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Enzymatic Hydrolysis of Ammonia-Treated Rice Straw

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Abstract

Rice straw pretreated with liquid anhydrous ammonia was hydrolyzed with cellulase, cellobiase, and hemicellulase. Ammonia-processing conditions were 1.5 g of NH_3/g of dry matter, 85°C, and several sample moisture contents. There were four ammonia addition time (min)-processing time (min) combinations. Sugars produced were analyzed as reducing sugars (dinitrosalicylic acid method) and by high-performance liquid chromatography. Monomeric sugars increased from 11% in the nontreated rice straw to 61% of theoretical in treated rice straw (79.2% conversion as reducing sugars). Production of monosaccharides was greater at higher moisture content and was processing time dependent. Glucose was the monosaccharide produced in greater amounts, 56.0%, followed by xylose, arabinose, and fructose, with 35.8, 6.6, and 1.4%, respectively.

Index Entries: Sugars; rice straw; ammonia treatment.

Introduction

Rice straw is one of the most abundant agricultural wastes in California and Central Venezuela, accounting for more than 1.5 and 0.9 million t/yr, respectively *(1,2)*. Brazil, Colombia, Peru, Mexico, and Argentina also have considerable amounts of rice straw. Recently, rice has been considered one of the four top crop priorities in Venezuela, which will make the quantity of straw increase.

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Rice straw has a high content of cellulose and hemicellulose (about 70%) with energetic values similar to those of corn. Unfortunately, these carbohydrates have no value either as animal feeds, since they are hardly digested by ruminants and cannot be digested by single gutted animals and humans, or as feedstocks for sugars production, because of a very low conversion if they are not chemically and physically pretreated. On the other hand, rice straw is generally burned *in situ* after cropping; however, several concerns about this practice have arisen in terms of air pollution, and, therefore, it is necessary to look for other uses for the straw. Several groups are trying to use rice straw as a feedstock for ethanol production in California.

Several treatments such as acid-catalyzed steam explosion, acid hydrolysis, and ammonia freeze-explosion (AFEX) have been used *(1)* to increase the susceptibility of rice straw to enzymatic hydrolysis. The first two treatments produced greater sugar yields. On the other hand, an ammonia treatment (pressurization depressurization with ammonia) has been applied to several lignocellulosic materials, such as dwarf elephant grass, alfalfa, and florigraze rhizoma peanut *(3–5)*, and has produced high sugar yields (70–95%). Preliminary work on rice straw showed that high conversions could be attained with ammonia treatments *(6)*. Therefore, it may be possible to convert the rice straw fibers into sugars by using the pressurization depressurization with ammonia treatment and appropriate enzymes. The sugars produced could be used as feeds and foods, as well as substrates for fermentation processes to produce alcohols, organic acids and other chemicals. The objective of the present study was to investigate the effect of pressurization depressurization with ammonia treatment on the extent of enzymatic hydrolysis and sugar profile of ammonia-treated rice straw.

Materials and Methods

Raw Material

Rice straw derived from a medium-grain rice crop from the northern part of California´s Central Valley was used.

Ammonia Processing

A laboratory-scale batch ammonia reactor unit consisting of a 4-L reactor with appropriate support equipment was used for the treatment of rice straw. A 15% moisture (wet weight basis [wwb]) rice straw was hammer milled to nominally 1-in. length and kept under refrigeration until used. Water (adjusted to experimental level) and liquid anhydrous ammonia were added to 80-g samples (dry matter [DM]), and the temperature was rapidly raised to 85°C. After the treatment time, pressure was suddenly released and the treated samples were collected and allowed to air-dry overnight. Moisture contents are expressed in wet weight basis.

Ammonia treatment conditions were as follows: 15, 35, and 60% moisture content; 1.5 g of NH_3/g of DM; and four ammonia delivery time-dwell

Table 1
NDF Solubles and Hemicellulose Contents
of Untreated and Ammonia-Treated Rice Straw[a]

Sample[b]	Solubles (%DM)	Hemicellulose (%DM)
Untreated	22.15	27.63
4-20-15	28.18	23.11
4-20-35	41.41	12.16
4-06-35	36.54	16.33
4-20-60	47.17	11.39
4-00-35	31.02	18.94
1-05-60	30.22	18.50
4-05-60	32.10	17.27
1-00-60	24.40	24.55
4-00-60	26.00	22.73

[a] Cellulose contents did not vary with treatment.
[b] Numbers refer to ammonia delivery time (min), dwell time (min), and moisture content (%, wwb).

time combinations (1-0, 4-0, 4-5, and 4-20). Delivery time refers to the time the required amount of ammonia takes to get into the reactor chamber. Dwell time refers to the time allowed after the ammonia gets in the reactor until decompression occurs, when ammonia is removed from the chamber. Treatments and analyses were carried out in duplicate. Untreated rice straw was used as a control for all experiments. The experimental conditions are presented in Table 1.

Fiber Fractionation Analysis

Neutral detergent fiber (NDF), acid detergent fiber, and acid detergent lignin were determined in triplicate to estimate cellulose, hemicellulose, and lignin by standard fiber analysis. Solubles were calculated as the fraction solubilized by the NDF solution and includes sugars, oligosaccharides, pectin, protein, and some minerals *(7)*.

Enzymatic Hydrolysis

Enzymatic hydrolysis was performed with unwashed pretreated solids using cellulase (Spezyme CP; Genencor, Rochester, NY), cellobiase (Novozym 188; Novo Nordisk, Franklinton, NC), and hemicellulase (Biofeed Plus CT; Novo Nordisk), at a solids loading of 5% (w/v), in 500-mL Erlenmeyer flasks containing 100 mL of 0.05 *M* citrate buffer (pH 4.8) placed at 50°C at 100 rpm for 48 h in an incubator shaker (Innova 4300; New Brunswick Scientific, Edison, NJ). Sodium azide was added for preservation (0.15%). The first experiment was set with enzyme dosages of 2 and 5 IU/g of DM using the ammonia-treated sample with the lowest NDF to determine the best dosage and hydrolysis time. Sugar production was measured as reducing sugars during 72 h with the dinitrosalicylic acid (DNS) method *(8)*.

Enzymatic hydrolysis of the rest of the samples was carried out at optimal enzyme dosage and hydrolysis time, as described in Results and Discussion. Sugars were measured both as reducing sugars and as individual sugars by high-performance liquid chromatography (HPLC) *(3)* before and after enzymatic hydrolysis. Standard sugars for HPLC were sucrose, glucose, xylose, arabinose, fructose, mannose, and galactose (Sigma, St. Louis, MO).

Soluble sugars and sugar composition of the rice straw fiber were determined by acid hydrolysis and HPLC *(3,9)*. Cellulose conversion to sugars with respect to theoretical was determined based on initial glucose content, whereas hemicellulose conversion was based on initial xylose + arabinose content. Total sugar conversions were based on total initial individual sugars determined by acid hydrolysis.

Results and Discussion

Solubles, cellulose, hemicellulose, lignin, and protein contents of untreated rice straw used were 22.1, 45.2, 27.6, 5.1, and 5%, respectively (NDF solubles include protein, so compositions total >100%). The ammonia treatment increased solubles while decreasing hemicellulose in the solid phase owing to solubilization (Table 1). Hemicellulose decreased by 58.8% in the 4-20-60 treatment; lignin decreased from 5.1 to 4.14% (18.8%). Both factors contributed to enhancing the susceptibility of the fibers to enzymatic hydrolysis, as occurred with dwarf elephant grass, alfalfa, and florigraze rhizome peanut *(3–5)*. Figure 1 shows that for solubles contents lower than approx 32%, there is a linear relationship between reduction in hemicellulose and increase in solubles. As the ammonia treatment conditions improved, the increase in solubles was greater than the decrease in hemicellulose, suggesting that other components become soluble.

The unwashed 4-20-60 treatment sample was used to investigate the optimal enzyme loading and hydrolysis time; results are presented in Fig. 2. After 24 h, the sugar production for an enzyme loading of 2 IU/g of DM stopped, whereas for an enzyme loading of 5 IU/g of DM it still increased by approx 8%. Sugar yield for 5 IU/g was higher than for 2 IU/g (25.5%). This was similar to alfalfa *(4)*. A grass such as dwarf elephant grass and a legume such as florigraze rhizome peanut *(3,5)* required less enzyme to produce even higher sugar yields in the case of the grass. Based on these results, an enzyme loading of 5 IU/g of DM for a 48-h hydrolysis was selected to determine the susceptibility of all treated and untreated samples to enzymatic hydrolysis. Initial sugar content in untreated rice straw, measured after a 5-h wetting period, was 10.4 mg/g of DM, which was mainly glucose (90%) and galactose. Vlasenko et al. *(1)* found a lower value in AFEX-treated rice straw, and xylose, arabinose, galactose, and mannose were also present, although in very low amounts. Xylose was not detected in the treated samples, indicating that hemicellulose was partly solubilized (likely owing to production of oligomers) but not hydrolyzed to monomers during ammoniation, as has occurred with other ammonia-treated materials *(3–5)*. The acid hydrolysis of the untreated sample yielded (in mg of sugar/g of DM)

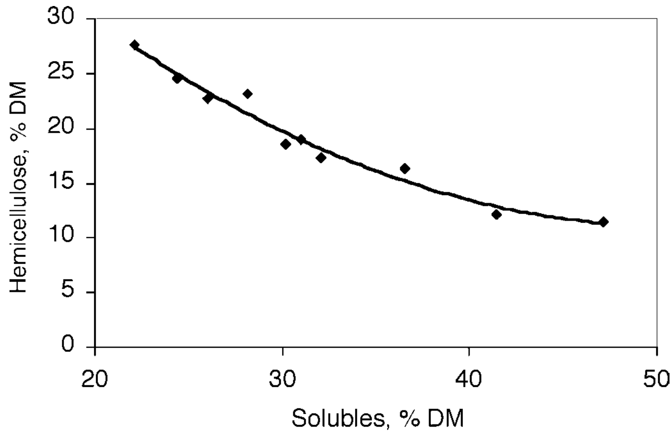

Fig. 1. Relationship between hemicellulose and solubles contents of rice straw samples.

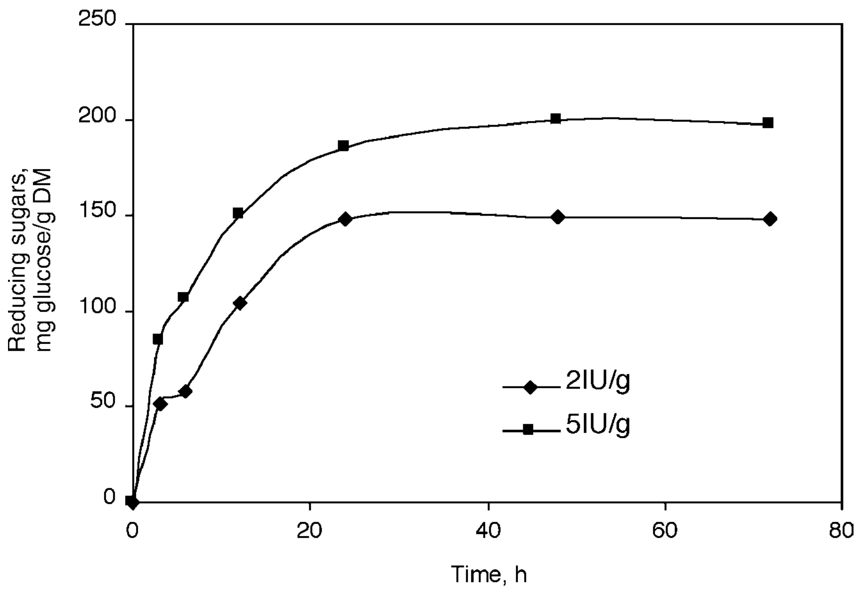

Fig. 2. Kinetics of enzymatic hydrolysis of rice straw measured as reducing sugars for 2 and 5 IU/g of Spezyme CP.

376.2 for glucose, 164.6 for xylose, 35.5 for galactose, and 42.4 for arabinose, making up a total of 618.7 mg/g of DM.

Table 2 presents the results of the sugar yields after enzymatic hydrolysis for all the unwashed treated samples and the untreated sample. Reducing sugars were always higher than individual sugars determined by HPLC, which indicates that there are dimers as well as oligomers.

Table 2
Reducing (DNS) and Individual Sugars (HPLC) from Enzymatic Hydrolysis of Untreated and Ammonia-Treated Rice Straw

	Sugars (mg/g DM)						
	HPLC[a]						
Sample[b]	G	X	Gal	A	F	Total sugars	DNS reducing sugars
Untreated	66.3	<0.4	2.8	<0.3	<0.4	69.1	82.4
4-20-15	90.4	63.1	<0.5	6.2	8.8	168.5	220.5
4-20-35	173.8	162.8	32.5	<0.3	8.5	377.6	482.8
4-06-35	152.5	138.9	<0.5	26.5	7.6	325.5	406.8
4-20-60	148.3	141.8	<0.5	27.5	7.1	324.7	450.5
4-00-35	126.0	68.5	1.7	14.2	6.9	217.3	231.7
1-05-60	177.4	102.4	<0.5	18.7	7.7	306.2	387.0
4-05-60	207.8	133.0	<0.5	24.4	5.9	371.1	489.8
1-00-60	150.0	39.9	<0.5	9.7	6.4	206.0	268.6
4-00-60	148.0	77.1	<0.5	16.7	7.9	249.7	302.3

[a] G, glucose; X, xylose; Gal, galactose; A, arabinose; F, fructose.
[b] Numbers refer to ammonia delivery time (min), dwell time (min), and moisture content (%, wwb).

The difference rose to 25% for high sugar conversion treatments. The relationship between both determinations is linear as shown in Fig. 3. Total reducing sugars increased from 82.4 mg/g of DM (13.3% conversion) in the untreated sample to 482.8 of mg/g DM in one treated sample (78.0% conversion), corresponding to a factor of 5.86, which shows the great efficiency of the treatment. Similarly, a factor of 5.46 for total individual sugars was found in the same treatment. Such treatment (4-20-35), which corresponds to a delivery time of 4 min and a dwell time of 20 min for a 35% rice straw moisture, was similar in sugar yield to a treatment (4-05-60) with the same delivery time and a shorter dwell time (5 min) but with a higher rice straw moisture (60%).

Complex interactions have been detected in several ammonia-treated materials; generally, the severity of the treatment increases as moisture content of the material increases—in other words, it appears that ammonia needs water to be effective *(3)*. In addition, since the dissolution is exothermal, biomass reaches a higher temperature than the reactor temperature, therefore increasing the severity. The fact that the sugar yield obtained in a treatment (1-05-60) with the same loading of ammonia, dwell time, rice straw moisture content but with a shorter ammonia delivery time (1 min) was 17.5% smaller (371.1 vs 306.2 mg/g of DM) appears to indicate that time is needed for ammonia to dissolve and to make contact with the material. Sugar production is linearly related to hemicellulose content (reduced by solubilization) by the treatment, as Fig. 4 indicates, which suggests that it can be used to predict sugar conversions from ammonia-treated materials.

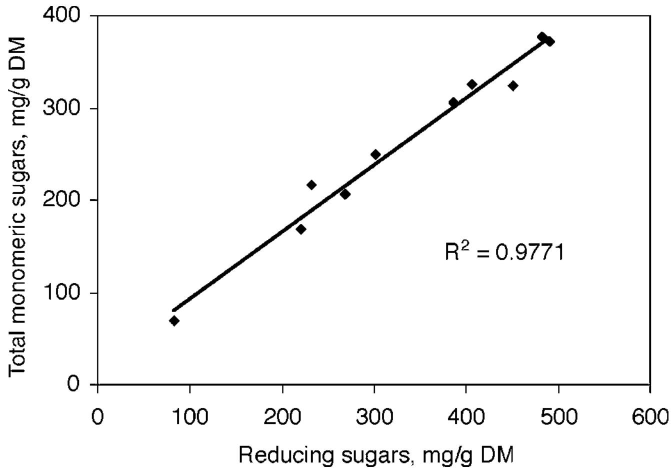

Fig. 3. Relationship between monomeric (HPLC) and reducing sugars produced by enzymatic hydrolysis of untreated and ammonia-treated rice straw.

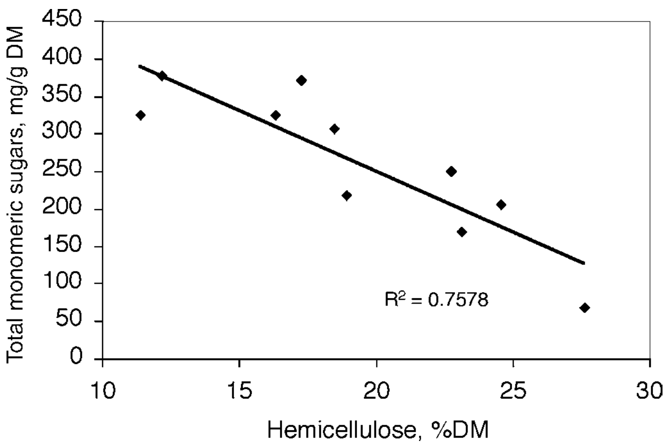

Fig. 4. Relationship between monomeric (HPLC) sugars produced by enzymatic hydrolysis and hemicellulose content of untreated and ammonia-treated rice straw.

Glucose yield increased from 66.3 mg/g of DM in the untreated rice straw to 207.8 mg/g of DM in the 4-05-60 treatment, or a 3.13 factor increase (55.2% of theoretical). On the other hand, ammonia treatment allowed other sugars such as xylose, arabinose, and fructose to appear. In the case of galactose, it was only separated from hemicellulose with the longest treatment (20 min). It is very interesting that this was true only at 35% moisture. The ammonia treatment used was considerably very efficient for hemicellulose hydrolysis (from negligible to 157.4 mg/g of DM), showing a conversion of 76.0% of theoretical. The maximum overall conversion based on total analyzed individual sugars (4-20-35 treatment, 377.6 mg/g of DM) was 61% of theoretical, whereas the one based on reducing sugars (4-05-60 treatment, 489.8 mg/g of DM) was 79.2%. The conversion of the nontreated sample (69.1 mg/g of DM) was just 11% of theoretical.

In previous works on rice straw, Dale et al. *(10)* obtained 95% conversion (based on reducing sugars) using an enzyme loading of 80 IU/g of DM on AFEX-treated rice straw, Vlasenko et al. *(1)* obtained 48.7% with an enzyme loading of 20 IU/g of DM, and Kaur et al. *(11)* achieved a maximum saccharification of 54.3% (cellulose basis) with a 26 filter paper units (FPU) of cellulose/g of rice straw treated with 4% sodium hydroxide in combination with 60 min of steam pressure. Therefore, the results obtained in our study with relatively high sugar conversions obtained with a very low enzyme loading and a 48-h hydrolysis time look very promising. The conversion that we obtained is similar to that obtained with an acid process and greater than that obtained with the Swan (acidified steam explosion) process *(1)*.

The release of monosaccharides was moisture dependent, as can be inferred when the 4-20-15, 4-20-35, and 4-20-60 treatments, which only differ in rice straw moisture content, are compared. The sugar yield for the higher moisture contents doubled that for 15% moisture.

Figures 5 and 6 show the effect of moisture as well as dwell time. For 35% moisture content, the optimal ammonia addition time-processing time was 4–20 min, while for 60% moisture content it was 4–5 min and produced the highest sugar yield. It can be seen that at 35% moisture, by increasing dwell time above 6 min, sugar yield increases slightly. However, since galactose was released, the sugar obtained at this condition is the maximum value for this moisture. A combination of high moisture content (60%) and long processing time (4–20 min) decreased glucose production, but it did not affect xylose production.

When enzymatic hydrolysis was applied to rice straw subjected to the optimal ammonia treatment, 4-05-60 (owing to lower dwell time), although similar to treatment 4-20-35, glucose was the monosaccharide produced in greater amounts (56.0%), followed by xylose (35.8%), arabinose (6.6%), and fructose (1.4%). Other monosaccharides were insignificantly produced. The sugars produced are fermentable and can be used to produce a wide variety of chemicals. In addition, all are digestible by animals, with only some limitations for pentoses in the case of feeding single gutted animals, however, the sugar profile is suitable for animal feeding. Glucose can be converted into fructose for sweetening, as it is currently done in the corn industry.

Conclusion

Enzymatic hydrolysis of ammonia-treated rice straw produced readily available material as monosaccharides useful for several purposes. The sugar yield values that we obtained are among the greatest values found in the literature. Sugar and glucose yields were moisture and dwell-time

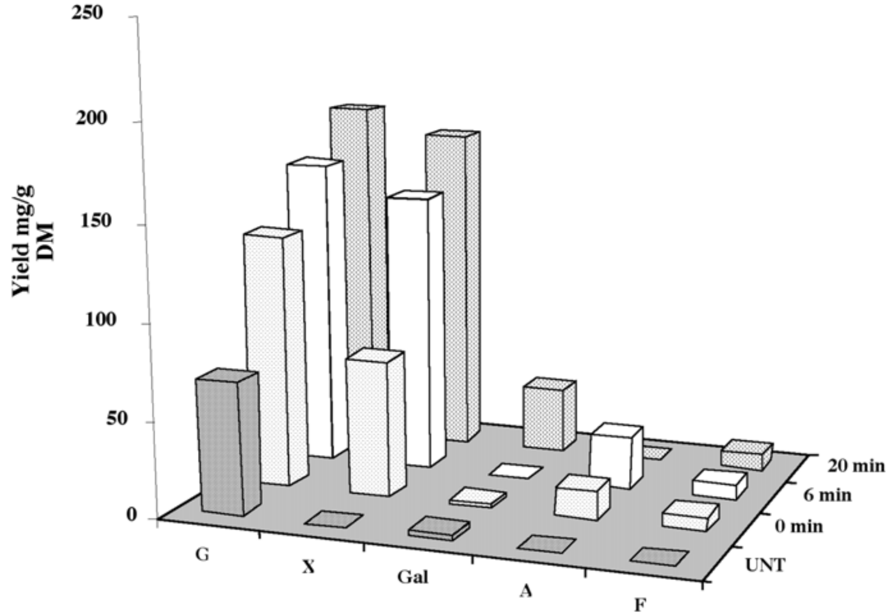

Fig. 5. Effect of ammonia dwell time on sugars yield from rice straw (35% moisture) for ammonia delivery time of 4 min. Unt, untreated; G, glucose, X, xylose; Gal, galactose; A, arabinose; F, fructose.

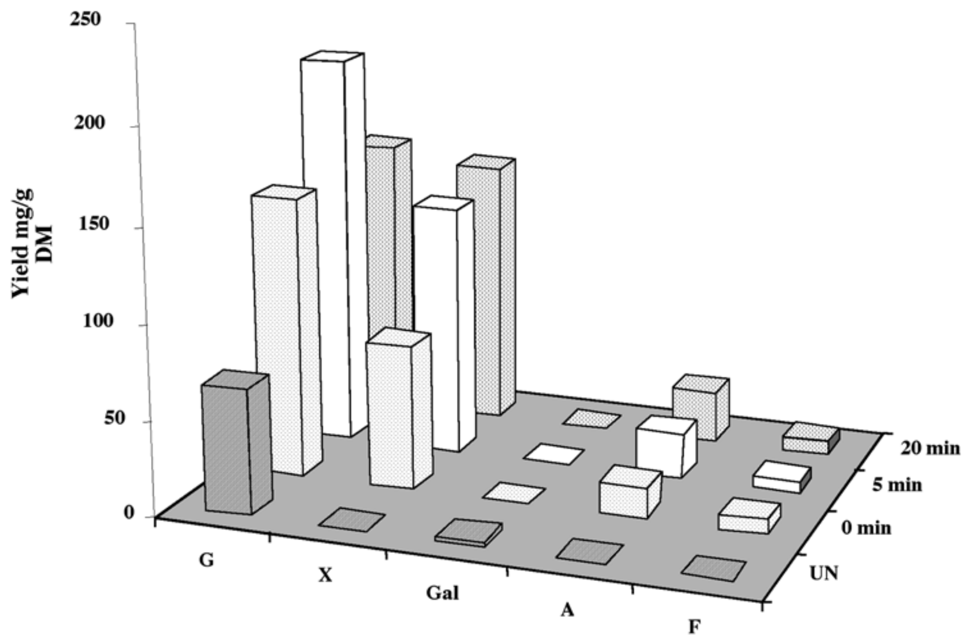

Fig. 6. Effect of ammonia dwell time on sugars yield from rice straw (60% moisture) for ammonia delivery time of 4 min. Unt, untreated; G, glucose, X, xylose; Gal, galactose; A, arabinose; F, fructose.

dependent. Hexoses represent >50% of the sugars. Ammonia treatment increased solubles and decreased hemicellulose content, which, in turn, are correlated with the sugar production. The optimal treatment condition was a 4-min ammonia delivery time, 5-min dwell time, 60% moisture content, and 1.5 g of ammonia/g of dry rice straw.

Acknowledgments

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