Bioresource Technology 135 (2013) 67–72

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

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

A novel surfactant-assisted ultrasound pretreatment of sugarcane tops for improved enzymatic release of sugars

Raveendran Sindhu, Mathiyazhakan Kuttiraja, Varghese Elizabeth Preeti, Sankar Vani, Rajeev K. Sukumaran, Parameswaran Binod *

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

HIGHLIGHTS

- First report on surfactant assisted ultrasound pretreatment of lignocellulosic biomass.
- SAUSP SCT removed lignin and hemicelluloses.
- Maximum sugar yield was 0.661 g/g of pretreated biomass.

ARTICLE INFO

Article history:
Available online 24 September 2012

Keywords:
Pretreatment
Hydrolysis
Sugarcane tops
Surfactant
Sonication

ABSTRACT

The aim of this study was to develop a novel surfactant-assisted ultrasound pretreatment of sugarcane tops as well as to optimize the effect of various operational parameters on pretreatment and hydrolysis. A novel surfactant-assisted ultrasound pretreatment was developed which could effectively remove hemicelluloses and lignin and improve the reducing sugar yield from sugarcane tops. Operational parameters for pretreatment and hydrolysis were studied and optimized. Under optimal hydrolysis conditions, 0.661 g of reducing sugar was produced per gram of pretreated biomass. The structural changes of native and pretreated biomass were investigated by Scanning electron microscopy (SEM), X-ray diffraction (XRD) and Fourier transform infrared analysis (FTIR). The results indicate that surfactant-assisted ultrasound pretreated sugarcane tops can be used as a potential feed stock for bioethanol production.

1. Introduction

Lignocellulosic biomass is available as a potential source for bioethanol production. Utilization of lignocellulosic feed stocks for biofuel production requires the development of pretreatment to break up lignin structures and to enhance enzymatic saccharification of cellulose (Brodeur et al., 2011). Agricultural residues serve as a low cost feed stock for the production of biofuels (Sindhu et al., 2011).

One of the major challenges in lignocelluloses to ethanol technology is the development of an efficient pretreatment method. Conventional pretreatment of lignocellulosic biomass is carried out using acid or alkali. This cannot be used as a common pretreatment strategy for all feedstock since there is large variation in composition between samples of the same biomass from different sources (Sindhu et al., 2012b). Hence there is a need for developing novel strategies for better removal of hemicelluloses and lignin.

According to NIIST–TIFAC survey, sugarcane tops (SCT) is the most available biomass in India (Sukumaran et al., 2010). It is commonly used as animal fodder or burnt in the field. Few reports were available on utilization of SCT for bioethanol production (Sindhu et al., 2011; 2012b).

Several reports were available on pretreatment of lignocellulosic biomass using acid (Sindhu et al., 2011), alkali (Preeti et al., 2012), ionic liquids (Weerachanchai et al., 2012), organosolvent (Sindhu et al., 2012a), organic acids (Sindhu et al., 2010), microwave (Binod et al., 2012), biological (Vaidya and Singh, 2012) and combined pretreatment (Vani et al., 2012). Surfactants have been known as a delignification agent. The advantages of ultrasound in pretreatment have been earlier reported by several authors (Suslick et al., 1999). Few reports were available on ultrasound pretreatment or ultrasound-assisted pretreatment of lignocellulosic biomass. Pretreatment of wood wastes (Kunaver et al., 2012), sugarcane bagasse (Velmurugan and Muthukumar, 2012; Li et al., 2012) and kenaf powder (Ninomiya et al., 2012) was done using ultrasound pretreatment or ultrasound assisted pretreatment. Ultrasound is commonly used for mixing. In addition to mixing effect, other effects are attributed to cavitation bubbles (Suslick et al., 1999). The main

* Corresponding author. Tel.: +91 471 2515361; fax: +91 471 2491712.
E-mail address: binodkannur@niist.res.in (P. Binod).

0960-8524/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biortech.2012.09.050

advantage of using ultrasound is that the acoustic waves can break the cohesion of a liquid and can create micro cavities. The implosion of cavitations bubbles leads to extremely high temperatures in the area of collapsed bubble. Each pretreatment has its own advantages and limitations.

The objective of the present study was to develop a novel process for hemicelluloses and lignin removal by adopting a surfactant-assisted ultrasound pretreatment (SAUSP) and to optimize the process parameters for pretreatment and hydrolysis. The structural features of native and pretreated samples were investigated by Scanning electron microscopy (SEM), X-ray diffraction (XRD) and Fourier transform infrared analysis (FTIR).

2. Methods

2.1. Feed stock

Sugarcane tops received from Godavari Biorefineries, Mumbai, Maharashtra, India were used in this study. Dried feed stock was milled in a knife mill to a size less than 1 mm. The dried material was stored at room temperature until further use. The compositional analysis of native and pretreated sugarcane tops was carried out by two stage acid hydrolysis protocol developed by National Renewable Energy Laboratory (Sluiter et al., 2011).

2.2. Pretreatment

2.2.1. Primary screening

Pretreatment was carried out in 150 ml stoppered conical flasks with a biomass loading of 10% w/w. The samples were soaked with different surfactants (1% w/w) like Tween-80, Tween-40, Tween-20, Triton X-100, PEG 6000, PEG 8000, SDS and CTAB for 60 min. The soaked samples were sonicated for 60 s. The sonication conditions were 40% amplitude, pulse 59 s on and 59 s off. After sonication, the samples were autoclaved at 121 °C, 15 lb pressure for 60 min, neutralized by washing with tap water and dried at room temperature. The pretreatment efficiency was measured based on reducing sugar yield after enzymatic saccharification of the pretreated sample.

2.2.2. Optimization of process parameters for pretreatment

SAUSP of SCT was carried out by statistical means. The design and planning of experiments were carried out by adopting a Taguchi design to generate experimental data as well as to understand the interaction between different process variables. The experimental set up of Taguchi design is shown in Table 1. The variables used in this model are surfactant concentration, sonication time, biomass loading and incubation time. The effects of four variables were studied at three different levels and a total of 16 runs were used in this study. After pretreatment, the samples were washed with tap water and dried at room temperature (32 ± 2 °C).

2.3. Characterization of native and pretreated sugarcane tops

2.3.1. SEM analysis

Scanning electron micrographs were taken at magnification 500× for both native and SAUSP SCT using a JEOL JSM-5600 Scanning electron microscope (Sindhu et al., 2010).

2.3.2. FTIR analysis

Fourier transform infrared spectra were studied using a Shimadzu spectrometer (Japan). It provides information about the chemical bonds and molecular structure of the material. The spectra were obtained with an average of 25 scans and a resolution of 4 cm^{-1} in the range of 4000–400 cm^{-1} (Sindhu et al., 2010; Kataoka and Kondo, 1998).

Table 1
Taguchi design for optimization of SAUSP SCT.

Run	Sonication time (sec)	Surfactant concentration (%)	Biomass loading (% w/w)	Residence time at 121 °C (min)	Reducing sugar (g/g)
1	30	1	5	0	0.467
2	30	2	10	15	0.563
3	30	3	15	30	0.574
4	30	4	20	45	0.631
5	60	1	10	30	0.565
6	60	2	5	45	0.623
7	60	3	20	0	0.426
8	60	4	15	15	0.530
9	90	1	15	45	0.638
10	90	2	20	30	0.639
11	90	3	5	15	0.605
12	90	4	10	0	0.402
13	120	1	20	15	0.093
14	120	2	15	0	0.464
15	120	3	10	45	0.569
16	120	4	5	30	0.596

2.3.3. XRD analysis

Crystallinity of native and SAUSP SCT were analyzed using a PANalytical (Netherlands) X-pert pro diffractometer (Sindhu et al., 2010). Crystallinity Index (CrI), degree of crystallinity and crystallite size were calculated as per protocols adopted by Segal et al. (1959), Zhou et al. (2005) and Oh et al. (2005).

2.4. Enzymatic hydrolysis

Enzymatic saccharification of SAUSP SCT was carried out in 150 ml stoppered conical flasks by incubating 10% w/w of biomass with commercial cellulase (Zytex India Private Limited, Mumbai, India). The enzyme loading was 60 FPU/g of pretreated biomass, 20 μl of 1× antibiotic solution (Penicillin Streptomycin cock tail) and 0.1% w/w surfactant (Tween-80) loading. The total reaction volume was made up to 20 ml with 0.1 M citrate buffer (pH 4.8). The samples were incubated at 50 °C in a shaking water bath (120 rpm) for 48 h. After incubation, the samples were centrifuged to remove the unhydrolyzed residue. The hydrolyzate was used for reducing sugar analysis by 2,5-dinitrosalicylic acid method (Miller, 1959).

2.5. Optimization of enzymatic saccharification by Box–Behnken design

A Box–Behnken design (Box and Behnken, 1960) was adopted to study the effects of independent variables on the response and factor interactions with different combinations of variables. The important parameters that affect enzymatic saccharification of the pretreated biomass were biomass loading, enzyme loading, surfactant concentration and incubation time. The effects of four variables were studied at three different levels. The three levels of variables were coded as −1, 0 and +1 which corresponds to lower, middle and higher values, respectively. The software Minitab 15 (Minitab Inc., USA) was used for experimental design, data analysis and quadratic model building. The experimental set up of Box–Behnken design is shown in Table 2. 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.