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Production of Reducing Sugars from Dilute Acid Hydrolysis of Rice Straw

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Abstract

Rice straw represents a huge percentage of agricultural residues across the world that is grossly underutilized and mostly burnt. This paper focuses on the use of dilute acids to hydrolyze rice straw with a focus of producing sugars that can be used for value added products. Acid hydrolysis was carried out at different concentrations of hydrochloric acid, sulfuric acid and acetic acid on 5% w/v and 7% w/v rice straw. Results show that maximum reducing sugar of 32.23% and 29.56% was obtained within 3 hours for 0.3% v/v HCl and H_2SO_4 respectively. Maximum reducing sugar obtained for 7% v/v acetic acid in 8 hours was 27.11%. Hydrolysis carried out at 5%w/v rice straw showed marginally better yields when compared with reactions carried out at 7% w/v rice straw. Results showed percentage hydrolysis yield of 29.56, 32.23 and 27.12 for 5% w/v rice straw and 27.21, 31.12 and 25.11 for 7% w/v rice straw for sulfuric acid, hydrochloric acid and acetic acids respectively.

Keywords: Acid Hydrolysis, Lignocellulose biomass, Reducing sugars, Rice straw

Introduction

Recent times have seen a dramatic increase in the production of rice in Nigeria. An estimated 5.7 million tonnes of rice was produced in 2017 [1]. A byproduct of rice production is the availability of rice straw and rice husk. Rice straw is one of the most abundant agricultural residues available in the world. It is estimated that 731 million tons per year rice straw is produced globally (Africa: 20.9 million tons, Asia: 667.6 million tons, Europe: 3.9 million tons, America: 37.2 million tons 61, and Oceania: 1.7 million tons) [2]. Rice straw is used as animal feed and as fertilizer for soil however its use is limited when compared to other straws. Its use as animal feed is limited by its characteristic low digestibility when compared to other straws. It also exhibits slow degradation in the soil. It has high mineral content and harbors rice related diseases therefore also making it unattractive for use as soil fertilizer. The utilization ratio of rice straw is low compared to other crop residues [3]. Open field burning is the major practice for its disposal leading to air pollution and its likely consequent effects on the environment. Rice straw like other lignocellulosic waste can be converted to value added products by hydrolysis. Rice straw contains 19–27% of hemicellulose, a heteropolymer composed mainly of xylose followed by arabinose [4-5]. Rice straw has low lignin content when compared to other lignocellulosic biomass such as wheat straw and corn stover. Therefore it is expected that it might be more amenable for hydrolytic degradation. Chemical hydrolysis using dilute acids can be used to produce reducing sugars which can utilize to produce biofuels and other valuable chemicals. In this work, dilute HCl, H_2SO_4 and CH_3COOH were used at different concentrations to produce reducing sugars. This paper examines the effect of concentration and residence time on the percentage yield of reducing sugars. The study will investigate dilute acid hydrolysis as a stand-alone process to see how much sugars can be produced. Several papers have looked at the effect of dilute acids [6-9]. However, most of these papers have investigated acid hydrolysis as a pretreatment option and combined it with either enzyme hydrolysis or a fermentation step to produce bioethanol. This paper will potentially reduce the enormous amount of straw that is left behind annually from the non-utilization of straw.

Materials and Methods

Rice straw was collected from a local farm in University of Agriculture Makurdi. It was sun dried and ground into small particles in the range of 0.36 mm to 1.00 mm with a mortar and pestle. It was then stored in a 10L glass container at room temperature of 25-30°C. Different concentrations of dilute acids were prepared by dissolving with distilled water. 1M HCl, H_2SO_4 and CH_3COOH were used for hydrolysis reactions. 5g of rice straw was weighed and transferred to a volumetric flask wrapped

with aluminum foil and then 100 mL of 1% v/v acid was slowly added to flask (5% w/v). Similarly, 10 g of rice straw was weighed and transferred to a volumetric flask and then 100 mL of 1%v/v acid was slowly added (10% w/v). Different concentrations of the dilute acids (HCl, H_2SO_4 and CH_3COOH) with serial dilutions of 0.1% v/v, 0.3% v/v, 0.3% v/v, 0.7%v/v and 1%v/v were also used for the reactions. Samples were collected at 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours and 8 hours respectively. 1 mL samples collected by means of a syringe were diluted with distilled water until it was neutral and then analyzed for reducing sugars. The reducing sugar produced from the acid treatment was measured by dinitrosalicylic acid (DNS) method. This involves mixing 3mL of diluted hydrolysate collected at different times and mixing with 3 mL of DNS reagent. This mixture is then heated in a test tube for 5 minutes and allowed to cool. The absorbance was then read by spectrum UV spectrophotometer at a wavelength 540 nm [10]. The concentration of reducing sugars was estimated from the calibration curve produced for glucose of concentration of between 0.1 g/L and 0.5g/L. Results were reported as mean ± SD for triplicate analyses.
The percentage reducing sugar was calculated as:

$$\% RS = \frac{[\text{sugar in solution}]\ (g/L)}{[\text{rice straw}]\ (g/L)} \times 100 \quad (1)$$

Where % RS is the percentage reducing sugar, the bracket, [ ] stands for concentration.

Results and Discussion

Figure 1 shows results of the acid hydrolysis carried out on 5% w/v and 7 % w/v rice straw using different concentrations of sulfuric acids and three different serial dilutions of 0.1% v/v, 0.3% v/v and 0.5% v/v of 1M H_2SO_4. Results show that increasing the concentration of sulfuric acid from 0.1% v/v to 0.5%v/v acid produces an increased reducing sugar yields with a maximum percentage reducing sugar of 29.56% and 27.21% for 5% w/v and 7% w/v rice straw respectively. There is however a reduction in the percentage reducing sugar when concentration of acid is increased from 0.3% v/v to 0.5% v/v. This result is similar to results obtained by Gupta et al. 2016 where % reducing sugar of 26.01% was obtained for H_2SO_4 sulfuric acid hydrolysis after 90 minutes [11]. The minor difference might be due to the longer retention time used in this experiment.
Results from Figure 1 also show that higher yields of sugars are obtained at 5%w/v rice straw concentration when compared to reactions carried out at 7% w/v. Acid concentration of 0.1% v/v showed the lowest reducing sugar yields of 12.93% and 13.11% at 5% w/v and 7% w/v rice straw respectively.

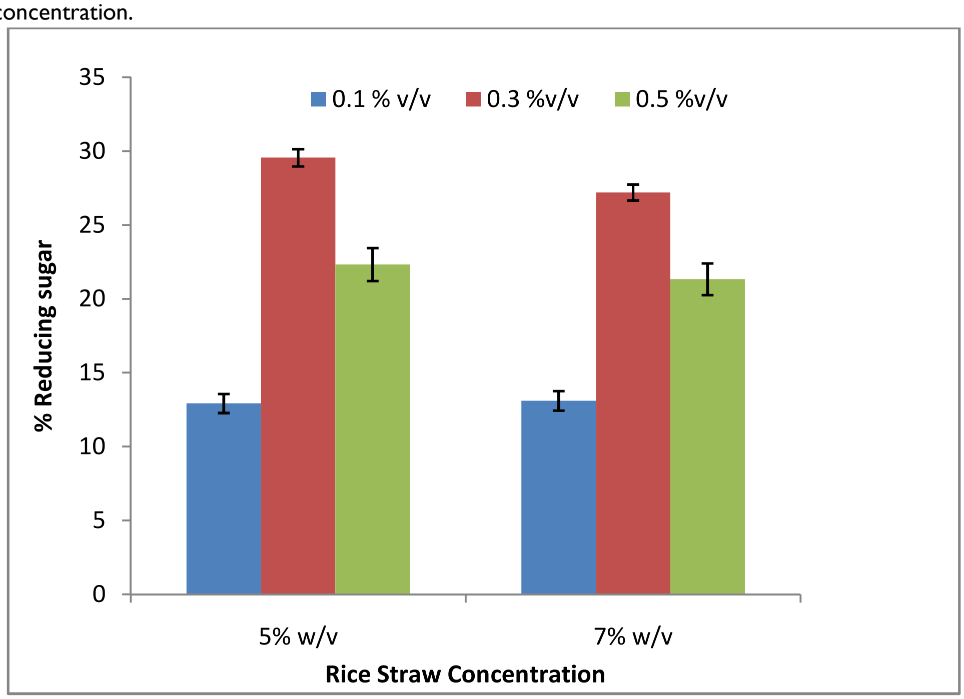

Figure 1: Acid hydrolysis of 5%w/v and 10% w/v rice straw at different sulfuric acid concentrations.

Results from Figure 2 show similar trends for acid hydrolysis of HCl acid when compared with hydrolysis by H_2SO_4. Maximum reducing sugar yield is obtained for 0.3% v/v HCl when compared to acid hydrolysis carried out at

0.1%v/v and 0.5% v/v HCl. Reactions carried out at 0.1% v/v HCl also produced the lowest reducing sugar yield. However, higher hydrolysis yield was obtained for hydrolysis with HCl than H_2SO_4 at corresponding rice straw concentration and acid concentration. While 5%w/v rice straw produced a reducing sugar yield of 32.23% for HCl acid at 0.3% v/v, sulfuric acid at 0.3% v/v gave a reducing sugar yield of 29.56%. Results at 0.5% v/v also show marginally higher reducing sugar for acid hydrolysis using HCl than hydrolysis using H_2SO_4.El-Tayeb *et al.* [12] reported a maximum reducing sugar of 12% when HCl was used to pretreat rice straw however differences in the reducing sugars obtained can be attributed to various factors (particle size, liquid to solid ratio, type and concentration of acid used, temperature, and reaction time) to influence monomer yield [13-14]. Reactions carried out at rice straw concentration of 7%w/v had lower hydrolysis yield when compared to reactions carried out at 5% w/v.

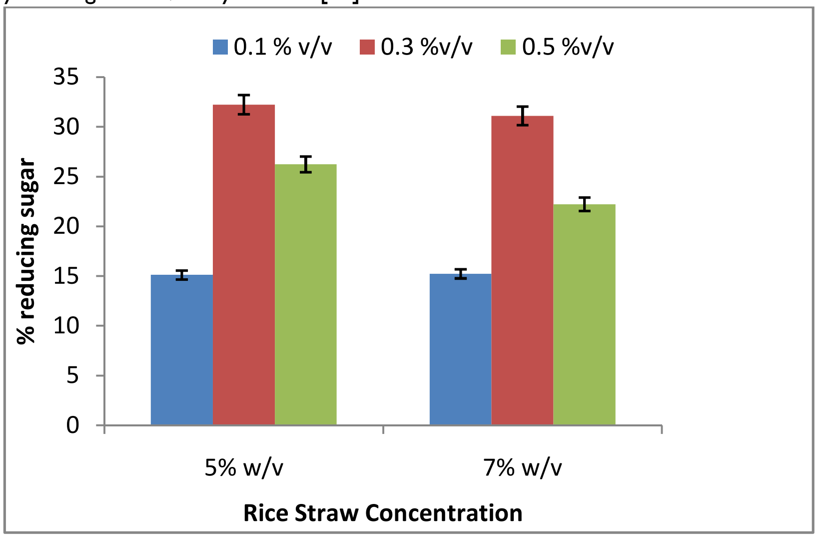

Figure 2: Acid hydrolysis of 5%w/v and 7% w/v rice straw at different Hydrochloric acid concentration

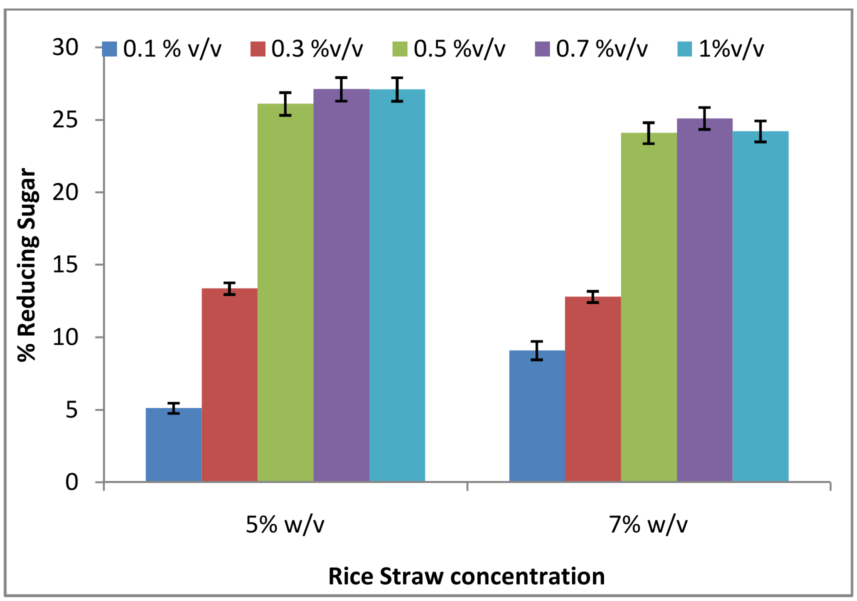

Figure 3: Acid hydrolysis of 5%w/v and 10% w/v rice straw at different acetic acid concentrations

However results in Figure 3 show that there is an increase in reducing sugar yield as the acid concentration is increased from 0.1%v/v to 0.7% v/v. Hydrolysis with CH_3COOHshows that a significantly lower reducing sugar is produced at 0.1% v/v and 0.3% v/v. There is however increased hydrolysis yields at acid concentrations of 0.5%v/v, 0.7%v/v and 1%v/v. The highest reducing sugar of 27.12% is produced at 0.7% v/v $CH_3COOH$when 5%w/v rice straw is used. The acid hydrolysis of rice straw using acetic acid also shows that hydrolysis carried out at 5%w/v rice straw produces marginally higher percentage reducing sugar when compared to hydrolysis at 7%w/v. This is observed when HCl, H_2SO_4 or CH_3COOH are used. Acid hydrolysis by acetic acid produced lower % reducing sugar yields when compared to HCl and H_2SO_4. This might be due to the fact that HCl and H_2SO_4 are much stronger acids with higher pKas when compared to CH_3COOH [15-16]. Figure 4 gives a comparison of the maximum reducing sugars produced for HCl, H_2SO_4 and CH_3COOH respectively. It can be observed that the maximum reducing sugar was produced for HCl and H_2SO_4 after 3 hours and then there is a drop in reducing sugar as the reaction time increases up to 8 hours. However, hydrolysis by CH_3COOHshows a steady increase in the hydrolysis yield as it increases from 30 minutes to 8 hours. The reduction in reducing sugar yield might be due to the degradation of the simple sugars by the stronger mineral acids. Monosaccharides are known to dehydrate to produce furfurals, hydroxyl methyl furfurals (HMF) and phenolics [17]. This trend is not observed for acetic acid hydrolysis. While maximum percentage reducing sugar is obtained at 27.12% at 7%v/v Acetic acid, maximum percentage reducing sugar obtained for reactions at 3%v/v acid concentration is 32.23% and 29.56% for HCl and H_2SO_4 respectively. This result shows that mineral acids produces higher sugars in 3 hours compared to acetic acids which produced its highest sugars in 8 hours.

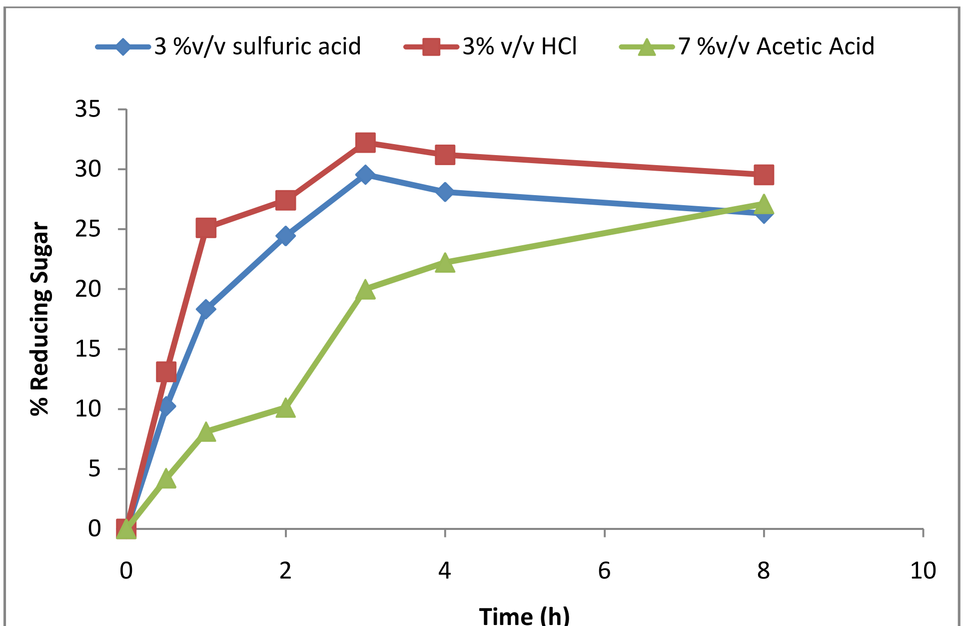

Figure 4: Comparison of the progress curve for the Acid hydrolysis of 5% w/v using Hydrochloric acid, sulfuric acid and acetic acid.

Mineral acids are stronger and produce more sugars at shorter retention time however it has been argued that organic acids might be suitable alternatives to mineral acids[18]. This is because they are environmentally friendly and improve the quality of by-products such as the remaining solids. The degradation of sugars observed in hydrolysis by mineral acids might produce substance that might be inhibitory to enzymes or microbes in the utilization of sugars to produce value added products in further saccharification or fermentation [19].

Table1: Table showing the percentage reducing sugar produced at different times for several concentrations of HCl, H_2SO_4 and CH_3COOH

Time (h)	Reducing Sugar Yield (%)										
	HCl			H_2SO_4			CH_3COOH				
	0.1% v/v	0.3%v/v	0.05%v/v	0.1% v/v	0.3%v/v	0.5%v/v	0.1% v/v	0.3%v/v	0.5%v/v	0.7% v/v	0.10% v/v
0.5	2.23	3.24	4.34	2.45	3.11	4.45	1.21	2.01	2.21	2.43	2.83
1	7.12	10.34	14.56	8.56	12.12	13.34	2.42	5.16	6.42	8.12	9.12
2	10.23	25.23	19.45	13.33	28.23	24.77	3.34	7.12	9.34	10.12	11.12
3	12.93	29.56	22.34	15.12	32.23	26.23	4.12	8.34	15.12	17.38	18.34
4	11.32	27.12	36.23	14.23	31.02	25.12	5.12	10.37	22.11	24.12	23.57
8	10.12	25.12	34.34	13.88	30.23	24.21	5.11	13.37	26.11	27.12	27.11

Table 1 shows results at different retention times for all acid hydrolysis reactions carried out. It confirms that after 3 hours there is a steady decrease in the reducing sugar yield when mineral acids (HCl and H_2SO_4) are used however this is not observed for acetic acid. Reactions continue and reach a maximum reducing sugar yield at 8 hours.

Conclusion

Dilute acid hydrolysis is a fast and convenient method to degrade biomass waste like rice straw and other lignocellulosic biomass. It has a dual advantage of solubilizing hemicelluloses and further converting it to fermentable sugars. Literature has suggested that dilute acid pretreatment disintegrates hemicelluloses structure and redistribute lignin present in the lignocellulosic structure of rice straw. This paper shows that mineral acids like sulfuric acid and hydrochloric acid can be used to effectively produce simple sugars within 3 hours while organic acids can be used to produce simple sugar at longer retention times of 8 hours. Results show that increasing the concentration of hydrochloric acid and sulfuric acid from 0.1% v/v to 0.5%v/v acid produces an increased reducing sugar yields with maximum reducing sugar yield produced at 0.3% v/v for both sulfuric acid and hydrochloric acid. On the other hand, the use of acetic acid- an organic acid shows that lower reducing sugar is produced at 0.1% v/v and 0.3% v/v. However, much higher hydrolysis yields are observed at acid concentrations of 0.5% v/v, 0.7% v/v and 1% v/v with at 0.7% v/v acetic producing the maximum reducing sugars. Comparison of substrate concentrations show that 5% w/v produced the highest reducing sugars when compared to substrate concentrations of 7% w/v.

It was earlier reported that mineral acids are known to cause the accumulation of compounds such as furfurals, hydroxyl methyl furfurals (HMF) and phenolics inhibiting microbial cell and affecting the specific growth rate and cell-mass yield of reactions downstream. It is therefore suggested that a two-step acid hydrolysis stage can be adopted that requires a separation of the sugars before further downstream activities.

Declaration of conflicting interests

The authors declared no potential conflicts of interest.

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