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Ultrasound assisted ammonia pretreatment of sugarcane bagasse for fermentable sugar production

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ABSTRACT

This study presents the ultrasound assisted ammonia pretreatment (UAAP) of sugarcane bagasse (SCB) and the influence of SCB particle size, liquid ammonia concentration, sonication time, temperature and liquid to solid ratio (LSR) on cellulose recovery and delignification. The maximum cellulose recovery and delignification observed at the optimum conditions (particle size 0.274 mm, sonication time 45 min, ammonia concentration 10%, LSR 10 mL/g and temperature 80 °C) were 95.78 and 58.14%, respectively. The dilute acid hydrolysis of pretreated SCB produced 16.58 g/L glucose, 8.21 g/L xylose, 2.78 g/L arabinose, 0.81 g/L furfural and 1.79 g/L acetic acid. The hydrolysate contained less inhibitors compared to the values reported in the literature during fermentable sugar production.

1. Introduction

Lignocellulosic biomass (LCB) is one of the renewable resources for the production of second generation biofuels and there is a growing interest in the developing countries to utilize LCB for the production of biofuels [1]. The various LCB used for the production of biofuels include agricultural residues(e.g., wheat straw, sugarcane bagasse, corn stover), forest products (hardwood and softwood), switch grass, etc., Among the various raw materials available, sugarcane bagasse (SCB) has been given much attention in the past. In addition to lignin, hemicellulose and cellulose, SCB contains proteins and ash. Lignin present in SCB is covalently bonded with hemicellulose, which prevents the entry of hydrolytic agents to convert the polymeric cellulose into monomeric sugars. Therefore, pretreatment of LCB is necessary prior to acid or enzymatic hydrolysis. The pretreatment process should: remove lignin; preserve hemicellulose sugars; reduce the formation of degradation products; and reduce the crystallinity of cellulose. It also alters the structure of cellulosic biomass and hence, the accessibility of acid or enzyme catalysts, that convert carbohydrate polymers into fermentable sugars [2]. The pretreatment processes available include mechanical pretreatment [3], strong acid pretreatment [4], alkaline pretreatment [5], ammonia fiber explosion (AFEX) [6], ultrasound assisted alkaline pretreatment [7], dilute acid (DA) and cellulose solvent based lignocellulose fractionation (COSLIF) pretreatment [8]. In ammonia fiber explosion (AFEX), biomass is exposed to high pressure (250–300 psi) in the presence of liquid ammonia at moderate temperatures (60–100 °C) [9]. It produces lignin depolymerization, hemicellulose solubilization and cellulose decrystallization [10]. This method was used to treat rice straw with 28% (w/v) ammonia at 60 °C for 7 days for the betterment of pretreatment process [11].

Ammonia is highly volatile and hence, the recovery is easy compared to other catalysts used. About 95% of ammonia used during the AFEX pretreatment can be recovered and reused [12]. In ammonia recycle percolation (ARP) method, LCB is treated with aqueous ammonia (10–15 wt.%) at high temperatures (150–170 °C) with a fluid velocity and residence time of 1 cm/min and 14 min, respectively. Under these conditions, aqueous ammonia reacts with lignin and cleaves the lignin-carbohydrate linkages, which causes depolymerization of lignin. There is no inhibitor formation in ARP method and after the process, ammonia can be separated and recycled [13]. The foregoing brief survey clearly indicates that the AFEX pretreatment can be used efficiently for biomass with less lignin content [3]. Therefore, there is a need for further improvement of this method to treat biomass with higher lignin content and hence, ultrasound was incorporated in addition to liquid ammonia. In this study, SCB was used as a biomass, which has a lignin content of more than 25%. Furthermore, the ultrasound was combined with ammonia to

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reduce the byproducts formation and to carry out the treatment at moderate temperature. In this study, the synergistic effect of ultrasound and ammonia on the removal of lignin and cellulose recovery was investigated. The pretreated SCB was subjected to ultrasound assisted dilute sulphuric acid hydrolysis.

2. Materials and methods

2.1. Materials

Sugarcane bagasse was collected from a sugarcane processing unit located in Chennai, India. It was washed with distilled water and dried to remove the moisture present. The dried SCB was milled and screened to select the particles having size lower than 1 mm (ASTM standard, mesh size >18), and then, stored in polyethylene bags at room temperature. The moisture content of sieved bagasse was about 4% on dry basis. The chemicals such as 3,5-dinitrosalicylic acid and liquid ammonia used in this study were of analytical grade and purchased from Sisco Research Laboratory Pvt., Ltd., Mumbai, India and sulphuric acid was purchased from Thermo Fischer Scientific India, Ltd., Mumbai, India.

2.2. Sonicator

Sonotrode H3 probe type model made up of titanium with tip diameter 3 mm and an approximate length of 100 mm (Hielscher Ultrasonic Processor UP400S, Germany) was used in this study. The operating power and frequency of the sonicator were 400 W and 24 kHz, respectively. The adjustable amplitude and pulse were maintained at 100% and the temperature was controlled using a water bath.

2.3. Taguchi approach for experimental design

Taguchi approach is a simple and robust technique used for the optimization of process parameters and to improve the product reliability and quality. In this method, the main influencing parameters are located at different rows in a designed array. The regression analysis gives the average change in the value of one variable when there is a change in the value of the other variable and it is used to neglect the insignificant factors from the experiment. In this study, five principle parameters such as ammonia concentration (v/v), SCB particle size (mm), sonication time (min), temperature (°C) and liquid to solid ratio (LSR in mL/g) were considered to analyze the pretreatment process performance. Taguchi approach was followed to obtain the optimal parameters through signal to noise (S/N) ratio and analysis of variance (ANOVA). Signal to noise ratio (S/N) is used for the analysis of results and in this, signal represents the desirable value (mean) and the noise represents the undesirable value (standard deviation from mean). In this study, "nominal the best" criterion was chosen for the calculation of S/N ratio. The S/N ratio was calculated from the equation:

$$\text{S/N ratio} = 10\,\text{Log}_{10}\left(\frac{\mu^2}{\sigma^2}\right) \tag{1}$$

where μ is the mean value of cellulose recovery obtained in the designed experiment and σ is the variance value of cellulose recovery. Analysis of the experimental data using the ANOVA and effects of the factors give the output that is statistically significant for finding the optimum levels [14]. The analysis was made using MINITAB version 15.1.0.0. Taguchi experimental design containing five factors and three levels was used and this suggested 27 experiments for the optimization of delignification. The factors and levels were selected based on the previous study [15].

2.4. Ultrasound assisted ammonia pretreatment

To perform ultrasound assisted ammonia pretreatment, 1 g SCB of desired size was added with liquid ammonia of desired concentration in a 100 mL Erlenmeyer flask and was irradiated with ultrasound at different operating conditions. The operating parameters such as liquid ammonia concentration (5, 10 and 15%, v/v), LSR (10:1, 15:1 and 20:1 mL/g), particle size (0.274, 0.460 and 0.925 mm), sonication time (15, 30 and 45 min) and temperature (40, 60 and 80 °C) were varied to obtain better conditions for the maximum recovery of cellulose and removal of lignin. The experiments were carried out in duplicate and average values were reported. The performance of ultrasound assisted ammonia pretreatment was compared with ultrasound pretreatment carried out for 45 min at 50 °C and liquid ammonia pretreatment carried out with 10% (v/v) for 30 min at 80 °C. After the pretreatment, the solids were separated by filtration using Whatman filter paper and washed with distilled water repeatedly until the pH of the filtrate reached neutral condition. The residue obtained was dried at 50 °C until constant weight was observed and then it was subjected to dilute acid hydrolysis. The % cellulose recovery in the solid content was calculated using the following equation:

$$\%\,\text{cellulose recovery} = \left(\frac{R_{\text{PT-SCB}}}{R_{\text{SCB}}}\right) \times 100 \tag{2}$$

where R_{SCB} is the amount of cellulose in native SCB, and $R_{\text{PT-SCB}}$ is the amount of cellulose in pretreated SCB measured in (g/g).

The % delignification was calculated using the following equation:

$$\%\,\text{Delignification} = \frac{D_{\text{SCB}} - D_{\text{PT-SCB}}}{D_{\text{SCB}}} \times 100 \tag{3}$$

where D_{SCB} is the amount of lignin present in native SCB, and $D_{\text{PT-SCB}}$ is the amount of lignin in pretreated SCB measured in (g/g).

2.5. Ultrasound assisted dilute acid hydrolysis

Ultrasound assisted dilute acid hydrolysis experiments were carried out in an Erlenmeyer flask (250 mL) containing pretreated SCB and 100 mL of 2% dilute sulphuric acid at 50 ± 10 °C. The ultrasonic waves were pulsed using cycle control system and the cycle was set at 50% for all experiments. The optimum hydrolysis conditions reported in the previous study were followed [7] and samples withdrawn at regular time intervals were centrifuged at 8000 rpm for 10 min. The pellet obtained was rinsed twice with distilled water and the supernatant obtained was subjected to the determination of glucose, xylose, arabinose, acetic acid and furfural analysis. All experiments were carried out in triplicate and the average values were reported. The catalytic efficiency $[E]$ of ultrasound assisted acid hydrolysis of pretreated SCB was calculated using the following equation from [16]

$$[E] = \frac{\sum S}{1 + \sum I} \tag{4}$$

where $\sum S$ is the sum of sugar concentrations in the hydrolyzate (glucose, xylose and arabinose) and $\sum I$ is the sum of inhibitor concentration in the hydrolyzate (acetic acid and furfural).

2.6. Analytical methods

Cellulose, hemicellulose, lignin and ash contents were estimated by detergent extraction method [17]. The reducing sugar concentration was determined using 3,5-dinitrosalicylic acid (DNS) method [18]. Glucose, xylose, arabinose and acetic acid concentrations in hydrolysate were determined using high performance liquid chromatography (HPLC) coupled with UV and refractive