Madras Agric. J., 2020; doi: 10.29321/MAJ.S.000445

RESEARCH ARTICLE

A Catalytic Hydrothermal Pretreatment Process for maximising ethanol production from Pearl Millet biomass

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ABSTRACT

The pretreatment efficiency was compared after enzymatic hydrolysis using a commercial cellulase and crude enzyme (IICT, Hyderabad). The optimized parameters for enzymatic saccharification were found to be lime + HTL pretreated bajra biomass showed a total reducing sugar yield of 745 mg/g of biomass with 84.96% of enzymatic saccharification. The enzymatic digestibility of the bajra biomass increased dramatically due to pretreatment. The overall cost-effective pretreatment and enzymatic saccharification process are lime + HTL pretreatment method and has been recommended as a suitable pretreatment method for cost-effective and safest method for bio-ethanol production. The actual value of total reducing sugar yield of 686.41mg/g of biomass and 22.65 g/L of bio-ethanol were obtained for the optimized process condition.

Keywords: *Bajra; Enzymatic hydrolysis; Saccharification; Sugar release; Cellulase*

INTRODUCTION

With the concern about the environmental impact of fossil fuel and depletion of energy, search for alternative bio-fuel has now become a mandate. Bio-ethanol and bio-diesel are the chief source of alternative liquid biofuel for transportation that can substitute for fossil fuel. The new EBP allows for bio-ethanol production directly from damaged or broken food grains (wheat and rice), molasses, and sugarcane juice from distillery units. In 2019, India reached around 5.8% of (EBP) bio-ethanol market saturation point compared to the 4.1% level of blending in the previous year. Oil Marketing Companies (OMC) procured up to 2.4 billion liters of bio-ethanol because of its favorable season for sugar crop cultivation with financial support. This quantity of bio-ethanol will reach up to 6.6% rate of blending. Bio-ethanol production was continued for five consecutive years for fuel and potable purposes. In India, supply of bio-ethanol for the potable and industry sector will be low due to higher prices, which will result in more energy demand being achieved by import in 2019. A new license has been introduced for importing of bio-ethanol (for non-fuel use) will mostly delay, if not prohibit imports. The United States is the bulk quantity of bio-ethanol suppliers, and it has grown up to 750 million liters in 2019. Pradhan Mantri JI-VAN Yojana program was launched for 2G ethanol production in Feb, 2019. This scheme will be providing financial support for nearly around $277 million for 3 years (2018-19 to 2023 - 2024) (Aradhey, 2018)

MATERIAL AND METHODS

In raw bajra biomass, cellulose and hemicellulose fractions are strongly linked by means of lignin matrix. Hence, a pretreatment experiment for bajra biomass was carried out with (Raw biomass, H_3PO_4+ HTL, AHP + HTL, and $Ca(OH)_2$) previously optimized chemical concentration (Maheshwari *et al.*, 2017). Combo catalytic hydrothermal pretreatment method was used for benchmark lignin reduction. After pretreatment, the reactors were immediately cooled by quenching in an ice water bath for 30 min. Pretreatment liquids were separated by centrifugation (12000 g, 5 min.) for further analysis. The pretreated residual solid bajra biomass was washed with deionized water for compositional analysis and enzymatic saccharification.

Optimizing process parameters for enzymatic saccharification

A statistical method was recently used as an alternative to improve the enzymatic hydrolysis process performance and to develop more economical approach. The optimization of the enzymatic hydrolysis parameters has been done using numerical optimization factorial optimal (custom) design tool in statistical software package

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named Design Expert by using desirability function.

After that pretreatment process, biomass proximate analysis was performed. Enzymatic saccharification experiment was measured by the standard procedure described in NREL/TP-510-42629 (Selig *et al.*, 2008). The filter paper assay for commercial cellulase enzyme and crude enzyme from IICT was performed according to the method of NREL/TP-510-42628 method (Adney and Baker, 2008) and expressed in filter paper units (FPU)

The pretreatment efficiency was compared after an enzymatic hydrolysis trial was performed on the neutralized residual bajra biomass obtained from each type of pre-treated (H_3PO_4 + HTL, AHP+ HTL, Ca(OH) +HTL and raw bajra) biomass, enzyme and dosage [cellulase 40, 50 and 60 FPU, xylanase (10, 20 and 30 U/ml), Crude enzyme 40, 50 and 60 FPU IICT (Indian Institute of Chemical Technology, Hyderabad), cellulase 40 FPU and xylanase 10 U/ml], agitation (200 rpm) solid loading (10%), reaction time (6, 12, 24, 48 and 72 h) and temperature (50˚C) .

Simultaneous Saccharification and Co-Fermentation (SSCF)

SSF experiment was carried out in 500 ml conical flask containing 200 ml of working volume with 10% of lime + HTL pretreated bajra biomass loading and other required nutrients like ammonium sulphate (2 g/L), KH_2PO_4 (5 g/L), yeast extract (5 g/L), calcium chloride (0.2 g/L) and magnesium sulphate (5 g/L) were added. The pH of the fermentation media was adjusted to 4, 4.5 and 5 in separated conical flask. This slurry was autoclaved at 121°C for 20 min and allowed it to for cooling at ambient temperature. After that sterilization, three enzymes (cellulase, xylanase and its combination) at enzyme dosage of 60 FPU /g were added in to the conical flask. In addition to that, two types of yeast strains namely *Pitchia stipitis* and *Saccharomyces cerevisiae* with 10% level of inoculums were added for standardization of fermentation. The yeast inoculums had a cell load of 1 x 10^9/ ml of broth. The slurry was incubated on incubator cum shaker at 50 °C at 20 rpm and allowed it to for fermentation. Samples were taken at particular (24, 48, 72 and 96 h) periodical intervals for determination of ethanol, and total reducing sugar. Ethanol content was recovered from fermented mixture by distillation method. The rate of bajra biomass theoretical ethanol yield or cellulose conversion was calculated from the following formula.

$$\text{Theoretical ethanol yield} = \frac{[EtOHyield]f - [EtOHyield]\ I}{0.51\ X\ (f[biomass]X\ 1.111}\ \text{X}\ 100$$

[EtOH yield]f	=	Ethanol yield at the end of the fermentation (g/L) minus any ethanol produced from the enzyme and medium
[EtOH yield]I	=	Ethanol yield at the beginning of the fermentation (g/L) which should be zero
Biomass	=	Dry weight of the pretreated biomass at starting point of the fermentation (g/L)
F	=	Cellulose fraction in the dried biomass (g/g)
0.51	=	Conversion factor from glucose to EtOH (based on stoichiometric biochemistry of yeast
1.111	=	Converts cellulose to equivalent glucose

RESULTS AND DISCUSSION

Bajra biomass with hydrothermal (HTL) pretreatment process was performed with previously

Table 1. Sugar release and enzymatic (cellulase) saccharification of best pretreated biomass

Pretreated biomass		Sugar release (mg/g)					Saccharification (%)				
		6h	12h	24h	48h	72h	6h	12h	24h	48h	72h
Raw biomass	Cellulase enzyme (40 FPU)	25 (0.02)	59 (0.05)	129 (0.04)	225 (0.04)	309 (0.03)	18.39 (0.06)	24.42 (0.27)	29.41 (0.13)	30.58 (0.04)	32.42 (0.10)
H_3PO_4 + HTL		68 (0.05)	142 (0.02)	197 (0.03)	305 (0.01)	403 (0.03)	42.43 (0.01)	54.38 (0.01)	65.34 (0.01)	71.26 (0.01)	72.65 (0.01)
AHP + HTL		88 (0.05)	175 (0.05)	274 (0.04)	315 (0.01)	456 (0.02)	43.44 (0.01)	55.28 (0.01)	66.34 (0.01)	72.14 (0.01)	73.57 (0.01)
Lime + HTL		95 (0.02)	186 (0.02)	305 (0.03)	404 (0.02)	501 (0.03)	45.75 (0.01)	57.36 (0.01)	68.40 (0.01)	74.80 (0.01)	75.76 (0.01)
Raw biomass	Cellulase enzyme (50 FPU)	25 (0.02)	59 (0.05)	129 (0.04)	225 (0.04)	309 (0.03)	20.27 (0.01)	25.48 (0.01)	30.11 (0.01)	31.38 (0.01)	32.88 (0.01)
H_3PO_4 + HTL		76 (0.05)	156 (0.03)	201 (0.03)	321 (0.05)	423 (0.03)	44.65 (0.01)	55.48 (0.01)	67.19 (0.01)	71.15 (0.01)	72.53 (0.01)
AHP + HTL		93 (0.09)	187 (0.09)	299 (0.03)	322 (0.04)	479 (0.02)	45.55 (0.02)	58.40 (0.01)	69.35 (0.01)	74.45 (0.01)	75.27 (0.01)
Lime + HTL		100 (0.08)	199 (0.05)	322 (0.03)	414 (0.07)	533 (0.06)	46.25 (0.13)	59.43 (0.12)	70.64 (0.31)	75.25 (0.23)	82.40 (0.11)
Raw biomass	Cellulase enzyme (60 FPU)	25 (0.02)	59 (0.05)	129 (0.04)	225 (0.04)	309 (0.03)	21.18 (0.01)	26.15 (0.01)	31.42 (0.01)	32.18 (0.01)	33.38 (0.01)
H_3PO_4 + HTL		99 (0.03)	175 (0.02)	238 (0.03)	353 (0.03)	466 (0.03)	47.29 (0.01)	53.09 (0.01)	61.27 (0.01)	71.41 (0.01)	73.49 (0.01)
AHP + HTL		105 (0.03)	214 (0.03)	312 (0.02)	418 (0.01)	502 (0.04)	45.86 (0.01)	55.76 (0.01)	63.49 (0.01)	75.40 (0.01)	76.24 (0.01)
Lime + HTL		122 (0.01)	242 (0.04)	349 (0.01)	467 (0.02)	698 (0.02)	47.46 (0.01)	58.43 (0.02)	72.57 (0.01)	83.37 (0.01)	84.96 (0.04)

optimized condition. The optimized parameters were found to be 1.1% lime concentration with 140˚C for 30 min. For avoiding the condensation of water in the reactor, steam should be directly passed through the jacket of the reactor. Otherwise, a sieve was fitted in the reactor for the collection of condensed water.

The optimization of the pretreatment parameters has been done using a numerical optimization tool in a statistical software package named Design Expert by using the desirability function. This design contains a total of 29 experimental trials. The dependent variables selected for this study were sugar yield (g/L), lignin reduction (per cent), and increased rate of cellulose content (per cent). The independent variables chosen were catalyst concentration, temperature, and time. The hydrothermal pretreatment is that a water vapor phase or moist steam is used to pretreat the bajra biomass. The hydrothermal pretreatment conditions were a combination of previously optimized chemical concentration, total solid loading with the temperature of 140, 150, and 160˚C and reaction time was 10, 20, and 30 min (Medina *et al.*, 2016). After treatment, the reactors were immediately cooled by quenching in an ice water bath for 30 min. Pretreatment liquids were separated by centrifugation (12000 g, 5 min) for further analysis. The solid bajra residues were washed with deionized water for compositional analysis and enzymatic saccharification.

Table 2. Sugar release and enzymatic (IICT, Crude enzyme) saccharification of best pretreated biomass

Pretreated Biomass		Sugar release (mg/g)					Saccharification (%)				
		6 h	12 h	24 h	48 h	72 h	6 h	12 h	24 h	48 h	72 h
Raw biomass	Crude enzyme IICT (40 FPU)	25 (0.02)	59 (0.05)	129 (0.04)	225 (0.04)	309 (0.03)	18.39 (0.06)	24.42 (0.13)	29.41 (0.22)	30.58 (0.11)	32.42 (0.07)
H3PO4 + HTL		101 (0.08)	199 (0.10)	321 (0.07)	442 (0.05)	501 (0.05)	32.61 (0.01)	50.09 (0.01)	59.74 (0.01)	68.72 (0.01)	69.24 (0.01)
AHP + HTL		129 (0.06)	243 (0.09)	408 (0.04)	499 (0.09)	568 (0.07)	40.24 (0.01)	52.19 (0.01)	60.35 (0.01)	69.44 (0.01)	70.16 (0.01)
Lime + HTL		155 (0.04)	269 (0.05)	412 (0.06)	534 (0.04)	614 (0.03)	42.29 (0.01)	52.55 (0.01)	61.35 (0.01)	71.94 (0.01)	72.27 (0.01)
Raw biomass	Crude enzyme IICT (50 FPU)	25 (0.02)	59 (0.05)	129 (0.04)	225 (0.04)	309 (0.03)	20.27 (0.01)	25.48 (0.01)	30.11 (0.01)	31.38 (0.01)	32.88 (0.01)
H3PO4 + HTL		122 (0.05)	218 (0.13)	352 (0.03)	483 (0.07)	554 (0.04)	44.65 (0.13)	52.48 (0.21)	60.19 (0.17)	70.15 (0.19)	71.53 (0.24)
AHP + HTL		146 (0.09)	309 (0.09)	436 (0.03)	510 (0.04)	628 (0.02)	41.33 (0.11)	53.25 (0.19)	62.21 (0.13)	70.40 (0.04)	71.89 (0.02)
Lime + HTL		179 (0.09)	365 (0.07)	485 (0.02)	562 (0.05)	666 (0.03)	46.25 (0.04)	57.43 (0.08)	68.64 (0.04)	79.25 (0.11)	82.40 (0.41)
Raw biomass	Crude enzyme IICT (60 FPU)	25 (0.02)	59 (0.05)	129 (0.04)	225 (0.04)	309 (0.03)	21.18 (0.01)	26.15 (0.01)	31.42 (0.01)	32.18 (0.01)	33.38 (0.01)
H3PO4 + HTL		137 (0.07)	259 (0.04)	552 (0.05)	643 (0.02)	654 (0.07)	47.29 (0.01)	55.09 (0.01)	66.27 (0.01)	72.41 (0.01)	73.49 (0.01)
AHP + HTL		175 (0.03)	339 (0.02)	636 (0.05)	710 (0.05)	728 (0.02)	43.71 (0.01)	54.33 (0.01)	65.08 (0.01)	72.19 (0.01)	73.56 (0.01)
Lime + HTL		213 (0.04)	475 (0.02)	685 (0.02)	732 (0.05)	745 (0.03)	47.77 (0.01)	61.52 (0.01)	74.45 (0.01)	83.05 (0.01)	84.36 (0.01)

The optimized parameters were found to be lime + HTL pretreated substrate with 60 FPU crude-enzyme loading for 72 h incubation period. The actual value of sugar yield 745 mg/g of biomass and the rate of saccharification (84.36 %) was obtained for the optimized condition. Pretreated biomass showed a total sugar yield of 25 to 745mg/g of biomass and enzymatic hydrolysis of 59.37 to 84.96%. The predicted responses were (712 mg/g of biomass) sugar recovery and 84.23% of enzymatic saccharification and furnished in Table 1, Table 2 and Fig.1.

HTL pretreated hydrolysate recorded TRS 4.092 g/L 100 g of biomass, lignin reduction (65.73%), removal of hemicellulose (34.27%) and 65.73% of cellulose content was obtained for the optimized condition. (Wood *et al.*, 2016) reported that steam (180 to 230 °C) pretreatment with rice husk and straw biomass recorded sugar yield (300 to 500 µg /g of biomass). In the present study, bajra biomass subjected to hydrothermal pretreated hydrolysate showed that glucose (2.973 g/L), galactose (1.191g/L), arabinose (0.714 g/L), mannose (0.542g/L) and xylose (0.692g/L) were obtained in the H_3PO_4+ HTL pretreated biomass. In case of steam explosion pretreatment with sugarcane biomass, sugar recovery [cellobiose (0.09 g/L), glucose (0.14 g/L), xylose (0.75 g/L) arabinose (0.09 g/L) and oligomers (2.10 g/L) were recorded] was low which was reported by (Cunha *et al.*, 2014).

Similar experimental results were noticed by Walker *et al.* (2018) where 75 to 95% of xylose were recovered from wheat straw, miscanthus, willow and corn stover by the steam explosion with phosphoric acid pretreatment. In this present study, 65.73% of cellulose content was obtained from the

HTL pretreated bajra biomass which was slightly low (50 to 64%) in wheat straw by steam explosion pretreatment (Ballesteros *et al.*, 2006).

Simultaneous saccharification and Co-fermentation (SSCF)

The SSCF efficiency of lime + HTL pretreated biomass loading (10%) in a conical flask containing three different enzymes (cellulose, xylanase and its combination) along with (60 FPU) three pH level (4, 4.5, 5), two yeast strains (*Pitchia stipitis and Saccharomyces cerevisiae*) and 10% level of yeast inoculums were standardized and furnished in Fig.2. Totally 91 experimental runs were performed. The simultaneous saccharification and co-fermentation (SSCF) process provided the option for conversion of the LCB sample into bio-ethanol because it enhanced bio-ethanol concentrations with less capital investment when compared to competing processes. In this study, bio-ethanol production from bajra biomass was carried out as per the optimized variables of the SSF process. The ethanol production and utilization of reducing sugars were recorded over fermentation time.

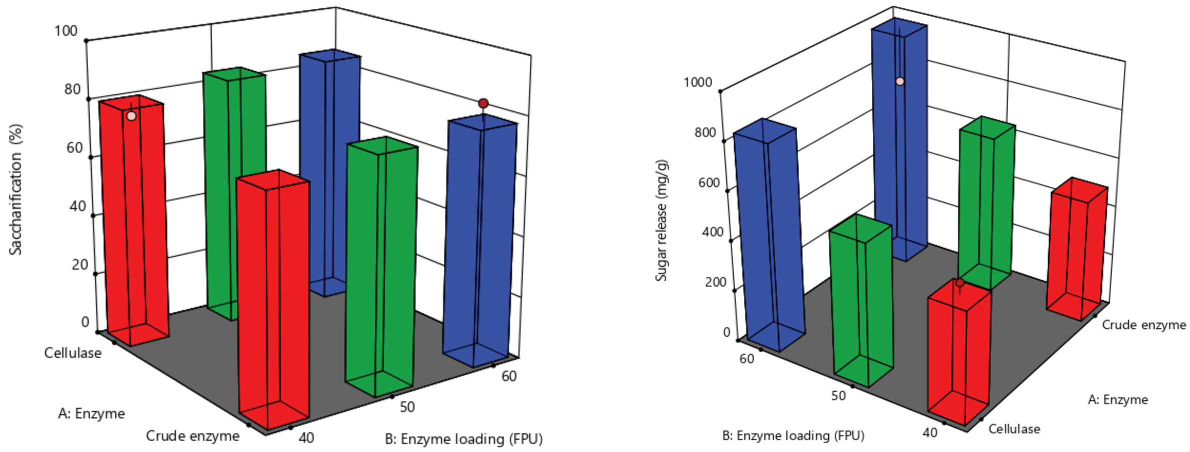

Figure 1. Sugar release and enzymatic saccharification of best pretreated biomass

The optimized parameters are found to be cellulase, pH (4.5), 96 h, and *Pitchia stipitis.* This condition shows the maximum desirability of 1.0. This model gave a good approximation of the temperature, time concentration of the substrate and chemical range where there action takes place during the pretreatment. The actual value of total reducing sugar yield of 686.41mg/g of biomass and 22.65 g/L of bio-ethanol were obtained for the optimized process condition. The predicted value of total reducing sugar yield 695mg/g of biomass and bio-ethanol 22.94 g/L was obtained for the optimized condition. The optimal conditions for LCB 15% (substrate loading) sample, cellulase enzyme with dosage of 100 FPU at 38.5°C for 57.2 h was obtained by Hari Krishna and Chowdry (2000) and in

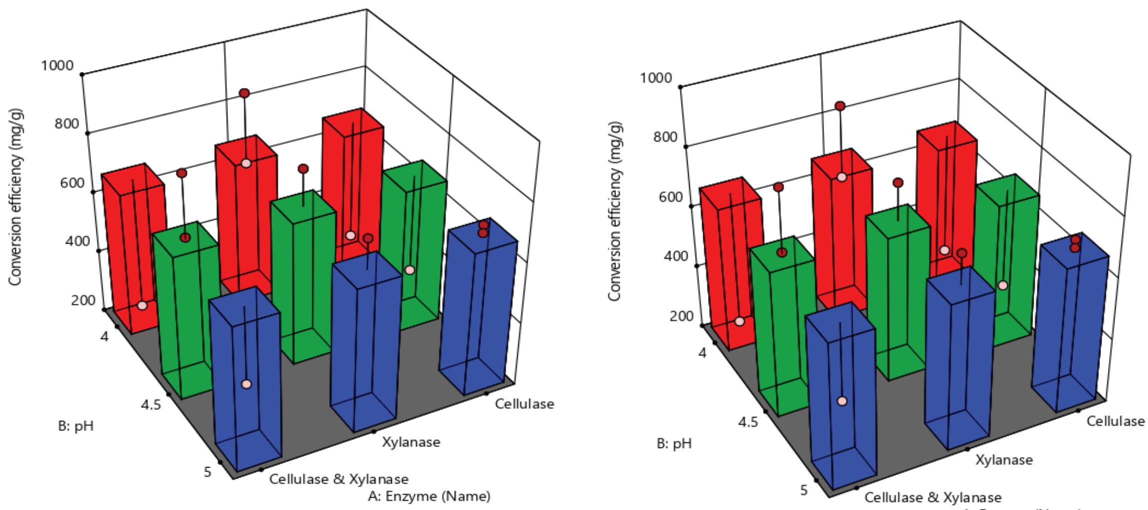

Figure 2. Simultaneous Saccharification and Co-Fermentation (SSCF) bio-ethanol yield

this study had cellulase enzyme (60 FPU), pH (4.5) 96 h and *Pitchia stipitis* at 50° C and 74 % of ethanol was obtained by Yasuda *et al*., 2014 from ammonia pretreated Napier grass with xylanase and cellulose for 96 h by using *S.cerevisiae* and *E.coli KOH.*

CONCLUSION

The pretreatment character should be low cost, application of industrial level, low cost of preparation, effectiveness in the wide range of LCB, complete recovery of fermentable sugars (desirable product) and providing a cellulose fraction possible to be enzymatically converted into glucose at a high rate. Among the pretreatment, acid pretreatment cost was high due to its high-cost acid. Among these chemicals, the lime cost is inexpensive when compared to other chemicals. Combo catalytic HTL pretreated biomass showed a higher rated of enzymatic saccharification than in other pretreatments. This is a promising technique for pretreatment of bajra biomass because of its low residential time, higher solid loading with lower energy requirement.

ACKNOWLEDGMENTS

The authors kindly acknowledge the funding granted by INDO-US Joint clean energy research and development centre (JCERDC) under the scheme "US-India consortium for development of Sustainable Advanced Lignocellulosic Biofuel Systems" (SALBS).

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