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Research paper

Material balance studies for the conversion of sorghum stover to bioethanol

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ABSTRACT

The conversion of lignocellulosic biomass to ethanol involves three major unit operations such as pretreatment, hydrolysis and fermentation. Each unit operation involves processing of biomass with changes in its structure, and release of fermentable and other sugars and lignin degrading compounds. The evaluation of biomass conversion processes through material balance is fundamentally crucial in its commercialization. This gives an idea about the transfer of biomass from one phase to another and hence eventually of the efficiency of the total process. In the present study, material balance has been evaluated in each unit operations for sorghum biomass to ethanol conversion. An account of carbohydrates in the native as well as pretreated sorghum biomass, the release of fermentable sugars and the conversion of sugars to ethanol was maintained and analysed. Ethanol yield of 91.94 g per kg sorghum was achieved without any detoxification and washing of pretreated biomass after mild acid pretreatment followed by enzymatic hydrolysis and fermentation.

1. Introduction

Demand for ethanol as an alternative fuel is steadily increasing due to depletion of oil reserves and the need for cleaner fuel [1]. Lignocellulosic feedstock offers a potential raw material for the production of ethanol. Dedicated energy crops including switchgrass, miscanthus, sugarcane, poplar and sweet sorghum have been used intensely for research. However, very little work has been done to investigate sorghum stover as a dedicated bioenergy crop. Based on multi-year agronomic data and theoretical ethanol production, sorghum can achieve more than 1300 gallons of ethanol per acre [2]. Sorghum stover is having many advantageous characteristics such as high cellulose and hemicellulose content that make it a potential candidate for biofuel. The primary objective of this study is to analyse the mass balance in each unit operations for sorghum biomass to ethanol conversion. Such analysis is highly needed in determining the commercial viability of the process. Loss of materials in every stage of operation is inevitable and this material loss should be accounted to draw a conclusive economic analysis. In this study, the material flow was evaluated at every step from pretreatment to ethanol production. Also, physicochemical changes in the biomass structure were analysed by SEM, XRD and FTIR to determine pretreatment efficacy.

2. Materials and methods

2.1. Feedstock and chemicals

Sorghum (*Sorghum bicolour*) biomass was obtained from The Directorate of Sorghum Research, Hyderabad, India. The biomass consisted of leaves and aerial stems. It was dried and milled to a size less than 10 mm. The milled samples were stored in airtight containers at room temperature until further use. The native sorghum was first subjected to composition analysis to know the percentage distribution of constitutive polymers. The compositional analysis was carried out by two-stage acid hydrolysis protocol developed by the National Renewable Energy Laboratory (NREL) [3].

2.2. Pretreatment

The pretreatment of sorghum stover was done using dilute sulphuric acid (0.37% (v/v)) in a high pressure reactor at a

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temperature of 150 °C for 15 min [4]. Initially, the milled biomass was analysed for moisture content and weighed accordingly. After the pretreatment, the pH of pretreated mixture was adjusted to 5 using sodium hydroxide (10 N). The amount of solution needed for neutralization was recorded. After neutralization, it was filtered through muslin cloth to separate solid and liquid fractions. Liquid fraction was centrifuged at 10000 rpm for 10 min to remove fine solid particles. The wet biomass was then air dried at room temperature, weighed and moisture was analysed to get dry weight of the biomass. The pretreated liquor was centrifuged at 10000 rpm for 10 min to separate the residual solids and the total volume of pretreated liquor was recorded. The liquor was subjected to sugar analysis by HPLC (Shimadzu) using Rezex RPM carbohydrate column (Phenomenex) with flow rate of 0.6 ml/min and deionised water as mobile phase at 80 °C. The pretreated biomass was subjected to composition estimation and SEM, XRD and FTIR analyses.

2.3. Characterisation studies

2.3.1. SEM analysis

The difference in the physical structure of biomass before and after acid pretreatment was observed by scanning electron microscopy (Zeiss model, Evo 18, special edition). The samples were mounted on a double-sided conductive tape on pre-cut brass sample stubs and the sputter coated with gold palladium using a fine coater. The images of native and pretreated sorghum were acquired with a 10–15 kV accelerating voltage and magnification 1000×.

2.3.2. XRD analysis

The crystallinity of native and pretreated samples were measured by X-ray diffraction using a PANalytical X-pert pro diffractometer (Netherlands), set at 40 kV, 30 mA; and radiation was Cu Kα ($\lambda = 1.54$ A°). The samples were scanned in a 2θ range from 10 to 30° and a step size of 0.03° was used for the analysis.

2.3.3. FTIR analysis

Fourier Transform Infrared spectroscopic studies of native and pretreated sorghum were carried out to detect changes in functional groups. FTIR spectrum was recorded between 4000 and 400 cm^{-1} using a Shimadzu Spectrometer with detector at 4 cm^{-1} resolution and 25 scans per sample. Discs have been prepared by mixing 3 mg of dried and finely ground sample with 300 mg of KBr (Spectroscopic grade) in an agate mortar. The resulting mixture was pressed at 10 MPa for 3 min.

2.4. Enzymatic hydrolysis

The pretreated sorghum was subjected to enzymatic saccharification using commercial cellulase (Zytex India Pvt. Ltd., Mumbai, India) which was having high cellulase activity and negligible xylanase and beta glucosidase activity. The enzyme was added in concentration of 20 FPU/g of substrate with substrate loading 10% (w/w). The hydrolysis mixture also contained standard antibiotic solution (1% v/v) and Tween 80 as surfactant (0.05% w/v) in 0.05 M citrate buffer solution (pH 4.8). The hydrolysis was carried out at 50 °C in a vessel set at 200 rpm for 48 h. The hydrolysate was harvested at the end of 48 h and the total volume was recorded. Hydrolysate was then centrifuged at 10000 rpm for 10 min and supernatant was analysed for sugars by HPLC. The unhydrolysed biomass was air dried, analysed for moisture and weighed.

2.5. Ethanol fermentation

The organism used for fermentation was *Saccharomyces cerevisiae*. Commercially available baker's yeast was used to obtain the organism. The dessicated culture was suspended in sterile saline, serially diluted and plated on PDA agar plates to get isolated colonies. A single isolated colony was picked and suspended in YPD broth and allowed to grow at 30 °C for 18 h. The 45 ml of hydrolysate was added with 5 ml of stock solution of yeast extract, ammonium sulphate and KH_2PO_4 in percent concentration 0.2, 0.04 and 0.5, respectively. The antibiotic solution (0.1% v/v) was added to avoid contamination. The 18 h old inoculum broth was centrifuged and the pellet obtained was inoculated (0.27 g/L on dry weight basis). The fermentation was carried in 50 ml capacity screw capped bottles at 30 °C in static condition up to 48 h. The uninoculated medium was kept as control. Samples were withdrawn at specific intervals and centrifuged and the supernatant was analysed for residual sugars and ethanol.

2.6. Ethanol estimation

Ethanol produced after fermentation was estimated by Gas Chromatography using Porapak Q column with FID detector. The carrier gas flow rate was 30 ml/min with oven temperature 155 °C, injector temperature 175 °C and detector temperature 250 °C. The samples were filtered through 0.2 μ filter before analysis.

3. Results and discussion

3.1. Characterisation studies

3.1.1. SEM analysis

Scanning electron microscopy was carried out to assess the morphological structural modifications. SEM images taken at 1000× magnification explained the changes occurred during pretreatment (Supplementary Fig. 1). In case of nonpretreated biomass, the structure observed was rigid, highly conserved, compact and nonporous. The pretreated sample was found to be distorted which may be due to breakdown of cellulose–hemicelluloses–lignin network leading to the removal of hemicellulose fraction. The same observation was found in the case of acid pretreated chilli residues [5]. During acid pretreatment, intermolecular hydrogen bonds within cellulose get partially disrupted and thus resulting in the porous structure with high surface area. This is beneficial for enzymes to penetrate, adsorb and easily hydrolyse the biomass during hydrolysis. Identical observations were earlier reported for sugarcane tops pretreated with acid [6,7].

3.1.2. XRD analysis

X-ray diffractogram studies revealed the changes of crystallinity during pretreatment of native sorghum samples. Crystallinity is defined as the ratio of the amount of crystalline cellulose to the total amount of sample material, including crystalline and amorphous cellulose [8]. The degree of crystallinity of lignocelluloses plays a significant role in the enzymatic hydrolysis. Crystallinity index was calculated based on the procedure adopted by Kim and Holtzapple [8]. The crystallinity indices of native and pretreated samples were 72.73% and 68.25%, respectively. Crystallinity part of the substrate is imparted by cellulose I_β and amorphous part by paracrystalline I_a celluloses. The decrease in index value after pretreatment showed the distortion of crystalline components in lignocellulosic structure. This in turn showed the increase in the efficiency of enzymatic hydrolysis, as the enzymes can efficiently hydrolyse the amorphous structure than more ordered and stable crystalline structure [9]. So it is of great importance for pretreatment methods to convert I_β cellulose to more easily digestible paracrystalline and amorphous celluloses. Contradictory reports have been observed for other biomass such as sugarcane tops [6]