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Optimization of xylose production from wheat straw using of chemicals and enzymatic catalases

امثل انتاج لسكر الزايلوز من تبن القمح باستخدام المحفزات الانزيمية والكيميائية

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Abstract. Wheat straw is one of the most abundant agricultural by product and it is an attractive source of lignocelluloses , which is a renewable resource for the production of fuels and chemicals, and therefore their exploitation could be economically feasible. Biotechnological conversion of biomass into fuels and chemicals requires hydrolysis of the polysaccharide fraction into monomeric sugars. In this study, the effects of various pretreatments (concentration different of NaOH, H_2SO_4) as well as solid to liquid ratio, reaction temperature and time and subsequent enzymatic hydrolysis of wheat straw using autoclave processing which is essential to hydrolysis of wheat straw for xylose release were examined. The results of hydrolysis degree indicated that the optimal reaction conditions for the recovery of xylose from wheat straw hemicellulose were obtained when autoclave wheat straw was treated with xylanase, ground wheat straw was autoclaved 10 parts water at 121°C and the liquid and solid phases separated. The maximum xylose concentration was 23.38 mg/mL at optimized reaction conditions of 40°C reaction temperature, 30 min. reaction time and liquid-solid ratio of 1:10 (volume-mass).

Keyword: wheat straw, lignocellulose, xylanase, xylose

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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. It is the most abundant biomass with an estimated annual production of 1011 t containing potential energy of about 2 × 1021 J (Binder and Raines 2010).

Lignocellulosic complex lattice biomolecule structures consist of three basic parts; cellulose (35-50%), hemicellulose (20- 35%) and lignin (5-30%), along with smaller amounts of pectin, protein, extractives and ash. Nevertheless, the composition of each constituent varies from one plant species to another, which are tightly bound together by hydrogen and covalent bonds. Due to its complex structure, lignocellulosic biomass cannot be directly utilized by most bioethanol producers and must be pretreated in several consecutive steps to achieve fermentable sugars(Lin et al., 2010).

Among biomass components, hemicelluloses which are mainly composed of xylans, provide an important source of interesting molecules such as xylose and xylooligosaccharides which have potential applications in different areas, notably in chemical, food and pharmaceutical Xylose can be used as substrate to produce a wide variety of compounds or fuel by chemical or biotechnological processes.

Xylose can be hydrolyzed from xylan-rich materials like rice husk, corn stalk, wheat straw and flax straw. Xylose is a sugar purified from plants, which constitutes the hemicellulose, one of the main components of lignocellulose (Carlos, et, al 2012). Xylose has wide food, medicinal, and industrial applications (Silva et al., 2015). For example, xylose is an ideal alternative to regular table sugar as it is safe, healthier and toxin-free, and is not associated with obesity or other medical conditions commonly associated with sugar such as diabetes. It has become popular in Europe, Japan, and the USA since the 1960s, and it received FDA approval (Wang and Lu, 2013).

Wheat straw contains about 32.8% cellulose, 36.8% hemicellulose and 16.8% lignin. These components cannot be easily separated without altering hemicellulose, which is closely bound to lignocellulosic components (Silva et al., 2015).

With hydrolytic enzymes i.e. hemicellulases and cellulases different carbohydrate components from lignocellulosic raw materials and pulps can be selectively degraded. The selectivity of enzymatic treatment makes it an interesting process step when designing new process concepts.

The enzymatic route has been receiving a great deal of attention as an alternative technology for hydrolysis of lignocellulosic biomass due to mild operation condition and minimal formation of degrading compounds (Silva et al., 2015). Enzymatic methods are desirable as they do not requires any special equipment to be operated at high temperatures (Wang and Lu, 2013), are able to perform reactions with lower energy consumption and lower environmental impact (Mussatto et al., 2008). However, the enzymatic digestion of hemicellulose fractions in natural biomass substrates are always limited by its complex structure and components (Lin et al., 2010). Hence, the optimization of critical reaction parameters including substrate concentrations (Hardt et al., 2014), enzyme loadings (Carlos et al., 2012), pH (Romsaiyud et al., 2009), temperature (Jin et al., 2011) and hydrolysis time (Akpinar et al., 2009; Ovissipour et al., 2009) should be performed in relation to different substrates.

Furfural and HMF are degradation products of the sugars during dilute acid hydrolysis with inhibitory effect during fermentation (inhibitors). These substances are considered the main inhibitors as their activity can be intense even at small concentrations (Silva et al., 2015).

Xylose is also a versatile sugar compound and has many applications such as sugar source for nonnutritive agent in pharmaceutical industry, additive in color photography and brightener in zinc electroplating (Jin et al., 2011).

There are several xylose purification operation from hemicellulose hydrolyzate, acid hydrolysis, enzymatic hydrolysis to produce xylose which could partially improve purity and yield in commercial xylose production.

Enzymatic hydrolysis can not only economize energy on account of the relatively mild reaction conditions, but also avoid using toxic and corrosive chemicals (Jin et al., 2011). Enzymatic hydrolysis of biomass hemicellulose does not produce toxic products. Alternatively, xylose can be produced by enzymatic hydrolysis xylan. Generally, in lignocellulosic biomass, xylan exists in xylan lignin complex and becomes resistant to hydrolysis (Hardt et al., 2014). Therefore xylose production is carried out in two stages: alkaline extraction of xylan from lignocellulosic biomass followed by enzymatic hydrolysis (Akpinar et al., 2009).The enzyme hydrolysis is 10 catalyzed by xylanase enzyme for xylose production. The main aim of this investigation was to optimize the best possible conditions for optimum pretreatment of wheat straw as a feedstock for the xylose production while focusing the effect of different variables during pretreatment process - i.e. temperature (◦c), acid concentration (%),and residence time (min).

Materials and methods:

Raw Material

Wheat straw (*Triticum aestivum*) was obtained from local producers in Iraq. The sunlight dried straw was milled, screened to select the fraction of particles with a size lower than 0.5 mm, homogenized into a single lot and stored until used. The moisture content was 9 %.

2. Chemicals and enzymes:

The standards used included D-xylose, D-glucose, and furfural (all > 99.0% purity), Xylanase which were obtained from Sigma- Aldrich. Other chemicals were reagent-grade birches from local markets. Water for general use was distilled in a glass unit.

Methods

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Acid Pre-treatment

10 g wheat straw were mixed with sulphuric acid (H_2SO_4) solution in a 250 mL flask. The following pre-treatment condition was applied: incubation time of 20 minutes, pre-treatment temperature (121; mix 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 pre-treatment was carried out in an 9 L table-top autoclave (CertoClav, Traun, Austria).

Alkaline Pre-treatment

10 g wheat straw were mixed with sodium hydroxide (NaOH) solution in a 250 mL flask. The following pre-treatment condition was applied: incubation time of 20 minutes, pre-treatment temperature (121; mix ratio of solid :liquid (1:10, 1:15 and 1:20) and concentrations of (NaOH) was (0 %, 6 % 8 and %,10 %. The pre-treatment was carried out in an 9 L table-top autoclave (CertoClav, Traun, Austria). The dependent variables were the yield of D-xylose .

Separation

The pre-treated wheat straw was separated into solid and liquid fractions by vacuum filtration (Filter paper MN 640 w, Düren, Germany). The solid fractions were washed with distilled water until the wash water was neutral.

2.6. Enzymatic Hydrolysis

Enzymatic hydrolysis is the main step for conversion of oligosaccharides into C5-sugars (Xylose). For the enzymatic hydrolysis, 10 g ground wheat straw was mixed with water in mixed ratio (1:2) in a 250 ml flask. The pre-treatment was carried out in an 9 L table-top autoclave (CertoClav, Traun, Austria).

After autoclave pre-treatment, wheat straw was mixed with water in mixed ratio (1:10) solid liquid in a 250 ml flask

The conversion of oligosaccharides into C5-sugars was carried out using xylanase. The amount of enzyme used was 1 mL for mixture of wheat straw. The incubation was carried out for 180 min. at 40 °C in a shaking incubator at rotational speed of 2.5 s-1. The supernatants were obtained by centrifugation. After removal of solid particles, the crude wheat straw hydrolysates were analyzed for sugar content by HPLC using a HPX-87P column (Biorad, Richmond,CA) and for reducing sugar by dinitrosalisalic acid reagent using xylose as standard (Miller, 1959). The hydrolysis of wheat straw was calculated in percentage of xylose extraction .

2.7. Chemical Analysis

Wheat Straw Composition. Moisture, protein, lipid, and ash contents were determined by Approved Methods of AACC International (2000). The polysaccharides in straw were hydrolyzed according to Browning (1976), and the monosaccharide composition determined. Ground wheat straw (1.0g) was mixed with 72% sulfuric acid (10 mL), and the mixture held at 30 °C for 1 h with stirring. The concentration of acid in the mixture was adjusted to 4.0% by adding water (170 mL), and the mixture heated at reflux for 2 h.

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An aliquot (20 µL) of the hydrolyzate was made to volume (10 mL) with water containing an internal standard, and the sugars were assayed as described below.

Statistical analysis:
The statistical analysis was carried out using single-factor analysis of variance (ANOVA), while multiple comparison tests were used to determine the statistical significance with a 95% confidence level. For the data analyses, GenStat software was used.

Results and desiccation:

Composition of Wheat Straw.
The results of wheat straw composition are presented in Table1 The results are given as percentages of the total mass on a dry basis. The percentage of cellulose, hemicellulose and lignin in the dried wheat straw was 33.2%, 26.8% and 23.4%, respectively. Wheat straw is primarily composed of cellulose, hemicellulose and lignin (Aman and Nordkovist 1983).

Table 1: percentage of cellulose , hemicellulose and lignin in wheat straw

Component Content	***Percentage %***
Cellulose	33.2
Hemicellulose	26.8
lignin	23.4

The lignocellulosic materials contain more cellulose than hemicellulose, the hemicellulose fraction is more accessible to hydrolysis than cellulose. Hemicellulose occurs mostly as amorphous xyloglucan in type I plant cell walls or as amorphous glucuronoarabinoxylan in type II walls in the grass family .

Protein and ash accounted for 4.8% and 7.6%, respectively, of wheat straw (**Table 2**). The protein was within the range of 2.4 to 5.8% given by Theander and Aman,(1984) but the ash content of 7.6% was higher than the 4–6% of Harper and Lynch. Table 2. Chemical composition of wheat straw (dry weight)

Table 2: chemicals composition of wheat straw

Component Content	***Percentage %***
Carbohydrates	62
Glucose	33.6
xylose	24.4
Protein	4.9
ash	7.6

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lipid	0.6

Optimum produce of D-Xylose from Straw by Acid Catalyzed Hydrolysis at autoclave pressure and atmospheric Pressure.

The statistics analysis was applied to the acid-catalyzed, autoclave pressure hydrolysis of straw to predict the highest yield of D-xylose with highest selectivity table 3. The second-order model predicted a maximum yield of 19.43% D-xylose (straw basis) at an acid concentration of 1.5%, temperature of 140 °C.

In the present report, acid hydrolysis of wheat straw has been investigated in the following factors:

Effect of solid ratios (wheat straw : acid solution) Based on the specified condition (0.5%, 1% and 1.5% H_2SO_4, 121°C and 20 min.), the solid ratios of 1:10, 1:15 and 1:20 were examined. The ratio of 1:15 and acid consternation 1.5% has been selected as optimal wheat straw concentration resulting in xylose concentrations of 19.43 mg/ml (Figure2).

Table 3: xylose yield with acid pretreatment

mix ratio	acid cons.%	Xylose mg/ml
01:10	0.5	17.56
01:10	1	18.86
01:10	1.5	19.22
01:15	0.5	17.56
01:15	1	18.96
01:15	1.5	19.43
01:20	0.5	17.01
01:20	1	18.3
01:20	1.5	18.67

Effect of sulfuric acid concentrations The H_2SO_4 concentrations of 0.5-1.5% and control (without acid) with 0.5 % - 1:15 solid ratio were tested under steaming at 121°C for 20 min. The higher acid concentrations showed seemly the better yield of xylose (table 3). In order to minimize acid consumption used for neutralization, 0.5% H_2SO_4 has been chosen with xylose concentrations of 17.01mg/ml.

Tortosa et al (1990) working with corn cobs, reported that hydrolysis at 100°Crequired sulfuric acid concentrations ranging from 9 - 12% to obtain good yields of Dxylose (~19%). However, the process was accompanied by a significant formation of furfural due to the prolonged reaction time (1.5 to 4 h) required.

The effect of NaOH concentration on the enzymatic hydrolysis:

Alkaline hydrolysis

This experiment was carried out under those conditions used in the dilute acid treatment.

The NaOH concentrations 6 to 10% were studied and found that less extraction of xylose was obtained (table 4). As compared to dilute acid treatment, this alkaline treatment under the above specified condition was not suitable for xylose extraction from wheat straw.

Table 4: xylose yield at alkaline pretreatment

mix ratio	NaOH cons.	xylose
01:10	6	18.56
01:10	8	18.86
01:10	10	19.00
01:15	6	18.56
01:15	8	18.96
01:15	10	19.03
01:20	6	17.81
01:20	8	18.3
01:20	10	18.67

On the other hand, xylose extraction has been fairly good, while increasing the NaOH concentration. At 10% NaOH, the concentrations of xylose 19.22

The mechanism of alkaline hydrolysis is believed to be saponification of intermolecular ester bonds, which crosslinks xylan hemicelluloses and other components, for example, lignin and other hemicellulose (Hardt et al., 2014).

Pretreatment with NaOH removed lignin and at the same time partially degraded the hemicellulose.

Effect of pretreatment conditions on yield of total sugars released by enzyme hydrolysis.
Since 15% of pentoses (mainly d-xylose and larabinose) can be found in alkali pretreated wheat straw, it would be economically viable to convert 3.3. Alkaline Pre-treatment Wheat straw was pre-treated with different concentrations of sodium hydroxide at 100 °C. The xylose concentration of the solid and the liquid fraction were determined.

Fig.5 shows that a pre-treatment temperature of 100 °C and a sodium hydroxide concentration up to 5 % results in a xylose concentration increase up to 59.2 g kg-1 xylose in the liquid fraction. Beyond these conditions, the xylose concentration is decreasing. In comparison to the total xylose concentration of untreated wheat straw, it can be shown that increasing concentrations of sodium hydroxide lead to a xylose loss.

Enzymatic hydrolysis
The yields of D-xylose released by xylanase hydrolysis of the wheat straw autoclaved are shown in **table 5**. The release of D-xylose after 120 min. xylanase digestion of the solid phase was the highest at 8% of straw when the pretreatment at 160 °C lasted 30 min. Beyond the 180 min. incubation with xylanase, there was essentially no increase in D-xylose yield
When straw was autoclave treated, xylanase digestion of the swollen solid phase yielded more D-xylose as pretreatment time increased from 15 to 120 min.

The enzyme reaction conditions are mild, although the pretreatment of straw requires mechanical and thermal energy.
Also, the saccharification of this commercial derived enzymes showed that the degree of hydrolysis (release of xylose) has been improved . further determined the effect of enzymatic reaction time on the efficiency of hydrolysis for the pretreated wheat straw. The pretreated wheat straws were mixed with xylanase for 15 to 180 min. The results showed that as the enzymatic reaction time was increased, the concentration of the released xylose was also increased. The content of xylose was gradually decreased after 150 min. of reaction. This might be due to the inhibition of the enzyme activity by the accumulated hydrolysis products.

Both pretreatment and better source of xylanases, as well as working under optimal conditions should be obviously considered for enzymatic hydrolysis of wheat straw for xylose production.

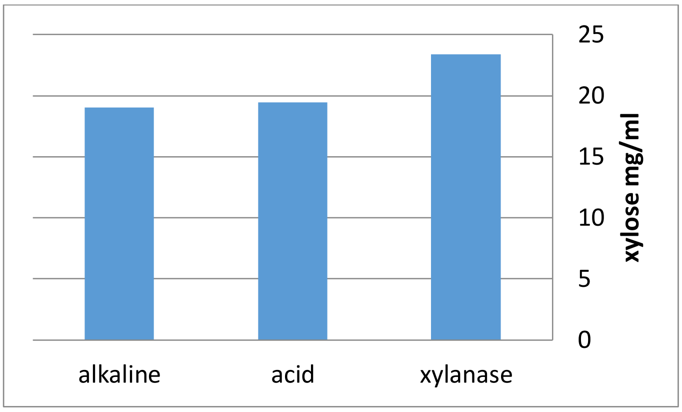
Table 5: xylose yield using of xylanase treatment

incub. time	**xylose con. Mg/ml**
30	20.74
60	21.25
90	21.97
120	23.38
150	22.24
180	22.16

3.4. Comparison of Applied Pre-Treatment Methods

The best results from all applied pre-treatment methods were compared to determine the most effective hemicellulose to xylose conversion process. Fig.1 shows that xylanase leads to the highest xylose concentration of 23.38, followed by a xylose concentration of 19.43 using acid treatment wheat straw . The worst yield is achieved by alkaline pre-treatment. The maximum yield of 23.38 D-xylose obtained by commercial xylanase hydrolysis was higher than the 19.43 mg/ml D-xylose obtained using sulfuric acid under pressure at 121 °C for 20 min.

Fig. 1 : compared of treatment methods for xylose production from wheat straw



CONCLUSION

From 3 different treatments of wheat straw for xylose extraction, enzymes treatment after autoclave (121C for 20 min.) was found to be the best alternative for use in wheat straw hydrolysate. preparation dilute acid (1.5% H2SO4, solid ratio of 1:15, particle size 0.5-1.4 mm, and 121°C for 30 min.). as well as the alkaline treatment under this study were not be suitable for xylose hydrolysate preparation, as compared with the dilute acid hydrolysis.

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الملخص العربي

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حسين فاضل , خالدة عبد الرحمن

جامعة بغداد- كلية الزراعة- قسم علوم الاغذية

تبن القمح هو واحد من أكثر المخلفات الزراعية وفرة ، وهو مصدر معتبر من المركبات اللكنوسيليلوز، و مورد من موارد الطاقة المتجددة لإنتاج الوقود والمواد الكيميائية، وبالتالي استغلالها يمكن أن تكون مجدية اقتصاديا. ويتطلب تحويل الكتلة الحيوية إلى وقود ومواد كيميائية بتحلل جزء السكريات المتعددة إلى سكرات أحادية. في هذه الدراسة، فإن تأثير المعاملات المختلفة (تركيز مختلف من هيدروكسيد الصوديوم وحامض الكبريتيك المخفف وكذلك نسبة صلبة إلى سائلة، درجة حرارة التفاعل والوقت) وكذلك التحلل الأنزيمي لتبن القمح بعد استخدام معالجة الأوتوكليف هو أمر ضروري للتحلل الهيميسيليلوز في تبن القمح الى سكر الزايلوز . وأشارت نتائج درجة التحلل إلى أن ظروف التفاعل المثلى لاستخلاص الزيلوز من هيميسيلولوز القمح القمح تم الحصول عليها عندما تم معالجة التبن المعامل بالأوتوكليف قبل المعاملة مع انزيم الزيلانيز 121 ◦C. كان الحد الأقصى للتركيز زيلوز 23.38 ملغ / مل في ظروف التفاعل الأمثل من 40 درجة مئوية درجة حرارة التفاعل، 30 دقيقة. زمن التفاعل ونسبة السائل الصلبة من 1:10 (حجم الكتلة).

الكلمة الرئيسية: تبن القمح، ليكنوسلولوز، زيلانيز، زيلوز