in the range defined previously. Regression coefficient was determined based on the predicted and the experimental responses.

2.5. Inhibitor analysis of the hydrolyzate

The hydrolyzate obtained after enzymatic saccharification of combined pretreated and enzymatically saccharified RS was evaluated for inhibitors like furfural, 5-hydroxymethyl furfural, citric acid, succinic acid, propionic acid, acetic acid and formic acid. The unhydrolyzed residue from the hydrolyzate was removed by centrifugation and filtered through a 0.2 μm filter (Pall, USA) and were analyzed by HPLC using a photodiode array detector kept at 55 °C. Rezex ROA column (Phenomenex) was used with an injection volume of 10 μl and flow rate was maintained at 0.6 ml/min. The concentrations of the inhibitors were analyzed using the standard curve.

2.6. Characterization of native and pretreated RS

2.6.1. Scanning electron microscopy (SEM)

Morphology of native and pretreated RS was monitored by SEM in a JEOL JSM – 5600 scanning electron microscope with an acceleration voltage of 15 kV. Images of native, control samples and residue left out after combined pretreated and hydrolysis of RS was taken at a magnification of 500×. The samples were sputter-coated with gold–palladium using a JEOL-JFC-1200 fine coater (Medina et al., 2016).

2.6.2. X-ray diffraction

The cellulose crystallinity index (CI) of native, control and residue left out after combined pretreated and hydrolysis of RS was analyzed by XRD using a PANalytical (Netherlands), x-pert pro diffractometer with a step size of 0.03° using a Cu-Ka radiation X-ray (k = 1.54 Å) at a voltage of 40 kV and current 30 mA. The crystallinity index defined as the crystalline to amorphous ratio was calculated based on the method proposed by Segal et al. (1959) using the formula:

$$CrI\ (\%) = [(I_{002} - I_{am})/I_{002}] \times 100$$

2.7. Fermentation

2.7.1. Bioethanol

Fermentation of the non-detoxified hydrolyzate obtained after combined pretreatment and hydrolysis was carried out using 18 h old *Saccharomyces cerevisiae* with an inoculums concentration of (2×10^7 cells/ml) and incubated at 30 °C for 72 h. Fermentation was carried out in 250 ml stoppered conical flasks containing 100 ml of hydrolyzate with a reducing sugar concentration of 37.4 mg/ml. After fermentation the samples were centrifuged at 10,000 rpm of 5 min at 4 °C and filtered through 0.4 μm filters (Pall, USA) and analyzed by Gas chromatography (Chemito, India) equipped with flame ionization detector (FID). The concentration of ethanol was calculated based on elution time and peak areas of known concentration of ethanol.

Please cite this article in press as: Sindhu, R., et al. Development of a combined pretreatment and hydrolysis strategy of rice straw for the production of bioethanol and biopolymer. Bioresour. Technol. (2016), http://dx.doi.org/10.1016/j.biortech.2016.02.080