Industrial Crops and Products 83 (2016) 104–112

Contents lists available at ScienceDirect

Industrial Crops and Products

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

Bench scale dilute acid pretreatment optimization for producing fermentable sugars from cotton stalk and physicochemical characterization

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ARTICLE INFO

Article history:
Received 28 July 2015
Received in revised form 2 November 2015
Accepted 23 November 2015
Available online 31 December 2015

Keywords:
Cotton stalk
Pretreatment
Saccharification
Response Surface Methodology
Characterization
Mass balance

ABSTRACT

Cotton stalk is a holocellulose rich, inexpensive agricultural residue available in surplus without any competitive uses neither as food nor as animal fodder. These aspectshold high potential for cotton stalk as a biomass to be suitable for ethanol production. Dilute acid pretreatment conditions on bench scale have been optimized for cotton stalk by Response Surface Methodology (RSM) using Central Composite Design (CCD). Effect of four pretreatment process variables viz. temperature, acid concentration, time of reaction and stirring speed has been optimized for maximum enzymatic sugar release during the subsequent enzymatic saccharification. Under the optimized pretreatment conditions, i.e., temperature: 157 °C, acid concentration: 1.07% (w/w),and time: 20 min, enzymatic sugar releasewas found to be 684 mg/g of dry pretreated biomass. A correlation of hemicellulose removal and inhibitor formation with combined severity factor (CSF) was drawn. Mass balance carried out for the pretreatment step under optimized conditions resulted in 68.35 and 8.31% of xylose and glucose saccharificationyieldsrespectively.Subsequent enzymatic saccharification yieldsofglucose and xylose were 93.56 and 19.93% respectively. The overall saccharification yield integrating pretreatment and enzymatic hydrolysis of cotton stalk was 91.06%. Physicochemical characterization of native and pretreated biomass was carried out by compositional analysis, FT-IR and XRD revealing significant changes in biomassproperties responsible for improved saccharification efficiency.

1. Introduction

Lignocellulosic materials (LCM) are most abundant andlowerin cost, thus,have been identified as promising feedstock for producing fermentable sugars (Kumar et al., 2014; Pu et al., 2013; Ragauskas et al., 2006). LCM is mainly composed of cellulose, hemicellulose and lignin. Cellulose is a homopolymer of C6 sugars predominantly glucose, whereas; hemicellulose is a hetero-polymer of xylose, arabinose, glucose andgalactose. Often these sugars are acetylated at primary hydroxyl groups. Lignin is an irregular and complex polyphenolicpolymer consisting of phenylpropanoid as a monomeric unit.The carbohydrates present in biomass can be converted to fermentable sugars by enzymatic hydrolysis (Aimia et al., 2015).However, this conversion poses technological challenges owing to the presence of recalcitrants like hemicelluloses, lignin etc.bonding strongly with cellulose (Gaur et al., 2015). Thus, LCM needs to be treated prior to enzymatic digestion to remove these recalcitrants leading to increased porosity and exposed cellulose (Chang and Holtzapple, 2000). Pretreatment is the most expensive step for the conversion of lignocellulosic biomass to biofuels and has a great potential for improvement in efficiency and cost reduction (Kumar and Murthy, 2011; Galbe and Zacchi, 2007). For this purpose, efficientpretreatment methods must be developed to maximize the production of fermentable sugar and minimize degradation products (fermentation inhibitors) like, acetic acid, furfural and 5-hydroxymethylfurfural (HMF).

Depending upon the pretreatment parameters, several key properties of biomass are altered and theseimpact the recalcitrance of pretreated biomass, including the chemical constituents, cellulose crystallinity and ultrastructure, lignin/hemicellulose structures, the cellulose degree of polymerization and accessibility (Gaur et al., 2015; Pu et al., 2013; Raj et al., 2015). Improvement in

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http://dx.doi.org/10.1016/j.indcrop.2015.11.056
0926-6690/

pretreatment efficiency may significantly lower the cost of lignocellulosic ethanol production. Various pretreatment technologies have been reported in literature but no single technology can be declared a "winner" due to the associated limitations. Physical pretreatment such as steam explosion does not require any chemicals and may be good for certain feedstocks (Sharma et al., 2014) but due to its limited ability to remove hemicellulose is not a good choice for long-term ethanol production. Biological pretreatment is not preferred as it is sluggish in nature. Mechanical communition is another method of exposing cellulose but is energy intensive and costly process thus, is seldom used. Ammonia treatment and Ammonia Fiber Expansion (AFEX) are efficient in lignin removal but the need for ammonia recovery and recycling makes this process very challenging. Among chemical pretreatments, alkaline pretreatment is a good alternative for lignin removal however, works better on herbaceous and agricultural residues with low lignin content (Aimia et al., 2015; Umagiliyagea et al., 2015). Dilute acid (DA) treatment is being preferred worldwide for commercialization of cellulosic ethanol due to its low cost and effectiveness (Mosier et al., 2005). It works by hydrolyzing the hemicelluloses (upto 80%) while keeping cellulose and lignin intact. The chances of oligomer formation are minimized by the use of optimized dilute acid pretreatment conditions. Moreover, DA pretreatment is a versatile technique and may be applied to variety of feed stocks including hardwood, softwood, agricultural residues, municipal solid waste, waste paper and herbaceous crops. The associated disadvantages are formation of inhibitors and corrosive nature of acid requiring appropriate metallurgy for pretreatment reactor.

In dilute acid pretreatment, there could be several key variables that need to be carefully manipulated to subsequently achieve maximum enzymatic sugar release. Varying these variables, oneby one could be time consuming and yet may not lead to desirable results. Statistical tool, Response Surface Methodology (RSM) has been successfully applied to biomass pretreatment by many researchers (Pu et al., 2013; Umagiliyagea et al., 2015; Hongbin et al., 2014). In the present study, four different variables (temperature, acid concentration, time of reaction and stirring speed) have been optimized using RSM to achievedesired targets. Under optimized conditions, process mass balance was conducted. Physicochemical properties of native and pretreated cotton stalk were also studied using FT-IR, XRD and compositional analyses.

Cotton is one of the most abundant crops in the tropical and subtropical regions.India occupies first place among the cotton growing countries (Binod et al., 2012). The increase in cotton planting is highly beneficial for economic development, butcotton stalk,an agricultural residue needs to be removed from the field after harvestingas it adversely delays the subsequent plantation (Kaur et al., 2012). Cotton stalk has a good potential as a raw material for cellulosic ethanol production owing to its high cellulose content. In India, about 29.0 million metric tons per annum (MMTPA) cotton stalk is generated out of which approximately 11.8 MMTPA is in surplus with no other competitive uses and bears a potential to generate 2.95 MMTPA ethanol (Binod et al., 2012). In the current study, cotton stalk has been exploited as a feedstock for producing fermentable sugars. The optimized process conditions will pave the way to attempt replication on pilot scale.

2. Materials and methods

2.1. General

Cotton (*Gossypiumhirsutum*) stalk was collected from Sirsa district, west of Haryana, India (29.14–30.0 North latitude and 74.29–75.18 East longitudes) at the time of crop harvesting (March 2013). The stalk was air dried and shredded to the particle size ~2 mm by knife mill and stored in airtight containers at 25 °C until further use. All the experiments were conducted using a single lot of biomass. Chemicals like xylose, glucose, arabinose acetic acid, 5-hydroxymethylfurfural (HMF), furfural and sulfuric acid were obtained from Merck(India) andwereof analyticalgrade. All chemicals were used without further purification. The cellulase preparation Cellic CTec3 was kindly gifted by M/sNovozymes (Mumbai, India). The initial activity of cellulase in the enzyme preparation was 154 FPU/ml.

2.2. Design of experiments using RSM

The RSM was employed for optimizing the operating conditions of acid pretreatment to achievehigherresponse value, i.e. enzymatic sugar release from pretreated biomass. The responsewas assumed to be affected by four pretreatment process variablesviz. temperature (x_1), acid concentration(x_2), time of reaction (x_3) and stirring speed (x_4). The enzymatic sugar release Y, can be assigned values as a function of four variables, i.e.,$Y = f(x_1, x_2, x_3, x_4)$.

For designing the set of experiments using RSM, upper and lower limits were defined for each independent variable and fed to the software Design Expert 9.0.4.1 (Statease, USA). The range and levels of independent variables were based on our preliminary studiesand the limits were: temperature (120 and 180 °C), acid concentration (0.25 and 2.0%, w/w), time of reaction (5 and 65 min) and stirring speed (200 and 600 RPM). To examine the individual and combined effects of these variables on response factor, central composite design were employed. A set of 30 experiments with six central points for replications was generated by the software.A polynomial quadratic equation predicting the response as a simultaneous function of these four variables may be represented as:

$$Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_4 x_4 + \beta_{11} x_{12} + \beta_{22} x_{22} + \beta_{33} x_{32} + \beta_{44} x_{42} + \beta_2 x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{14} x_1 x_4 + \beta_{23} x_2 x_3 + \beta_{24} x_2 x_4 + \beta_{34} x_3 x_4 \quad (1)$$

where, Y is the enzymatic sugar release, β_0 is a constant, β_1, β_2, β_3, β_4 is linear coefficients, β_{12}, β_{13}, β_{14}, β_{23}, β_{24}, β_{34} , are interaction coefficients and β_{11}, β_{22}, β_{33} and β_{44} are quadratic coefficients. The significance of the model and regression coefficient was tested by the analysis of variance (ANOVA).

2.3. Pretreatment of biomass

Perusal of the published work on pretreatment of biomass using pressure reactor indicated that the biomass was heated to the desired temperature along with the catalyst (acid) already added to it and then cooled after the completion of the reaction. However, such experimental conditions were inappropriate, as the exact time of the reaction which is a very important variable could not be controlled.

In view of this, we designed a special bench scale 2 l high pressure reactor having a facility to add acid to the biomass exactly at the desired temperature bypressure equalizing mechanism and then cooled very rapidly.The reactor was fitted with a stirrer of length 290 mm and diameter 50 mm. The heating time for our reactor was ≤9 min for minimum operational temperature (120 °C) and ≤12 min for maximum operational temperature i.e. 180 °C used for this study. Cooling was achieved using high power chiller and the time required to bring the reactor temperature down to ~35 °C (low enough to open the reactor) was ≤12 min. throughout. We believe that this much control over the heating and cooling temperatures is appreciable. Moreover, addition of acid to each set of experiment was carried out as soon as the desired temperature was reached. It was desirable to achieve high concentration of hemicellulose sugars in the liquid stream. Preliminary studies showed that