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DILUTE ACID HYDROLYSIS OF RICE STRAW FOR EFFICIENT RECOVERY OF XYLOSE

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Abstract: Lignocellulosic biomass is unique among the entire renewable energy spectrum as it is the only source of carbon that can be converted into convenient solid, liquid, and gaseous fuels. The technologies for biofuels and bioenergy are developing rapidly and are fast becoming an important business area. Rice straw is a by-product of rice production and is a great bio-resource. However a huge quantity of the rice straws is not used and burnt in the open fields causing air pollution problem. It is one of the abundant lignocellulosic waste materials available worldwide, which were evaluated for the production of xylose, which can be used as a raw material and converted to high value added products such as furfural, xylitol and microbial cell growth. The objective of the present study was to determine the effect of H_2SO_4 concentration, temperature and retention time on the production of sugars in the form of xylose. The process conditions were optimized in respect of conc. of sulphuric acid, temperature and retention time. The pretreatment of biomass with H_2SO_4 (0.5% v/v) at 140°C for 90 minutes were found to be optimum. These optimized process conditions for hydrolysis of rice straw resulted 80-95% recovery of pentose sugar in aqueous fraction consisting of xylose as major constituent. The xylose thus produced could be utilized as source of value added products with form of furfurals and xylitol etc.
Keywords: Rice straw, Chemical Characterization, Xylose, Dilute acid hydrolysis.
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INTRODUCTION

Lignocellulosic biomass is unique among the entire renewable energy spectrum as it is the only source of carbon that can be converted into convenient solid, liquid, and gaseous fuels. The technologies for biofuels and bioenergy are developing rapidly and are fast becoming an important business area. The use of biomass crops in producing fuels has many advantages *viz.* non-food crop of renewable nature, reduced greenhouse gas production, recyclability of nutrients (lower inputs required), longer growing season (more carbon fixed) in some cases etc. As compared to fossil fuel, most of the biofuels are environmentally benign with the emission of very little sulfur and non-toxic chemicals. However, the development of bio-fuels from lignocellulosic biomass is a major step towards harnessing one of the world's most prevalent, yet least-utilized renewable energy resources (Kadam *et. al.,* 2000). Rice straw is a renewable, abundant and inexpensive lignocellulosic material in China, at an output of 178 million metric tons per year. Paddy occupies the largest acreage in India with production over 135 million tons of rice and over 250 million tons of rice straw in 2010-2011 (FAOSTAT, 2009). Up to now, an efficient way to utilize it yet to be discovered is disposed by burning, resulting in a huge environmental pollution. Rice straw is an agricultural residue containing xylose, which represents up to 90% of the total sugar present in the hemicellulosic fraction of this residue and can be converted to different products. One of these products, xylitol, is widely used in food and pharmaceutical industries. Xylitol production by fermentation may be an attractive alternative to the traditional process employing chemical synthesis (Roberto *et.al*. 2003). Lignocelluloses are mainly comprised of cellulose, a polymer of six-carbon sugar, glucose; hemicellulose, a branched polymer comprised of xylose and other 5-carbon sugars and lignin consisting of phenyl propane units. The presence of lignin limits the fullest usage of cellulose and hemicellulose. To convert these energy rich molecules into simpler forms, it is necessary to remove the lignin from lignocellulosic materials. A number of pretreatment methods such as concentrated acid hydrolysis (Liao *et.al*.2006), dilute acid hydrolysis (Cara *et.al*. 2008), alkali treatment (Carrillo *et.al,*.2005), sodium sulphite treatment (Kuhad *et.al*.1999), sodium chlorite treatment (Sun *et.al.,* 2004), steam explosion (Ohgren *et.al.,* 2005), ammonia fiber explosion (Teymouri *et.al.,* 2005) lime treatment (Kim *et.al.,* 2005), and organic solvent treatment (Xu *et.al.,* 2006) have been used frequently to remove lignin and improve the saccharification of the cell wall carbohydrates.

Of these methods, dilute acid treatment and enzymatic hydrolysis have been the most popular ones. Dilute acid hydrolysis is a fast and convenient method to perform but it leads to the accumulation of fermentation inhibitory compounds such as furfurals, hydroxyl methyl furfurals (HMF) and phenolics. These compounds, depending on their concentration in the fermentation media, can inhibit microbial cell and affect the specific growth rate and cell-mass yield. The acid hydrolysis pretreatment removes the hemicellulosic portion and some fraction of lignin but rest of the lignin remains intact to the cellulosic substrate. During enzymatic hydrolysis of lignocellulosic biomass cellulase components, b-glucosidase and endoglucanase have more binding affinity towards lignin than to the carbohydrates, resulting in lower efficiency of saccharification. Hence, to achieve maximum hydrolysis of cellulosics, which is a prerequisite for ethanol fermentation, an appropriate delignification treatment of biomass is required (Kaya *et.al*.2000). The present work was conducted to select better Dilute acid pretreatment process conditions to achieve maximum sugars in form of Xylose from rice straw.

EXPERIMENTAL

Raw material and chemicals: Rice Straw was collected from agricultural fields of Saharanpur UP (India). After collection, the rice straw was air-dried and procured and subjected to chopping to attain a particle size 2-4 cm. All the chemicals used during these researches were of analytical grade manufacture by Sigma and Merk.

Physico-chemical Characterization of Raw material: Physico-chemical analysis of raw material such as Ash, Pentosan, Klasan Lignin, Hollocellulose, alpha cellulose, beta cellulose and gamma cellulose was carried out by TAPPI (TAPPI, 1992) standard protocols (alpha cellulose- TAPPI Method T203 om -83; Klasion lignin –TAPPI Method T222 om- 83; Pentosans –TAPPI Method T223 hm- 84; Ash-TAPPI Method 211 om-93).

Dilute acid pretreatment: The hydrolysis of rice straw was done at different acid concentrations (0.1%-0.6% v/v). For the maximum breakdown of hemicelluloses, the process of acid hydrolysis was optimized at different temperatures (120°C, 140°C and 160°C) and at different retention times (60, 90 and 120 min.). The bath ratio was fixed at 1:8 in all the tested samples at given conditions. The acid hydrolyzate after treatment was recovered by filtering the contents through double-layered muslin cloth. The remaining rice straw was washed with tap water till neutral pH. The hydrolyzate was analyzed for sugars and biomass was dried overnight till constant weight and used for further experiments.

Sugar Analysis: Reducing sugars were analyzed by DNS method (Miller *et.al*.1959) and xylose sugars were estimated by p-bromoaniline method (Bala, 2004).

RESULTS AND DISCUSSION

Physico-chemical Characterization of Raw material

Table 1 represents the composition of rice straw which shows high pentosan content *i.e.* 18.83% followed by ash 16.73%, lignin 14.32%

with fair and good amount of hollocellulose 63.20% consists of 40.15 % α-cellulose, 14.23% β-cellulose and 7.82% of γ-cellulose.

Table 1. Physico- Chemical Composition of Rice Straw

Parameters	Unit, %
Ash, %	16.73
Pentosan, %	18.83
K. Lignin, %	14.32
Hollocellulose, %	63.2
α-cellulose	40.15
β-cellulose	14.23
γ-cellulose	7.82

Dilute acid pretreatment of Rice straw

In the present study, the acid treatment was given to the all the tested samples of rice straw to breakdown of hemicelluloses to monomers. At lowest temperature (120⁰C), acid hydrolysis of rice straw was increased with the increase in acid concentration from 0.1% to 0.5% v/v dilute H_2SO_4 with bath ratio of 1:8, while a slight reduction was observed in hydrolysis rate at highest acid concentration *i.e.* 0.6% v/v. A maximum amount of TRS (20.46%) and pentose sugar (11.08%) was observed at 0.5% v/v acid treatment with 90 min. of retention time and followed by 19.10% of TRS and 10.31% of xylose at 0.5% v/v acid treatment with 60 min. of retention time and 18.90% of TRS and 10.50% of xylose at 0.5% v/v acid treatment with 120 min. of retention time. The increase in temperature from 120⁰C to 140⁰C had a positive impact on hydrolysis of rice straw in all the optimized conditions (Table 2). A maximum amount of TRS (26.01%) and pentose sugar (15.40%) was observed at 0.5% v/v acid treatment with 90 min. of retention time and followed by 23.54% of TRS and 12.70% of xylose at 0.5% v/v acid treatment with 60 min. of retention time and 22.90% of TRS and 10.80% of xylose at 0.5% v/v acid treatment with 120 min. of retention time. A further increase in temperature from 140⁰C to 160⁰C could not cause any positive impact on hydrolysis rate. The hydrolysis rate was reduced at 160⁰C in comparison to other used temperatures (120⁰C and 140⁰C) (Table 2). The increase in temperature produced a slightly different picture of hydrolysis and maximum amount of TRS (17.63%) was observed at 0.3% v/v acid concentration with 120 min retention time while maximum pentose sugar (13.65%) was observed at 0.5% v/v acid treatment with 60 min. of retention time.

Table 2. Effect of different variables (acid concentration, temperature and retention time) on release of sugars during the acid pretreatment of Rice straw

Temp. ⁰C	Acid Conc., % v/v with bath ratio 1:8	Retention Time 60 min.			Retention Time 90 min.			Retention Time 120 min.		
		Residual Biomass, (%)	Reducing Sugars, (%)	Xylose, (%)	Residual Biomass, (%)	Reducing Sugars, (%)	Xylose, (%)	Residual Biomass, (%)	Reducing Sugars ,(%)	Xylose, (%)
120	0.1	97.31	1.84	0.1	97.06	1.84	0.02	80.45	1.62	0.6
	0.2	92.77	2.73	0.25	89.24	3.55	0.69	74.17	3.34	1.05
	0.3	85.9	7.38	0.48	82.76	9.07	1.77	72.14	8.04	2.42
	0.4	81.68	13.4	3.36	79.77	15.5	7.42	69.33	14.29	1.84
	0.5	80.23	**19.1**	**10.31**	75.40	**20.46**	**11.08**	68.99	**18.9**	**10.5**
	0.6	77.1	18.76	8.1	76.48	18.43	10.1	68.47	17.27	7.1
140	0.1	90.58	2.21	0.25	90.58	3.1	1.26	91.14	3.48	0.72
	0.2	83.00	9.86	0.63	80.33	10.26	4.14	82.53	8.25	2.23
	0.3	80.36	18	6.22	77.90	18.98	9.74	78.57	17.18	8.01
	0.4	77.92	18.92	7.86	73.86	23.01	10.41	75.53	**22.84**	**10.9**
	0.5	72.00	**23.54**	**12.7**	68.85	**26.01**	**15.4**	68.49	22.69	10.8
	0.6	72.84	21.2	10.6	67.20	23.41	10.22	67.20	22.61	10.1
160	0.1	80.45	6.15	0.41	78.87	7.62	0.5	76.87	7.1	0.45
	0.2	74.17	12.63	5.49	73.42	12.96	4.53	72.42	12.43	3.8
	0.3	72.14	14.53	10.14	71.48	15.35	5.9	70.10	**17.63**	**5.25**
	0.4	69.33	15.56	11.47	68.92	**17.2**	**9.65**	68.30	14.5	4.12
	0.5	68.47	**16.44**	**13.65**	67.80	16.38	9.3	67.10	11.8	3.25

	0.6	67.60	15.96	12.26	67.00	15.72	8.15	66.30	11.42	3.1

Physico-chemical Characterization of Pretreated Rice straw

Table 3 represents the composition of pretreated rice straw after dilute acid hydrolysis (0.50% H_2SO_4 concentration, 140^{0}C temperature and 90 minutes retention time) which shows K. lignin content was very high *i.e.* 21.85% followed by ash 19.10% , pentosan 3.40% with fair and good amount of hollocellulose 51.40% consists of 38.60% α-cellulose, 8.50 % β-cellulose and 4.30% of γ-cellulose.

Table 3. Composition of Rice Straw after Pretreatment

Parameters	Percentage
Ash	19.10
Pentosan	3.40
K. Lignin	21.85
Hollocellulose	51.40
α-cellulose	38.60
β-cellulose	8.50
γ-cellulose	4.30

In the present work, dilute H_2S0_4 pretreatment of rice straw was optimized to achieve maximum sugars yield in the form of xylose at minimum severity conditions. The optimum pretreatment conditions on the basis of above result *i.e.* 0.50% H_2S0_4 with bath ratio 1:8 at 140^{0}C for 90 minute, gave the maximum reducing sugars (26.01%) and maximum xylose (15.40%). These optimized process conditions for hydrolysis of rice straw resulted 80-95% recovery of pentose sugar in aqueous fraction consisting of xylose as major constituent. Jain *et al*. (2014) reported the pre-hydrolysis of rice straw at 140°C with 0.6% v/v dilute sulfuric acid for 90 min. holding time in a solid: liquid ratio of 1:8 to get maximum reducing sugar 12.52%. Thakur *et al.* (2011) reported the pre-hydrolysis of bagasse pith with 8% sulfuric acid, for 90 min retention time and 120°C in a solid : liquid ratio of 1:10 to get maximum reducing sugar 28.6 g/L. Dasgupta *et al*. (2013) also studied the acid (8%) pretreatment conditions using sugarcane baggase as a raw material in a solid: liquid ratio of 1:10 at 120°C for 90 min to extract pentose rich fraction 20 g/L. Gunja *et al*. (2016) reported the pretreatment of water hyacinth with 5% dilute sulfuric acid for 90 min of retention time at 140°C to get 19.85 g/L reducing sugar and 11.23 g/L xylose sugar. The xylose yield decreased owing to xylose degradation into furfural which can interfere the microorganism growing in fermentation process (Ezeji *et.al.* 2007). While any further increase in pretreatment stringency caused the released of oligosaccharides and toxic compound without much effect on sugar yield. The chemical delignification of lignocellulosic biomass has previously been reported to achieve better enzymatic saccharification as compared to untreated sample (Mussatto *et.al.* 2003; Mosier *et.al.,* 2005).

CONCLUSION

Dilute acid pretreatment method is used to saccharification of any lignocellulosic biomass. It has dual advantage of solubilizing hemicelluloses and further converting itto fermentable sugars. The process conditions were optimized in respect of conc. of sulphuric acid, temperature and retention time. The pretreatment of biomass with H_2S0_4 (0.5% v/v) at 140^{o}C for 90 minutes were found to be optimum. These optimized process conditions for hydrolysis of rice straw resulted 80-95 % recovery of pentose sugar in aqueous fraction consisting of xylose as major constituent. The xylose thus produced could be utilized as source of value added products with form of furfurals and xylitol.

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