HYDROLYSIS OF WHEAT STRAW UNDER ALKALI CONDITIONS

H. F. AL-Rubaia'ay* **K. A. Shakir**
Researcher **Prof.**
Dept. Food Science –Coll. Agric- Univ. of Baghdad
husseinf313@yahoo.com **dr_khalida55@yahoo.com**

ABSTRACT

Ground wheat straw autoclaved and non-autoclaved (l0g) was treated with 6, 8 and 10% sodium hydroxide of 1:10, 1:15 and1:20 mixing ratio for 1, 2 and 3hrs as reaction time. Results revealed a maximum value (18.79 %) of xylose were found in hydrolysate when autoclaved wheat straw was treated with 8% NaOH for 2hrs , whereas the lowest value was (13.52) % when the non autoclaved wheat straw (6% sodium hydroxide concentration , 1h reaction time and 1:10 solid: liquid) used. Also, results indicated that the highest level of furfural (0.34) % was observed when 10 % sodium hydroxide solution over 3 hrs incubation was used. while, the lowest level (0.11)% was observed when 6% sodium hydroxide after 1hr reaction was used, demonstrating that the reaction time and alkaline concentration has very significant role in furfural formation .

Key words: Alkaline pretreatment, lignocellulose, wheat straw, monosaccharaides, furfural.
***Part of Ph.D. Dissertation of the first author.**

تحلل تبن القمح تحت الظروف القاعدية

***حسين فاضل خليف الربيعي** **خالدة عبد الرحمن شاكر**
الباحث **استاذ**
قسم علوم الاغذية– كمية الزراعة – جامعة بغداد

المستخلص

تضمنت الدراسة معاملة مسحوق تبن القمح المعامل وغير معامل اوتوكليف بتراكيز مختلفة من محلول هيدروكسيد الصوديوم (6، 8 و10%) وبنسب خلط مختلفة (1:10، 1: 15 و1:20) (و/ح) وتركت لمدة 3 ساعة لغرض اجراء عملية التحلل وانتاج الزايلوز. اشارت النتائج المستحصلة ان اعلى قيمة من الزايلوز تحررت وبواقع (18.79%) مع محلول 8% هيدروكسيد الصوديوم بنسبة خلط 1:15 وبعد ساعتين من معاملة تبن القمح المعامل بالاوتوكليف. في حين كانت اقل نسبة زايلوز بواقع (13.52)% وفي المعاملة التي شملت 6% من هيدروكسيد الصوديوم بنسبة خلط 1:10 وبعد ساعة واحدة من بدء عملية التحلل. كما اشارت النتائج ان اعلى قيمة في الفورفورال المتحرر كانت 0.34% عندما كان تركيز هيدروكسيد الصوديوم 10% وبنسبة خلط 1:20 وبعد 3 ساعات من بداء التفاعل. اما اقل نسبة فورفورال (0.11)% ظهرت عند معاملة تبن القمح بالقاعدة بتركيز 6% بعد ساعة واحدة من بدء التفاعل مبينا ان تركيز القاعدة وزمن التفاعل فضلا عن نسبة الخلط لها دور كبير في نسبة الزايلوز المتحرر.

الكلمات الافتتاحية: تبن القمح، الهيميسيليبوز، التحلل القاعدي، الزايلوز والفورفورال.
البحث جزء من اطروحة الدكتوراه للباحث الاول

***Received:16/7/2017, Accepted:15/10/2017**

INTRODUCTION

Lignocellulose is an attractive renewable biomass for the production of various value added products that compose about half of the plant matter. Since lignocellulosic materials are highly abundant, plentiful and largely available in nature, it can be utilized to produce various beneficial products such as biofuels, animal feeds, enzyme, and healthy food products (30). Among the agricultural residues, wheat straw is the most abundant and it is being considered as one of the main renewable feedstocks for conversion into fuels and chemicals. It is principally constituted of cellulose, hemicellulose and lignin. Among these constituents, hemicellulose is of particular interest because of its unique properties and composition. In the last two decades of research has been witnessed the technological development for the hemicellulose depolymerization into its monomeric constituents, mainly xylose, and their subsequent conversion into value-added products via microbial fermentation (4). Pretreatment process plays an important role in the lignocellulosic conversion because it helps in breaking the structure of lignin and disrupting the crystalline structure of cellulose and hemicelluloses thus improving enzyme accessibility during hydrolysis process (22). Alkaline pretreatment is one of the major chemical pretreatment technologies by using various bases, including sodium hydroxide (21). Alkaline pretreatment using sodium hydroxide (NaOH) shows great advantages in breaking down the internal structure of lignocelluloses component. Moreover, alkaline can degrade the ester and glycosidic chains and alter the structure of lignin, causing cellulose swelling and partial decrystallization of cellulose (14, 20). In many years, sodium hydroxide also has been widely studied, and it has been proven capable to break the lignin structure of the biomass, thus increasing the enzymes accessibility to degrade cellulose and hemicelluloses (31). To take full advantage of these alkaline pretreatment effects, critical process parameters such as alkaline concentration, pretreatment temperature and time must be optimized (21). Alkaline biomass pretreatment is normally performed at a lower temperature and pressure than other pretreatment methods (11) and is suitable for processing of agricultural residues such as wheat straw (19). During alkaline pretreatment, ester bonds, which cross-link lignin and xylan, are degraded by a similar mechanism as in soda or kraft processing (22). In this process, glycosidic linkages in the lignocellulosic cell wall matrix are broken down, resulting in alteration of the structure of lignin to polymeric lignin-like compounds (4), reduction of the lignin hemicellulose complex, cellulose swelling, and the partial decrystallization of cellulose (10). Alkaline also neutralizes the organic acids and phenols normally formed during this process, less sugar is degraded and fewer inhibitors are formed compared to acid pretreatment. Dilute NaOH) application loosens the biomass structure, separate the bonds between the lignin and the carbohydrates, increases the internal surface area, decreases the degree of polymerization and crystallinity, and disrupts the lignin structure (30).== This study was aimed to investigate the optimum conditions for alkali hydrolysis of wheat straw to achieve the highest xylose release.

MATERIALS AND METHODS

Sample collection

Wheat straw was collected from farm of Wassit .The samples were chopped into small pieces, and dried before being ground and sieved to a particle size smaller than 0.5mm. Dried samples were stored in sealed plastic bags at room temperature. All other chemicals used in the experiment were of analytically pure grade.

Alkali extraction:

The dried wheat straw samples were mixed with 20 ml of water then autoclaved at 121^{o}C for 20 min, after autoclaved wheat straw were mixed with sodium hydroxide (NaOH) solution in a 250 mL flask. The following pre-treatment condition was applied: incubation time of (1, 2 and 3h), treatment temperature (65 °C); mixing ratio of solid :liquid (1:10, 1:15 and 1:20) and concentrations of (NaOH) was (6, 8 and 10 %). The dependent variables were the yield of xylose. Also, non-autoclaved wheat straw was treated with sodium hydroxide in same conditions of autoclaved wheat straw.

Analytical method

Dry mater was measured according to the method described by Yoney *et al* (29): 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 (2).

Determination of Cellulose, hemicellulose and lignin

Cellulose, hemicellulose and lignin were determined as using by (26) Neutral Detergent Fiber (NDF), Acid-Detergent Fiber (ADF) and acid detergent lignin (ADL) methods was carried out in central lab. in agriculture college. Hemicellulose was calculated as NDF – ADF and cellulose as ADF – ADL The amount of reducing sugar was determined by using 3,5-dinitrosalicyclic acid (DNS) method with xylose as a standard. (15). Xylose concentration were colorimetrically measured at 671 nm using Bial's reagent Orcinol (3,5-dihidroxytoluene), according to method described by (3).

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 (6). 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 (12).

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

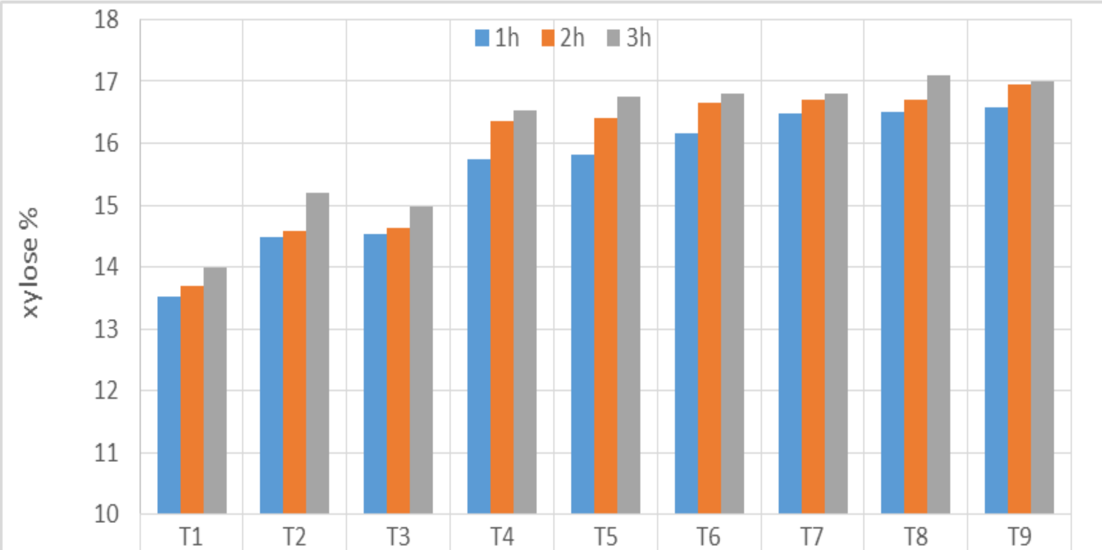
Composition of wheat straw: Table 1 lists the main chemical proportions of the wheat straw. The percentage of xylose and glucose in the experimental wheat straw as compared to these which was mentioned in the literature studies. It has been noticed that the experimental wheat straw contained 41.42% glucose and 24.13%, xylose

Table 1 chemicals composition based on dry weight of wheat straw compare with other references

Component	Percentage %	Percentage % in references	References
Protein	4.15± 0.32	2.4 - 5.8	(1)
Ash	7.25±0.41	4–6	(1)
lipid	0.48±0.11	0.6	(1)
Lignin	20.33±0.63	14–25	(11)
Pentosan asXylose	24.13±0.54	23.4-26	(13)
Glucose	41.42±0.47	41.3	(1)
Cellulose	37.46	34–43	(22)
Hemicellulose	34.97	26–35%	(22)

These results indicated that based on dry. Wheat straw is mostly composed out of lignocellulose; cellulose, hemicellulose and lignin. Results in Table 1 showed that the percentage of cellulose and hemicellulose was 37.46 and 34.97 respectively. Apart from those compounds it also contains some proteins, sugars, organic acids, ash, wax and little to no lipids (18). Because the crops grow under multiple unique conditions, it results in a wide diversity when it comes to the quantitative composition of straw (16). Wheat straw is an agricultural by-product, which is mainly composed of cellulose (34–43%), hemicellulose (26–35%) and lignin (14–21%) (22). Also, Bohdan and Yaser (5) reported that wheat straw presents many interesting characteristics and consists mainly of cellulose

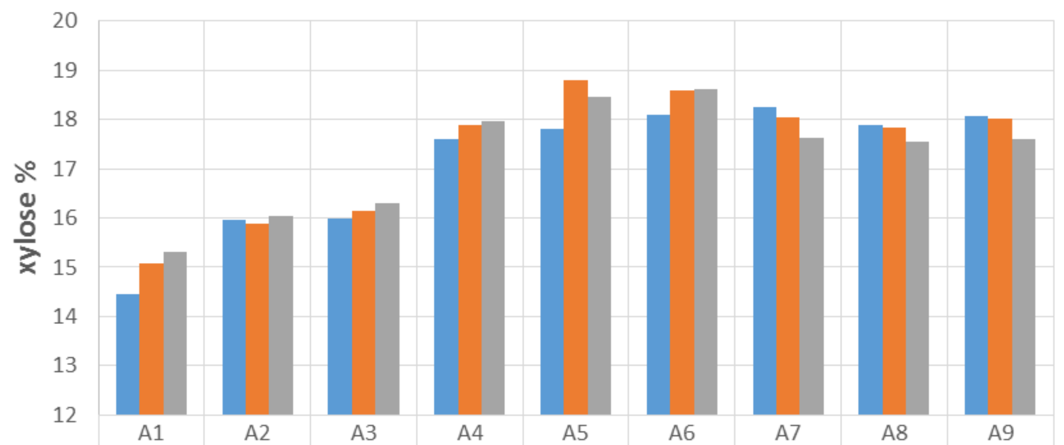
(30–40%), hemicellulose (20–35%) and lignin (15–25%), and the most abundant hemicellulosic polymers are xylans. Ortiz *et al* (17) stated that the main components of wheat straw are cellulose ~34-43%, hemicelluloses ~26-35% and lignin around ~14-21%, which differ slightly in composition owing to dissimilar 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 (1). 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. Figure 1. Represents the percentage of xylose obtained from alkaline hydrolysis of non -autoclaved wheat straw using different concentration of sodium hydroxide (6, 8 and 10) and different of mixing ratio (1:10, 1:15 and1:20) for 1, 2 and 3hrs. The amount of xylose released by alkali treatment of wheat straw varied from 13.52% to 17.1% depending on the pretreatment conditions.



	T1	T2	T3	T4	T5	T6	T7	T8	T9
NaOH %	6	6	6	8	8	8	10	10	10
Mix. ratio	01:10	01:10	01:10	01:15	01:15	01:15	01:20	01:20	01:20
1h	13.52	14.48	14.53	15.75	15.81	16.16	16.49	16.51	16.58
2h	13.69	14.58	14.64	16.37	16.4	16.65	16.7	16.71	16.94
3h	14	15.2	14.97	16.53	16.75	16.8	16.81	17.1	16.99

Figure 1. Xylose recovery from non- autoclaved alkaline treated wheat straw using different concentration of sodium hydroxide (6, 8 and 10%) during different (1, 2 and 3 hrs) of reaction time. (using L. S. D = 0.77 in P<0.5)

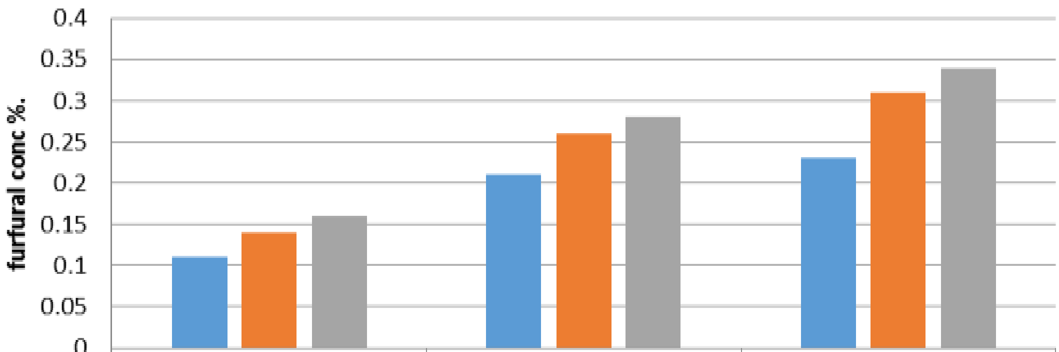
According to, (Fig. 1) the obtained results revealed that the xylose recovery from non-autoclaved samples was positively proportional to sodium hydroxide concentration and reaction time in all treatments (T1 –T8). As illustrated in figure 1, non- autoclaved alkaline treated wheat straw, the xylose recovery was at lowest level 13.52%, when the lower in treatment (T1 6% sodium hydroxide concentration , 1h reaction time and 1:10 solid liquid) and at highest level with 17.1% for T8 (8% alkaline concentration , 3h reaction time and 1:20 solid liquid). Also, Carvalheiro *et al*., (8) stated that alkaline pretreatment increases the accessibility of enzyme to cellulose and is more effective for lignin and hemicelluloses solubilisation.



	A1	A2	A3	A4	A5	A6	A7	A8	A9
NaOH%	6%	6	6	8	8	8	10	10	10
Mix. Ratio	01:10	01:15	01:20	01:10	01:15	01:20	01:10	01:15	01:20
1h	14.46	15.96	15.98	17.61	17.82	18.09	18.25	17.89	18.06
2h	15.09	15.88	16.143	17.88	18.79	18.58	18.05	17.84	18.03
3h	15.3	16.03	16.29	17.98	18.46	18.62	17.63	17.56	17.59

Figure 2. Xylose recovery from autoclaved alkaline treated wheat straw using different concentration of sodium hydroxide (6, 8 and 10%) during different (1, 2 and 3 hrs) of reaction time. (using L. S. D = 0.8 in P<0.5)

Meanwhile, on autoclaved alkaline treated samples fig. 2. The amount of xylose released by alkali treatment of wheat straw varied from 14.46 % to 18.79 % depending on the pretreatment conditions. The results revealed from fig. 2 . the xylose concentration increased up to its maximum value (A5) as alkaline concentration, mixing ratio and reaction time increased, whereas, at A6- A9 gradually decrease was noticed as those factors increased this could be as a result of fast degradation of xylose into furfural at high alkaline concentration. Regarding solid –liquid ratio, the xylose recovery was at lowest level with 1:10 ratio, (6% sodium hydroxide and 1hr) and at highest level with 1:15 ratio (8% sodium hydroxide and 2hr). Also results showed (fig 2) that in autoclaved samples, lower concentration of alkaline, 8% was required for higher xylose recovery (18.79%) as compared to non autoclave samples which were (17.1%) using 10% sodium hydroxide. Regarding the alkaline concentration effect, it has been noticed that alkaline concentration at a fixed mixing ratio and reaction time, it had a significant effect on xylose concentration. The obtained results indicated that the xylose production was strongly affected by the alkaline concentration and reaction time, whereas the effect of liquid-solid ratio was minimal. Pretreatment process plays an important role in the lignocellulosic conversion because it helps in breaking the structure of lignin and disrupting the crystalline structure of cellulose and hemicelluloses thus improving enzyme accessibility during hydrolysis process (23). According to figure 2, again xylose concentration was positively proportional to each of alkaline concentration, and reaction time, as happened with previous acidic treatment, in this experiment xylose concentration also increased up to a maximum value (18.79) in (8% NaOH, 1:20 solid : liquid ratio over 2h reaction time) and after that it is gradually decreased with increasing alkaline concentration, and reaction time, (A6, A7, A8 and A9), and no Significant differences of yield in xylose observed for the same this four treatments (A6, A7, A8 and A9). In respect to any fixed reaction time, high alkaline concentration decreased xylose concentration this due to degradation of xylose into furfural. In general, it has been noticed that the xylose yield obtained from autoclaved wheat straw was significantly higher than that of non - autoclaved wheat straw for all alkaline treatments. This is because that autoclaving processing makes wheat straw more accessible and increase surface area for hydrolysis. Wu *et al* (27) reported that alkaline treatment helps in dissolving lignin and part of the hemicellulose, whereas steam explosion dissolves mainly hemicellulose, so the reaming solid fraction after treatment by both methods mainly cellulose. In previews studies, physical treatments using to increase accessible surface area of biomass is usually followed by physiochemical pretreatments. Alkaline biomass pretreatment is normally performed at a lower temperature and pressure than other pretreatment methods (11) and is suitable for processing of agricultural residues such as l decrystallization of cellulose (14 ,20). Tongjun *et al.*, (25) reported that a relatively low alkali pre-extraction allows for several advantageous potential process outcomes, including highly selective lignin removal versus xylan. Further, it decreases alkali consumption and substantially decreases the required alkali recovery, which decreases the capital requirements. Although lignin removal helps improve hydrolysis yields, xylan retention improves the overall sugar yields for wheat straw (19). Alkaline hydrolysis of autoclaved wheat straw resulted in minimal sugar degradation and no inhibitory compounds formed as compared to that of acidic hydrolysis. Alkaline pretreatment using sodium hydroxide (NaOH) shows great advantages in breaking down the internal structure of lignocelluloses component. Moreover, alkali can degrade the ester and glycosidic chains and alter the structure of lignin, causing cellulose swelling and partia the subsequent hydrolysis. Figure 4 showed that the levels of furfural concentration was proportional to reaction time and alkaline concentration, the highest level of furfural (0.34%) was observed when 10% sodium hydroxide was used for 3hrs reaction time, while, the lowest level (0.11%) was observed when 6% sodium hydroxide was used for 1hr reaction time demonstrating that the reaction time and alkaline concentration has very significant role on concentration of furfural.



reaction time	1h	2h	3h
6%	0.11	0.21	0.23
8%	0.14	0.26	0.31
10%	0.16	0.28	0.34

Figure 4. Concentration of furfural formed during alkaline hydrolysis of autoclaved wheat straw using (6,8 and 10%) of NaOH solution and 1:15 solid: liquid ratio through 3hrs hydrolysis.

The alkaline pretreatment is regarded as one of the most efficient methods and sodium hydroxide (NaOH) is widely used for its outstanding delignification capacity and simple operation. Other researchers (7, 25) indicated that the hydrolysis degree of wheat straw pretreated by NaOH was more than three times that compared with untreated wheat straw. NaOH pretreatment was proved effective in treating biomass, particularly for lignocellulose (9). It may cause lignocellulose to change in both chemical property and physical structure, for instance, swell leading to an increase in the internal surface area, a decrease in the degree of polymerization and crystallinity, separation of structural linkages between lignin and carbohydrates, and disruption of the lignin structure (27). These changes will release a mass of organic matter (lipid, monose, and other carbohydrates) into liquid. On the other hand, NaOH pretreatment can enhance hydrolysis of substrates and improve yield of sugar (25). At mild temperatures (< 100°C), treatment of grasses with NaOH induces significant solubilization of xylan and lignin relative to dicots and thus, has been proposed as a standalone pretreatment for grasses including corn stover, wheat straw, sweet sorghum and switch grass (28). There are various pretreatment strategies that have been building up for the pretreatment of wheat straw. This study has summarized some of the main strategies that are presently being used and has provided several ways as to which biomass types have been shown to be more suitable with each pretreatment. The results obtained from this study are highly beneficial in identifying the optimum condition for alkaline pretreatment in treating the wheat straw. The experimental findings in NaOH pretreatment of wheat straw are in good agreement with the model predictions with less than 10% thus signifying the adequacy of the models employed in optimizing the NaOH pretreatment conditions parameters. There are several other pretreatment approaches that have also find out, several of which are dissimilarity of the processes described here. The selection of pretreatment is basically related on biomass properties, availability to materials required for the process, expenditure of reagents desired, and the price of equipment to operate for that procedure.

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