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Physicochemical characterization of alkali pretreated sugarcane tops and optimization of enzymatic saccharification using response surface methodology

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ABSTRACT

Alkali pretreatment of sugarcane tops was carried out with 3% NaOH for 60 min at 121 °C in a laboratory autoclave. The effect of solid loading, enzyme loading, incubation time and surfactant concentration on enzymatic saccharification was studied using a response surface method according to Box–Behnken design. Under optimized conditions 77.5% sugar was recovered from the pretreated biomass. This yield was seven times higher than that obtained with untreated sugarcane tops. A substantial amount of lignin (90%) was removed by this pretreatment method. Physicochemical characterization of native and alkali pretreated sugarcane tops were carried out by XRD, FTIR and SEM and the changes in the chemical composition were also monitored. The X-ray diffraction profile showed that the degree of crystallinity was higher for alkali pretreated biomass than that for native.

1. Introduction

Increased concerns on depending fossil fuels and their environmental issues lead to search for alternative fuels. A solution for this problem is utilization of lignocellulosic biomass for the production of bioethanol. Lignocellulosic materials are composed of high energy bonds and can be used for the production of biofuels, instead they are commonly disposed by open air burning [1].

Among the various agricultural crop residues, sugarcane tops (SCT) is one of the most surplus available biomass in India. SCT is an attractive raw material for fermentable sugar and ethanol production due to its high content of glucan and xylan. It represents the leaves plus top portion of the plant after harvesting. For each 1 MT of sugarcane produced, SCT is accounted for about 0.25–0.30 MT [2]. SCT are usually stored in open fields and burnt. They can also be used as animal fodder before the leaves started rotting [3]. The ability to produce biofuels and value added products from lignocellulosic biomass depends on the ability to remove hemicelluloses and lignin from the lignocellulosic biomass [4]. Different protocols for conversion of lignocellulosic biomass into fermentable sugars have been demonstrated on laboratory and pilot scales. Three main processes are involved in the conversion of lignocellulosic biomass to ethanol – pretreatment, hydrolysis and ferme ntation. An effective pretreatment is essential to make the substrate suitable for enzymatic saccharification by altering crystallinity as well as lignin content.

The prime factors that influence enzymatic saccharification of pretreated biomass were biomass loading, enzyme loading, surfactant concentration and incubation time. Response surface methodology (RSM) is a statistical technique used for modeling and optimization of multiple variables and determines optimum process conditions by combining experimental results. Based on the experimental results, RSM could tell the optimum conditions to obtain the desired responses, as well as the mathematical model in explaining the relationship between the experimental variables and its responses. RSM has been successfully used for the optimization of pretreatment and enzymatic saccharification of wheat straw [5], switch grass [6], prairie cord grass [7], rice straw [8] and sugarcane tops [9].

To date, there are limited studies on pretreatment and optimization of enzymatic hydrolysis of sugarcane tops (SCT) [9,10]. In the present study, SCT was investigated for bioethanol production using alkali pretreatment. Enzymatic hydrolysis is the key step in the conversion of cellulose into ethanol. Optimization of enzymatic hydrolysis of SCT by classical method involve changing one independent variable at a time, while maintaining other parameters at a

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fixed level which is extremely time consuming and expensive for a number of variables. To improve yield and rate of enzymatic hydrolysis research has been focused on optimization of hydrolytic process. Box–Behnken design and response surface methodologies were employed for optimization of hydrolysis conditions. The effect of biomass loading, enzyme loading, surfactant concentration and incubation time on reducing sugar yield as well as physicochemical characterization of native and alkali pretreated SCT were carried out.

2. Materials and methods

2.1. Feedstock

Sugarcane tops used in this study was received from Godavari Sugar Mills, Maharashtra, India. The raw materials were dried and milled using a knife mill to a particle size less than 1 mm, homogenized and stored at room temperature until used. Native SCT contains 12% moisture content.

2.2. Compositional analysis

The composition of native and alkali pretreated samples were determined according to National Renewable Energy Laboratory (NREL) analytical methods for biomass [11]. SCT (300 mg) was hydrolyzed with 72% (v/v) H_2SO_4 for 1 h at 30 °C. After first hydrolysis, the acid was diluted to 4% concentration by adding distilled water. Second hydrolysis was carried out by autoclaving the reaction mixture at 121 °C for 1 h. Filtration of the autoclaved solution was carried out through 0.2μ filters for HPLC analysis and the solid residues remained after filtration was used to determine the acid insoluble lignin. HPLC (Shimadzu LC-2010) analysis was carried out using degassed Milli Q water as mobile phase with a flow rate of 0.8 ml/min and Rezex RPM-Monosaccharide Pb+2 (8%) (300 × 7.8 mm) column was used. Oven temperature was maintained at 80 °C and the sugars were detected using (Refractive Index) RI detector. Glucose, xylose and arabinose were used as standards fort HPLC analysis.

2.3. Alkali pretreatment of sugarcane tops

Pretreatment was carried out in 250 ml Erlenmeyer flasks with 15% w/w biomass loading, 3% NaOH and autoclaved (121 °C, 15lb pressure) for 60 min. The pretreated samples were neutralized with 1N H_2SO_4, followed by washing with tap water and dried at room temperature (30 ± 2 °C).

2.4. Physicochemical characterization of the feedstock

In order to investigate changes in biomass physical and chemical features after pretreatment, characterizations were performed on native and alkali pretreated SCT. Chemical composition as well as cellulose crystallinity, FTIR responses and scanning electron microscopy visualizations were carried out.

2.4.1. SEM analysis

SEM analysis was used to investigate the structural transformations of native and alkali pretreated SCT. The analysis was performed using a scanning electron microscope (JEOL JSM − 5600) [10].

2.4.2. XRD analysis

Crystallinity index of native and alkali pretreated SCT was determined by X-ray diffraction methods using X-pert pro diffractometer (PANalytical, Netherlands). The X-ray diffractograms were recorded from 10 to 30° with a step size of 0.03° using a Cu–Kα radiation X-ray ($\lambda = 1.54$ Å) generated at a voltage of 40 kV and a current 30 mA. The crystallinity index of each sample was expressed as the crystallinity index using the following equation [12].

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

I_{002} is the intensity of 002 peak at $2\theta = 22.4°$ and I_{am} is the intensity of the background scatter at $2\theta = 18.0°$.

The crystallite size was calculated based on the following equation

$$D(\mathrm{hkl}) = \frac{k\lambda}{\beta_0 \cos\theta}$$

D(hkl) is the size of the crystallite (nm), k is the Scherrer constant (0.94), λ is X-ray wavelength (for copper, 0.15418 nm). β_0 is the full-width at half-maximum of the reflection hkl, measured at 2θ which is the corresponding Bragg angle [13].

The degree of crystallinity was calculated using the following equation [14].

$$\chi_c = F_c/(F_a + F_c) \times 100\%$$

F_c and F_a are the area of crystalline and non-crystalline regions.

2.4.3. FTIR analysis

FTIR Spectroscopy provides information about the physicochemical and conformational properties of polysaccharide. Relative absorbances of bands were detected by a baseline correction method. IR spectra were examined using a Shimadzu spectrometer (Japan) [10].

2.5. Enzymatic hydrolysis

Enzymatic saccharification of alkali pretreated SCT was performed in 150 ml stoppered conical flasks by incubating 1.4 g of biomass in 100 mM citrate buffer (pH 4.8). Hydrolysis was performed using a commercial cellulase (Zytex India Private limited, Mumbai). The samples were incubated at 50 °C for 48 h in a shaking water bath. The hydrolyzate was used for reducing sugar (glucose, xylose, arabinose and mannose) analysis by 2,5-dinitrosalicylic acid method [15].

2.5.1. Optimization of hydrolysis by RSM

Box–Behnken design was used to study the effect of independent variables on the response and factor interactions with different combinations of variables. Variables selected were biomass loading, enzyme loading, surfactant concentration and incubation time. The effect of four variables was studied at three different levels and a total of 27 runs were used for the study. The software Minitab 15 (Minitab Inc, USA) was used for experimental design, data analysis and quadratic model building. The experimental setup of RSM is shown in Table 1.

3. Results and discussion

3.1. Pretreatment, compositional analysis and characterization of native and alkali pretreated biomass

The alkali pretreated SCT upon enzymatic hydrolysis yielded 0.684 g of reducing sugar per g of pretreated biomass. The cellulose, hemicelluloses, total lignin and other contents obtained from 1 g of native and pretreated SCT were presented in Table 2. The cellulose and hemicelluloses content was lower and lignin content was