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Ultrasound-assisted alkaline pretreatment of sugarcane bagasse for fermentable sugar production: Optimization through response surface methodology

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ABSTRACT

Ultrasound-assisted alkaline pretreatment of sugarcane bagasse (SCB) for fermentable sugar production was carried out and the influence of particle size, liquid to solid ratio (LSR), NaOH concentration, temperature and sonication time on delignification and reducing sugar production was ascertained with Placket–Burman design. The best combination of each significant factor was determined by a central composite design (CCD) and optimum pretreatment conditions for maximum reducing sugar yield (96.27%) were particle size of 0.27 mm, LSR of 25 ml/g, NaOH concentration of 2.89% (w/v), temperature of 70.15 °C and pretreatment time of 47.42 min. Under these conditions, 92.11% of theoretical reducing sugar yield was observed experimentally. The substantial reduction in pretreatment time and temperature with improved efficiency is the most attractive features of the ultrasound-assisted alkaline pretreatment.

1. Introduction

Enzymatic hydrolysis is regarded as the most promising technique for converting lignocellulosic compounds into fermentable sugars (Wooley et al., 1999; Wright, 1998); however, this approach requires biomass in small particles and removal of lignin (Dasari and Berson, 2007; Pan, 2008). Pretreatments for lignocellulosic materials include steam explosion (Hernandez-Salas et al., 2009), dilute acid hydrolysis (Hendriks and Zeeman, 2009), alkaline pretreatment (Zhang and Cai, 2008; Hendriks and Zeeman, 2009) and wet oxidation (Rosgaard et al., 2007). Alkaline pretreatments show less sugar degradation and furan derivatives formation (Gonzalez et al., 1986) than thermal and acid pretreatments (Fengel and Wegener, 1984). The alkaline pretreatment process can be improved further by the application of ultrasound (Filson and Dawson-Andoh, 2009). The ultrasonic treatment of aqueous media produces cavitation, which generates high temperature, pressure and extreme shear forces. The decomposition of water molecules into free radicals by cavitation aids in cleaving the linkages in lignin and xylan networks (Chuanyun et al., 2004; Yaldagard et al., 2008). Sun et al. (2004) treated sugarcane bagasse (SCB) with alkali/alkaline peroxide and ultrasound for the extraction of hemicellulose from SCB and reported 90% hemicellulose and lignin removal, but more detailed analyses of factors influencing delignification or cellulose recovery are needed. Therefore, the aim of the present study was to optimize particle size, liquid to solid ratio (LSR), NaOH concentration and sonication time on ultrasound-assisted alkaline pretreatment by response surface methodology (RSM) with the intention to improve the delignification and reducing sugar yield.

2. Methods

2.1. Materials

Sugarcane bagasse was obtained from a sugarcane juice shop in Chennai, India. The SCB was air dried, milled using a laboratory blender (Remi Anupam Mixie Ltd., Mumbai) and screened to obtain particles of different size ranges. The particle size ranges are average values of upper and lower sieves openings of ASTM and BS sieves. Determination of the main fractions (cellulose, hemicelluloses, and lignin) was carried out by detergent extraction methods (Goering and Vansoest, 1970).

2.2. Sonochemical reactor

The ultrasonic treatment was carried out using a titanium probe-type sonolyzer (Hielscher UP 400S, Germany). The operating frequency and power of the sonolyzer were 24 kHz and 400 W, respectively. The amplitude was maintained at 100% and the temperature was controlled using a water bath.

2.3. Design of experiments

The entire design was carried out in two stages. Initially, five variables were screened to identify significant factors using a

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two-level Plackett–Burman design (PBD) and in the second stage the level of these factors were optimized using a central composite design (CCD).

2.3.1. Screening of parameters

The effect of particle size, LSR, NaOH concentration, temperature and sonication time on reducing sugar production was evaluated using PBD in twelve experimental runs. The software used for PBD was MINITAB 16. The variables with a confidence level higher than 90% were considered significant for both delignification and reducing sugar production. All experiments were carried out in duplicate and the averages were taken as responses.

2.3.2. Optimization of significant parameters

Effects of the variables such as particle size, LSR, NaOH concentration, temperature and sonication time on response were fit with the second-order polynomial model given in Eq. (1).

$$Y = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_4 D + \beta_5 E + \beta_{11} A_1^2 + \beta_{22} A_2^2 + \beta_{33} A_3^2 + \beta_{44} A_4^2 + \beta_{55} A_5^2 + \beta_{12} AB + \beta_{13} AC + \beta_{14} AD + \beta_{15} AE + \beta_{23} BC + \beta_{24} BD + \beta_{25} BE + \beta_{34} CD + \beta_{35} CE + \beta_{45} DE \quad (1)$$

where Y is the predicted response; β_0 is the constant; β_1, β_2, β_3, β_4, and β_5 are linear coefficients; β_{11}, β_{22}, β_{33}, β_{44}, and β_{55} are quadratic coefficients; β_{12}, β_{13}, β_{14}, β_{15}, β_{23}, β_{24}, β_{25}, β_{34}, β_{35}, and β_{45} are interaction coefficients; A, B, C, D, and E are factors representing particle size, liquid to solid ratio, NaOH concentration, temperature and sonication time, respectively.

Five significant variables were considered for the optimization using a five-level CCD. ANOVA and regression analyses were carried out to evaluate the effects of variables and their interactive effects. Coefficients of the full model were analyzed for their significance and the insignificant ones were eliminated from the model. The predicted response and optimal levels of the variables were found by solving the second-order polynomial model for maximization of response using MINITAB 16 software (Lu et al., 2009).

2.4. Experimental

SCB biomass was dispersed in 100 ml of desired concentration of NaOH solution (0.25%, 1.00%, 1.75%, 2.50% and 3.25%) in an Erlenmeyer flask and treated with ultrasound at different operating conditions based on the experimental design. The performance of ultrasound-assisted alkaline pretreatment was compared with those of alkaline pretreatment carried out at 30 ± 1 °C, ultrasound treatment in the absence of NaOH and commercial pretreatment (2% NaOH in an autoclave at121 °C for 1 h) (Zhang and Cai, 2008; Hendriks and Zeeman, 2009). After the treatment, the contents were filtered through Whatman No. 1 filter paper and the supernatant was subjected to sugar, acetic acid and furfural analyses. The solid residue was repeatedly washed with water until the filtrate was neutral. The residue was dried at 50 °C until constant weight was observed and used for saccharification experiments. Experiments were performed in triplicate and mean values were employed in the analyses. The sugar concentration in terms of gram per gram was calculated by dividing the sugar concentration in pretreated solids by the total solids recovered after pretreatment. The polysaccharide (glucan, xylan and arabinan) recovery in the solid content was calculated using the following equation:

$$\%\ \text{recovered} = \frac{P_{\text{PT-SCB}}}{P_{\text{SCB}}} \times 100 \quad (2)$$

where P_{SCB} is the concentration of polysaccharide in SCB, $P_{\text{PT-SCB}}$ is the concentration of polysaccharide in pretreated SCB, which is calculated by multiplying estimated sugar concentration (g/g) by recovered solids after pretreatment.

The % delignification was calculated using the following equation:

$$\%\ \text{delignification} = \frac{L_{\text{SCB}} - L_{\text{PT-SCB}}}{L_{\text{SCB}}} \times 100 \quad (3)$$

where L_{SCB} is the concentration of lignin in SCB, $L_{\text{PT-SCB}}$ is the concentration of lignin in pretreated SCB, which was calculated by multiplying estimated lignin concentration (g/g) by recovered solids after the pretreatment.

2.5. Enzymatic convertibility

Pretreated SCBs were weighed and suspended in 100 ml of 50 mM sodium citrate buffer (pH 4.8) supplemented with 0.02% sodium azide under aseptic conditions. The contents were mixed with 25 FPU/g dry matter and 0.46 CBU/g cellulose of commercially available cellulase and β-glucosidase (Sisco Research Laboratory (SRL), India), respectively. The reaction mixture was incubated in a shaking incubator at 45 °C and 150 rpm for 26 h (Aswathy et al., 2010). The release of soluble reducing sugars was periodically measured with the dinitrosalicylic acid (DNS) method (Miller, 1959).

2.6. Analytical methods

2.6.1. Physical characterization

The morphological differences of SCB pretreated by various methods were examined using scanning electron microscopy (SEM, JEOL Ltd., Tokyo, Japan). The crystalline nature of the raw and treated SCB was analyzed using a Rigaku RINT-TTR3 X-ray diffractometer (Rigaku Co., Tokyo, Japan). The nickel-filtered Cu Kα radiation (λ = 0.1542 nm) was applied at 50 kV and 30 mA. Samples were scanned over the range of 2θ = 5–50° and the crystallinity index (*CrI*) was determined using Eq. (4) (Segal et al., 1959):

$$CrI = \frac{I_{\text{Crystalline}} - I_{\text{Amorphous}}}{I_{\text{Crystalline}}} \times 100\ \% \quad (4)$$

where, $I_{\text{crystaline}}$ = intensity at 21° and $I_{\text{amorphous}}$ = intensity at 18.8°.

2.6.2. Chemical characterization

The total reducing sugars in enzymatic hydrolysates of SCB was determined by the DNS method (Miller, 1959) and cellulose concentration was estimated spectrophotometrically by the anthrone method (Updegroff, 1969). Anthrone and DNS reagents were obtained from SRL, India. The concentration of sugars, acetic acid and furfural was determined using high performance liquid chromatography (HPLC). The HPLC system (Shimadzu, CA, USA) consisted of a liquid pump (LC-10AD), a refractive index detector (RID-6A) and a system controller with Shimadzu EZS software. The monosaccharides (glucose, xylose and arabinose) were separated in an ion-exchange column (Aminex HPX-87P Bio-Rad Laboratories, Hercules, CA) at 85 °C. Ultra-pure water was used as mobile phase at a flow rate of 0.6 ml/min (Sluiter et al., 2008). The degradative products (acetic acid and furfural) were separated using an ion exchange column (Aminex HPX-87H) at 65 °C. The mobile phase, 5 mM H_2SO_4, was used at a flow rate of 0.6 ml/min (Sluiter et al., 2008). The composition of raw and pretreated SCBs was determined by strong acid hydrolysis as described previously (Velmurugan and Muthukumar, 2011).The conversion factors for hexose (glucose) and pentoses (xylose and arabinose) were 1.111 and 1.136, respectively (Demirbas, 2005). Hemicellulose, lignin and ash contents were estimated by methods described by Goering and Vansoest (1970) and APHA (2005). Cellulase activity