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RESEARCH OF ENZYMATIC HYDROLYSIS PROCESSES OF WHEAT'S STRAW BY LIQUID CELLULOLYTIC ENZYMATIC AGENTS

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ABSTRACT

Objective of this research was to investigate process of enzymatic hydrolysis of wheat's straw by liquid cellulolytic enzymatic preparations. At the first stage wheat straw has been subjected to autohydrolysis then the fibrous weight was washed out by water with reception of sugar solution. The following stage included extraction of disrupt lignin and modelling experiments for studying enzymatic kinetics was the last stage. As a result of the carried out researches it has been established, that that the maximum exit of reacting substances is observed 4,8 %, the minimum exit is 2,2 %. The general maintenance of carbohydrates in wheaten straw has made 83,3 % from weight of wheaten straw. Among monosaccharides in straw are prevailed xylose and glucose. Measurement of pH of the investigated wheaten straw in water at the hydromodule 1 : 5,8 has shown, that pH makes $7,2840 \pm 11$. pH of straw presoaked in 0,59 % of weights to sulphurous acid has made $2,24 \pm 0,11$. pH of the straw presoaked in 1,18 % of weights to sulphurous acid has made $1,48 \pm 0,10$.

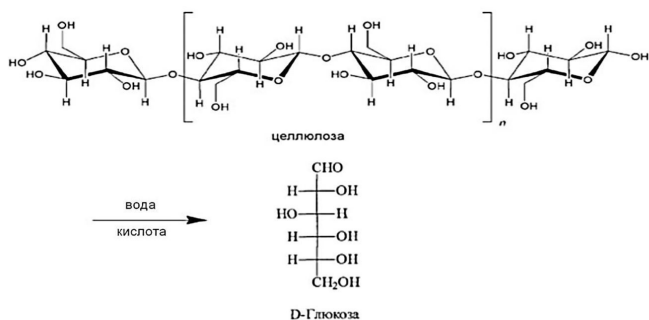
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Materials and Methods

At the first stage, wheat straw was subjected to autohydrolysis, after which the pulp was washed with water to obtain a solution of sugars (Fig. 1). When water extraction in the solution goes up to 90% of hemicelluloses.



Picture 1. Getting D-glucose from cellulose

The next step involved the extraction of degraded lignin. Lignin solvents were, by analogy with native lignin, dioxane-water (9: 1), ethanol-water (9: 1), they remove up to 90% of lignin. In addition, a NaOH solution with a concentration from 0.4 to 2.0% was used as a solvent.

Thus, after a two-stage extraction, a product was obtained consisting mainly of cellulose. Such cellulose was used as a raw material for the production of glucose. The treatment of cellulose was carried out in the temperature range 190-250 ° C with varying concentration of sulfurous acid from 0.6 to 2.5% by weight.

The optimum temperature and duration of cellulose hydrolysis with sulfuric acid were 160 ° C – 170 ° C and 30–80 minutes, respectively. With an increase in the concentration of sulfurous acid, an increase in the rate of decomposition of sugars is observed.

The optimum temperature and duration of cellulose hydrolysis with sulfuric acid were 160 ° C - 170 ° C and 30–80 minutes, respectively. The amount of decomposition of sugars is observed.

Model experiments for the study of enzymatic kinetics were carried out using paper and cotton wool as a source of cellulose. In experimental processes of fermentolysis, pre-ground, sifted and dried to a constant value in a drying oven at a temperature of 120 ° C for 2 hours were used, wheat straw was pre-steamed in an autoclave at an excess pressure of 0.05-0.1 MPa for 0.5 - 1 hour

The processes of fermentolysis were carried out in accordance with the characteristics of the used enzyme preparations while maintaining an active acidity in the range of 4.9-5.0 units. pH and temperature 49 ° C.

The duration of the processes of fermentolysis was 7-10 hours.

The hydrolysis of wheat straw with sulfurous acid at a concentration of 0.59-3.00% of the mass was carried out with varying temperatures in the range of 150-190 ° C. In the study of wheat straw hydrolysates, we limited ourselves to determining the content of PB and dry substances [85, 86, 145].

The obtained hydrolysates were used in the composition of nutrient media during the cultivation of various strains of microorganisms [130-140], while the qualitative composition of carbohydrates in hydrolysates was not evaluated. For the development and improvement of the method of wheat straw hydrolysis with sulfuric acid, we studied the effect of temperature and duration of treatment on the composition and carbohydrate content of hydrolyzates.

To measure the pH of the hydrolysates, a pH meter of multitest ion meter-oximeter IPL-513 was used.

Results and discussion

Enzyme hydrolysis was performed by enzymatic hydrolysis of straw at various concentrations of the enzyme preparation PSA 03197-1.0 ENA cellerage CB100. The results of the experiment are shown in table 1.

Table 1. Results of fermentation of wheat straw in the fermenter

Experience number	The amount of enzyme units activity	Maximum concentration PB, %	Output PB, %
1	62-75,8	0,43	4,3
2	62-75,8	0,46	4,6
3	6,2-17,6	0,46	4,6
4	31-37,9	0,48	4,8
5	62-75,8	0,32	3,2
6	1,6-1,9	0,26	2,6
7	3,0-3,8	0,26	2,6
8	4,6-5,7	0,22	2,2

From table 3 it follows that the maximum yield of PB is observed in experiment 4 and is 4.8%, while the minimum yield is noted in experiment 8 and is 2.2%.

The results of wheat straw hydrolysis showed that the ash content of the studied wheat straw was $5.60 \pm 0.25\%$ - this is a relatively high figure for wheat straw. The straw was previously dried to equilibrium moisture.

To characterize the feedstock, the qualitative and quantitative composition of wheat straw polysaccharides was determined (Table 2). The composition and content of carbohydrates found in the wheat straw under study was consistent with the literature data [2].

The total carbohydrate content in wheat straw was 83.3% of the mass of wheat straw. Among monosaccharides, xylose and glucose prevailed in straw.

Table 2. Composition of hardly hydrolysable and leggohydrolysable wheat straw polysaccharides

Monosaccharides	Easily hydrolyzed polysaccharides, % of wheat straw *	Hard hydrolysable polysaccharides, % of wheat straw *
Rhamnose	1,8	0,0
Arabinose	10,4	0,2
Galactose	3,8	traces
Glucose	13,3	14,1
Xylose	36,5	0,4
Galacturonic acid	1,5	traces
Glucuronic acid	1,3	traces
Total amount	68,6	14,7

* the table shows the average values, the standard deviation did not exceed $\pm 2.5\%$.

The total content of arabinose in the straw under study was 10.6%. The high content of xylose and arabinose is due to the fact that glucurono-arabinoxylan predominates among wheat glycan binding glycans [3]. Hard-to-digest polysaccharides of wheat straw are represented by crystalline cellulose [4], while glucose present in the fraction of lightly hydrolysable polysaccharides is contained in amorphous cellulose or glucan with a mixed type of bonds — the characteristic polysaccharides of cereals [5].

High-temperature hydrolysis of wheat straw was carried out in the temperature range of 150–200 °C with a sulfurous acid concentration of 0.59 and 1.18% of the mass and a water ratio of 1: 5.8 (Table 3). An increase in temperature and acid concentration led to a significant increase in the degree of depolymerization of polysaccharides and a reduction in the time to achieve the highest yield of PB.

Table 3. Changes in the concentration of PB during the pretreatment of wheat straw with sulfurous acid

Температура, °C	Концентрация кислоты	Концентрация РВ, %								
		Время гидролиза, мин.								
		10	20	30	40	50	60	70	80	90
150	0,59	0,3±0,04	1,1±0,03	1,2±0,54	1,5±0,05	1,5±0,04	1,4±0,16	2,3±0,51	2,1±0,32	2,3±0,32
	1,18	0,4±0,14	1,3±0,05	1,5±0,08	1,9±0,10	2,0±0,06	2,0±0,14	2,2±0,17	2,1±0,10	2,1±0
160	0,59	0,4±0,03	1,3±0,03	1,8±0,03	1,8±0,03	1,9±0,11	1,7±0,03	1,7±0,03	1,6±0,03	1,6±0,03
	1,18	0,7±0,26	1,7±0,37	2,1±0,56	2,2±0,31	2,1±0,23	1,9±0,20	2,0±0,07	2,0±0,08	2,0±0,16
170	0,59	0,7±0,5	1,6±0,03		1,9±0,04	2,1±0,02	2,1±0,07	2,0±0,02	1,9±0,03	
	1,18	1,2±0,03	2,2±0,03	2,3±0,03	2,5±0,03	2,3±0,21	2,2±0,26	2,2±0,19	2,1±0,20	1,8±0,03
180	0,59	0,6±0,02	1,8±0,27	1,7±0,44	2,2±0,30	2,1 ±0,3 6	2,0±0,26	1,9±0,11	1,8±0,14	1,6±0,16
	1,18	1,0±0,44	2,2±0,18	2,5±0,23	2,7±0,20	2,0±0,25	2,6±0,25	2,4±0,29	2,4±0,31	
190	0,59	1,4±0,42		2,2±0,05	2,0±0,25	1,9±0,25	1,5±0,40	1,4±0,34	1,3±0,28	1,3±0,17
	1,18	1,4±0,03	2,7±0,03	2,7±0,03	2,6±0,03	2,4±0,03	1,9±0,03	1,9±0,03		
200	0,59	0,6±0,03	1,8±0,03	2,1±0,03	1,8±0,03	1,9±0,03	1,6±0,03	1,5±0,03	1,5±0,03	1,4±0,03
	1,18	2,0±0,03	3,0±0,03	2,7±0,03	2,2±0,03	1,6±0,03				

In general, the dynamics of RV changes in the process of hydrolysis is of an extreme nature, which is due to the irreversible processes of the destruction of carbohydrates occurring under the influence of acid and high temperatures. In addition to sugars, other degradation products of plant tissue are formed in the process of acid hydrolysis [6]. In addition, most natural biomass has a certain level of buffer capacity.

Therefore, the actual pH obtained during acid hydrolysis at high temperature may vary. In this regard, studies of the effect of the neutralizing ability of biomass on the effective acid concentration are important.

Measuring the pH of the test wheat straw in water at a water ratio of 1: 5.8 showed that the pH is 7.2840.11. The pH of the straw soaked in 0.59% by mass of sulfurous acid was 2.24 ± 0.11 . The pH of the straw soaked in 1.18% by mass of sulfurous acid was 1.48 ± 0.10 .

In the first minutes of hydrolysis with an increase in the RV content in the hydrolyzate, a decrease in the pH of the hydrolyzate is observed,

which may be due to the accumulation in the hydrolyzate of uronic acids, as well as acetic acid contained in the raw materials.

Thus, during the chemical analysis of wheat straw for the depolymerization processes of the polysaccharides contained in it, we found that this type of waste from the agricultural sector can be effectively processed into bioavailable sugars.

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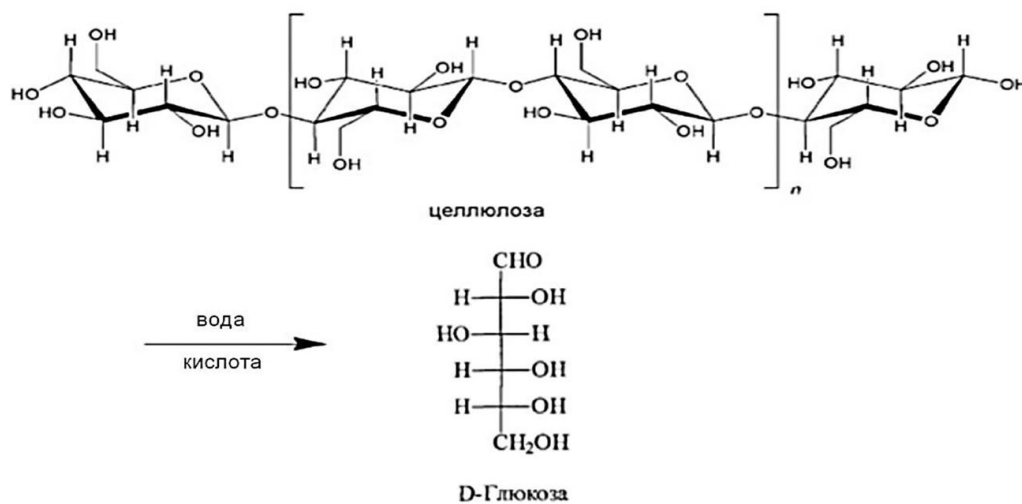
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Tables and Figures



Picture 1. Getting D-glucose from cellulose

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	1,18	2,0±0,03	3,0±0,03	2,7±0,03	2,2±0,03	1,6±0,03					