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Development of a novel ultrasound-assisted alkali pretreatment strategy for the production of bioethanol and xylanases from chili post harvest residue

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HIGHLIGHTS

- First report on ultrasound assisted alkali pretreatment of chili residue.
- Effective removal of hemicelluloses and lignin.
- Pretreated biomass suitable for xylanase production.
- Absence of major fermentation inhibitors.
- Fermentation of hydrolyzate yielded 1.94% of bioethanol.

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ABSTRACT

A novel ultrasound-assisted alkali pretreatment strategy was developed which could effectively remove lignin and hemicelluloses and improve the sugar yield from chili post harvest residue. Operational parameters that affect the pretreatment efficiency were studied and optimized. Inhibitor analysis of the hydrolyzate revealed that major fermentation inhibitors like furfural, 5-hydroxymethyl furfural as well as organic acids like citric acid, succinic acid and propionic acid were absent. Hence fermentation can be carried out without detoxification of the hydrolyzate. Changes in structural properties of the biomass were studied in relation to the pretreatment process using Scanning Electron Microscopy (SEM) and the changes in chemical composition were also monitored. The biomass pretreated with the optimized novel method could yield 0.428 g/g of reducing sugars upon enzymatic hydrolysis. The hydrolyzate obtained by this novel pretreatment strategy was found to be suitable for bioethanol and xylanase production.

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1. Introduction

Increase in consumption of fossil fuels and industrialization as well as accumulation of green house gases leads to search for alternative strategies of energy. Lignocellulosic biomass is the most abundant and renewable resource consisting mainly of cellulose and hemicelluloses and serves as a potential candidate for future renewable energy. Bioethanol is considered as promising alternative to fossil fuels due to several advantages of like clean burning, high octane number and high heat of vaporization when compared to gasoline (Sun et al., 2016).

Lignocellulosic biomass is composed of cellulose, hemicelluloses, lignin and extractives. These components are interconnected by several covalent and non-covalent bonds. One of the major hindrances for bioethanol production from lignocellulosic biomass is the recalcitrance nature. The main physical barrier preventing enzymatic saccharification of cellulose is lignin and hemicelluloses. Hence some pretreatment of lignocellulosic feedstocks to be carried out to break the recalcitrance nature and remove the barriers of hemicelluloses and lignin for improving the enzymatic saccharification rate (Zakaria et al., 2015). Lignin also acts as a bar-

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rier by unproductive binding of cellulase and preventing enzymatic accessibility of cellulose and hemicelluloses (Kim et al., 2003).

India is one of the chief producers of chili in the world. After harvesting of chili, the residue which is left out is creating several socio-economic and environmental concerns. Hence utilization of this residue for the production of bioethanol or other value added products seems promising. Till date only few reports were available for the utilization of this feedstock for ethanol or other value added products. The carbohydrate polymer matrix in CPHR is strongly bounded and cross-linked to lignin. This prevents enzymatic saccharification. Hence a pretreatment strategy has to be applied for the breaking of biomass rigid structure and for more efficient enzymatic saccharification. This includes acid and alkali pretreatment (Preeti et al., 2012), microwave assisted surfactant pretreatment (Sindhu et al., 2017), sono-assisted acid pretreatment (Sindhu et al., 2016) and crude glycerol assisted surfactant pretreatment of chili post harvest residue (Sindhu et al., 2015).

Among the common chemical pretreatments, alkali pretreatment exhibits efficient delignification by lignin solubilisation and cellulose swelling in room temperature. It is the most commonly used method to remove lignin and hemicelluloses from lignocellulosic biomass. During alkali pretreatment the ester linkages in lignin and hemicelluloses are broken down (Sun et al., 2016). If the alkaline pretreatment is carried out at higher temperature the ether linkages in lignin and hemicelluloses are also broken down. Other effects of alkaline treatment includes alteration of structure of lignocellulosic matrix by swelling cellulose which results in the interruption of crystalline structure which in turn leads to decrease of crystallinity index. Ultrasonic waves serves as an efficient source for the production of fermentable sugars from biomass. The main effect of ultrasonic waves is cavitation and acoustic streaming of biomass. This will reduce the particle size which in turn improves the enzymatic saccharification rate. Only two reports were available on ultrasound assisted alkali pretreatment. This includes ultrasound assisted alkali pretreatment of corncobs (Soontornchaiboon et al., 2016; Subedhar and Gogate 2014). No reports were available on ultrasound assisted alkali pretreatment of CPHR.

Most of the conventional pretreatment strategies can only weaken partial factors that limit the enzymatic saccharification of lignocellulosic biomass. Hence combined pretreatment strategies are considered to be effectively remove hemicelluloses and lignin and improve enzymatic saccharification of pretreated biomass (Sun et al., 2016). Most of the physical pretreatment strategies improve the accessible surface area to varying degrees (Sun et al., 2016). Hence exploiting the potential of alkali as a cost effective agent for delignification, combined with ultrasound are likely to be a promising hybrid strategy for pretreatment of biomass.

The objective of the present study was to select the best alkali for ultrasound assisted alkali pretreatment of chili post harvest residue (CPHR) and to optimize various process parameters affecting ultrasound assisted alkali pretreatment of CPHR (USAAP CPHR) and utilization of the hydrolyzate obtained after enzymatic saccharification for the production of bioethanol and xylanases.

2. Materials and methods

2.1. Feedstock

Chili post-harvest residue (CPHR) received from Virudhanagar, Tamil Nadu, India was used in this study. The samples were dried (at room temperature 30 °C) and milled using a knife mill. The milled biomass was stored at room temperature until use. Compositional analysis of native and pretreated samples was carried out by adopting NREL protocol (Sluiter et al., 2008).

2.2. Screening of various alkali for USAAP CPHR

Different alkalis (NaOH, KOH and Ca $(OH)_2$) at 2% w/w level were used for initial screening. Biomass (solid) loading of 10% w/w was used for pretreatment. Ultrasound pretreatment was carried out with a frequency of 80 Hz for 30 min followed by hydrothermal pretreatment in a laboratory autoclave at 121 °C for 60 min. After pretreatment the samples were used for hydrolysis after washing and drying.

2.3. Optimization of various process variables affecting USAAP CPHR

Various process parameters affecting USAAP CPHR were optimized by adopting a Taguchi design. Four parameters were selected at four different levels. The parameters selected were biomass loading, sonication time, alkali concentration and pretreatment time. The experiment consists of a total of 16 runs. The details were presented in Table 1. After pre-treatment, the biomasses were neutralized with IN H_2SO_4, washed with tap water and air dried at room temperature.

2.4. Validations of optimized conditions of pre-treatment

For the validation of the model, three confirmation experiments were carried out within the range defined previously. Correlation analyses were carried out based on the predicted and experimental values.

2.5. Enzymatic saccharification

Enzymatic saccharification of the pretreated biomass was carried out in 150 ml stoppered hydrolysis flasks by incubating 10% w/w of pretreated biomass with commercial cellulase (Zytek India Ltd, Mumbai, India). The enzyme loading was 20 FPU per g of pretreated dry biomass, 0.1% w/w of Tween 80 was used as surfactant, 200 μl of antibiotic solution (Penicillin-Streptomycin cocktail, Himedia, India) were added and the total reaction volume was made up to 30 ml with 0.1 M citrate buffer (pH 4.8). The samples were incubated in a shaking water bath at 50 °C for 48 h. After incubation the samples were centrifuged to remove the un-hydrolyzed biomass. Reducing sugar was analyzed by 3, 5-dinitrosalicylic acid method (Miller,1959).

2.6. Scanning electron microscopy

Physical changes of the native and USAAP CPHR were observed by scanning electron microscope (JEOL JSM-5600). Scanning electron micrographs were taken at a magnification of 1000X. The samples were mounted on a double sided conductive tape on precut brass stubs and sputter coated with gold palladium using a JEOL JFC-1200 fine coater. The images were taken with a 10–15 kV accelerating voltage.

2.7. Inhibitor analysis of the hydrolysate

The hydrolyzate obtained after enzymatic saccharification, was centrifuged to remove the un-hydrolyzed residue and filtered through 0.2 μm PES membrane filters (Pall, USA) and the filtrate was evaluated for inhibitors such as furfural, 5-hydroxymethylfurfural, citric acid, succinic acid, propionic acid, acetic acid and formic acid by HPLC. The inhibitors were analyzed using a photodiode array detector set at 254 nm. Rezex ROA columns (Phenomenex) were used with an injection volume of 10 μl and flow rate was maintained at 0.6 ml/min. The concentrations of inhibitors were analyzed using the standard curve.

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