PRODUCTION OF XYLOSE USING ACID HYDROLYSIS OF WHEAT STRAW

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ABSTRACT

This study was conducted to produce xylose by acid hydrolysis from lignocellulose by product . The process of acid hydrolysis carried out using dilute H_2SO_4 to hydrolyze the available sources of lignocellulosics in Province of Wassit being (corn stover, rice husk, reed and wheat straw) ,wheat straw was selected as the best source for hemicellulose (35)% as compared to the above mentioned sources. The hydrolysis process included two separated experiments ,acid hydrolysis of autoclaved and non- autoclaved wheat straw using the following reaction conditions [(0.5, 1 and 1.5%) sulfuric acid solution, (1, 2 and 3hrs) hydrolysis time and (1:10, 1:15 and 1:20) solid to liquid ratio]. The results indicated that the optimal reaction conditions for the recovery of xylose from autoclaved wheat straw was observed when 1% sulfuric acid solution, 1:15 solid: liquid ratio over 1 hr. incubation time was used . From scaling up to using 10 g wheat straw as substrate, an average xylose yield was 20.61g xylose/100 g of wheat straw (dry basis) . Also, results indicated that the highest level of furfural (0.77) % was observed when 1.5% sulfuric acid solution over 3hrs hydrolysis. while, the lowest level (0.22)% was observed when 0.5% sulfuric acid after 1hr reaction time used, demonstrating that the reaction time and acid concentration has very significant role on concentration of furfural formation.

Key words: acid hydrolysis hemicellulose, wheat straw, monosaccharaides, furfural, lignocellulose

***Part of Ph.D. Dissertation of the first author.**

انتاج الزايلوز من المخلفات الزراعية بالتحلل الحامضي

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المستخلص

اجريت الدراسة الحالية لانتاج سكر الزايلوز من المواد اللكنوسيليلوزية بطريقة التحلل الحامضي. اذ استخدمة تراكيز مختلفة من محلول حامض الكبريتيك المخفف (1,0.1و1.5 %) لتحليل كل من (تبن القمح وسبوس الرز وكوالح الذرة الصفراء والقصب). وقد جريت عملية التحلل على تبن القمح المعاملة بالاوتوكليف وتلك غير المعاملة بالاوتوكليف لكونها المصدر الافضل لانتاج الزايلوز. مزج تبن القمح مع محلول حامض الكبريتيك المخفف بنسب خلط مختلفة كانت بواقع (1:10، 1:1.5 و 1:20) (و/ح) وتركت لمدة 3 ساعات وكانت النماذج تؤخذ بعد كل ساعة لفحص نسبة الزايلوز المتحرر. اشارت النتائج المستحصلة الى ان افضل نسبة زايلوز كانت بواقع (20.61 غم/لتر) من المعاملة التي استخدم فيها 1% محلول حامض الكبريتيك المخفف ونسبة خلط 1:1.5 وبعد 2 ساعة من بداء التفاعل. ووجد ان نسبة الفورفورال الناتجة كانت (0.77 %) من تركيز الزايلوز الناتج وبعد 3 ساعات من بدء التفاعل عند استخدام محلول حامض الكبريتيك بنسبة 1.5% في حين اقل نسبة فورفورال كانت بواقع (0.22)% عند استخدام حامض الكبريتيك 0.5% وبعد ساعة واحدة من بدء التفاعل وبنسبة خلط 1:10.

الكلمات الافتتاحية: المواد اللكنوسيليلوزية، الهيميسيليبوز، التحلل الحامضي والزايلوز.

***البحث مستل من اطروحة دكتوراه للباحث الاول.**

***Received:16/7/2017, Accepted:19/10/2017**

INTRODUCTION

Xylose is a sugar purified from plants, which constitutes the hemicellulose, one of the main components of lignocellulose (36). Xylose has wide food, medicinal, and industrial applications (32). It has become popular in Europe, Japan, and the USA since the 1960s, and it received FDA approval (46). In addition to its uses in food applications, in the past decades, xylose from lignocellulose has been used extensively to produce a wide variety of fuels or chemical compounds by chemical or biotechnological processes, such as xylitol, green surfactant, ethanol, furfural, 2,3-butanediol, hydroxymethylfurfural (HMF), and furan resins (44). Several Researchers (20) reported that the carbohydrate fraction of the plant cell wall can be converted into fermentable monomeric sugars. Nevertheless, the composition of each constituent varies from one plant species to another. Since the main component of the hemicellulosic fraction is xylan, this heteropolymers has been converted into many different value-added products such as xylose, xylitol, antioxidants and xylooligosaccharides (1). There is a lot of lignocellulosic biomass available all around the world as agro-residues (sugarcane bagasse, wheat straw, rice straw, corn stover, etc.). Among lignocellulosic biomass resources, wheat straw is one of the largest biomass feedstock in the world. FAO statistics reported a world annual wheat production in 2014 of 728 million tons, satisfy the growing demand of human consumption (28). The average ratio of wheat straw is 1.3 kg of straw per kg of wheat grain (16, 23), this means more than 900 million tons of wheat straw produce in 2014. The dilute acid hydrolysis of the biomass is, by far, the oldest technology for converting the biomass to chemical products. Other Researchers (29) stated that the use of dilute acids to catalyze the hydrolysis of hemicelluloses to its constituents is a well-known and effective method. When this method is used, the composition and concentration of the hydrolysis product depends on the type of raw material used and the operational conditions employed. Compared with other hydrolysis methods, dilute acid hydrolysis is especially useful for the conversion of xylan in hemicellulose to xylose, which can be further fermented to xylitol or ethanol by many microbial strains.(39). The major disadvantage of dilute acid hydrolysis over enzymatic treatment is that it generates a hydrolysate that contains not only the sugar needed for bioconversion but also the degradation products of sugar and lignin as well as acetic acid, which could slow down or prevent the bioconversion of hydrolysate (27). Hence, it is important to choose less severe conditions that will maximize the yield of xylose while minimizing the formation of by-products such as furfural, hydroxyl methyl furfural (HMF), acetic acid, and lignin degradation products. Several Researchers (11, 45) Reported that in acidic pretreatment , hydrogen ions mainly catalyze the hydrolysis of glycosidic bonds and monosaccharide dehydration, resulting in hemicellulose degradation and furan production. A previous study by the authors showed that the different acids influence the degradation of xylan due to the dissociation constant (k_d) of each acid (31). As known , the Ka value of weak acid is changed as temperature changes. Several Researchers (16) have reported that more than 80% yield of xylose is obtained when the hydrolysis of hardwood hemicellulose is performed at 120–140 ^oC using 2.5% H_2SO_4. This study was aimed to investigate the optimum conditions for acidic hydrolysis of lignocellulosic materials to achieve the highest xylose release.

MATERIAL AND METHODS

Samples of agricultural wastes i.e. wheat straw, rice husks, corn cops and reed were collected from a local farm in Wassit and used as raw materials in this study . All the agricultural wastes samples were sunlight-dried, milled, screened to select the fraction of particles with a size lower than 0.5 mm, homogenized into a single lot and stored at room temperature until used. The dried wheat straw samples were mixed with 20 ml of water then autoclaved at 121^oC for 20 min, after autoclaving process wheat straw were mixed with sulphuric acid solution in a 250 mL flask. The following treatment condition was applied: incubation time of 1, 2 and 3h (65°C); mixing ratio of solid :liquid (1:10, 1:15 and 1:20) and concentrations of (H_2SO_4) was (0 %, 0.5 % 1 %, and 1.5 %). The dependent variables were the yield of xylose. Also non autoclaved wheat straw, treated with dilute sulfuric acid under same conditions as that of autoclaved wheat straw. After hydrolysis, the liquid fraction (wheat straw hydrolysate) was filtered through Whatman No: 1 filter paper and the pH was raised to 9 with calcium oxide and decreased to pH 6 with sulfuric acid consecutively. The hydrolysate was concentrated under vacuum at 70°C to increase xylose concentration. After these treatments, the hydrolysate was mixed with 10% activated charcoal, agitated (200 rpm, 30°C, 1 h) and then filtered. the filtrate was analyzed for solubilized sugars (xylose) and byproducts (furfural).

Analytical methods

Dry mater was measured according to the method described by Several Researchers (48): The material was dried at 60 °C until dry mater content of (90-92)% Protein content of wheat straw was determined by the Kjeldahl method (protein as 6.25 x N). Moisture, lipid and ash content were determined according to the method described by AACC (3).=

Reducing Sugar Measurement

Total reducing sugars were quantified according to the dinitrosalicylic acid (DNS) method using a modified DNS reagent (21). A standard curve for the DNS assay was prepared using xylose as a suitable standard and expressing the activity in terms of reducing sugars released as D-xylose equivalents. The assay was performed in triplicates. (cellulose, hemicellulose And lignin) was determined according to the method described by Several Researchers (43).

Charcoal adsorption

Powdered charcoal (Probus, Madrid, Spain) was activated with hot water and dried at room temperature. Charcoal detoxification of hydrolyzates was carried out by mixing hydrolyzates with charcoal (mass ratio: 10 g /l) at room temperature under stirring for one hour (7). The liquid phase was recovered by filtration and used for making culture media.

Colorimetric assay of furfural

The determination of furfural concentration of the plant samples was carried out spectrophotometrically based on measuring the absorbance at 277 nm using the standard curve derived from known levels of furfural in water (10).

Statistical analyses

The collected data were statistically analyzed using analysis of variance (ANOVA) by GENSTAT computer software package. Differences between treatment means were compared using Least Significant Difference (LSD) ≤ 0.05 probability level .

RESULTS AND DISSECTION

Composition of lignocellulosic materials The results of chemical composition (cellulose, hemicellulose and lignin) analysis of raw materials used in this study are given in Table 1. Lignocellulosic materials are principally composed of cellulose, hemicellulose and lignin. The difference in these main components might be due to the genetics, location and growth conditions. Among these constituents, hemicellulose is of particular interest because of its unique properties for xylose production and composition.

Table 1. percentage of cellulose, hemicellulose, lignin and ash in experimental lignocellulose material(corn cob, wheat straw, rice husk and reed)

Parameters (%)	Corn cob	Wheat Straw	Rice Husk	Reed
Dry mater	92.32	92.35	93.41	93.52
Cellulose	36.60	37.46	38.43	45.16
Hemicellulose	33.77	34.97	22.60	16.40
Lignin	21.20	20.53	16.67	24.73
Ash	4.93	4.43	11.80	5.13

Results in Table 1 showed that the percentage of hemicellulose contents in wheat straw, corn cob , rice husks and reed were 34.97, 33.77, 22.6 and 16.40 %, respectively. Hemicellulose is the second major compound of these biomass sources, varied between 16.40 and 34.97 % of dry mass. These composition similar to those reported in literature for the same material, whose values vary from 39 to 45 % for cellulose, 26–36 % for hemicellulose and 11–25 % for lignin (6). These contents differ between raw materials and also with plant variety, location, time of year and collection system, as well as with the particular analytical methods used. A large number of types of lignocellulose biomass has been evaluated as xylan-rich; therefore, the selection of biomass should meet three criteria: it must be abundant and located within the transportation radius, it must contain high amounts of xylan and xylose, and it should not contain too many impurities that will increase the risk of contamination during bioconversion and purification (49). High amounts of pentosans are also present in agricultural residues, such as in sugarcane bagasse, corn cobs, corn fiber as well as in wheat and rice straw ((38). Also, (8) reported that corn cobs, sugarcane bagasse, and wheat/ rice straw are the major materials that have been investigated as biomass for the production of xylitol.

Composition of wheat straw

Table 2. lists the main chemical proportions of the wheat straw. The percentage of xylose in the experimental wheat straw as compared to these which was mentioned in the literature studies. It has been noticed that the experimental wheat straw contained 41.42% glucose and 24.13%, xylose. Whet straw is mostly composed out of lignocellulose; cellulose, hemicellulose and lignin. Part from those compounds it also contains some proteins, sugars, organic acids, ash, wax and little to no lipids (30). Because the crops grow under multiple unique conditions, it results in a wide diversity when it comes to the quantitative composition of straw (22). Wheat straw is an agricultural byproduct, which is mainly composed of cellulose (34–43%), hemicellulose (26–35%) and lignin (14–21%) (39). Also, Bohdan and Dahman (4) reported that wheat straw presents many interesting characteristics and consists mainly of cellulose (30–40%), hemicellulose (20–35%) and lignin (15–25%), and the most abundant hemicellulosic polymers are xylans. Ortiz *et al* (26) stated that the main components of wheat straw are cellulose ~34-43%, hemicelluloses ~26-35% and lignin around ~14-21%, which differ slightly in composition owing to dissimilar varietal, geographical, and climatic influences in the growth of wheat straws . In addition, wheat straw contains nearly as much D-xylose in polysaccharide form as corncobs and wood (2). The considerably high hemicelluloses content in wheat straw compared to other raw materials, indicates the potential of this material for production of xylose, which may be used as a raw material for production of xylitol.

Table 2 Percentage of chemicals composition based weight dry of wheat straw compare with other references

Component	Percentage %	Percentage % in references	References
Protein	**4.15± 0.32**	**2.4 - 5.8**	**(2)**
Ash	**7.25±0.41**	**4–6**	**(2)**
lipid	**0.48±0.11**	**0.6**	**(2)**
Lignin	**20.33±0.63**	**14–25**	**(4)**
Pentosan as Xylose	**24.13±0.54**	**23.4-26**	**(12)**
Glucose	**41.42±0.47**	**41.3**	**(2)**

Pretreatment of wheat straw

Acidic treatments

Table 3 and 4. Were shows the amount of xylose in autoclaved and non autoclaved acid treated wheat straw hydrolysate through 1, 2, and 3 hrs hydrolysis using different concentration of sulfuric acid (0.5, 1 and 1.5%), it was found that the reaction time, mixing ratio and acid concentration were important factors influencing the hydrolysis process.

Table 3. Xylose concentration of acid hydrolysate from autoclaved wheat straw treated with different concentration of H_2SO_4 at different mixing ratio and incubated for 1,2and 3 hrs at 65C.

NO.Treat.	Mix. ratio	H_2SO_4 %	Time /h		
			1	2	3
				Xylose %	
T1	1:10	0.5	16.28± 0.32a *	16.91± 0.035a	17.12±0.41 b
T2	1:15	0.5	17.78± 0.51b	17.70± 0.26b	17.85± 0.28b
T3	1:20	0.5	17.80±0.73 b	17.96± 0.62b	18.11± 0.53b
T4	1:10	1	19.43± 0.15c	19.70± 0.05c	19.80± 0.02c
T5	1:15	1	19.64± c0.40	20.61±0.16 d	20.28±0.12 c
T6	1:20	1	19.91±0.24 c	20.40±0.38d	20.44±0.38 c
T7	1:5	1.5	20.07± 0.24d	19.87± 0.53c	19.45±0.16 d
T8	1:15	1.5	19.71±0.16 c	19.66±0.24 c	19.38±0.21 d
T9	1:20	1.5	19.88±0.24 c	19.85±0.12c	19.41± 0.32d

***Means followed by different letters between raws and columns are significantly different ($p<0.05$)**

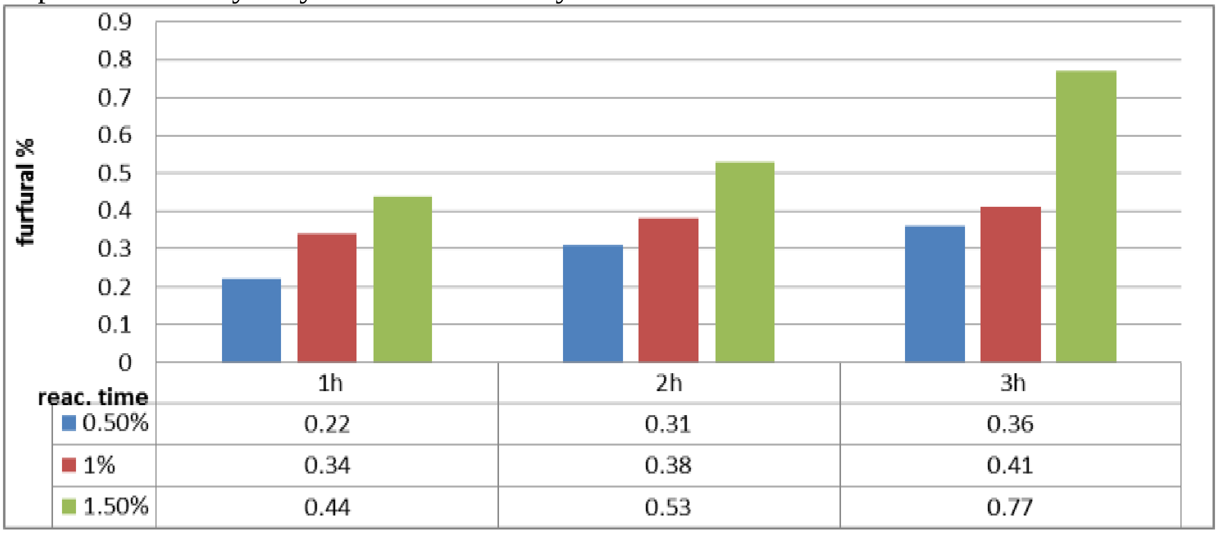
The effect of chemical reagents on the lignocellulose material can be attributed to the fluctuation of pH values as a resent of difference hydrogen ion or hydroxyl ion concentration. It has been noticed from table (3) that the yield of xylose in autoclaved acid treated wheat straw hydrolysate varied from 16.28% to 20.61%, and the highest value was in T5 depending on the treatment condition. For non- autoclaved hydrolysate (table 4) those values ranged from 14.64% to 18.22%, and the highest value was observed in T6. Table 3, also show that the amount of xylose released was significantly affected by autoclave treatment in contrast to non autoclave treatment. On the other hand, the reaction time with autoclaved straw was relatively shorter, typically 2 h, compared to 3h in non autoclaved treatments. Ranganathan *et al* (34) found that autoclaving wheat straw at temperatures 120–210 °C and treating with 0.5–1.5% acid, was able to produce 80–95% xylose.

Table 4. Xylose concentration of acid hydrolysate from non- autoclaved wheat straw treated with different concentration of H_2SO_4 at different mixing ratio and incubated for 1,2 and 3 hr at 65°C.

NO. Treat.	mix. ratio	H_2SO_4 %	Time /h		
			1	2	3
				Xylose %	
A1	1:10	0.5	14.64±0.22a *	14.81±0.18 a	15.12±0.43 a
A2	1:15	0.5	15.60±0.35 b	15.70±0.27 b	16.32±0.36 c
A3	1:20	0.5	15.65± 0.51b	15.76±0.48 b	16.09±0.38 c
A4	1:10	1	16.87±0.46 c	17.49± 0.17d	17.65± 0.21d
A5	1:15	1	16.93±0.12 c	17.52± 0.24d	17.87± 0.29d
A6	1:20	1	17.28±0.77 d	17.77± 0.16d	18.22± 0.30e
A7	1:10	1.5	17.61± 0.24d	17.82±0.32 d	18.11± 0.69e
A8	1:15	1.5	17.63±0.17 d	17.83±0.08 d	17.92± 0.48e
A9	1:20	1.5	17.70±0.47 d	18.06±0.26 e	17.93±0.42 d

***Means followed by different letters between rase and column are significantly different ($p<0.05$).**

In general, the statistical analysis results showed significant differences between the highest xylose yield which obtained from autoclaved acid treated wheat straw compared to that from non –autoclaved acid treated wheat straw. This because that autoclave processing makes wheat straw more accessible and increase surface area for hydrolysis. In previews studies, physical treatments using to increase accessible surface area of biomass is usually followed by physiochemical pretreatments. Bohdan and Dahman (4) stated that A pretreatment is required to break up the recalcitrant structure of lignocellulose and improve the accessibility of hydrolytic enzymes to their substrates. Steam explosion is the most effective pretreatment of lignocellulosic biomass that is currently used for commercial ethanol production from wheat straw (41,17). Several Researchers (13) found that the hemicellulose fraction can be easily removed by acid treatment, and that the resulting hydrolysate is rich in fermentable sugars, primarily xylose, which could serve as a fermentation medium for the production of xylitol. Table 4 also illustrated that, in non-autoclaved wheat straw, xylose yield varied from 14.64 %, when the hydrolysis carried out with low acid concentration (0.5%) for 1h (A1), to 18.22 for 1% acid/3h (A6). Meanwhile, it has been noticed that as the reaction time, mixing ratio and acid concentration increased, xylose concentration also increased up to a maximum value in (A6), and after that it is gradually decreased reaction time ,mixing ratio and acid concentration increased, this due to fast degradation of xylose into furfural at higher acid concentration. It was found that the sugar concentration increased with increasing of hydrolysis time, This is due to the hydronium ion of acid attack to ester bond in lignin-carbohydrate complex, resulting the releasing of sugar monomer into the solution (33). After 60 min, the sugar concentration remarkably reduced with increasing of reaction time. This is because of its toxic compound (FIMF and Lewlinic acid). The furfural was increased with increasing of hydrolysis time (37). Also Table 4 shows the effect of the solid to liquid ratio on the amount of xylose recovered from wheat straw hydrolysate. The lowest xylose concentration (14.64%) was obtained when 10 g of wheat straw treated with 100 ml of 0.5% sulfuric acid for 1h. While the highest xylose concentration (18.22%) was obtained when 200 ml of 2% sulfuric acid was reacted with 10 g of dry wheat straw for 3hrs. This result is in agreement with that found in the literature: The concentration of released sugars during pretreatment process is directly dependent upon the type of lignocellulosic material, composition of substrates, temperature, reaction time, acid concentration, solid-to-liquid ratio and the reactors employed in the process (1, 15, 19, 24). Table 3 indicated that autoclaving wheat straw resulted different xylose concentration as compared to non-autoclaved wheat straw upon acid hydrolysis for (1, 2 and 3) hrs. It is obvious in Table 3, xylose concentration was positively proportional to acid concentration with the increase in acid concentration, reaction time, and the highest value (20.61%) was noticed with (T5) treatment (1% acid concentration, 2h reaction time and 1:15 mixing ratio), after that xylose concentration gradually decreased as the acid concentration, reaction time, increased (T6, T7, T8 and T9), in those treatments the highest yield of xylose observed after 1h of reaction time, whereas after 3h those values were at lower level (Table 3). In autoclaved samples, through any reaction time , as acid concentration increased xylose concentration decreased and this could be due to fast degradation of xylose into furfural at high acid concentration. Karimi *et al*., (15) illustrated that the acid hydrolysis is the most widely used pretreatment process for hydrolyzing biomass to produce monomeric sugars. In hydrolysis step, acid releases protons that break heterocyclic ether bonds between the different sugar monomers in the polymeric chains formed by the celluloses and hemicelluloses producing pentose and hexose sugars. In dehydration step, sugars dehydrate to form degradation products such as furfural. Among the factors that influence the efficiency of acid hydrolysis, temperature, reaction time and acid concentration are the most widely investigated factors. However, some authors investigated the influence of particle size of biomass and solid/liquid ratio (14). The acid concentration is considered one of the most important factors regarding the release of sugars. High concentrations of acid may decompose the hemicellulosic structure, producing inhibitors and also causing damage to the equipment used. Therefore, an appropriate acid concentration is essential for acid hydrolysis of lignocellulose at industrial scale (40). Roberto *et al.,* (35) stated that the optimum condition for xylose production was 1% H_2SO_4 and reaction time of 27 min was found of corn cob hydrolysate. In general, it is observed that mild temperature led to a significant recovery of sugars while higher temperatures caused more sugar degradation, aiding the formation of inhibitors (47). According to Gírio *et al*. (9) findings, sulphuric acid/hydrochloric acid concentrations for hemicellulose hydrolysis are in the range of 0.5–1.5 % and temperatures between 121°C and 160 °C. The pretreatment conditions will depend on the type and species of the used vegetal biomass. Neureiter *et al*. (25) found that the acid concentration is the most significant factor for hemicellulose hydrolysis. During acid pretreatment, more severe processes cause fast sugar degradation and thus gave poor hydrolytic efficiency (5). Roberto *et al*. (35) investigated the conversion of rice straw into reducing sugar (xylose) in a semi-pilot reactor Hydrolysis of rice straw by dilute sulfuric acid at different time were investigated. It can be seen from figure 2. that the levels of furfural increased with the increase in reaction time and acid concentration, the highest level of furfural (0.77) % was observed when 1.5% sulfuric acid was used for 3hrs reaction time, while, the lowest level (0.22)% was observed when 0.5% sulfuric acid was used for 1hr reaction time , demonstrating that the reaction time and acid concentration has very significant role on concentration of furfural formation



reac. time	1h	2h	3h
0.50%	0.22	0.31	0.36
1%	0.34	0.38	0.41
1.50%	0.44	0.53	0.77

Figure 2. Concentration of furfural at different reaction conditions (0.5, 1 and 1.5% of H_2SO_4, 1,2 and 3 hrs and 1:15 solid: liquid ratio) acidic hydrolysis of autoclaved wheat straw

However, when the sulfuric acid concentration was increased more than 1% the concentration of the xylose obtained decreased slightly. This reduction in xylose may have occurred as a result of degradation of xylose to furfural, which would be consistent with previous reports. Both Lee *et al* (18) and Torget *et al*., (42) reported that xylose is more sensitive to degradation to furfural, particularly at acid concentrations over 1% and reaction temperatures higher than 120°C. The results of acids hydrolysis of wheat straw indicated that the optimal reaction conditions for the recovery of xylose from wheat straw hemicellulosic fraction was an 1% sulfuric acid concentration, a reaction time of 1h and a solid to liquid ratio of 1:15. High xylose yields 20.61% were attained when the wheat straw was pretreated (autoclaved). The results of this study are of considerable importance to the commercial production of xylose using agricultural residue as resources more abundant.

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