Industrial Crops and Products 99 (2017) 7–18

Contents lists available at ScienceDirect

Industrial Crops and Products

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

Evaluation of OrganoCat process as a pretreatment during bioconversion of rice straw

Amruta Morone[a], R.A. Pandey[a,*], Tapan Chakrabarti[b]

[a] CSIR-National Environmental Engineering Research Institute (CSIR-NEERI), Nagpur, Maharashtra, India
[b] Visvesvaraya National Institute of Technology (VNIT), Nagpur, Maharashtra, India

ARTICLE INFO

Article history:
Received 16 August 2016
Received in revised form
29 December 2016
Accepted 21 January 2017

Keywords:
Rice straw
OrganoCat pretreatment
Optimization
Saccharification
Structural changes
Process economics

ABSTRACT

Rice straw is deemed as an attractive feedstock for biofuel and platform chemical production owing to its renewability and availability on regional as well as global scale. However, the recalcitrance of rice straw compels an ancillary pretreatment step in the bioconversion process that effectively fractionates the lignocellulosic components. The present article evaluates the OrganoCat process as a pretreatment for rice straw and its effect on the subsequent enzymatic hydrolysis owing to the novel application of OrganoCat pretreatment to rice straw. The maximum cellulose recovery of 98.99%, hemicellulose solubilization and lignin removal of 88.79% and 71.46% respectively was achieved during the OrganoCat pretreatment of rice straw along with improved cellulose accessibility leading to maximum glucose release of 160.57 ± 2.7 g/kg untreated rice straw at different pretreatment conditions included under the range of operating conditions examined in the present study. OrganoCat pretreatment also led to effective fractionation and cell wall breakdown, thereby, enhancing the cellulose accessibility as distinctly evident from the adsorption studies and indirectly deduced from SEM and FT-IR analysis. The material balance of the input & output streams indicated an efficient one-step fractionation with 50% catalyst recovery and 80% solvent recovery. The significance of the obtained results are reported and discussed to gain an insight into the overall potential of the process.

1. Introduction

The dwindling fossil fuel reserves, increasing CO_2 emissions and growing demand for liquid transportation fuels from the transportation sector has led to exploration of renewable resources for biofuel production in order to switch from petroleum-based fuels to renewable biomass-based biofuels and platform chemicals (Morone et al., 2015). International Energy Agency (IEA) has predicted a continual increase in the use of biomass-based fuels which will cater up to 27% of the transportation fuels in 2050 (IEA Report, 2011). Lignocellulosic biomass (LCB), for e.g. rice straw is deemed to be an attractive feedstock owing to its renewable nature, abundant availability and high carbohydrate content. In addition, the open-field burning of this abundant raw material as a waste causes environmental pollution and affects the ecosystem health (Zhu et al., 2015). Therefore, utilizing this under-utilized feedstock for energy, biofuel & bio-based chemical production would allow to tap its unexplored potential. However, recalcitrance of rice straw makes pretreatment an obligatory step in the bioconversion process. While various pretreatment strategies like acid or alkaline hydrolysis (Loow et al., 2016), organosolv (Sindhu et al., 2012), ionic liquids (Xu et al., 2016), wet explosion (Salehi et al., 2012) have been explored for rice straw; the investigation on OrganoCat or biphasic solvent pretreatment still lags behind.

OrganoCat is Organosolv-like process but employs a biphasic solvent system in order to allow easy separation of components unlike in Organosolv. OrganoCat process is a catalytic process for biomass fractionation and involves an organic acid catalyst (for e.g. oxalic acid) which selectively depolymerizes the carbohydrates and a green bio-based organic solvent (for e.g. 2-methyltetrahydrofuran) which allows selective solvolysis of lignin (Pace et al., 2012; Rinaldi, 2014). Although OrganoCat pretreatment has been examined for beech wood, reed and spruce (vom Stein et al., 2011; Grande et al., 2015), OrganoCat pretreatment has not yet been investigated for rice straw. Moreover, the hydrolysis behavior of each LCB is different for the same pretreatment which calls a need to study the individual behavior of biomass (Morone and Pandey, 2014). Therefore, the present study evaluates the effect of process parameters involved in OrganoCat pretreatment process for rice straw through a statistical approach. Further, the efficacy

* Corresponding author at: CSIR-NEERI, Nagpur-440020, Maharashtra, India.
E-mail address: ra_pandey@neeri.res.in (R.A. Pandey).

http://dx.doi.org/10.1016/j.indcrop.2017.01.036
0926-6690/

Table 1
Statistical 2^3-factorial design for OrganoCat pretreatment of rice straw.

Factors	Low level	High level
Temperature (°C)	125	160
Pressure (bar)	10	20
Reaction time (min)	25	45

of pretreatment for rice straw is examined on the basis of cellulose recovery, hemicellulose solubilization, lignin removal, inhibitor formation and the subsequent%saccharification. The present article also attempts to analyze the structural and surface changes occurring in rice straw during the OrganoCat process allied with the reaction chemistry involved in the pretreatment process. It further presents an overview of its potential and contribution to plausible economic improvement.

2. Materials and methods

2.1. Materials

Rice (*Oryza sativa)* straw was collected from the fields in Kanhan, Maharashtra, India and was dried at 45 °C for 48 h followed by grinding and sieving with +20/−80 mesh (ASTM specification) to obtain a particle size of 0.18–0.85 mm. All the chemicals (i.e. oxalic acid, 2-methyltetrahydrofuran, etc.) used during the study were of analytical grade (Sisco Research Laboratories, Mumbai, India). The enzymes cellulase and β-glucosidase were procured from Sigma Aldrich, U.S.A.

2.2. OrganoCat pretreatment

Rice straw was subjected to a biphasic solvent pretreatment wherein 20 g rice straw was suspended in 125 mL aqueous phase containing 0.1 M oxalic acid catalyst to extract hemicellulose and 125 mL organic phase containing 2-methyltetrahydrofuran (MTHF) for extraction of lignin. The reaction was conducted in a stainless steel reactor (0.75L) at a solid loading of 80 g/L. The reactor contents were stirred at an agitation speed of 150 rpm and the reactor was sealed to ascertain a leakage-proof reactor. The reaction mixture was pressurized with CO_2 gas (10–20 bar). CO_2 gas was used to pressurize the reaction mixture importantly to maintain the organic solvent into liquid phase at higher temperatures (i.e. at temperatures above the solvent's boiling point). Moreover, according to the material safety datasheet of 2-MTHF, the vapors of MTHF may form an explosive mixture with air and can lead to severe hazards when exposed to oxidizers. On the other hand, CO_2 is non-flammable and moderately reactive, thereby, making it a suitable choice. The reactor contents were then heated to the desired temperature at the heating rate of 4.71 °C/min and held for a particular time period. This was followed by cooling and separation of solid and liquid fractions. The solid pulp was washed with ethanol (20%) followed by water to eliminate any residual 2-MTHF and was subsequently dried, weighed and analyzed for composition. The biphasic liquor was separated into aqueous phase and organic phase. Lignin was extracted from 2-MTHF using a rotary evaporator and the solvent was recovered for reuse. The schematic representation of OrganoCat process is depicted in Fig. 1.

2.3. Statistical design and optimization

The experimental trials were designed using a 2^3-statistical design in order to optimize the OrganoCat process for rice straw. Temperature, pressure and time were selected as significant factors at two levels generating 8 different experiments (Table 1). The variables viz. temperature, pressure and time were designated as X_1, X_2, X_3 respectively. A model was constructed as a function of these independent variables on the predicted response which was as follows:

$$y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{1,2} X_1 X_2 + \beta_{1,3} X_1 X_3 + \beta_{2,3} X_2 X_3 \quad (1)$$

Where, y is the predicted response; X_1-X_3 are the independent variables influencing the response variable y; β_0 is the baseline term; $\beta_{1\text{-}3}$ are the linear regression coefficients; and $\beta_{1,2}$, $\beta_{1,3}$, $\beta_{2,3}$ are the interaction coefficients. The analysis of variance (ANOVA), regression coefficients and the polynomial regression equation were obtained by using MINITAB 16.0 software (PA, USA). The experimental trials were performed in a random manner and a single operator performed all the trials to minimize block effect.

2.4. Enzymatic hydrolysis

The pretreated rice straw was subjected to enzymatic hydrolysis at the solid loading of 60 g/L and 80 g/L separately in sodium citrate buffer (0.1 M, pH-4.8) along with 15FPU/g dry matter (DM) cellulase and 7.5CBU/g DM β-glucosidase. The saccharification was carried out for 72 h at 50 °C with an agitation speed of 100 rpm. The enzymatic hydrolysis at 60 g/L solid loading was performed in order to restrict the mass transfer limitations during the study of adsorption of enzymes onto the substrate. The samples were collected at definite time interval and centrifuged at 14,089 × *g* (7500 rpm) for 10mins to eliminate residual fibers. The glucose, total reducing sugar (TRS) yields and free protein were monitored in the supernatant and%enzymatic cellulose convertibility (%ECC) were calculated as amount of cellulose digested to cellulose added multiplied by 100. The protein content was estimated by Folin-Lowry method using bovine serum albumin (BSA) as standard. The samples were precipitated with two volumes of chilled acetone in order to remove the interference of sugars in protein analysis. This was followed by centrifugation at 18,785 x g for 10 mins at 4 °C and dissolution of the pellet in 0.05 M sodium citrate buffer (pH 4.8) (Ghose, 1987). Adsorbed protein was calculated based on the initial protein added and free protein available at different time intervals. These data were used to further calculate the adsorption parameters like maximum adsorption capacity (σ), equilibrium constant (K_d), affinity constant (A), and binding strength (S). These adsorption parameters were estimated through a non-linear regression of adsorption data using the following equation (Pareek et al., 2013)

$$[E_{ads}] = \frac{\sigma [S] [E_f]}{K_d} + [E_f] \quad (2)$$

Where $[E_{ads}]$ is the concentration of enzyme adsorbed (mg/mL), σ is the maximum adsorption capacity (mg/mg substrate), [S] is the concentration of substrate (mg/mL), $[E_f]$ is the concentration of free enzyme and K_d is the equilibrium constant (mg enzyme/mL). On the basis of this data, A, the affinity constant given by $A = 1/K_d$ and the binding strength ($S = \sigma^*A$) were calculated.

2.5. Analytical methods

2.5.1. Compositional analysis

Rice straw was analyzed for composition i.e. moisture, ash, extractives and lignin pre- and post-pretreatment using NREL protocols (Sluiter and Sluiter, 2011). Cellulose and hemicellulose were determined gravimetrically (Foyle et al., 2007; Kristensen et al., 2008). Moreover, sugar composition was also estimated based on the acid hydrolysis of rice straw pre- and post-hydrolysis. TRS in the aqueous fraction of OrganoCat process and enzymatic hydrolysate of rice straw was estimated using dinitrosalicylic acid method. The