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Improved Sugars Release from Chili Post-harvest Residues by Dilute Acid Assisted Lime Pretreatment

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ABSTRACT

Lignocellulosic biomass seems promising renewable feedstocks for the production of biofuels and other value-added products. It is composed of cellulose, hemicelluloses and lignin, which are connected by several intra- and inter-polymer linkages. For effective utilization of these components, it is necessary to de-construct the biomass in to individual components, which is carried out by various pretreatment techniques. In this work, chili post-harvest residues were used for the generation of sugars with ultimate aim of producing bioethanol. Optimization of various pretreatment process parameters affecting dilute acid assisted lime pretreatment were carried out by adopting a Taguchi design. Results showed best conditions of pretreatment as biomass loading of 10 % (w/w), incubation time of 30 min, $Ca(OH)_2$ concentration of 2 % (w/w) and HNO_3 concentration of 5 % (w/w), which resulted a maximum of reducing sugar yield of 0.622 g/g.

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1. Introduction

Depletion of fossil fuels and increase in environmental concerns like greenhouse gas emissions leads to search for alternative strategies of energy. Bioethanol serves as a potential alternative fuel. Lignocellulosic biomass serves as a potential renewable substrate for the production of bioethanol (Rezania et al., 2017). Usage of fossil fuels in automobiles release large amount of CO_2 which is a major cause of global warming.

Production of bioethanol from lignocellulosic biomass involves several unit operations like pretreatment, neutralization, enzymatic saccharification, detoxification and fermentation. One of the most energy intensive processes is the pretreatment. Several research and developmental activities are going on in this direction to develop strategies which are economically viable. Most of the strategies currently in practice are energy intensive and economically nonviable.

Conventional pretreatment of lignocellulosic biomass is carried out by using either acid or alkali. During acid pretreatment, hemicelluloses are removed from the biomass in the liquid stream while in alkali pretreatment lignin is removed from the biomass in the liquid stream. An integrated process employing the benefits of acid and alkali pretreatment followed by enzymatic hydrolysis would be promising for better hemicelluloses and lignin removal. Chili post-harvest residue is an underexploited lignocellulosic biomass and India has an availability of 0.6 million tons of this residue (Pandey et al., 2009). Till date only few reports were available for its utilization for the production of bioethanol and other value added products (Sindhu et al., 2015, Sindhu et al., 2017, Sindhu et al., 2018). Some kind of pretreatment must be carried to make it accessible for enzymatic saccharification by removing hemicelluloses and lignin. Though several conventional as well as alternative strategies have been developed addressing biomass recalcitrance, most of them are a failure or economically nonviable as an industrial process. The benefits of combined acid and alkali pretreatment was earlier reported by Zhou et al., 2013 for pretreatment of spent mushroom substrate for reducing sugar and biofertilizer production.

The objective of the present study was to select the best dilute acid for dilute acid assisted lime pretreatment of chili post-harvest residue (CPHR) and to optimize various process parameters affecting dilute acid assisted lime pretreatment of CPHR (DAALP CPHR) as well as characterization of native and the pretreated samples by SEM and XRD.

2. Materials and methods

2.1. Feed stock

Chili post-harvest residue (CPHR) received from Virudhanagar, Tamil Nadu, India was used in this study. The samples were dried and milled using a knife mill.

2.2. Screening of various acids for dilute acid assisted lime pretreatment of chili post-harvest residue (DAALP CPHR)

Initial screening experiments were carried out with four different acids

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(H_2SO_4, HCl, HNO_3 and H_3PO_4) at an initial concentration of 2 % w/w, biomass (solid) loading of 10% w/w and $Ca(OH)_2$ concentration of 2% w/w. Pretreatment was carried out 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 parameters affecting DAALP of CPHR*

Optimization of various process parameters affecting dilute acid assisted lime pretreatment of CPHR was carried out by adopting a Taguchi design. The experiment consists of a total of 16 runs. The parameters selected were biomass (solid) loading, HNO_3 concentration, Ca $(OH)_2$ concentration and pretreatment time. Parameters like biomass loading, HNO_3 concentration, $Ca(OH)_2$ concentration and pretreatment time were selected at four levels. Details were presented in Table 1.

Table 1: Taguchi design for optimization of various process parameters affecting hydrolysis of dilute acid assisted lime pretreatment of chili post harvest residue.

Run No:	Biomass Loading (% w/w)	Incubation Time (min)	$Ca(OH)_2$ Conc. (% w/w)	HNO_3 Conc. (% w/w)	Reducing Sugar (g/g)
1	5	15	2	2	0.153
2	5	30	3	3	0.180
3	5	45	4	4	0.096
4	5	60	5	5	0.037
5	10	15	3	4	0.620
6	10	30	2	5	0.622
7	10	45	5	2	0.496
8	10	60	4	3	0.481
9	15	15	4	5	0.123
10	15	30	5	4	0.173
11	15	45	2	3	0.111
12	15	60	3	2	0.094
13	20	15	5	3	0.167
14	20	30	4	2	0.154
15	20	45	3	5	0.102
16	20	60	2	4	0.096

2.4. *Compositional analysis, characterization of native and pretreated biomass by SEM and XRD*

Compositional analysis of native and pretreated samples was carried out by adopting NREL protocol (Sluiter et al., 2008). To investigate physical and chemical changes of lignocellulosic biomass before and after pretreatment, characterizations were performed. In addition to compositional data, physical attributions like morphological changes, cellulose crystallinity changes were also monitored. SEM and XRD analysis were performed as per the protocol adopted by Binod et al., 2012.

3. Results and discussion

3.1. *Compositional analysis of native and pretreated CPHR*

Compositional analysis of the biomass revealed that the native biomass contained 39.95 % cellulose, 17.85 % hemicelluloses and 25.32 % lignin. Control 1 (water alone) contained 41.05 % of cellulose, 16.79 % of hemicelluloses and 24.11 % of lignin. DAALP CPHR contained 43.89 % of cellulose, 10.27 % of hemicelluloses and 14.37 % of lignin. Mass balance analysis revealed a 34 % loss of biomass during the pretreatment process. DAALP was found to be effective in removing hemicelluloses and lignin.

3.2. *Screening profile of various acids for DAALP of CPHR*

Four different acids – H_2SO_4, HCl, HNO_3 and H_3PO_4 at 2 % (w/w) were used for initial screening to select the best acid for DAALP of CPHR. Control experiments were carried out with water alone Initial screening were carried out with 10 % (w/w) of biomass (solid) loading, dilute acid concentration of 2 % (w/w), lime concentration of 2 % (w/w) and pretreatment time of 60 min in a laboratory autoclave at 121 °C. Control samples were the pretreatment carried with water alone gave a reducing sugar yield of 0.05 g/g. H_2SO_4, HCl, HNO_3 and H_3PO_4 assisted lime pretreatment gave a reducing sugar yield of 0.253, 0.214, 0.284 and 0.204 g/g respectively. DAALP CPHR gave a better reducing sugar yield when compared to dilute acid pretreated alone or lime pretreated alone samples. Since HNO_3 assisted $Ca(OH)_2$ pretreated samples gave higher reducing sugar yield it was selected for further optimization of different process parameters affecting DAALP of CPHR by adopting a Taguchi design. Effect of HNO_3 on pretreatment of biomass was earlier reported by Fariaz-Sanchez et al., 2015 for the pretreatment of *Pinus pseudostrobus* wood, where better lignin and hemicelluloses removal were observed with a combined pretreatment with HNO_3 and NaOH. Delignification effect of $Ca(OH)_2$ on sweet sorghum biomass was earlier reported by Kurian et al., 2014. One of the main advantages of $Ca(OH)_2$ is that it removes 80 % lignin without affecting the carbohydrate portion and also it removes 95 % of minerals from the biomass (Agbor et al., 2011).

3.3. *Effect of different process parameters on DAALP of CPHR*

Maximum reducing sugar yield (0.622 g/g) was observed in Run No: 6 where the conditions of pretreatment were $Ca(OH)_2$ concentration of 2 % (w/w), HNO_3 concentration of 5 % (w/w), biomass loading of 10 % (w/w) and pretreatment time for 30 min. Figure 1 (a-d) shows interaction between various process parameters on reducing sugar yield of DAALP CPHR.

Figure 1a shows an interaction between pretreatment time and biomass loading on reducing sugar yield of DAALP CPHR. At low to middle levels of pretreatment time (15 – 35 min) the reducing sugar yield is high (0.60 g/g). It decreases with increase of pretreatment time (40 - 60 min). At low levels of biomass loading (5.0 – 7.5 % w/w) the reducing sugar yield is low (0.40 g/g); it increases with increase of biomass loading (7.5 -11.5 % w/w). Maximum reducing sugar yield (0.60 g/g) was observed with middle levels of biomass loading (9.5 -10 % w/w) and low to middle levels of pretreatment time (15 - 30 min). For improving the economics of pretreatment, it is desirable to carry pretreatment with higher biomass loading. In the present study, pretreatment is not effective at higher biomass loading, this may be due to a negative impact on rheological properties at higher biomass loading and requires specific reactors like helical stirring reactors for proper mixing of biomass at high solid loading (Wu et al., 2011; Zhang et al., 2015).

Figure 1b depicts interaction between biomass loading and $Ca(OH)_2$ concentration on reducing sugar yield of DAALP CPHR. Maximum reducing sugar yield (0.6 g/g) was observed with low to middle levels of $Ca(OH)_2$ concentration (2.0 – 3.0 % w/w) and low levels of biomass loading (8.0 – 10 % w/w). Reducing sugar yield decreased with high levels of biomass loading and $Ca(OH)_2$ concentration.

Figure 1c represents interaction between biomass loading and HNO_3 concentration on reducing sugar yield of DAALP CPHR. At low levels of HNO_3 concentration (2.0 - 3.0 % w/w) reducing sugar yield is low (0.3 g/g). Maximum reducing sugar yield (0.6 g/g) was observed with high levels of HNO_3 concentration and low levels of biomass loading (7.5 – 8.0 % w/w). Importance of HNO_3 as a pretreatment agent was reported by Tutt et al., 2012, where different dilute acids like H_2SO_4, HCl and HNO_3 were used for pretreatment of wheat straw. The study revealed that highest glucose to cellulose conversion rate was observed with HNO_3 (316. 7 g/kg). The samples pretreated with HNO_3 showed highest hydrolysis (57.5 %) and fermentation efficiency (68.0 %) when compared to other acids.

Figure 1d presents interaction between $Ca(OH)_2$ concentration and HNO_3 concentration on reducing sugar yield of DAALP CPHR. At low levels of HNO_3 concentration the reducing sugar yield is low (0.2 g/g). Maximum reducing sugar yield (0.6 g/g) was observed at high levels of HNO_3 concentration (4.0 – 4.5 % w/w) and low levels of $Ca(OH)_2$ concentration (2.5 – 3.0 % w/w).

3.4. *Characterization of native and pretreated biomass by SEM and XRD*

Characterizations of native and pretreated biomass were carried out by Scanning electron microscopy (SEM) and X-ray diffractogram (XRD). The results indicate that there is difference between the native and pretreated samples. Figure 2 (A-E) shows the scanning electron micrographs of native, hydrothermal, dilute HNO_3, $Ca(OH)_2$ and DAALP CPHR. The native samples showed a highly ordered and compact structure while the pretreated samples showed a highly distorted structure. Identical observations were earlier reported by Bak et al., 2009 and Ko et al., 2009.

Details of crystallinity index (CrI), crystallinity size (nm) and crystalline degree are presented in Table 2. The results indicate that the CrI (%) and crystalline degree were higher for pretreated samples when compared to

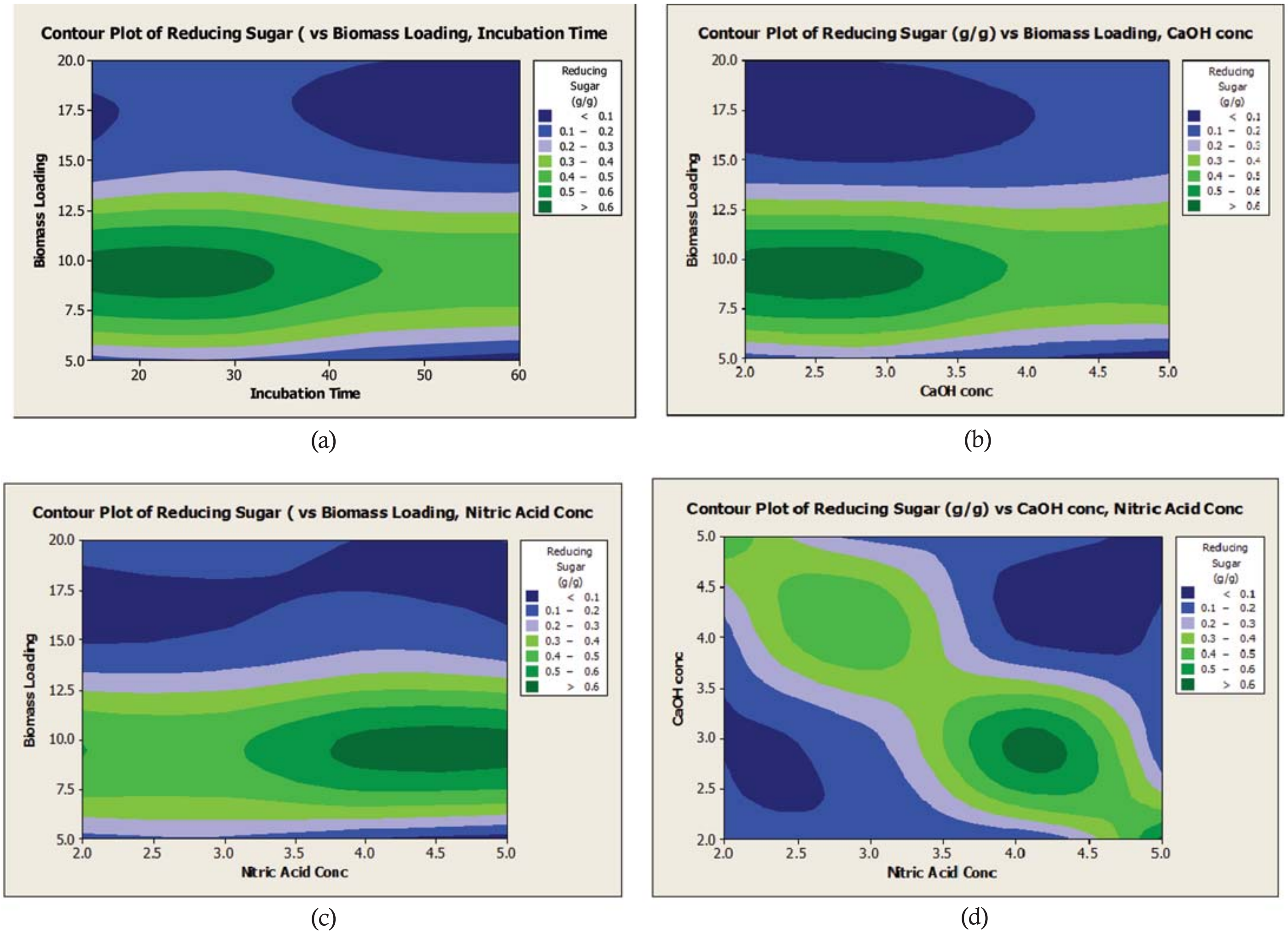

Fig.1. Contour plots showing interaction between (a) pretreatment time and biomass loading (b) Biomass loading and $Ca(OH)_2$ concentration (c) biomass loading and HNO_3 concentration (d) $Ca(OH)_2$ concentration and HNO_3 concentration on reducing sugar yield

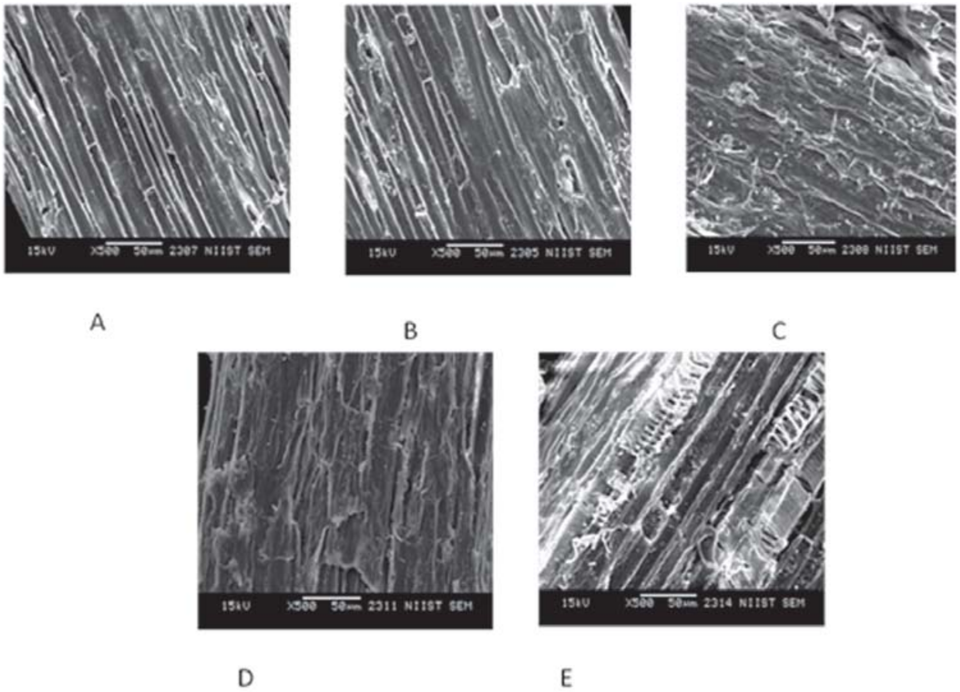

Fig.2. Scanning electron micrographs of native and pretreated samples A- Native B- Hydrothermal pretreated C- HNO_3 pretreated D- $Ca(OH)_2$ pretreated and E- Dilute acid assisted lime pretreated CPHR

Table 2: Crystallinity index, crystalline size and crystalline degree of native and pretreated CPHR

Sample	Crystallinity Index (%)	Crystalline Size (nm)	Crystalline Degree
Native CPHR	23.17	0.9239	44.56
Hydrothermal Pretreated CPHR	28.17	0.4324	45.19
HNO_3 Pretreated CPHR	39.09	0.1856	45.78
$Ca(OH)_2$ pretreated CPHR	33.33	0.2231	45.29
DAALP CPHR	43.69	0.1681	47.15

native CPHR. The crystalline size was also higher for native biomass when compared to pretreated biomass. Identical observations were earlier reported by Binod et al. (2012) for microwave assisted pretreatment of sugarcane bagasse.

4. Conclusion

Compositional analysis data revealed that hemicelluloses and lignin were removed during DAALP of CPHR. The optimum conditions of pretreatment were $Ca(OH)_2$ concentration of 2 % (w/w), HNO_3 concentration of 5 % (w/w), biomass loading of 10 % (w/w) and pretreatment time for 30 min. Maximum reducing sugars yield of 0.622 g/g was observed after optimization of various process parameters affecting DAALP of CPHR. To the best of our knowledge, this is the first report on DAALP of CPHR. Fine tuning of various process parameters with scale-up data and their integration could make the process economically viable.

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