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Bioethanol Production from Ammonia Pretreated Rice Straw

Shaik Muzammil Pasha[1], P. Navya[1], Shaik Musfera[2], Y.S. Goutham[1] and Dr. Chand Pasha[1*]
[1]Department of Microbiology, Nizam College, Osmania University, Hyderabad, INDIA.
[2]Department of Microbiology, Shantinikethan Women's College, Osmania University, Hyderabad, INDIA.

[*]Corresponding Author: cpasha21@gmail.com

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ABSTRACT

Rice straw is produced in large quantity throughout the world. Rice straw is a leading feedstock for bioethanol production. Diluted ammonia pretreatment for one week at room temperature was found to be effective pretreatment. This pretreated rice straw was acid hydrolyzed and subsequently fermented with *Saccharomyces cerevisiae* CP11 strain. 1.5% ammonia pretreatment at room temperature for one week resulted 82.4% delignification and 78.49% of acid hydrolysis. Acid hydrolysate was fermented with maximum ethanol concentration 5.70 % with an ethanol yield of 0.46g/g and fermentation efficiency of 90.6%. Diluted ammonia pretreatment at higher temperature has reduced delignification, saccharification and fermentation efficiency with more phenols and furfurals.

Keywords- Rice straw, ammonia pretreatment, acid hydrolysis, detoxification, fermentation efficiency.

I. INTRODUCTION

Increasing demand, price, and pollution from petroleum products drive the search for alternative renewable fuel resources. Bioethanol is pure fuel source derived by fermenting cellulosic biomass (1). Raw materials for producing Bioethanol are residues of agricultural crops like rice straw, sugarcane bagasse, beet pulp, elephant grass, and wood. 80% of the lignocelluloses are polysaccharides (2). Production of bioethanol does not add carbon dioxide to the environment; thus, it does not contribute to global warming and eco- friendly. Burning of bioethanol results in less consumption of escape organic compounds like nitrogen oxide, carbon monoxide. The method of producing bioethanol using lignocellulosic biomass has wide interest (3). Rice straw is the most abundant lignocellulosic material in the world, and is the outcome of rice production. Rice straw is produced at a rate of approximately 731 million tonnes per year, with Africa accounting for 20.9 million tons, Asia accounting for 667.6 million tons, and Europe accounting for 3.9 million tonnes (4). 205 billion litres of bioethanol per year can be produced from rice straw. It is such a large production from a single biomass source (5). Rice straw contains cellulose (32-47%), hemicelluloses (19-27%), lignin (5-24%), and ashes (18.8%). Mostly, the rice straw is considered useless and burned in fields (6). As the cellulose is embedded in the lignin matrix, there is a need to improve the acquisition of this cellulose, for the conversion of cellulose to glucose. Ammonia is an effective delignifying agent. Soaking in aqueous ammonia is an alkaline treatment that leads to delignification of the biomass and has a high impact on substrates with low lignin content (7). Our aim is to develop a method for pretreatment of rice straw with ammonia and acid hydrolysis of cellulose and hemicellulose in rice straw into simple sugars, simultaneously the fermentation of hydrolysate to bioethanol.

II. MATERIALS AND METHODS

1.1. Raw materials and reagents:

Rice straw was collected from the paddy fields of Jallapally village, Nizamabad district, Telangana, India in June 2022. The moisture content was removed by drying in a hot air oven at $85^{o}C$ for 24hrs. The dried

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rice straw was cut into small size using manual miller and sieved by 20 and 80 meshes to obtain a size range of 0.19 mm-0.90 mm. The rice straws were then stored at room temperature till further use. All chemicals were analytical grade, obtained from MERK.

1.2. Compositional analysis:

The composition of rice straw was analyzed for moisture, cellulose, hemicellulose, lignin and ash content**.** The moisture content was estimated using dry weight method (8). Cellulose content was obtained using cold anthrone reagent (9). Hemicellulose was calculated by using a standard fiber analysis procedure (10, 11). Lignin content and ash content was determined using TAPPI (Technical Association of Pulp and Paper Institute, Atlanta, Georgia, USA, 1992) standard methods (12).

1.3. Pretreatment of rice straw:

Rice straw was pretreated with ammonia at different concentrations 0%, 0.5%, 1%, 1.5% (v/v) at a solid loading of 10% (w/v). Treatments were done in duplicates at room temperature for a week and in an autoclave at 121^oC with 15psi pressure for 60min holding time.

The pretreated straw was filtered, washed properly with deionized water, dried in an air-circulated oven for 15hr at 90^oC, and utilized for further hydrolysis and fermentation procedures.

1.4. Acid hydrolysis and detoxification of rice straw:

The pre-treated rice straw was subjected to sulfuric acid hydrolysis. The pre-treated biomass 10% (w/v) in 0.5M sulfuric acid solution was autoclaved at 121^oC for 10 minutes, then 10% pretreated biomass was added and autoclaved at 121^oC for 30 minutes. After two batches subjected to heat treatment in water bath at 90^oC for 4 hrs. The acid hydrolysate acquired was detoxified with dry lime up to pH 10 for 1hr, filtered and pH was readjusted to 6 with acid. This was succeeded by treating with 2% (w/v) charcoal for 30min by stirring and filtering. The obtained filtrate solution was utilized as a sole source of carbon for fermentation experiments.

1.5. Fermentation:

Saccharomyces cerevisiae CP11 strain, isolated and maintained in our laboratory was used in this study. The inoculum was prepared by growing yeast on YPD (Yeast extract, peptone and dextrose) media, at 30^oC for 24hr. The prepared culture of *Saccharomyces cerevisiae* CP11 was used as inoculum in fermentation.

To 100ml of acid hydrolysate, 1.5g yeast extract, 1g of each peptone and $(NH_4)2SO_4$, 0.5g of each K_2HPO_4, $MgSO_4.H_2O$ and $MnSO_4$ at pH 5.5 were added to prepare fermentation media. The media was autoclaved at 110^oC for 30min. After cooling the media, 3% of inoculum was added. The fermentation was carried out at 30^oC, with agitation of 100rpm for 4hrs, and then kept at static conditions for 92hrs. For every 24hr intervals, samples were collected, analyzed for ethanol content and reducing sugars.

Analytical methods:

Ethanol estimation in fermentation broth was carried out by gas chromatography. The method involved a SHIMADZU GC 2010 with a flame ionization detector. GC was carried out according to NREL procedure LAP # 011, using ZB-Wax column (30mm × 0.25mm).

Estimation of total sugars by Anthron method and reducing sugars was done by DNS method of Miller GL. (13).

Total content of phenolic compound in hydrolysate was determined by FolinCiocalteus (FC) method (14).

The concentration of furfural in the acid hydrolysate was determined by measuring the absorbance of difference spectrum at 277 nm, the characteristic absorption maxima for furfural (15).

III. RESULTS

a. Chemical composition of rice straw:

The compositional analysis of rice straw showed that, it contained 9.75 ± 0.25% moisture, 37.65 ± 0.81% cellulose, 27.05 ± 0.96% hemicellulose, 11.35 ± 0.47% lignin, and 20.24 ± 0.21% ash. Holocellulose was 64.7 ± 1.77%, which was the combination of cellulose and hemicellulose.

b. Pre-treatment of rice straw:

The results depicted that in pretreatment at room temperature for one week, lignin content decreased with increase in concentration of ammonia of pretreatment. The rice straw soaked at a concentration of 1.5% at room temperature displayed the highest delignification rate (82.46%) and high cellulose content (64.24%) (Table1).

Table 1: Rice straws composition after pretreatment at room temperature

Concentration of NH_3 (%)	Cellulose (%)	Hemicellulose (%)	Holocellulose (%)	Lignin (%)	Delignification (%)	Ash (%)
Control NaOH	47.12	29.87	76.99	8.57	24.49	11.47
0	37.65	27.05	64.7	11.35	0	13.21
0.5	49.24	29.25	78.49	7.21	36.47	12.11
1	53.15	22.17	75.32	4.21	62.90	17.25
1.5	64.24	22.01	86.25	1.99	82.46	10.65

In pretreatment at higher temperature for 60 minutes, lignin content decreased with increase in concentration of ammonia but delignification is less than room temperature method. The rice straw soaked at a concentration of 1.5% at 121^oC displayed the highest delignification rate (78.41%) and high cellulose content (56.21) (Table 2).

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Table 2: Rice straw composition after pretreatment in autoclave:

Concentration of NH_3 (%)	Cellulose (%)	Hemicellulose (%)	Holocellulose (%)	Lignin (%)	Delignification (%)	Ash (%)
Control NaOH	48.21	31.24	79.45	8.75	22.90	11.21
0	41.26	30.87	72.13	10.01	11.80	14.78
0.5	47.65	29.55	77.2	7.68	32.33	17.24
1	49.25	26.11	75.35	4.72	58.41	18.65
1.5	56.21	24.98	81.19	2.45	78.41	13.25

c. Acid hydrolysis and detoxification:

Rice straws after pretreatment at room temperature:

The reducing sugars were 135.4; total sugars were 158g in 1 liter acid hydrolysate of 200g substrate. The maximum saccharification efficiency was 78.49%. Total phenols were 24mg and furfural content was 10.87mg in liter acid hydrolysate.

Rice straw composition after pretreatment in autoclave:

The reducing sugars were 112.32; total sugars were 144g in I liter acid hydrolysate of 200g substrate. The maximum saccharification efficiency was 69.17%. Total phenols were 124mg and furfural content was 106.47mg in liter acid hydrolysate.

d. Fermentation:

Rice straw after pretreatment at room temperature:

Total reducing sugars in 100 ml fermentation medium was 13.54 grams. Maximum ethanol concentration and reducing sugar consumption was found at 48 h of fermentation. The leftover sugar was 1.21g and the consumed sugar was 12.3g/100ml fermentation medium. The maximum ethanol concentration produced was 5.70 % with an ethanol yield of 0.46g/g. A fermentation efficiency of 90.6% was achieved.

Rice straw composition after pretreatment in autoclave:

Total reducing sugars in 100 ml fermentation medium was 11.23 grams. Maximum ethanol concentration and reducing sugar consumption was found at 48 h of fermentation. The leftover sugar was 2.81g and the consumed sugar was 8.422g/100 ml fermentation medium. The maximum ethanol concentration produced was 3.75 % with an ethanol yield of 0.445/g. A fermentation efficiency of 87% was achieved.

IV. DISCUSSION

The compositional analysis of rice straw in this study was moisture (9.75%), cellulose (37.65%), hemicellulose (27.05%), lignin (11.35%) and ash (20.24%), which was similar to other studies conducted in Nigeria (4), India (16), China (17) and Egypt (18). Though considering different regions, the chemical composition of rice straw didn't vary to a great extent, and the results were almost similar. Hence, bioethanol technology, once developed can be used globally. The rice straw was delignified with different concentrations of NH_3 0, 0.5, 1 and 1.5 at 121ºC for 30 minutes and at room temperature for a week in this study. 1.5% ammonia pretreatment at room temperature for one week caused 82.4% delignification and 78.49% of acid hydrolysis. Rice straw was pretreated with dilute ammonia (6) and 70ºC for extended time to give a 70% to 80% glucose yield (19). Rice straw was combined with pretreated dilute sulfuric acid and ammonia and used for enzyme hydrolysis. The rice straw efficiently yielded fermentable sugar and achieved almost the same crystallinity index as that of α-cellulose. (20). The acid pre-treatment and subsequent enzyme treatment yielded a high amount of reducing sugars, which were fermented to yield ethanol. The final glucose concentration decreased, due to enzyme inhibition by glucose accumulation (5). Similarly, Kolusheva and Marinova (21) concluded that elevated concentration of glucose significantly decreased the hydrolysis rate and affected the enzyme inhibition. Vlasenko et al., (22) have found that after acid pretreatment (10% solid mater, 0.8% acid and 160 °C) followed by enzyme treatment, the yield of glucose was 43 g L^{-1} or 43% conversion of cellulose to glucose. 67.7g/L reducing sugars and 79g/L total sugars were obtained by ammonia pretreatment and acid hydrolysis. The results in present study are higher than reported. This is may be due to effective pretreatment and acid hydrolysis. A study on sugarcane bagasse showed 88.6% of production yield with M1SAA pretreatment at solid: liquid ratio of 1:6 (19). Kim et al., (23) reported 88.5% yield using ammonia-recycled percolation (ARP) and hot water pretreatment, 99% and 92.5% yield using ARP process with 60PFU and 10PFU cellulose, respectively. Soaking in ethanolic aqueous ammonia (SEAA) pretreatment of corn stover resulted in almost 90% yield (24; 25; 7). Poornejad et al., (26) reported a yield of 93.3% with N-oxide pretreatment of rice straw. Li et al., (27) reported a production yield of 84% using SAA-pretreated corn stover in two-phase SSF process. Kim and Lee, (28) reported an SSF yield of 84% with ARP pretreated rice straws. The present study reports 90.06% ethanol fermentation efficiency without cellulases, due to few inhibitors and efficient delignification. In this study, the fermentation was carried out using *Saccharomyces cerevisiae*, as it's efficient than other yeast species (29). Rice straw pretreated at room temperature produced more ethanol (5.7%) than rice straws pretreated in an autoclave (3.75%) because there is a formation of furfurals at larger amounts in the case of autoclave pretreated rice straws. Usual pre-treatment methods at higher temperatures are releasing more furfurals, which inhibit fermentation efficacy. As a result, it is required that pretreatment be performed at room temperature with

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ammonia for an extended period of time. This had yielded high efficacy in pretreatment, acid hydrolysis and ethanol fermentation efficiency.

V. CONCLUSION

Diluted ammonia pretreated rice straw was acid hydrolyzed and subsequently fermented with *Saccharomyces cerevisiae* CP11 strain. 1.5% ammonia pretreatment at room temperature for one week caused 82.4% delignification and 78.49% of acid hydrolysis. Acid hydrolysate was fermented with an ethanol yield of 0.458g/g of consumed sugar with fermentation efficiency of 90.06%.

REFERENCES

[1] Y. Wang et al. Cascading of engineered bioenergy plants and fungi sustainable for low-cost bioethanol and high-value biomaterials under green-like biomass processing. Renew. Sustain. Energy Rev. 2021; vol. 137.
[2] Kaparaju P, Serrano M, Thansen AB, Kongian P, Angelidai I. (2009). Bioethanol, biohydrogen and biogas production from wheat straw in a biorefinery concept. Bioresour Technol, 100, 2562-2568.
[3] Krishna, S. H., Janardhan, R. T. & Chowdary, G. V. (2001). Simultaneous saccharification and fermentation of lignocellulosic waste to ethanol using a thermotolerant yeast. Bioresource Technology, 77, 193-196.
[4] D. Y. Tsunatu, K. G. Atiku, T. T. Samuel, B. I. Hamidu & D. I. Dahutu. (2017). Production of bioethanol from rice straw using yeast extracts peptone dextrose. Nigerian Journal of Technology, Vol. 36, No. 1, 296 – 301.
[5] Elsayed B. Belal. (2013). Bioethanol production from rice straw residues. Brazilian Journal of Microbiology, 44, 1, 225-234.
[6] Ko, J. K., Bak, J. S., Jung, M. W., Lee, H. J., Choi, I., Kim, T. H., & Kim, K.H. (2009). Ethanol production from rice straw using optimized aqueous-ammonia soaking pretreatment and simultaneous saccharification and fermentation processes. Bioresour. Technol., 100 (19), 4374-4380.
[7] Kim, T.H., Gupta, R., & Lee, Y.Y. (2009b). Pretreatment of biomass by aqueous ammonia for bioethanol production. Methods. Mol. Biol., 581, 79-91.
[8] Mike Lawrence, Andrew Heath & Pete Walker. (2009). Determining moisture levels in straw bale construction. Construction and Building Materials, 23, 2763–2768.
[9] D.M.Updegraff. (1969). Semimicro determination of cellulose in biological materials. Analytical Biochemistry, 32, 420–424.
[10] Banchorndhevakul, S. (2002). Effect of urea and urea-gamma treatments on cellulose degradation of Thai rice straw and corn stalk. Radiat. Phys. Chem., 64(5-6), 417-422.
[11] Sulbaran-De-Ferrer, B., Aristiguieta, M., Dale, B. E., Ferrer, A., & Ojeda-De-Rodriguez, G. (2003). Enzymatic hydrolysis of ammonia-treated rice straw. Appl. Biochem. Biotechnol., 105-108, 155-164.
[12] TAPPI Committee: TAPPI test methods. (1992). Atlanta, GA (USA): TAPPI Press.
[13] Miller GL. (1959). Use of dinitro salicylic acid reagent for determination of reducing sugar. Anal Chem, 31, 426-428.
[14] Singleton, V.L., Rossi, J.A. (1965). Colorimetric of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Viticult., 16, 144-158.
[15] Congcong Chi , Zeng Zhang , Hou-min Chang & Hasan Jameel. (2009). Determination of Furfural and Hydroxymethylfurfural Formed From Biomass Under Acidic Conditions. Journal of Wood Chemistry and Technology, 29, 4, 265-276..
[16] Malarmathi Kavindapadi Balasubramaniam & Ravikumar Rajarathinam. (2013). Implementation of White rot fungal Pretreated Rice straw for Sustainable Bioethanol Production by Saccharomyces cerevisiae. International Journal of Engineering Research & Technology, Vol. 2, Issue 11.
[17] Shengying Jin & Hongzhang Chen. (2007). Near-infrared analysis of the chemical composition of rice straw. Industrial Crops and Products, 26, 207–211.
[18] M. Hashem, E. H. Ali, & R. Abdel-Basset. (2013). Recycling Rice Straw into Biofuel "Ethanol" by Saccharomyces cerevisiae and Pichia guilliermondii. J. Agr. Sci. Tech., Vol. 15, 709-721.
[19] Hadayatkhah, A., Motamedi, H., NajafzadehVarzi, H., Ghezelbash, G., Amopour Bahnamiry, M., & Karimi, K. (2013). Improvement of hydrolysis and fermentation of sugarcane bagasse by soaking in aqueous ammonia and methanolic ammonia. Biosci. Biotechnol. Biochem., 77 (7), 1379-1383.
[20] Kim et al. (2013). Pretreatment of rice straw with combined process using dilute sulfuric acid and aqueous ammonia. Biotechnology for Biofuels, 6, 109.
[21] Kolusheva T & Marinova A. (2007). A study of the optimal conditions for starch hydrolysis through thermostable a-amylase. Journal of the University of Chemical Technology and Metallurgy, 42, 93-96.
[22] Vlasenko EY, Ding H, Labavitch JM, Shoemaker SP. (1997). Enzymatic hydrolysis of pretreated rice straw. Califor Inst Food Agric Res, 59, 109-119.
[23] Kim, T., Nghiem, N., & Hicks, K. B. (2009a). Pretreatment and fractionation of corn stover by soaking in ethanol and aqueous ammonia. Appl. Biochem. Biotechnol., 153(1-3), 171-179.
[24] Kim, T. H., Kim, J. S., Sunwoo, C., & Lee, Y. Y. (2003). Pretreatment of corn stover by aqueous ammonia. Bioresour. Technol., 90(1), 39-47.
[25] Kim, T. H., & Lee, Y. Y. (2005). Pretreatment and fractionation of corn stover by ammonia recycle percolation process. Bioresour. Techol., 96(18), 2007-2013.
[26] Poornejad, N., Karimi, K., & Behzad, T. (2013).

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https://doi.org/10.55544/jrasb.1.5.13

Improvement of saccharification and ethanol production from rice straw by NMMO and (BMIM) (OAc) pretreatments. Ind. Crop. Prod., 41(6), 408-413.
[27] Li, X., Kim, T. H., & Nghiem, N. P. (2010). Bioethanol production from corn stover using aqueous ammonia pretreatment and two-phase simultaneous saccharification and fermentation (TPSSF). Bioresour. Technol., 101(15), 5910-5916.
[28] Kim, T., & Lee, Y. (2007). Pretreatment of corn stover by soaking in aqueous ammonia at moderate temperatures. Appl. Biochem. Biotechnol., 137-140(1-12), 81-92.
[29] S. Bhattacharyya, A K Chandel, M. Taherzadeh, C. Bhattacharjee. (2022). Enhance the bioethanol production from the extracted fermentable sugar by sonication. Journal of Phase Change Materials, 2(1).