



## ASSESSMENT OF CHANGES IN THE CHEMICAL COMPOSITION OF OAT HUSK DURING PRETREATMENT BY AUTOHYDROLYSIS

I. N. Pavlov

Igor N. Pavlov, PhD in Engineering, Senior Research Scientist<sup>1</sup>, Associate Professor<sup>2</sup>

<sup>1</sup>Institute for Problems of Chemical and Energetic Technologies, Siberian Branch of the Russian Academy of Sciences (IPCET SB RAS), Socialisticheskaya st., 1, Biysk, Russia, 659322

<sup>2</sup>Department of Machines and Apparatuses of Chemical and Food Production, Biysk Technological Institute (branch) of Altai State Technical University named after I.I. Polzunov, Hero of the Soviet Union Trofimov st., 27, Biysk, Russia, 659305

E-mail: pawlow-in@mail.ru

---

### Keywords:

oat husk, pretreatment, auto-hydrolysis, hydro-thermal treatment, cel-lulose, enzymatic hy-drolysis

*We carry out the preliminary processing of oat husk biomass by autohydrolysis. There was a change in the process temperature in the range of 160-220 °C, the processing time in the range of 5-60 minutes and the treatment hydromodule 1:10. To characterize the pre-treatment conditions, we use the autohydrolysis severity factor, which varied from 2.50 to 5.37. We assessed the effectiveness of the autohydrolysis performed by changing the chemical composition of the solid phase of the treated oat husk. The auto-hydrolysis of oat husk biomass with increasing hardness of the treatment conditions leads to a significant decline of hemicelluloses in the solid phase when the severity factor reaches 4.17 at the first stage and to a subsequent almost complete removal. Also, at the first stage, there is a slight change in the concentration of lignin and an increase in the proportion of cellulose in the solid fraction. A further increase in the severity of the conditions at the second stage with a change in the severity factor from 4.7 to 5.37 leads to the accumulation of lignin in the solid residue up to a maximum value of 45.7% as a result of the lignin condensation reaction. Due to the increase in the lignin content, the proportion of cellulose in the solid fraction decreases. The authors assessed the effectiveness of the pretreatment by the final accumulation of reducing substances obtained as a result of enzymatic hydrolysis using an enzyme complex consisting of the preparations "Cellolux-A" and "Bruzime BGX. At the first stage, with a decrease in the concentration of hemicelluloses, the availability of cellulose for enzymes significantly increases and the yield of reducing substances reaches a maximum value of 66.7% with a severity factor of autohydrolysis of 4.17. At the second stage, an increase of the severity of the processing conditions to a value of a factor of 5.37 leads to the accumulation of lignin in the fixed residue, which limits the effect of enzymes. It reduced the yield of substances during enzymatic hydrolysis to 30.0%.*

---

### Introduction

Lignocellulosic biomass is the renewable resource. The cellulose and hemicelluloses are extracted from it and used to produce various value-added products [1]. Oat husk is one of the widespread and accessible raw material sources in agricultural regions and is a zero-cost agro-industrial product. The use of cellulose and hemicelluloses as substrates in an enzymatic process treated with selected enzyme preparations makes it possible to obtain benign hydrolysates for



use as nutrient media in bacterial cellulose biosynthesis technology [2, 3] or for further digestion in bioethanol production technology. In lignocellulosic biomass technology pretreatment can affect the physico-chemical properties of the biomass and its structure. The efficiency of the subsequent enzymatic hydrolysis and biosynthesis processes depends on the pretreatment conditions [4, 5]. Nowadays there are various pretreatment methods [6]. Hydrothermal pretreatment (autohydrolysis) methods are widely used. By the process, the biomass is treated at high temperatures under increased pressure. As a result, a significant increase of the availability of the cellulose surface for the action of enzyme preparations can be achieved [7, 8]. Autohydrolysis involves the use of technology that treats the original biomass using water as the main reaction medium. Processing under these conditions allows complete removal of hemicelluloses, partial degradation of lignin and changes of cellulose structure [9]. This increases the availability of cellulose for the action of enzymes and its susceptibility to enzymatic hydrolysis.

The purpose of this paper is to investigate the effect of treatment temperature and residence time of oat husk (*Avena Sativa*) pretreatment by hydrothermal treatment (autohydrolysis) on the composition of the solid fractions of the biomass. We assessed the effectiveness of pretreatment by the results of enzymatic hydrolysis of the solid fraction of the treated biomass compared to the hydrolysis of untreated oat husk biomass.

### Experimental part

The study contains the information about the processing of lignocellulosic raw material of oat husk (*Avena Sativa*). Oat husk is an agro-industrial product that is an oblong particle of 8-10 mm in size and does not require pre-shredding, and is used for processing in its native form. The chemical composition of oat husk is shown in Table 1.

**Table 1.** Chemical composition of native oat husk biomass

Biomass	Mass fraction, %		
	Kürchner cellulose	hemicellulose	lignin
Oat husk	49.26	31.83	16.86

We use 2.3 litre stainless steel reactor for the hydrothermal treatment (autohydrolysis). The reactor allows the process to be carried out at a maximum temperature of 300 °C and an operating pressure of up to 10 MPa. The reactor has an external electric heating element, the set temperature of the oat husk biomass treatment is achieved by it. The hinged bearing of reactor allows its rolling motion with an amplitude of 150° [10]. We premixed the oat husk with purified water in a liquid-solid ratio of 10:1. We placed the suspension in the reactor, hermetically sealed it and made an overpressure of 30 bar in a CO<sub>2</sub> atmosphere to keep the water in liquid form at high temperatures during the treatment process. Then we heated the suspension to the target temperature. When the temperature reached the desired value, we connected the reactor rolling system and pumped the suspension at maximum amplitude to mix the reaction mass and stabilise the temperature in the reactor. We treated the biomass of oat husk in the temperature range 160-220 °C, with holding times ranging from 5 to 60 min. After the assigned reaction time, we cooled the reactor down to below 100 °C and drained the reaction mass. We



separated the reaction mixture into liquid and solid fractions by filtration. We pre-washed the solid fraction with water, dried to a moisture content of 7-10% and used for chemical analysis

Then we carried out the enzymatic hydrolysis. And use the solid fraction as a substrate to study the reactivity to enzymatic hydrolysis using the industrial enzyme preparations "Cellolux-A" and "Bruisheim BGX". The enzymatic activity of the individual preparations is shown in Table 2.

**Table 2.** The enzymatic activity of the individual preparations

Name of the medicine	The enzymatic activity
"Cellolux-A" (standardised for cellulase) PA Sibbiofarm LLC, Russia	cellulase: (2000±200) units ClS/g xylanase: (8000±800) units KS/g β-Glucanase: up to (1500±150) units β-GcS/g
"Bruisheim BGX" (standardised for hemicellulase) «Polfa Tarchomin Pharmaceutical Works S.A.», Poland	cellulase: (2100±105) units ClS/g xylanase: (4200±210) units KS/g; β-Glucanase: (530±26.5) units β-GcS/g.

For the enzymatic hydrolysis, we placed a substrate with a mass of 5 g (absolute dry matter) into a 0.5 l conical flask. Also, we dissolve the enzyme preparations in acetate buffer solution (pH=4.7), each in an amount of 0.04 g/g substrate and placed the buffer solution into a flask to obtain 150 ml of substrate with a concentration of 33 g/l. Then we carried out the hydrolysis under constant stirring on a "PE-6410 M" platform (Russia) with a frequency of oscillation of 150 min<sup>-1</sup> for 72 h. During hydrolysis, there was a constant temperature of (45±2) °C to obtain the maximum yield of reducing substances at pH=4.6 [11] and the samples of the hydrolysates were taken every 8 hours. We identified the concentrations of reducing substances in the hydrolysate in terms of glucose by spectrophotometric method using 3,5-dinitrosalicylic acid reagent (Panreac, Spain) on a UNICOUV-2804 spectrophotometer (United products & instruments, USA).

Reducing substances (by weight of substrate, %) were calculated by the formula

$$n_{rs} = \frac{C_{rs} \cdot V_h}{m_s} \cdot 0,9 \cdot 100,$$

where  $C_{rs}$  is final concentration of reducing substances, g/l;  $V_h$  - hydrolysate volume, l;  $m_s$  - mass of substrate for fermentation, g; 0.9 - coefficient due to attachment of water molecule to anhydroglucose residues of corresponding monomeric links as a result of enzymatic hydrolysis.

The authors identified the chemical composition of the raw material and substrate samples by GOSTs and special methods for the analysis of plant raw materials [12]. The mass fraction of cellulose by Kürchner was analysed by treating the cellulose with an alcoholic solution of nitric acid and quantifying the undissolved residue. The mass fraction of acid-soluble lignin was analysed by the Komarov's method [13]. The mass fraction of hemicelluloses was analysed by the iron orsine method according to GOST 10820-75. The mass fraction of ash was analysed by the combustion method according to GOST 18461-93. We calculated the yield of the solid fraction per mass of raw material by the formula

$$n = \frac{m_{sf} \frac{(100-W)}{100}}{m_m} \cdot 100,$$



where  $m_{sf}$  - mass of solid fraction, g;  $m_m$  - mass of the original oat husk biomass sample, g;  $W$  - mass fraction of moisture in the solid fraction, %.

We analysed the mass fraction of moisture in the solid fraction by "MB23" moisture analyser (Ohaus, USA).

To assess the severity factor of the hydrothermal treatment conditions, which is a function of residence time and treatment temperature [14]:

$$R = \log \left( t \cdot \exp \left( \frac{T - T_R}{14,75} \right) \right),$$

where  $t$  is the treatment time, min;  $T$  - treatment temperature, °C;  $T_R$  - reference reaction temperature (equal to 100 °C). The value 14.75 is an empirical parameter depending on activation energy and temperature.

The research was carried out using equipment from the Biysk Regional Center for Collective Use of the Siberian Branch of the Russian Academy of Sciences (IPCET SB RAS, Biysk).

## Results and discussion

The effect of the auto-hydrolysis conditions influenced on changing of the solid fractions composition after pre-treatment of the oat husk. The change in solid fractions composition after treatment as a function of temperature and treatment time is shown in Table 3. The severity factor was 2.50-5.37 during the treatment.

**Table 3.** Solid fractions composition after pre-treatment

Temperature, °C	160			180			200			220		
Processing time, min	5	25	60	5	25	60	5	25	60	5	25	60
Auto-hydrolysis severity factor $R$	2.50	3.20	3.58	3.10	3.79	4.17	3.69	4.39	4.77	4.29	4.99	5.37
Yield, %	69.3	66.0	64.8	48.9	46.1	43.8	47.1	44.1	42.7	41.5	38.5	35.5
Mass fraction, %:												
- cellulose	47.3	62.3	60.6	70.6	75.7	62.3	70.2	65.0	53.8	62.2	58.3	50.1
- lignin	15.9	18.9	21.2	20.9	18.0	33.6	36.2	32.4	43.5	35.0	40.6	47.5
- hemicellulose	30.0	16.8	13.1	7.1	3.3	2.1	1.7	1.0	0.7	0.9	0.5	0.4
pH Level	4.21	4.15	4.03	3.98	3.94	3.30	3.44	3.14	3.08	3.22	3.06	3.02

When the treatment temperature is increased, more biomass is removed compared to the treatment time is increased. As the temperature increases, the water ion content of the solution increases. As a result, water acts as a weak polar solvent. Therefore, increasing the treatment temperature primarily removes hemicelluloses [15], which is reflected in a decrease in biomass yield at an average treatment time of 25 min from 66.0% at 160 °C to 38.5% at 220 °C.

We use the severity factor to assess the influence of the treatment conditions during autohydrolysis on the change in the solid fraction composition. The change of hemicelluloses content in the solid fraction as a result of the treatment is shown in Fig. 1.

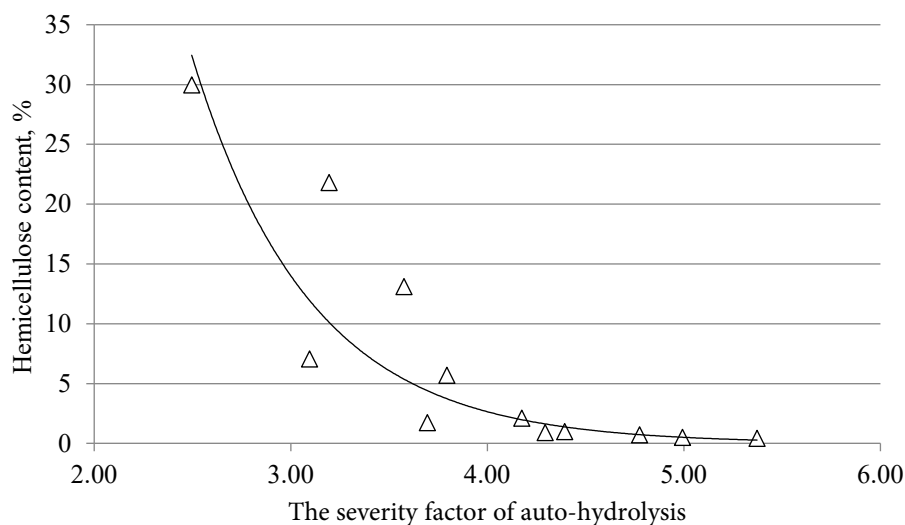
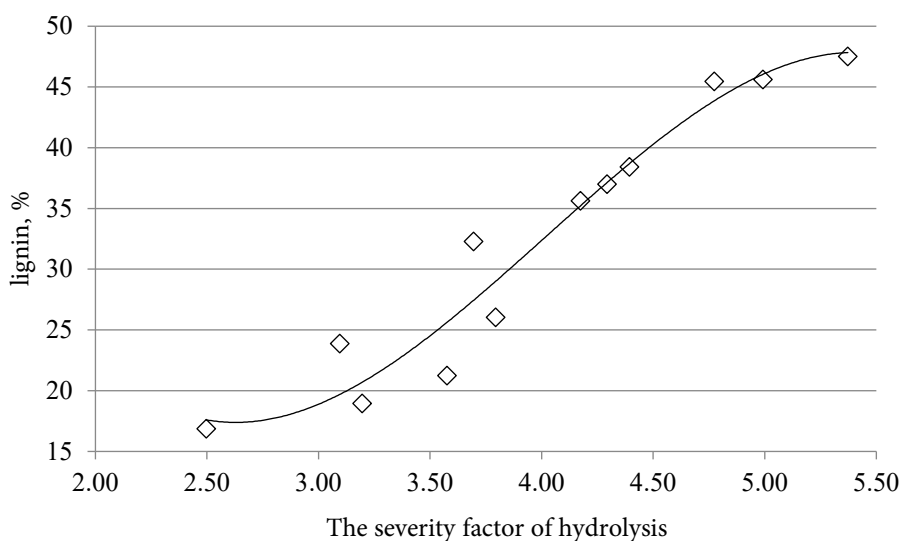


Fig. 1. The change of hemicellulose content depending on the auto-hydrolysis severity conditions

Autohydrolysis of oat husks is followed by almost complete removal of hemicelluloses from an initial content of 31.83% in the original oat husk to 0.4% under the most severe processing conditions. The greatest effect on the loss of hemicelluloses has the temperature at equal treatment time (see Table 3). At the initial auto-hydrolysis stage, the most intense removal of hemicelluloses occurs when the severity factor changes from an initial value of 2.50 to a value of 3.79. The concentration of hemicelluloses in the solid phase decreases from 31.83 to 1.7% under these processing conditions. The treatment conditions increase further from an auto-hydrolysis severity factor value of 3.69 to a value of 5.37 as the removal rate decreases, and the hemicellulose content decreases from 1.7 to 0.4%. The most severe treatment conditions with an autohydrolysis severity factor greater than 4.29 lead to almost complete removal of hemicelluloses to a final content of 0.4%. A similar effect has been recorded by many authors carried out treatments by auto-hydrolysis [16]. Hemicelluloses move to the liquid phase with increasing temperature and processing time, where they are decomposed to organic acids such as formic acid, acetic acid, lactic acid and glycolic acid. As the severity of the auto-hydrolysis conditions increases, the concentration of acids in the liquid phase increases, as indicated by the change in acidity of the medium from an initial pH value of 4.21 to a final pH value of 3.02 under the chosen treatment conditions (see Table 3), which is also in accordance with the studies of many authors [13]. Many works indicate a positive change in the structure of lignocellulosic plant raw materials when combining the effects of autohydrolysis pretreatment with additional mechanical treatment, which provides a positive change in the structure of the feedstock [17].

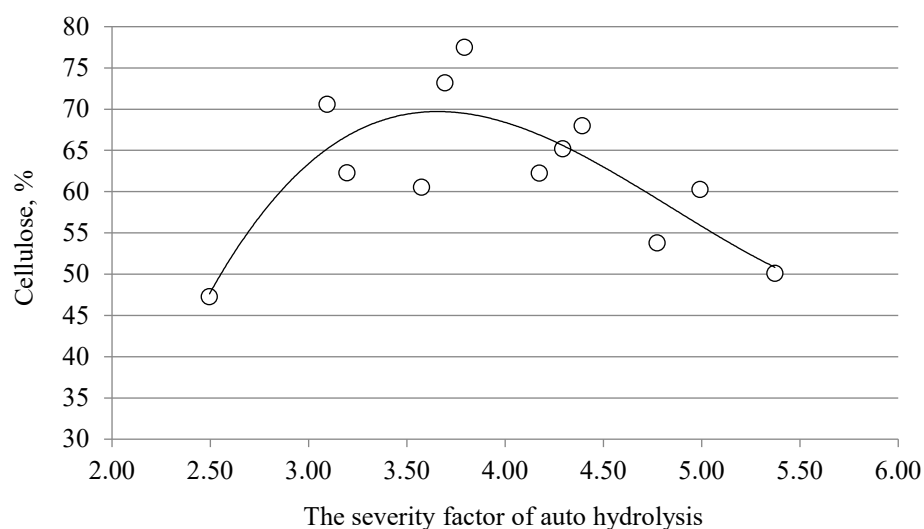
We assessed the effect of the treatment conditions on the change of lignin content after autohydrolysis in the solid phase. The change of lignin concentration with increasing severity of treatment conditions is shown in Fig. 2.



**Fig. 2.** The change of hemicellulose content depending on the auto-hydrolysis severity conditions

During the treatment, the severity of the auto-hydrolysis conditions at an initial treatment severity of 2.50 results in a slight reduction of the lignin concentration to 15.9% relative to the initial lignin content of the native oat husk. However, a subsequent increase in the processing conditions in the severity factor range of 3.20 to 5.37, which occurs especially with an increase in the process temperature, leads to a reverse accumulation of lignin in the solid fraction. It was confirmed by many researchers and related to the fact that a lignin condensation reaction may take place when the severity of the treatment is increased. The severe processing conditions cause a lignin condensation reaction on the surface of the solid fraction, at a rate that prevails over the rate of lignin hydrolysis. This reaction results in the formation of pseudolignin in the solid fraction [18].

The effect of the treatment conditions on the cellulose in the solids fraction is shown in Fig. 3 as a function of the auto-hydrolysis severity factor.



**Fig. 3.** The change of cellulose content depending on the auto-hydrolysis severity conditions

Studies of different raw materials show that the impact on cellulose during autohydrolysis depends primarily on the origin of the lignocellulosic raw material. For example, in case of



woody raw materials, researchers note that cellulose hydrolysis is negligible, whereas in the case of herbaceous raw materials and agricultural residues intensive cellulose hydrolysis may take place. This work shows the concentration of cellulose in the solid fraction increases when the severity factor of the auto-hydrolysis conditions rises from 2.50 to 3.79 (see Fig. 3) during the oak husk treatment. Under processing conditions in the temperature range 160–180 °C, irrespective of processing time, cellulose hydrolysis is negligible. This increases the concentration in the solid phase as most of the hemicelluloses are removed (see Fig. 1). At the severity of the processing conditions, corresponding to a hardness factor value of 3.79, the highest cellulose concentration of 75.7% is achieved, which corresponds to the processing conditions at 180 °C and a holding time of 25 min (see Table 3). Conducting the treatment at high temperatures of 200 to 220 °C and increasing the processing conditions, also involving longer processing times, results in a reduction of the cellulose concentration in the solid phase. Thus, with an increase in the severity factor of the conditions from 3.79 to 5.37, the cellulose content of the solid fraction decreases from 75.7 to 50.1%. This indicates that hydrolysis of the cellulose of the oat husk is taking place. Under these conditions the large amount of energy introduced into the reaction mass allows to break the strong cellulose structure [19]. Increasing the severity of the processing conditions promotes cellulose fibre rupture and its concentration in the solid fraction is reduced to 50.1%.

The pretreatment should allow the enzyme preparations to gain access to the cellulose for further hydrolysis to reducing substances. The solid fraction was therefore subsequently used as a substrate for enzymatic hydrolysis. The concentration of reducing substances after hydrolysis of the treated husk biomass compared to the untreated one was used to assess the efficiency of auto-hydrolysis. The dependence of the concentration of reducing substances after enzymatic hydrolysis of the solid fraction on the severity of the conditions is shown in Fig. 4. Initial intensification of the processing conditions provides improved conditions for enzyme access to the cellulose surface.

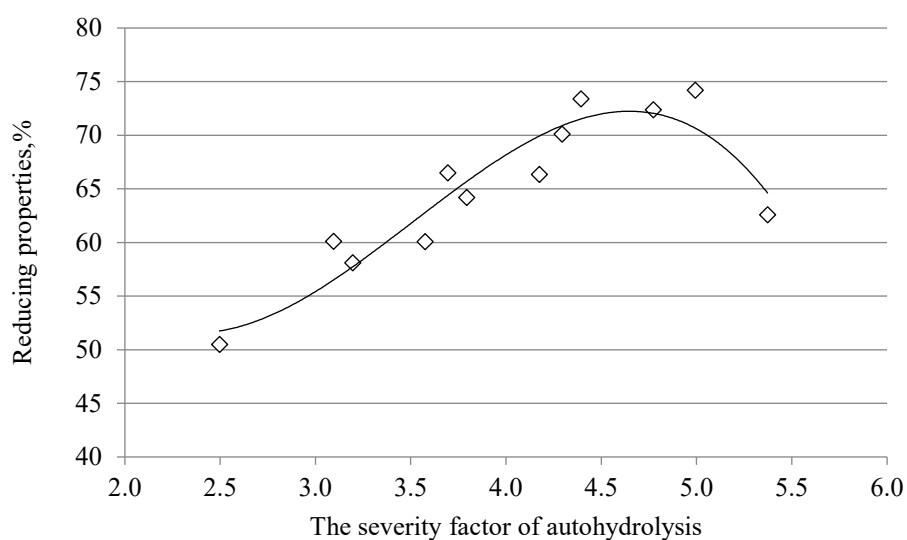


Fig. 4. Influence of the severity of the auto-hydrolysis conditions on the enzymatic hydrolysis of solid fractions



After hydrolysis of the untreated biomass, a reducing agent concentration of 8.5% was obtained after 72 h. By increasing the severity factor from an initial value of 2.50 to 4.17, the action of the enzymes is intensified, resulting in an increase in the yield of reducing substances to a maximum of 66.4%. The maximum yield is 7.8 times higher than the yield of reducing agents from untreated oat husk biomass.

At this stage the effect of the treatment conditions is primarily due to the intensive removal of hemicelluloses, which is reflected in the dependence plot of the hemicelluloses content when their level is reduced to 2.1% (see Fig. 1). The accumulation of lignin concentration as a result of the condensation reaction in this treatment step is also negligible, up to 30.0% (see Fig. 2). A further increase in temperature and treatment time is accompanied by a severer auto-hydrolysis condition with an increase in the severity factor to a maximum value of 5.37. At the same time, there is a reduction in the concentration of reducing substances accumulated up to 42.6%. At this stage, the biomass of oat husk is subjected to excessively severity processing conditions, leading to condensation of lignin and deposition of soluble lignin compounds on the cellulose surface [20]. The confirmation of the condensation reaction is the intensive accumulation of lignin in the solid fraction when the severity factor rises above the value of 4.17 (see Fig. 2) and the fact that the reactivity of cellulose decreased, resulting in a reduced accumulation of reducing substances in the enzymatic hydrolysis (see Fig. 4).

## Conclusions

Pre-treatment by auto-hydrolysis, carried out under increased water pressure conditions, provided favourable conditions for the treatment of oat husk biomass. An increase of the acidity of the medium to pH of 3.02 with a more severe processing condition of 5.37 indicates an increase in the acidity of the reaction mixture, due to which the hydrolysis process of the oat husk biomass takes place. The severity of the processing conditions is increasing of processing temperature and time. Increasing the severity of the treatment conditions in the first stage in the range of severity factors from 2.50 to 4.17 leads to almost complete removal of hemicelluloses, resulting in a higher cellulose concentration in the solid phase after treatment with a low lignin concentration. This processing step improves the availability of cellulose for the action of enzymes. As a result of the enzymatic hydrolysis of solid residues, a maximum yield of reducing substances of 66.4% was achieved, which is 7.8 times higher than the yield after fermentation of untreated oat husk biomass. The further increase of the severity of the treatment conditions in the second stage from a severity factor value of 4.17 to 5.37 results to poorer conditions of enzymatic hydrolysis and a reduction of substances yield to 42.6%. The reason is the decrease of the cellulose surface availability for the action of enzymes due to the condensation reaction of lignin and its deposition on the surface of the cellulose fibre.

*The research was carried out within the framework of the state task of IPCET SB RAS "Fundamental bases of creation of integrated technology for processing light renewable non-food plant raw materials into products demanded by the Russian economy" with the state registration number of the project 121061500030-3.*





## References

1. Liu C.G., Xiao Y., Xia X.X., Zhao X.Q., Peng L., Srinophakun P., Bai F.W. Cellulosic ethanol production: Progress, challenges and strategies for solutions. *Biotechnology Advances*. 2019. V. 37. N 3. P. 491-504. DOI: 10.1016/j.biotechadv.2019.03.002
2. Gladysheva E.K., Golubev D.S., Skiba E.A. Investigation of bacterial nanocellulose biosynthesis by *Medusomyces gisevii* Sa-12 from enzymatic hydrolyzate obtained by alkaline delignification of miscanthus. *Izvestiya vuzov. Prikladnaya khimiya i biotekhnologiya*. 2019. V. 9. N 2. P. 260–269. DOI: 10.21285/2227-2925-2019-9-2-260-269 (in Russian).
3. Kashcheyeva E.I., Gismatulina Y.A., Budaeva V.V. Pretreatments of Non-Woody Cellulosic Feedstocks for Bacterial Cellulose Synthesis. *Polymers*. 2019. V. 11. P. 1645. DOI:10.3390/polym11101645.
4. Chashchilov D.V. Experience of pulping process research and equipment analysis: from laboratory bench to industrial plant. *Ot khimii k tekhnologii shag za shagom*. V. 2. N 1. 2021. P. 29-39. DOI: 10.52957/27821900\_2021\_01\_29 (in Russian).
5. Bychkov A.L., Podgorbunskikh E.M., Ryabchikova E.I., Lomovsky O.I. The role of mechanical action in the process of the thermomechanical isolation of lignin. *Cellulose*. 2018. V. 25. P. 1-5. DOI: 10.1007/s10570-017-1536-y
6. Makarova E.I., Budaeva V.V. Bioconversion of non-food cellulosic biomass. Part 1. *Izvestiya vuzov. Prikladnaya khimiya i biotekhnologiya*. 2016. T. 6. N 2. P. 43-50. DOI:10.21285/2227-2925-2016-6-2-43-50 (in Russian).
7. Jiang K., Li L., Long L., Ding S. Comprehensive evaluation of combining hydrothermal pretreatment (autohydrolysis) with enzymatic hydrolysis for efficient release of monosaccharides and ferulic acid from corn bran. *Industrial Crops and Products*. 2018. V. 113. P. 348-357. DOI: 10.1016/j.indcrop.2018.01.047.
8. Lyu H., Zhou J., Geng Z., Lyu C., Li Y. Two-stage processing of liquid hot water pretreatment for recovering C5 and C6 sugars from cassava straw. *Process Biochemistry*. 2018. V. 75. P. 202-211. DOI: 10.1016/j.procbio.2018.10.003.
9. Cardona E., Llano B., Penuela M., Juan Pena J., Rios L.A. Liquid-hot-water pretreatment of palm-oil residues for ethanol production: An economic approach to the selection of the processing conditions. *Energy*. 2018. V. 160. P. 441-451. DOI: 10.1016/j.energy.2018.07.045.
10. Pavlov I.N., Denisova M.N., Makarova E.I., Budaeva V.V., Sakovich G.V. Versatile thermobaric setup and production of hydrotropic cellulose therein. *Cellulose Chemistry and Technology*. 2015. V. 49. N 9-10. P. 847-852.
11. Kashcheyeva E.I., Budaeva V.V. Determination of the reactivity of cellulosic substrates towards enzymatic hydrolysis. *Zavoskaya laboratoriya. Diagnostika materialov*. 2018. V. 84. N 10. P. 5-11. DOI: 0.26896/1028-6861-2018-84-10-5-11 (in Russian).
12. Obolenskaya A.V., Elnitskaya Z.P., Leonovich A.A. Laboratory work in wood and cellulose chemistry. M.: *Ecologiya*, 1991. 320 p. (in Russian).
13. Liu L., Liu W., Hou Q., Chen J., Xu N. Understanding of pH value and its effect on autohydrolysis pretreatment prior to poplar chemi-thermomechanical pulping. *Bioresource Technology*. 2015. V. 196. P. 662–667. DOI: 10.1016/j.biortech.2015.08.034.
14. Batista G., Souza R.B.A., Pratto B., Dos Santos-Rocha M.S.R., Cruz A.J.G. Effect of severity factor on the hydrothermal pretreatment of sugarcane straw. *Bioresource Technology*. 2019. V. 275. P. 321-327.
15. Michelin M., Teixeira J.A. Liquid hot water pretreatment of multi feedstocks and enzymatic hydrolysis of solids obtained thereof. *Bioresource Technology*. 2016. V. 216. P. 862–869. DOI: 10.1016/j.biortech.2016.06.018.
16. Moniz P., Pereira H., Duarte L.C., Carvalho F. Hydrothermal production and gel filtration purification of xylo-oligosaccharides from rice straw. *Industrial Crops and Products*. 2014. V. 62. P. 460-465. DOI: 10.1016/j.indcrop.2014.09.020.
17. Podgorbunskikh E.M., Ryabchikova E.I., Bychkov A.L., Lomovskii O.I. Changes in the structure of cell wall polymers in thermomechanical treatment of highly lignified plant feedstock. *Doklady Physical Chemistry*. 2017. V. 473. Part 1. P. 49-51. DOI: <https://doi.org/10.1134/S0012501617030046>.



18. **Ko J.K., Kim Y, Ximenes E, Ladisch M.R.** Effect of liquid hot water pretreatment severity on properties of hardwood lignin and enzymatic hydrolysis of cellulose. *Biotechnology and Bioengineering*. 2015. V. 112. N 2. P. 252-262. DOI: 10.1002/bit.25349.
19. **Zhu R., Yadama V.** Effects of hot water extraction pretreatment on physicochemical changes of Douglas fir. *Biomass and Bioenergy*. 2016. V. 90. P. 78-89. DOI: 10.1016/j.biombioe.2016.03.028.
20. **Chen T-Y., Wen J-L., Wang B., Wang H-M., Liu C-F., Sun R-C.** Assessment of integrated process based on autohydrolysis and robust delignification process for enzymatic saccharification of bamboo. *Bioresource Technology*. 2017. V. 244. P. 717-725. DOI: 10.1016/j.biortech.2017.08.032.

*Received 07.09.2021*

*Accepted 14.09.2021*