



RESEARCH ARTICLE

Effective Chemical Pretreatment for Recovery of Fermentable Sugars from Pearl Millet biomass

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ABSTRACT

Enzymatic hydrolysis rate ranged from 34.17 to 71.28 % in pre treated bajra biomass than in raw biomass. The enzymatic hydrolysis of the pretreated biomass resulted in more sugar release than in raw biomass. The hydrolysis rate is hindered by various factors such as substrates, composition (lignin and hemicellulose fraction), inhibitor concentration, reaction time, pH and enzyme loading, and its activity. Bajra biomass substrate sugar release and saccharification rate was increased with the enzyme dosage level and incubation time. Even though, lime pretreated biomass with cellulase and IICT crude enzyme at dosage level of 60 FPU/g of the substrate, for 72 h showed that higher saccharification rate (67.6 and 71.28 %) and sugar release (414 and 639 mg/g of substrate).

Key words: *Bajra; Enzymatic hydrolysis; Cellulase enzyme; Saccharification; Sugar release*

INTRODUCTION

The major energy demand is still supplied from conventional fuels such as oil, coal, and natural gas. The utilization of fossil fuel has drastically increased the level of greenhouse gases (GHGs) in the earth's atmosphere. Increased public concern about global warming and over-dependence on imported oil has led to the development of renewable energy. Henceforth, bio-ethanol is utilized as effective fuel, blended with gasoline with the name of gasohol. Bio- ethanol can be produced by fermentation of carbohydrates like starch or cellulose that can be converted into sugars. Currently, bio-ethanol is produced from sugar and starchy materials. However, the raw biomass is insufficient to meet the increasing demand for fuels. The demand of bio-fuel range is defined by WEO (World Energy Outlook) 450 Scenario and Current Policies Scenarios. It is most likely to be doubled over these 25 years (IEA, 2013). Each and every country has been setting the target for bio-fuel blending production. In this context, India has an indicative target of 20 % blending of bio-ethanol (EBP) and 5 % HSD of BOB with diesel by 2030. In theory, all ethanol available in 2019, if used completely for EBP, would meet 6.6% blend rate. In India, the National bio-fuel policy (NBP) searches for alternative sources for the achievement of E20 for gasohol and B5 for bio-diesel in 2030 (Saravanan et al., 2018). The majority of the National Bio-fuel Programs have been started to cost-effective ethanol

production from various resources. India is initiating the use of ethanol as an automotive fuel for reducing fuel energy demand. Bio-fuel can be produced from three different kinds of biomass feedstock, which are rich in reducing sugars (sugarcane, sugar beet, molasses and fruit), starch (grains, potatoes and root crops) and cellulosic biomass (municipal solid waste, paper waste, forest and agricultural crop residues). The feasibility of a new energy crop largely depended on its production costs, availability, conversion efficiency and cost of existing fuels. These characters are present in the bajra crop, which makes the crop suitable as a bioenergy crop (Maheshwari et al., 2018). Pearl millet [*Pennisetum glaucum* (L.) R.Br.] is an annual C4 crop, locally known as bajra and it is a nutritious coarse grain cereal. Pearl millet is the fourth most important grain crop in India, which can be predominantly grown as stable food, feed and fodder (Sathya et al., 2013). An effective pretreatment process can be evaluated through sugar release, enzymatic hydrolysis, enzyme dosage, easy to handle, recycling of chemical and waste management (Kumar and Sharma, 2017). An effective pretreatment process is required to alter the composition of the LCB and prepare the biomass for enzymatic hydrolysis. The recalcitrance nature of biomass might be due to the presence of hemicellulose and lignin, crystallinity, degree of polymerization of cellulose, less availability of accessible surface area, particle size, and porosity (Alvira et al., 2010).

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MATERIALS AND METHODS

In raw pearl millet biomass cellulose, hemicellulose fractions are strongly linked by means of lignin matrix. A pretreatment experiment for bajra biomass was carried out with (H_3PO_4 , AHP and $Ca(OH)_2$) various chemical concentrations (Maheshwari *et al.*, 2017). After that pretreatment process, biomass cellulose content was analyzed. The pretreatment efficiency was compared after the enzymatic hydrolysis experimental trial was performed on the neutralized residual bajra biomass. Enzymatic saccharification experiment was measured by standard procedure described in NREL/TP-510-42629 (Selig *et al.*, 2008). The pretreatment parameters are bajra biomass obtained from each type of pre-treated (H_3PO_4 , AHP, $Ca(OH)_2$ 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, 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). Enzymatic activity was measured for the estimation of its purity in the mixture. The enzymatic activity of the biomass is constant in its pure form. Hence, 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). DNSA method was followed further to assess the amount of sugars released by the cellulase (Miller, 1959).

RESULT AND DISCUSSION

The proximate analysis of the raw and pretreated bajra biomass was carried out and given in the previous article. The pre-treatments efficiency was compared after enzymatic hydrolysis using a commercial cellulase and crude enzyme (IICT, Hyderabad).

Table 1. Enzymatic hydrolysis of best pretreated biomass with cellulase enzyme

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	Cellulase enzyme (40 FPU)	25	59	129	225	309	18.39	24.42	29.41	30.58	32.42	
		(0.02)	(0.05)	(0.04)	(0.04)		(0.06)	(0.27)	(0.13)	(0.04)		
		H_3PO_4	29	69	101	199	275	39.28	46.42	57.36	65.79	66.61
		(0.02)	(0.05)	(0.01)	(0.04)		(0.21)	(0.41)	(0.22)	(0.12)		
		AHP	39	86	174	203	384	40.32	48.18	58.15	66.21	69.40
(0.02)	(0.06)	(0.05)	(0.03)		(0.11)	(0.24)	(0.32)	(0.27)				
Lime	56	99	200	304	399	41.86	51.36	63.36	70.48	71.34		
(0.01)	(0.05)	(0.04)	(0.03)		(0.25)	(0.12)	(0.31)	(0.41)				
Raw biomass	Cellulase enzyme (50 FPU)	25	59	129	225	309	20.27	25.48	30.11	31.38	32.88	
		(0.02)	(0.05)	(0.04)	(0.04)	(0.03)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	
		H_3PO_4	30	72	115	202	289	40.42	48.57	59.24	66.40	67.54
		(0.01)		(0.02)	(0.04)	(0.02)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.02)
		AHP	44	97	183	216	399	41.56	50.34	61.35	68.60	69.37
(0.03)		(0.02)	(0.02)	(0.03)	(0.02)	(0.01)	(0.01)	(0.01)	(0.02)	(0.01)		
Lime	69	112	214	311	400	42.48	52.34	63.74	69.61	70.09		
(0.07)	(0.04)	(0.03)	(0.06)	(0.02)	(0.01)	(0.01)	(0.01)	(0.01)	(0.02)	(0.01)		
Raw biomass	Cellulase enzyme (60 FPU)	25	59	129	225	309	21.18	26.15	31.42	32.18	33.38	
		(0.02)	(0.05)	(0.04)	(0.04)	(0.03)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	
		H_3PO_4	39	85	172	219	301	41.55	45.52	55.74	68.45	69.40
		(0.04)		(0.08)	(0.04)	(0.04)	(0.08)	(0.07)	(0.09)	(0.21)	(0.33)	
		AHP	55	101	197	298	400	42.48	47.91	58.36	69.25	70.28
(0.08)	(0.04)	(0.01)	(0.03)	(0.03)	(0.02)		(0.21)	(0.32)	(0.11)	(0.241)		
Lime	72	132	224	323	414	43.45	50.19	59.34	70.57	71.28		
(0.02)	(0.04)	(0.02)	(0.02)	(0.03)	(0.21)		(0.35)	(0.42)	(0.16)	(0.22)		

The activity of the cellulase and the crude enzyme was determined as (123 FPU/ mL) and (185 FPU/ mL) with 1 unit of activity liberating 2 mg of reducing sugar expressed as glucose equivalent. In raw biomass cellulose, hemicellulose fractions are tightly packed with lignin matrix. The raw bajra biomass showed very low percentage of (32.42%) enzymatic hydrolysis because of its complex in

nature of biomass. Lignin is a formidable barrier for enzyme adsorption on to the substrate. Lignin encloses the cellulose in the cell wall hindering cellulase from reaching cellulosic fibrils.

Enzymatic hydrolysis rate ranged from 34.17 to 71.28 % in pretreated bajra biomass than in raw biomass and given in Table 1., Table 2, Fig.1, and Fig.2. The enzymatic hydrolysis of the pretreated

Table 2. Enzymatic hydrolysis of best pretreated biomass with IICT crude enzyme

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	59	129	225	309	18.39	24.42	29.41	30.58	32.42	
		(0.02)	(0.05)	(0.04)	(0.04)	(0.03)	(0.06)	(0.13)	(0.22)	(0.11)	(0.07)	
		H ₃ PO ₄	47	94	187	289	312	25.22	39.46	49.61	58.21	59.37
		(0.03)	(0.06)	(0.04)	(0.02)	(0.08)	(0.04)	(0.07)	(0.06)	(0.09)	(0.11)	
		AHP	69	113	202	317	411	27.76	41.28	52.70	61.78	62.54
(0.05)	(0.04)	(0.07)	(0.04)	(0.03)	(0.05)	(0.02)	(0.13)	(0.14)	(0.32)			
Lime	95	146	247	369	457	29.63	45.17	55.03	64.23	64.34		
(0.06)	(0.07)	(0.06)	(0.05)	(0.02)	(0.04)	(0.08)	(0.05)	(0.06)	(0.03)			
Raw biomass	Crude enzyme IICT (50 FPU)	25	59	129	225	309	20.27	25.48	30.11	31.38	32.88	
		(0.02)	(0.05)	(0.04)	(0.04)	(0.03)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	
		H ₃ PO ₄	50	102	211	312	457	34.17	40.18	45.27	55.29	56.16
		(0.06)	(0.07)	(0.03)	(0.03)	(0.05)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	
		AHP	76	124	256	413	515	35.32	47.78	53.59	60.24	61.50
(0.03)	0.06)	(0.02)	(0.04)	(0.02)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)			
Lime	104	199	314	452	544	40.12	52.27	59.23	65.34	66.58		
(0.05)	(0.09)	(0.04)	(0.05)	(0.02)	(0.09)	(0.11)	(0.17)	(0.12)	(0.04)			
Raw biomass	Crude enzyme IICT (60 FPU)	25	59	129	225	309	21.18	26.15	31.42	32.18	33.38	
		(0.02)	(0.05)	(0.04)	(0.04)	(0.03)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	
		H ₃ PO ₄	75	166	328	444	547	35.48	42.51	51.16	60.68	61.42
		(0.04)	(0.02)	(0.07)	(0.06)	(0.06)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	
		AHP	106	201	429	576	632	36.27	43.65	51.24	63.24	64.36
(0.05)	(0.07)	(0.05)	(0.04)	(0.05)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)			
Lime	124	216	514	602	639	41.30	53.49	64.32	66.49	67.60		
(0.05)	(0.05)	(0.05)	(0.04)	(0.07)	(0.11)	(0.09)	(0.04)	(0.06)	(0.02)			

biomass resulted in more sugar release than in raw biomass. The hydrolysis rate is hindered by various factors such as substrates, composition (lignin and

hemicellulose fraction), inhibitor concentration, reaction time, pH and enzyme loading and its activity (Igarashi et al., 2006). The enzymatic accessible

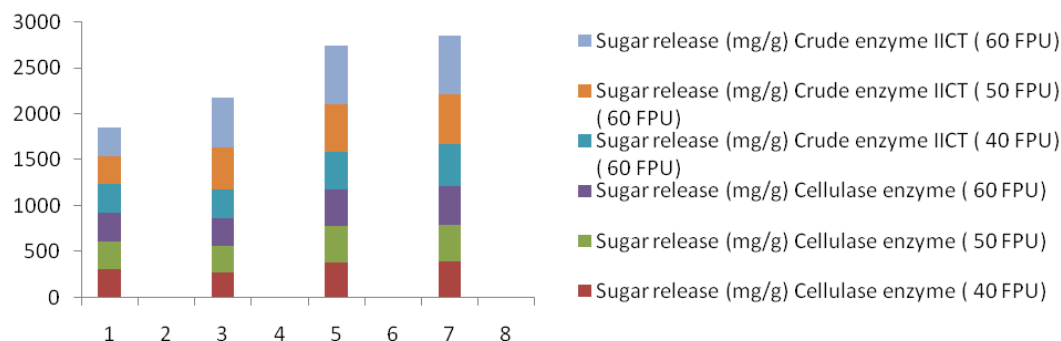


Figure 1. Sugar release from enzymatic hydrolysis of pretreated pearl millet biomass

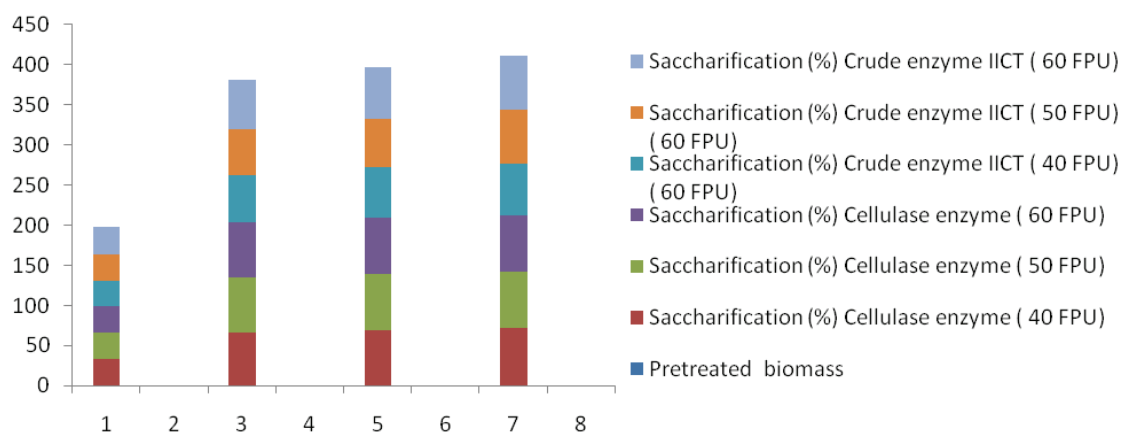


Figure 2. Saccharification rate of best pretreatment pearl millet biomass

area plays an important role in enzymatic saccharification. The amorphous form of cellulose is more accessible than in crystalline form of cellulose (Kumar *et al.*, 2012). In the present study, enzymatic hydrolysis rate was high in the lime pretreated sample when compared to other pretreatments. From this table, bajra substrate sugar release and saccharification rate was increased with the enzyme dosage level and incubation time. Even though, lime pretreated biomass with cellulase enzyme at dosage level of 60 FPU/g of substrate, for 72 h showed that higher saccharification rate (71.28 %) and sugar release (414 mg/g of substrate). Similar results were reported by Sun *et al.* (2016) in sugarcane bagasse showed high rate of enzymatic hydrolysis (80%) with 72 h residential time at 6 FPU/g of dried sugarcane bagasse substrate. This hydrolysis process is quickly occurring in the pretreated bajra biomass and is considered as a key parameter to reveal the pretreatment efficiency (Kim *et al.*, 2014) .

CONCLUSION

The major objective of this current investigation is, water and time consumption, be environmentally friendly and cost-effective. The enzymatic hydrolysis rate was high in the lime pretreated biomass and it consumes lower energy input cost. Enzymatic saccharification is required to utilize or degrade carbohydrate polymers prior to fermentation. The enzyme (Cellulase and IICT crude enzyme) cost is also one of the most important technical barriers for LCB ethanol production. To reduce the enzyme cost and maximum recovery of fermentation is mainly depends upon temperature, substrate loading, pH, mixing rate, enzyme loading, surfactant and incubation time. The process integration approach is highly reduces the capital cost. Successful breakthrough in the application of this method to pearl millet biomass can expand the way through which bio-ethanol production capacity can be increased greatly. The cost for lime pretreatment is lower when compared to other pretreatments applied in this study.

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