Hydrolysis of wheat straw hemicelluloses for maximum xylose extraction

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Abstract. The aim of the study is to select reaction conditions for hydrolysis of wheat straw with dilute sulfuric acid for maximum xylose extraction under mild conditions (at atmospheric pressure and temperature of 100°C). The authors found that maximum glucose yield (72.4-77.1 weight % of the initial content of hemicelluloses in wheat straw) is achieved at a concentration of H2SO4 2-3 weight % and the hydrolysis process duration of 5 hours. Analysis of the obtained hydrolysates showed that they contain cellulose (56.8-70.4 weight %), lignin (19.8-28.8 weight %) and hemicelluloses (2.8-15.3 weight %).

1. Introduction
The processes of acid-catalytic depolymerization of hemicelluloses (HC) are used to obtain xylose and other valuable products based on it, as well as to remove hemicelluloses in order to increase the efficiency of processes for obtaining high-quality glucose hydrolysates from lignocellulosic raw materials and to implement complex processing of the main biomass components into demanded chemical products [1-4]. Xylose is mainly obtained by acid-catalytic hydrolysis of plant raw materials’ hemicelluloses. Among the types of plant raw materials growing in Kazakhstan, wheat straw have the highest hemicellulose content (up to 30 weight %). The presence of a large amount of hemicellulose and a high density of wheat straw make it difficult to use traditional technologies for its processing into cellulose and glucose hydrolysates for bio-alcohols synthesis. Taking into account the presence of significant amounts of pentosans in wheat straw, it is economically expedient to carry out its complex processing with preliminary xylose extraction as one of the target products. The work [5] selected conditions for acid hydrolysis of wheat straw, providing maximum pentosan extraction. The studies showed that wheat straw hydrolysis at a temperature of 60°C for 2-3 hours does not allow achieving a high degree of pentosan extraction even at a sulfuric acid concentration in the solution of 20%. An increase in the acid concentration to 32% increases the pentosan yield up to 20-25% for the same hydrolysis duration. A similar effect is achieved with an increase in the hydrolysis temperature to 98°C at a sulfuric acid concentration of 3 weight % and the process duration 2-3 hours. The work [6], when obtaining hydrolysates from wheat straw, first carried out water extraction of straw in an autoclave at 165°C, and then hydrolysis of 72% H2SO4 at 25°C for 2 hours. After that, the mixture was diluted with water to a concentration of 40% H2SO4 and inversion of oligosaccharides was carried out at 60 and 80°C for 10 and 60 minutes. In this case, the straw conversion reached 23.7-27.4 weight %. In the composition of soluble products, about 4% of lignin, 3% of uronic acids and 11.3-20.0% of dissolved monosaccharides were identified, of which 15.5% is xylose. Complete xylose extraction in the straw processing with superheated steam at temperatures of 180-240°C was
achieved due to the loss of about 15 weight % in the cellulose yield [7]. In addition to xylooligosaccharides and xylose monomers, significant amounts of furfural and acetic acid were found in the aqueous extract. When processing wheat straw impregnated with a solution of 0.14 weight % H$_2$SO$_4$, hemicelluloses are mainly hydrolyzed to water-soluble products, and lignin remains in the solid fraction of the raw material, with water vapor at 200°C for 5 min under conditions of explosive autohydrolysis [8]. When processing autohydrolyzed straw with 0.25 M Na$_2$CO$_3$ at 120°C for 20 hours, most of lignin becomes soluble, and hemicelluloses remain in the solid fraction [8, 9]. The proposed method [10] for xylose extraction from wheat straw involves straw hydrolysis with 0.01 M solution of acetic acid at 140°C for 1 hour and the subsequent hemicellulose extraction with 1 M NaOH for 16 hours. The resulting fraction is a mixture of xylose and lignin and needs in complex sequential lignin purification and precipitation from a solution of sugars. Prehydrolysis of straw diluted with sulfuric or other acids at elevated temperatures provides a sufficiently high yield of sugars [11], and the use of dilute sulfuric acid is economically feasible [12]. It should be noted that a certain part of the hemicelluloses is very difficult to remove from the straw by hydrolysis. Residual hemicelluloses, co-crystallized with cellulose, which are difficult to hydrolyze, always remain in cellulose [13]. Thus, acid-catalytic hydrolysis of raw materials gives a set of C$_5$ and C$_6$ sugars and various water-soluble compounds formed during the depolymerization of hemicelluloses, amorphous part of cellulose, reactive part of lignin, and also due to secondary transformations of hydrolysis products [14]. To ensure selective HC depolymerization, it is necessary to select optimal conditions for hydrolytic straw conversion (temperature, composition of the reaction medium, etc.), at which conversions of other components are minimized.

2. Experimental Part

2.1. Initial raw material
In the experiments, air-dry wastes (wheat straw), growing in the vicinity of Shymkent (fraction of 2-5 mm), were used. The wheat straw composition determined by standard methods [% of the weight of absolutely dry straw]: cellulose - 46.8; lignin - 21.7; hemicellulose - 27.3; extractives - 3.2.

2.2. Wheat straw hydrolysis
A portion of air-dry wheat straw in an amount of 10 g was placed in a 500 ml three-necked round-bottom flask made of heat-resistant glass, equipped with a stirrer, a reflux condenser, and a thermometer. Then 80 ml of an aqueous solution of 1-4 weight % H$_2$SO$_4$ was poured (chemically pure grade, GOST 4204-77). The hydrolysis was carried out at atmospheric pressure, a temperature of 100°C, for 1-5 hours, with a stirring intensity of 14 revolutions per second. After a specified time, the resulting hydrolysate was cooled to room temperature, filtered on a Buchner funnel on a paper filter under vacuum, poured into a bottle, covered with a ground-in lid and analyzed on a VARIAN-450 gas chromatograph. The non-hydrolyzed residue was washed with distilled water until the wash water was neutral and dried in an oven at 103°C. The straw conversion was determined by the gravimetric method with an accuracy of ± 2-3%.

2.3. Analysis of hydrolysates
The individual composition and content of monosaccharides in the hydrolysates were determined by chromatography using a VARIAN-450 GC gas chromatograph with a flame ionization detector. A VF-624ms capillary column 30 m long and 0.32 mm inner diameter was used. Chromatographic conditions: the carrier gas was helium; the injector temperature was 250°C; the initial column thermostat temperature was 50°C (5 min), the temperature rose to 180°C at a rate of 10°C/min, holding at 180°C for 37 minutes. The detector temperature was 280°C. The duration of the chromatographic separation of monosaccharides was 55 minutes. To register the yield of oligosaccharides after 55 min of analysis, the thermostat temperature was raised to 250°C (10°C/min) and the column was kept at this temperature for 30 minutes. The total analysis time was 92 minutes. The hydrolysate sample was preliminarily subjected to derivatization as described in [16] with formation of trimethylsilyl derivatives. For this, a sample of neutralized hydrolysate containing about 10 mg of sugars and 4 mg of sorbitol was introduced into a round-bottom flask with a capacity of 15 cm$^3$ (the sorbitol solution was prepared earlier). The solution was stripped to dryness at 40-42°C. To remove water traces, 1 ml of an alcohol-toluene mixture (4:1) was added twice to the stripped sample, which was also removed by stripping. 1 ml of a freshly distilled dry
pyridine was added to the dry residue. If the analyzed sample was poorly dissolved, the flask was heated in a water bath at a temperature of 75-85°C for 2-3 minutes. To establish a stable equilibrium between the tautomeric forms of monosaccharides, a solution of the stripped hydrolysate sample in pyridine was kept for 12-20 hours. Then 0.3 ml of hexamethyldisilazane and 0.15 ml of trimethylchlorosilane were added to the solution. The flask was closed with a stopper, vigorously shaken for 30 seconds, and the reaction mixture was kept at room temperature for 10 minutes. When adding trimethylchlorosilane, the solution became cloudy due to the precipitation of ammonium chloride. Pyridine was stripped from the reaction mixture using a water-jet pump at a temperature of 40°C. Then, 1 ml of n-hexane was added to the flask; the resulting solution was filtered through a cone-shaped glass funnel with a paper filter and subjected to chromatography. The resulting chromatogram included the peaks of all monosaccharide tautomers in the hydrolysate sample. The peaks were identified using the retention times of tautomeric forms of monosaccharides previously established for these chromatographic conditions [16]. The ratio of the sum of the monosaccharide characteristic peak areas to the peak area of the internal standard (Si/St) was calculated. These ratios were used to find the weight of each monosaccharide in the hydrolysate sample using a calibration graph (calibration graphs for standard solutions of monosaccharides were built earlier). The following monosugars were used as standards for the analysis: crystalline hydrated glucose (GOST 975-88), D-xylose 142080.1208 (Panreac), D-mannose 373195.1208 (Panreac), D-sorbitol (Panreac).

2.4. Analysis of hydrolyzed wheat straw

The content of easily hydrolysable polysaccharides, difficult hydrolysable polysaccharides, and Klason lignin (modified by Komarov) was determined by standard methods in the solid residues of hydrolyzed straw samples [15]. X-ray diffraction patterns of straw samples were obtained using a Dron-3M diffractometer with CuKα radiation (λ = 0.154 nm). The diffraction patterns were recorded in the 2θ range of angles from 5 to 70° with a step of 0.01°. The crystallinity index of cellulose was calculated in accordance with the procedure described in [17].

3. Results and Discussions

To select the optimal conditions for the hydrolysis of wheat straw, providing the maximum xylose yield, the sulfuric acid catalyst concentration was varied from 1 to 4 weight % and the process duration was from 1 to 5 hours at a temperature of 100°C, hydromodule 8 and atmospheric pressure. It was found that the wheat straw conversion degree increases with an increase in the sulfuric acid catalyst concentration and the hydrolysis duration, and the content of residual hemicelluloses decreases from 27.3 weight % in the initial straw up to 2.8 weight % in lignocellulose obtained by the straw hydrolysis with 4% H₂SO₄ for 5 hours (Table 1). The chemical analysis of the lignocellulosic product showed that the minimum content of residual hemicelluloses in it is 2.8-3.1 weight % (Table 2). As follows from the data given in Table 2, hemicelluloses undergo the greatest changes in the hydrolysis process. Considering that their content in the initial straw is 27.3 weight %, after 5 hours of hydrolysis of 1-4% H₂SO₄, 5.5-2.8 weight % of hemicelluloses remain in the lignocellulose product. Moreover, with an increase in the hydrolysis duration, the content of hemicelluloses decreases, regardless of the sulfuric acid catalyst concentration. Cellulose in the acid hydrolysis process at a temperature of 100°C undergoes conversion to a lesser extent than hemicellulose. The cellulose content in the lignocellulosic product does not decrease with an increase in the hydrolysis duration, however, on the contrary, increases to 70.2-70.4 weight %. The increase in the cellulose content in the product is associated with the predominant removal of hemicelluloses during the hydrolysis process. A slight increase in the lignin content in the lignocellulose in comparison with the initial straw is obviously associated with the formation of lignin-like substances (so-called pseudo-lignin) during the hydrolysis of raw materials [18]. The target product yield of the wheat straw hydrolysis – xylose – depends on the sulfuric acid catalyst concentration and the process duration. The maximum xylose yields (72.4-77.1 weight % from the initial content of hemicelluloses) were obtained by the straw hydrolysis for 5 hours using 2 and 3% H₂SO₄, respectively. The increased catalyst concentration (4% H₂SO₄) with the hydrolysis duration of more than 2 hours does not allow obtaining a high xylose yield, which does not exceed 58-59 weight %. The hydrolysis duration increase to 5 hours decreases the xylose yield to 48 weight %. The xylose concentration decrease is due to the reactions of its secondary transformations. The resulting monosaccharides undergo multistep
Transformations, which lead to a decrease in the yield of sugars and formation of furan derivatives and other inhibitors of biochemical processes [19]. According to the XRD data, the straw hydrolysis in the dilute solutions of sulfuric acid leads to an increase in intensity and a decrease in the width of peaks with the 002 index. The width of X-ray diffraction lines depends on the size of crystallinity regions (coherent scattering regions): the larger the size of these regions, the less the width of the peak on the roentgenogram. A well-crystallized and lattice-homogeneous material gives narrow and high diffraction peaks (lines 2 and 3 in Figure 1).

Table 1. Influence of the sulfuric acid catalyst concentration and the wheat straw hydrolysis duration on its conversion and the content of hemicelluloses in the lignocellulose product (temperature 100°C)

| Catalyst concentration | Hydrolysis duration, hours | 1 | 2 | 3 | 4 | 5 |
|------------------------|----------------------------|---|---|---|---|---|
| 1% H₂SO₄ | Straw conversion* | 9.3 | 17.3 | 14.1 | 12.7 | 16.2 | 7.3 | 22.6 | 6.4 | 29.3 | 5.5 |
|  | Residual hemicelluloses ** | | | | | | | | | | |
| 2% H₂SO₄ | Straw conversion* | 15.9 | 12.2 | 18.3 | 8.6 | 22.8 | 4.8 | 29.1 | 3.8 | 33.3 | 4.2 |
|  | Residual hemicelluloses ** | | | | | | | | | | |
| 3% H₂SO₄ | Straw conversion* | 16.4 | 10.0 | 21.2 | 6.3 | 22.5 | 5.2 | 32.3 | 4.5 | 35.1 | 3.1 |
|  | Residual hemicelluloses ** | | | | | | | | | | |
| 4% H₂SO₄ | Straw conversion* | 22.3 | 7.9 | 27.4 | 5.1 | 26.2 | 3.4 | 28.6 | 3.2 | 36.9 | 2.8 |
|  | Residual hemicelluloses ** | | | | | | | | | | |

*Conversion, % from the weight of air-dry straw; **- from the weight of air-dry lignocellulosic product

Table 2. Influence of the sulfuric acid catalyst concentration on the lignocellulosic product composition of the wheat straw hydrolysis at a temperature of 100°C

| Raw material | Hydrolysis duration, hours | Absent | 1 | 2 | 3 | 4 | 5 |
|--------------|----------------------------|--------|---|---|---|---|---|
| Initial wheat straw * | Straw after hydrolysis with | 27.3 | 46.8 | 21.7 |
|  | 2 | 17.3 | 56.8 | 20.2 |
|  | 3 | 12.7 | 61.4 | 20.8 |
|  | 4 | 7.3 | 64.8 | 22.0 |
|  | 5 | 6.4 | 65.7 | 22.5 |
|  | 2 | 5.5 | 67.0 | 23.7 |
|  | 3 | 12.2 | 59.9 | 19.8 |
|  | 4 | 8.6 | 63.5 | 21.0 |
|  | 5 | 4.8 | 67.3 | 23.4 |
|  | 2 | 3.8 | 68.3 | 23.6 |
|  | 3 | 4.2 | 68.8 | 25.0 |
|  | 4 | 10.0 | 63.2 | 21.3 |
|  | 5 | 6.3 | 65.8 | 23.0 |
|  | 3 | 5.2 | 66.9 | 25.2 |
|  | 4 | 4.5 | 67.8 | 26.0 |
|  | 5 | 3.1 | 69.0 | 27.1 |
|  | 1 | 7.9 | 64.2 | 22.3 |
|  | 2 | 5.1 | 67.0 | 25.7 |
|  | 3 | 3.4 | 68.9 | 27.6 |
|  | 4 | 3.2 | 70.2 | 28.4 |
|  | 5 | 2.8 | 70.4 | 28.8 |

* - From the weight of air-dry straw; ** - from the weight of air-dry lignocellulosic product
The characteristic peaks in the region $2\theta = 15-16^\circ$ are due to X-ray diffraction from the planes 101 and 10-1 of the crystal lattice. The reflection intensity in the region $2\theta = 22.5^\circ$ corresponds to the plane 002 of the crystal lattice of natural cellulose I \cite{20, 21}. Thus, as a result of the preliminary initial straw hydrolysis in the solutions with the sulfuric acid content of 3 weight \%, the removal of easily hydrolysable polysaccharides of irregular structure occurred. The remaining ordered part of the hydrolyzed straw solid residue supramolecular structure corresponds to the crystal lattice of cellulose I. The crystallinity index of the initial wheat straw calculated from the diffractograms is 0.49, and for the samples hydrolyzed with 3 weight \% $\text{H}_2\text{SO}_4$, at a temperature of 100$^\circ$C for 2 and 5 hours, respectively 0.59 and 0.63. The solid residue of the wheat straw hydrolysis with the sulfuric acid solutions contains cellulose, lignin, and residues of hard-to-extract hemicelluloses.

![Figure 1. Diffraction patterns of the initial wheat straw (1), after the hydrolysis with 3\% $\text{H}_2\text{SO}_4$ for 2 hours (2), after the hydrolysis with 3\% $\text{H}_2\text{SO}_4$ for 5 hours (3)](image)

The analysis of the wheat straw hydrolysates by the gas chromatography showed the presence of not only the target product, xylose, in the solutions, but also small amounts of mannose and glucose. Mannose and glucose are products of the hydrolysis of hemicelluloses and easily hydrolysable part of cellulose, and mannose can be formed as a result of catalytic epimerization of glucose. It is known \cite{22} that the main components of hydrolysates formed during the hydrolysis of hemicelluloses are monosaccharides, and the formation of soluble oligomeric products occurs, the main of which is xylobiose. The following monosugars were determined in the composition of the dissolved products: xylose (2.3-77.1 weight \%), mannose (0.12-3.6 weight \%) and glucose (0.9-12.7 weight \%).

4. Conclusions

The process of hydrolysis of wheat straw with dilute sulfuric acid at a temperature of 100$^\circ$C and atmospheric pressure was studied. It was found that the maximum xylose yield (72.4 and 77.1 weight \% from the initial content of hemicelluloses) was obtained by the straw hydrolysis for 5 hours, 2 and 3\% $\text{H}_2\text{SO}_4$, respectively. The gas chromatographic analysis of the obtained hydrolysates showed that their main product is xylose, and that mannose and glucose are also present in small amounts. The solid residue from the hydrolysis of wheat straw diluted with sulfuric acid contains cellulose (56.8-70.4 weight \%), lignin (19.8-28.8 weight \%) and residual hemicelluloses (2.8-15.3 weight \%). According to the XRD data, the crystallinity indices of the hydrolyzed wheat straw are higher than those of the initial one, and amount to 0.59-0.63.
References

[1] Carvalho ML, Sousa JrR, Rodríguez-Zúñiga UF, Suarez CAG, Rodrigues DS, Giordano RC 2013 Kinetic study of the enzymatic hydrolysis of sugarcane bagasse Brazilian Journal of Chemical 3 437–47.

[2] Amezcua-Allieri MA, Sánchez Durán T, Aburto J 2017 Study of Chemical and Enzymatic Hydrolysis of Cellulosic Material to Obtain Fermentable Sugars Journal of Chemistry 2017 1–9.

[3] Bychkov AL, Bukhtoyarov VA, Lomovskii OI 2015 Stabilization of cellulolytic enzymes by sorption on the plant raw materials surface Russian Chemical Bulletin 64(5) 1189–1191.

[4] Andersen N 2007 Enzymatic Hydrolysis of Cellulose. Experimental and Modeling Studies, PhD Thesis, Technical University of Denmark, Lyngby.

[5] Kedelbaev B, Lakhanova K, Makhatov Z, Makhatov B, Sarsembayeva M 2018 Study of obtaining process of industrial significant products from wheat straw and guza-paya Journal of Biotechnology 280 28.

[6] Ballesteros M 2010 Enzymatic hydrolysis of lignocellulosic biomass Bioalcohol Production 159–77.

[7] Al-Kharousi MM, Sivakumar N, Elshafie A 2015 Characterization of cellulase enzyme produced by chaetomium sp. Isolated from books and archives Eurasian Journal of BioSciences 9 52–60.

[8] Qi H 2016 Cellulose-Based Functional and Smart Materials Springer Briefs in Applied Sciences and Technology 12 45–67.

[9] Makhatov ZhB, Kedelbayev BS, Doltayeva BZ, Orazova MM, Taizhanova MA, Mamatkulova FA, Arystanova SD 2018 Research of enzymatic hydrolysis processes of wheat's straw by liquid cellulolytic enzymatic agents Actual problems of applied sciences Journal World 10 11-17.

[10] Hu F, Ragauskas A 2012 Pretreatment and Lignocellulosic Chemistry BioEnergy Research 5(4) 1043–66.

[11] Lynd LR, Weimer PJ, van Zyl WH, Pretorius IS 2002 Microbial cellulose utilization: fundamentals and biotechnology Microbiology and molecular biology reviews: MMBR 66(3) 506–577.

[12] Makhatov ZhB, Kedelbayev BS, Dzhakashyeva M, Daulbai A, Doltayeva BZ, Akhanov U 2019 Investigation of Acid and Enzyme Wheat Straw Hydrolysis for Obtaining Polysaccharides Oriental Journal of Chemistry 35(2) 766–772.

[13] Yang P, Kobayashi H, Fukuoka A 2011 Recent Developments in the Catalytic Conversion of Cellulose into Valuable Chemicals Chinese Journal of Catalysis 32(5) 716–722.

[14] Somogyi M 1952 Notes on sugar determination J. Biol. Chem. 195 19-23.

[15] Kadic A 2018 The effects of mixing on the enzymatic hydrolysis of lignocellulosic biomass, PhD Thesis, Lund University, Lund.

[16] Coelho RR, Rosa PJ, Souza FR, Fróes MA 2013 Methods to Determine Chitinolytic Activity Methods to Determine Enzymatic Activity 22 39–67.

[17] GOST 2014 № 55298-2012. Enzyme preparations for the food industry. Methods for determining pectolytic activity, Standartinform.24, Moscow.

[18] Olsson C 2014 Cellulose processing in ionic liquid based solvents, PhD Thesis, Chalmers University of Technology, Gothenburg.

[19] Onda A, Ochi T, Yanagisawa K 2008 Selective hydrolysis of cellulose into glucose over solid acid catalysts Green Chemistry 10(10) 1033.

[20] Yoshida M, Liu Y, Uchida S, Kawarada K, Ukagami Y, Ichinose H 2008 Effects of Cellulose Crystallinity, Hemicellulose, and Lignin on the Enzymatic Hydrolysis of Miscanthus sinensis Monosaccharides Bioscience Biotechnology and Biochemistry 72(3) 805–810.

[21] Nascimento PR, Gravina-Oliveira PM, Coelho RR 2013 Methods to Determine Xylanolytic Activity Methods to Determine Enzymatic Activity 125–159.

[22] Palkovits R, Tajvidi K, Procelewska J, Rinaldi R, Ruppert A 2010 Hydrogenolysis of cellulose combining mineral acids and hydrogenation catalysts Green Chemistry 12(6) 972.