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Bioconversion of oat hull and miscanthus cellulose to glucose solutions

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Abstract: *Cellulose-containing raw materials are currently considered to be among the most promising types of raw materials for the production of value-added bio-products. However, for the implementation of the developed process, the application of universal pre-processing methods is a prerequisite. In the present paper, a study into the bioconversion of oat hull and miscanthus cellulose samples by enzymatic hydrolysis into glucose solutions is presented. The substrates were obtained by two-stage processing of raw materials with dilute solutions of nitric acid and sodium hydroxide. Enzymatic hydrolysis was carried out using the Cellolux-A and Ultraflo Core enzyme preparations at an initial substrate concentration of 40 g/L. At the first stage, the reactivity of the substrates to enzymatic hydrolysis in an acetate buffer solution was studied over a period of 72 hours. Cellulose samples from both types of raw materials were established to possess equally high reactivity in terms of the reducing substances. Glucose obtained from the substrate yielded 94–95 % and 88–91 %, respectively. This indicates the universality of the pre-treatment method used for raw materials having a cellulose content of 35–45 %. At the second stage, hydrolysis of the substrates was carried out using a pilot fermenter in an aqueous medium with an excess of enzyme preparations. Over a period of 32 hours, aqueous hydrolysates were obtained with a concentration of reducing substances and glucose equal to 42 g/L (94 % yield from the substrate) and 33–35 g/L (74–78 % yield from the substrate), respectively. The glucose (79–83 %) and pentose (1–2 %) content of the reducing substances indicates glucose to predominate in the composition of the resulting solutions. The high bioconversion efficiency rate is additionally demonstrated by a comparative analysis of scanning electron spectroscopy results for substrates and residues following hydrolysis in a pilot fermenter. Glucose solutions obtained in an aqueous medium are emerging as promising materials for the preparation of culture media and the synthesis of valuable metabolites.*

Keywords: *bioconversion, oat hulls, miscanthus, cellulose, enzymatic hydrolysis, glucose solution, culture medium*

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Биоконверсия шелухи овса и мискантуса в глюкозные растворы

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Резюме*: Целлюлозосодержащее сырье в настоящее время считается наиболее перспективным видом сырья для получения биопродуктов с добавленной стоимостью. Применение универсальных способов предварительной обработки является необходимым условием для реализации разраба-*

тываемого процесса. В данной работе была исследована биоконверсия образцов целлюлозы шелухи овса и мискантуса в глюкозные растворы путем ферментативного гидролиза. Субстраты получены двухстадийной обработкой сырья разбавленными растворами азотной кислоты и гидроксида натрия. Ферментативный гидролиз проводился с помощью ферментных препаратов «Целлолюкс-А» и «Ультрафло Коре» при начальной концентрации субстрата 40 г/л. На первом этапе исследована реакционная способность субстратов к ферментативному гидролизу в среде ацетатного буферного раствора в течение 72 ч. Установлено, что образцы целлюлозы из обоих видов сырья имеют одинаково высокую реакционную способность: выход редуцирующих веществ и глюкозы от массы субстрата составил 94–95 % и 88–91 % соответственно. Это свидетельствует об универсальности способа предварительной обработки, используемого для сырья с содержанием целлюлозы от 35 до 45 %. На втором этапе проведен гидролиз субстратов в водной среде в пилотном ферментере при избытке ферментных препаратов. За 32 ч. получены водные гидролизаты с концентрацией редуцирующих веществ 42 г/л (выход от массы субстрата 94 %) и глюкозы 33–35 г/л (выход от массы субстрата 74–78 %). Содержание глюкозы (79–83 %) и пентоз (1–2 %) в редуцирующих веществах свидетельствует о преимущественно глюкозном составе полученных растворов. Сравнительный анализ результатов растровой электронной спектроскопии субстратов и остатков после гидролиза в пилотном ферментере также демонстрирует высокую эффективность биоконверсии. Полученные в водной среде глюкозные растворы рекомендуются для приготовления питательных сред и дальнейшего их использования для синтеза ценных метаболитов.

***Ключевые слова**: биоконверсия; шелуха овса; мискантус; целлюлоза; ферментативный гидролиз; глюкозный раствор; питательная среда*

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INTRODUCTION

Cellulose-containing raw materials (CCRM) comprised of wood, algae, as well as energy and agricultural crops, are currently considered as among the most promising types of raw materials for various value-added products [1–6]. Vegetable CCRM is characterised by widespread distribution, abundance, renewability and low cost, as well as additionally not being a competitor with the production of food and feed [7].

CCRM mainly consists of three biopolymers, namely cellulose (40–50 %), hemicelluloses (25–30 %) and lignin (15–20 %) [2]. Cellulose and hemicelluloses represent sources of simple sugars obtained by enzymatic hydrolysis of raw materials and subsequently used as carbon sources for the synthesis of valuable metabolites and bioproducts. Enzymatic hydrolysates of cellulose and hemicellulose are appropriate in the technology of alcohols, polymers, acids, amino acids, enzymes, as well as for the production of microorganism biomass [1, 5, 6, 8–12]. The bioconversion of cellulose and hemicelluloses includes the following main processes: grinding and preliminary processing of raw materials in order to increase the availability of polysaccharides for enzyme action; enzymatic hydrolysis of cellulose/hemicelluloses into monosaccharides; obtaining of bioproducts from monosaccharides (biosynthesis); isolation and purification of bioproducts. In the CCRM bioconversion technology, the pre-treatment becomes a key stage, since it determines the efficiency of subsequent hydrolysis and biosynthesis processes [3, 8, 13]. Currently, various methods of pre-processing CCRM are available [2, 14–16]. In this study, a two-stage sequential processing of raw materials with dilute solutions of nitric acid and sodium hydroxide is proposed in terms of a pre-treatment method. Previously, this method was demonstrated to provide for the obtaining of high-quality cellulose characterised by a cellulose content of more than 90 % and a small amount of non-hydrolysable components (less than 5 % in total) [17, 18].

As CCRM, agricultural wastes, such as oat hulls (*Avena Sativa*) and the miscanthus energy culture (*Miscanthus sacchariflorus* (Maxim.)) was used. Presenting a zero-cost by-product of the agro-industrial complex, oat hulls are a widespread and affordable raw material source in agricultural regions of the world [8]. In accordance to the Federal State Statistics Service, the gross oat harvest in Russia amounted to 4.72 million tonnes in 2018 [19]. The hull fraction comprises 25–30 % of the dry grain mass; the sugar content in the oat hulls reaches 70 % [20, 21]. Miscanthus is a fast-growing perennial herbaceous plant with outstandingly high productivity (10–15 t/ha of dry

biomass per year) over the period of the last 15–20 years and requiring no special growing conditions [22–24]. Thus, the availability and low cost of these raw materials determines their potential use as substrates for biotechnological processes in the production of high value-added products having a high cellulose content.

The study was aimed at the bioconversion of oat hull and miscanthus cellulose samples into glucose solutions by enzymatic hydrolysis.

The research objectives included studying the enzymatic hydrolysis reactivity of oat hull and miscanthus cellulose samples in an acetate buffer solution, obtaining glucose enzymatic hydrolysates of the same samples in an aqueous medium using a pilot fermenter, assessing the degree of substrate conversion by evaluating the separate accumulation of reducing substances (RS) and glucose, as well as by analysing changes in the morphology of the substrates.

EXPERIMENTAL PART

The substrates presented by oat hull or miscanthus cellulose samples were obtained at the pilot plant of the Institute for Problems of Chemical and Energetic Technologies SB RAS (IPCET SB RAS) in a 250-litre apparatus by sequential processing of raw materials with dilute solutions of nitric acid and sodium hydroxide. This method involves the processing of raw materials with a 3–6 % solution of nitric acid at a temperature of 90–95 °C for 10–12 h (diversion duty 1 : 15) and subsequent processing with a 1–3 % solution of sodium hydroxide at a temperature of 90–95 °C within 2–4 h (diversion duty 1:15) for obtaining the target cellulose [17]. One part of the obtained cellulose samples was air-dried for the further analysis of the chemical composition, while the other part was subjected to enzymatic hydrolysis in the wet state.

The moisture content of the oat hull and miscanthus cellulose samples was determined using an MB23 moisture content analyser (Ohaus, USA). An analysis of the chemical composition of the substrates was carried out according to generally accepted methods for the analysis of vegetable material. The content of α-cellulose was evaluated by quantitative determination of the substrate residue undissolved in the 17.5 % sodium hydroxide solution[1]. The mass fraction of residual lignin was evaluated in accordance with the procedure described in [25]. The mass fraction of pentosans was determined by a UNICO UV-2804 spectrophotometer (United Products and Instruments, USA) using an orcin solution[1]. Ash content was estimated by ashing the substrate at 600 °C for 3 h [26]. The degree of cellulose polymerisation in substrates was calculated by analysing the cadoxene viscosity of solutions in accordance with the procedure presented in [27].

At the first stage, the enzymatic hydrolysis reactivity of wet substrates was evaluated in accordance with the procedure described in [28]. For hydrolysis, Cellolux-A (Sibbiopharm Ltd, Russia) and Ultraflo Core (Novozymes A/S, Denmark) enzyme preparations were applied. Characteristics of the preparations are presented in Table 1.

Table 1
Characteristics of the enzymes used

Таблица 1
Характеристики ферментных препаратов

Preparation	Producer	Passport Activity
Cellolux-A	*Trichoderma viride*	cellulase: 2000±200 CU/g xylanase: 8000 XU/g β-glucanase: up to 1500 BGU/g
Ultraflo Core	*Trichoderma reesei*	β-glucanase: 1930±97 BGU/mL cellulase: 700 CU/mL xylanase (optional)

For enzymatic hydrolysis, a 6 g sample of the substrate referred to an absolutely dry substance was placed in a 500 mL conical flask and filled with 0.1 M acetate buffer solution (pH = 4.6) containing dissolved enzyme preparations. Enzyme preparations were added in the following amount: Cellolux-A – 0.054 g/g of the substrate, Ultraflo Core – 0.165 mL/g of the substrate. The required volume of acetate buffer was calculated taking both the initial concentration of the substrate (40 g/L) and the mass fraction of water in the wet substrate into account. A total liquid phase volume of 150 mL was required. The flask with the suspension was placed on a PE-6410M mixing platform (Ekros, Russia) with an oscillation frequency of 150 min^{-1}. Hydrolysis was carried out at a temperature of 45±2 °C and pH = 4.6±0.1 providing the maximum yield of RS. An assessment of the degree of substrate hydrolysis was carried out by determining changes in the hydrolysate concentrations of RS and glucose. For this, 5 mL samples were taken-followed by filtration. The concentration of RS in filtrate was determined by UNICO UV-2804 spectrophotometer using a reagent based on 3,5-dinitrosalicylic acid (Panreac, Spain) [29]. Glucose concentration was obtained using the spectrophotometric glucose oxidase-peroxidase method using reagents from the Photoglucose kit (Impact LLC, Russia) [30]. After 72 hours of hydrolysis, the

[1] Obolenskaya AV, El'nitskaya ZP, Leonovich AA. Laboratory work in the chemistry of wood and cellulose. Moscow: Ekologiya; 1991. / Оболенская А.В., Ельницкая З.П., Леонович А.А. Лабораторные работы по химии древесины и целлюлозы: учеб. пособие для вузов. М.: Экология. 1991. 320 с.

suspension was vacuum filtered and the filtrate concentrations of RS and glucose were analysed. According to the results of the analysis, the final RS yield in relation to the mass of the substrate and hydrolysable components, along with the yield of glucose relative to the substrate and cellulose masses, was calculated according to the formulas:

$$\eta_S = [(C_{end} \cdot V) / m_s] \cdot 0.9 \cdot 100; \quad (1)$$

$$\eta_{HC} = \frac{C_{end} \cdot V}{m_s \cdot (100 - L - A)} \cdot 0.9 \cdot 100; \quad (2)$$

$$\eta_S = [(C_{end} \cdot V) / m_s \cdot C] \cdot 0.9 \cdot 100; \quad (3)$$

where η_S is the yield of RS (glucose) relative to the mass of the substrate, %;

η_{HC} is the yield of RS relative to the content of hydrolysable components in the substrate, %;

η_C is the glucose yield relative to the cellulose content in the substrate, %;

C_{end} is the final concentration of RS (glucose) in the hydrolysate, g/L;

V is the hydrolysate volume, L;

0.9 is the coefficient due to the addition of a water molecule to the anhydroglucose residues of the corresponding monomer units as a result of enzymatic hydrolysis;

m_S is the mass of the substrate for enzymatic hydrolysis, g;

L is the mass fraction of residual lignin in the substrate, %;

A is the mass fraction of ash in the substrate, %;

C is the mass fraction of α-cellulose in the substrate, %.

At the second stage, the hydrolysis of the substrates in an aqueous medium was carried out in a pilot fermenter. The 11 L fermenter (working volume of 7–9 L) is equipped with a mixing device, a heat exchange element, a device for supplying the necessary components to the process and a sampler [31]. The following hydrolysis conditions in the fermenter were used: substrate concentration of 40 g/L, temperature of 47±1 °C and pH = 4.6±0.1. The mixing frequency was decreased from 500 to 250 rpm as the reaction mass with a volume of 8 L became liquefacted. Enzyme preparations were added in excess to increase the rate of hydrolysis, amounting to 0.1 g/g of the substrate and 0.3 mL/g of the substrate for Cellolux-A and Ultraflo Core, respectively. The volume of distilled water for hydrolysis was calculated taking the moisture content of the substrate into account. 8 hours after the RS concentration stopped increasing, the process was terminated. In the process of hydrolysis, the pH level was measured every 2 hours and adjusted, if necessary by solutions of ammonia and phosphoric acid. At the end of the process, the reaction mass was separated in a TsLU-6-3 laboratory centrifuge (PJSC Dolgoprudny Scientific-Production Enterprise, Russia) at 3500 rpm for 10 minutes. The concentration of hydrolysate supernatant pentoses was determined in terms of the equivalent amount of xylose, using an orcin solution according to the procedure described in [1]. Based on the experimental results, the final yield of RS and glucose was calculated in accordance with formulas (1)–(3).

The morphology of substrates, as well as unreacted residues after their hydrolysis in a fermenter, was studied by JSM-840 scanning electron microscopy (SEM) instrument (Jeol, Japan) [32].

The studies were carried out using the equipment of the Biysk Regional Centre for Collective Use SB RAS (IPCET SB RAS, Biysk).

RESULTS AND DISCUSSION

The characteristics of the oat hull and miscanthus cellulose samples are presented in Table 2.

Cellulose samples obtained from oat hulls and miscanthus are characterised by a high content of α-cellulose (93–94 %), as well as pentosans (1–2 %), while the amount of non-hydrolysable components (lignin, ash) is not more than 5.3 %. The substrates have close degree of polymerisation (DP) values for cellulose.

Figure 1 presents the results of evaluating the enzymatic hydrolysis reactivity of cellulose samples, namely, the dependency of the RS and glucose concentration on the duration of hydrolysis in an acetate buffer solution (Stage 1).

As can be seen from the plots presented in Fig. 1, the cellulose samples of both raw material types clearly possess almost the same reactivity. In both cases, the accumulation of glucose in comparison with the accumulation of RS is slower. At the same time, the proportion of glucose in RS tends to grow constantly comprising 63, 80–81 and 93–95 % after 7, 32 and 72 hours of hydrolysis, respectively. The difference in the concentrations

Table 2

Characteristics of the substrates for enzymatic hydrolysis a

Таблица 2

Характеристики субстратов для ферментативного гидролиз

Substrate	Mass fraction, %				Cellulose degree of polymerisation (DP)
	α-cellulose	residual lignin	pentosans	ash	
Oat hull cellulose sample	93.6	3.7	2.1	1.0	1450
Miscanthus cellulose sample	92.8	4.0	1.0	1.3	1150

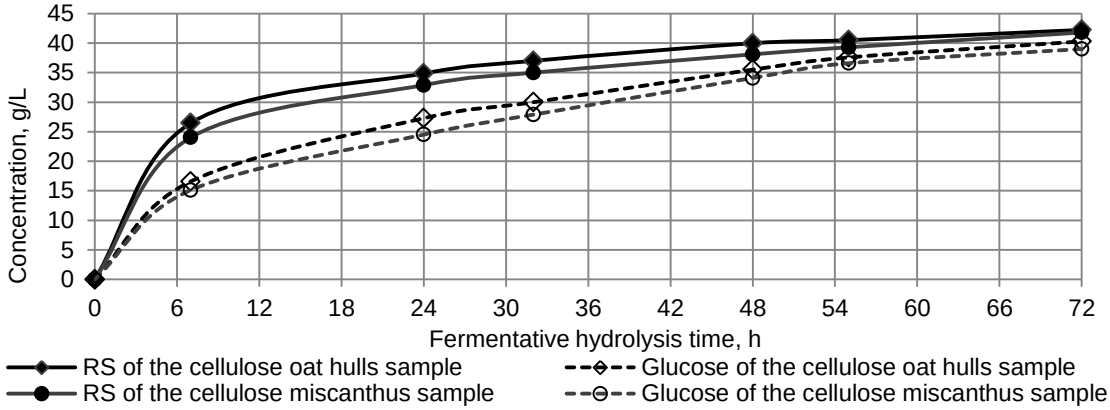

Fig. 1. Concentrations of reducing sugars (RS) and of glucose plotted against the time of enzymatic hydrolysis of the substrates of oat hulls and miscanthus in acetate buffer solution (Stage 1)

Рис. 1. Зависимость концентрации РВ и глюкозы от продолжительности ферментативного гидролиза субстратов из шелухи овса и мискантуса в среде ацетатного буферного раствора (этап 1)

of RS and glucose during the first 32 hours may be attributed to the presence in the reaction mass of cellobiose, a glucose dimer converting to glucose during hydrolysis and providing a glucose yield of 95–97 % relative to the cellulose content (Table 3). The close reactivity of the substrates indicates the versatility and high efficiency of the pre-treatment method used. Based on the presented data, the possible application of this method can be considered for pre-processing a number of sources of raw materials having an initial cellulose content in the range of 35–45 %.

At the second stage of the study, the enzymatic hydrolysis of substrates in an aqueous medium was studied in a pilot fermenter. Along with changing the buffer medium to water, the procedure of transferring to larger devices is acknowledged to decrease the hydrolysis efficiency due to sharp fluctuations in pH, insufficient mass transfer and circulation characteristics of the apparatus [33]. In this case, a reduction in the microbiological stability of the hydrolysis process is possible considering the biological purity of the enzymatic cellulose hydrolysates easily contaminated by extraneous microflora. Therefore, the high level of microbiological stability of enzymatic hydrolysis is retained by reduction of the process duration. In this connection, enzyme preparations were used in excess during hydrolysis in a fermenter holding an aqueous medium for the purpose of increasing the initial speed and reducing the duration of the process.

Figure 2 represents the dependency of the concentration of RS and glucose on the duration of hydrolysis of cellulose samples in an aqueous medium of a pilot fermenter (Stage 2).

Both substrates are converted at almost the same rate; their hydrolysis is characterised by a sharp increase in the concentration of RS (37 g/L) during the first 8 hours and a further slowdown of the process with a maximum concentration of 42 g/L. Due to the concentration of RS remaining constant between the 24th and 32nd hours of hydrolysis, the process was stopped. In contrast to

Table 3

Results of enzymatic hydrolysis of the substrates of oat hulls and miscanthus

Таблица 3

Результаты ферментативного гидролиза субстратов из шелухи овса и мискантуса

Hydrolysate characteristics	Stage 1				Stage 2	
	32 h		72 h		32 h	
	COH	CM	COH	CM	COH	CM
RS:						
concentration, g/L	37.0	35.0	42.2	41.8	41.8	41.7
yield relative to the substrate mass, %	83.3	78.8	95.0	94.1	94.1	93.8
yield relative to the content of hydrolysable components, %	87.0	84.0	99.6	99.3	98.7	99.1
Glucose:						
concentration, g/L	30.0	27.9	40.3	39.0	33.0	34.7
yield relative to substrate mass, %	67.5	62.8	90.7	87.8	74.3	78.1
yield relative to cellulose content,%	72.1	67.7	96.9	94.6	79.3	84.1
Pentose concentration, g/L	–	–	0.8	0.4	0.9	0.4

Note. COH denotes a cellulose sample of oat hulls; CM is a miscanthus cellulose sample.

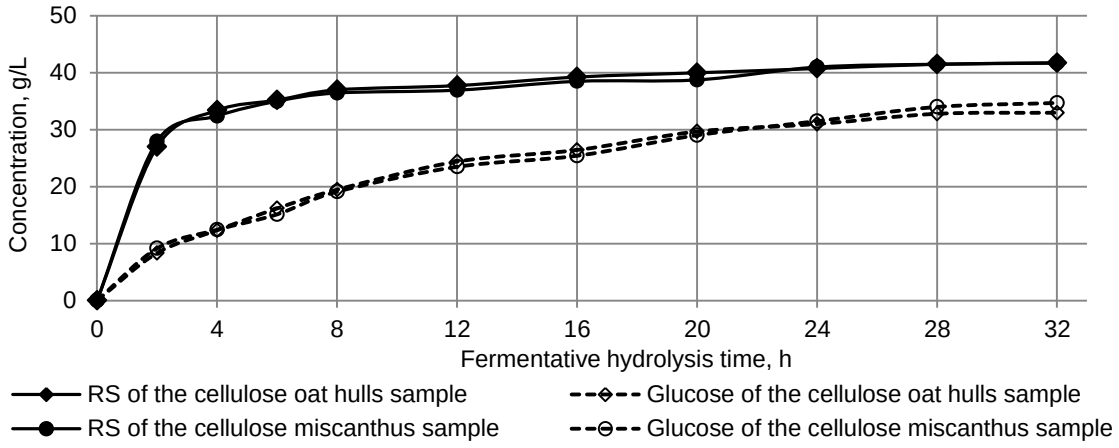

Fig. 2. Concentrations of reducing sugars (RS) and of glucose plotted against the time of enzymatic hydrolysis of the substrates of oat hulls and miscanthus in the fermenter (Stage 2)

Рис. 2. Зависимость концентрации РВ и глюкозы от продолжительности ферментативного гидролиза субстратов из шелухи овса и мискантуса в ферментере (этап 2)

the concentration of RS, the glucose concentration slowly increases throughout the process comprising 19 and 33–35 g/L after 8 and 32 hours for both substrates, respectively. An increase in the dosage of enzyme preparations led to a significant increase in the rate of RS formation compared to glucose. This is probably due to an insufficient cellobiase content in the enzyme preparations used.

The generalised results for the enzymatic hydrolysis of cellulose samples in an acetate buffer solution (Stage 1) and in an aqueous medium (Stage 2) presented in Table 3 include the intermediate results of Stage 1 after 32 hours. Comparison of the RS and glucose yield obtained in buffer and aqueous media after 32 h proves the predominant formation of RS in relation to glucose with 79–83 % and 94 % of RS and 63–68 % and 74–78 % of glucose contained in the buffer solution and in the aqueous medium, respectively. Thus, an increase in the dosage of enzyme preparations at Stage 2 contributed to an increase in the yield of RS and glucose from the substrate by 11–15 % and by 7–15 %, respectively.

At Stage 2, the RS yield relative to the content of hydrolysable components (99%) indicates the complete hydrolysis of the accessible part of the substrates into the RS. In this case, the glucose yield relative to the cellulose content (79–84 %) demonstrates the incomplete conversion of cellulose to glucose compared with the results of Stage 1 after 72 hours (95–97 %). The glucose (79–83 %) and pentose (1–2 %) content in RS characterises aqueous hydrolysates as predominantly glucose-based.

To visualise the results of enzymatic hydrolysis, the substrates were studied by SEM before and after hydrolysis in the same way as in works [34, 35]. Figure 3 contains SEM images of oat hull and miscanthus cellulose samples, as well as unreacted residues following hydrolysis in a pilot fermenter.

SEM results demonstrate the oat hull cellulose sample to consist of flat fibres having a hypertrophied surface of 2–5 μm thick, 20–0 μm wide and 300–800 μm long (Fig. 3, *a*, *b*). The lamellar shape of the fibres is due to the morphology of the raw materials, since the oat hulls are represented by multilayer flakes of lignocellulose coating the grain. The miscanthus cellulose sample (Fig. 3, *c*, *d*) is composed by heterogeneous fibres of different length and width, associated with using the different parts of the plant, i.e. stem and leaf. The unreacted residue after hydrolysis of an oat hull cellulose sample (Fig. 3, *e*) involves porous particles with pore sizes up to 10 μm, including single fibres. In contrast, a disordered mixture of fibres (up to 10 μm in diameter, up to 1000 μm in length) and irregularly shaped particles of 1–10 μm in size forms the residue after hydrolysis of a miscanthus cellulose sample (Fig. 3, *f*). According to the SEM results, small portions of the fibres remain at the end of the enzymatic hydrolysis, thereby confirming the effectiveness of the process with respect to the investigated substrates. Although the mass of unreacted residues following hydrolysis is very small due to the high degree of conversion of the substrates, further study of the composition and properties of the residues may be the subject of separate studies, as in the works of [35, 36].

As a result of hydrolysis of cellulose samples of oat hulls and miscanthus in a pilot fermenter, 8 L of aqueous glucose solutions having a concentration of 33 and 35 g/L, respectively, were obtained. Glucose solutions obtained in an aqueous medium are recommended for the preparation of culture media and the synthesis of valuable metabolites.

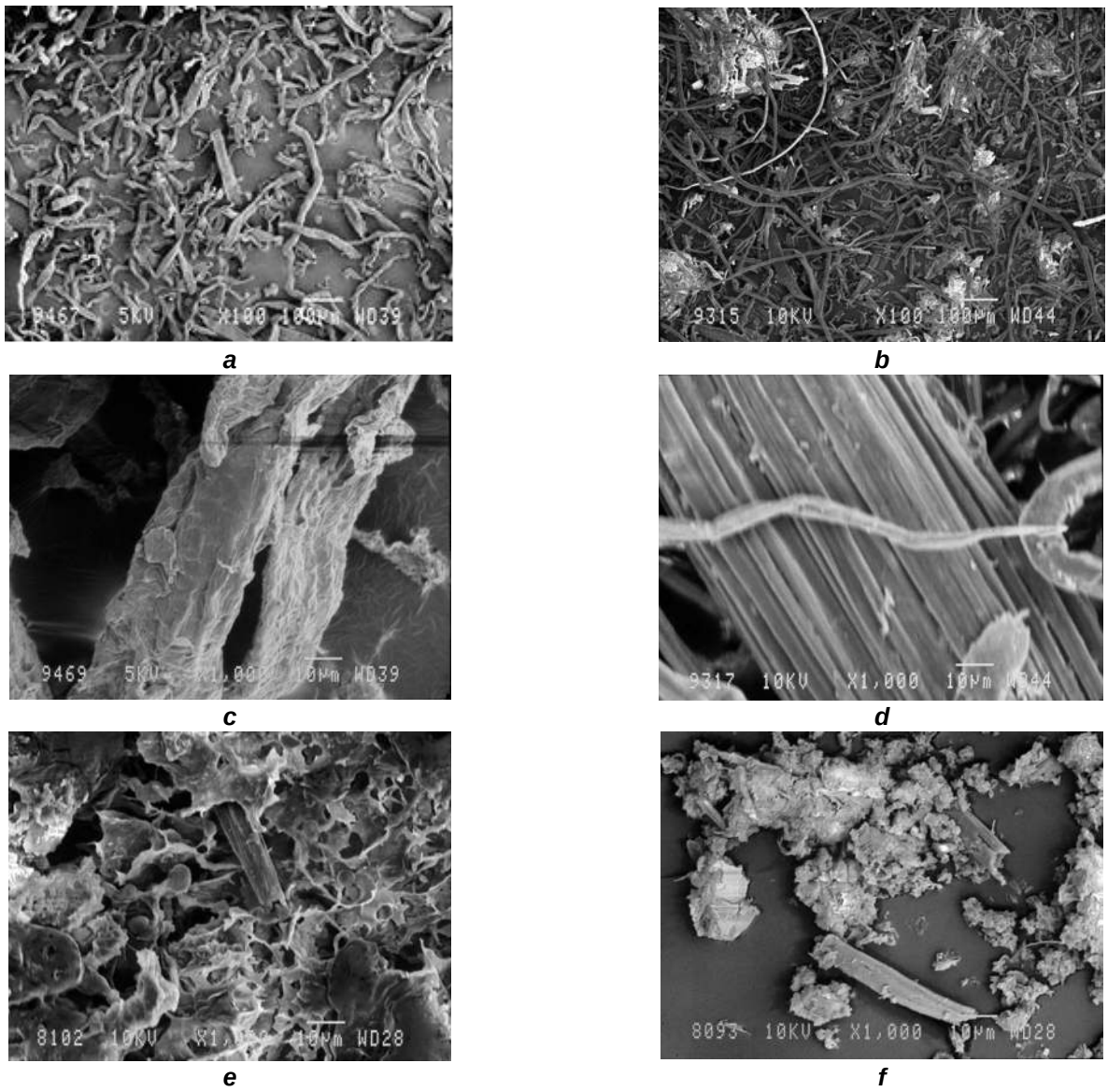

Fig. 3. SEM images of cellulose samples from oat hulls (a, b) at zooms ×100 and miscanthus (c, d) at zooms ×1000 and of the unreacted residues after hydrolysis of oat hulls (e) and miscanthus (f) celluloses at zooms ×1000

Рис. 3. Микрофотографии РЭМ образцов целлюлозы шелухи овса (a, b) при 100-кратном увеличении и мискантуса (c, d) при 1000-кратном увеличении и непрореагировавших остатков после гидролиза целлюлозы шелухи овса (e) и мискантуса (f) при 1000-кратном увеличении

CONCLUSIONS

The substrates of the oat hulls and miscanthus obtained by sequential treatment with dilute solutions of nitric acid and sodium hydroxide were established to be promising for the production of glucose solutions. As a result of evaluating the enzymatic hydrolysis reactivity of the studied substrates, the equal reactivity for two types of raw materials was established with the yield relative to the substrate mass comprising 94–95 and 88–91 % after 72 hours of hydrolysis for RS and glucose, respectively. Hydrolysis of substrates in an 11 L pilot fermenter provides for obtaining aqueous hydrolysates with RS and glucose concentration of 42 and 33–35 g/L, corresponding to the RS and glucose yield relative to the substrate mass equal to 94 and 74–78 %, respectively. The glucose content of 79–83 %, as well as the pentose amount of 1–2 % in RS, indicates the predominance of glucose in the composition of the obtained aqueous hydrolysates and appears to be essential for the synthesis of a number of metabolites. The high efficiency of enzymatic hydrolysis is also confirmed by the SEM results for unreacted residues of cellulose samples following hydrolysis in the fermenter as compared to substrates.

REFERENCES

1. Arevalo-Gallegos A, Ahmad Z, Asgher M, Parra-Saldivar R, Iqbal HMN. Lignocellulose: A sustainable material to produce value-added products with a zero waste approach – A review. *International Journal of Biological Macromolecules*. 2017;99:308–318. https://doi.org/10.1016/j.ijbiomac.2017. 02.097

2. Chen H, Liu J, Chang X, Chen D, Xue Y, Liu P, et al. A review on the pretreatment of lignocellulose for high-value chemicals. *Fuel Processing Technology.* 2017;160:196–206. https://doi.org/10. 1016/j.fuproc.2016.12.007

3. Diaz AB, Blandino A, Caro I. Value added products from fermentation of sugars derived from agro-food residues. *Trends in Food Science and Technology.* 2018;71:52–64. https://doi.org/10.1016/ j.tifs.2017.10.016

4. Liu CG, Xiao Y, Xia XX, Zhao XQ, Peng L, Srinophakun P, et al. Cellulosic ethanol production: Progress, challenges and strategies for solutions. *Biotechnology Advances.* 2019;37(3):491–504. https://doi.org/10.1016/j.biotechadv.2019.03.002

5. Kumar A, Gautam A, Khan H, Dutt D. Chapter 9. Organic Acids: Microbial Sources, Production and Applications. In: *Microbial Enzymes and Additives for the Food Industry*. New York: Nova Science Publishers; 2019.

6. Ha DT, Kanarskaya ZA, Kanarsky AV, Shcherbakov AV, Shcherbakova EN. The effect of carbon source on the biomass and exopolysaccharide synthesis by Paenibacillus mucilaginosus bacteria. *Izvestiya Vuzov. Prikladnaya Khimiya i Biotekhnologiya* = Proceedings of Universities. Applied Chemistry and Biotechnology. 2019;9(3):509–518. (In Russian) https://doi.org/10.21285/2227-2925-2019-9-3-509-51

7. Hu F, Ragauskas A. Pretreatment and lignocellulosic chemistry. *Bioenergy Research*. 2012;5: 1043–1066. https://doi.org/10.1007/s12155-012-9208-0

8. Demirel F, Germec M, Coban HB, Turhan I. Optimization of dilute acid pretreatment of barley husk and oat husk and determination of their chemical composition. *Cellulose.* 2018;25(11):6377–6393. https://doi.org/10.1007/s10570-018-2022-x

9. Gupta A, Verma JP. Sustainable bio-ethanol production from agro-residues: A review. *Renewable and Sustainable Energy Reviews.* 2015;41:550–567. https://doi.org/10.1016/j.rser.2014.08.032

10. Cheng Z, Yang R, Liu X, Liu X, Chen H. Green synthesis of bacterial cellulose via acetic acid pre-hydrolysis liquor of agricultural corn stalk used as carbon source. *Bioresource Technology.* 2017;234: 8–14. https://doi.org/10.1016/j.biortech.2017.02.131

11. Ong KL, Li C, Li X, Zhang Y, Xu J, Lin CSK. Co-fermentation of glucose and xylose from sugarcane bagasse into succinic acid by *Yarrowia lipolytica. Biochemical Engineering Journal.* 2019;148: 108–115. https://doi.org/10.1016/j.bej.2019.05.004

12. Cubas-Cano E, González-Fernández C, Ballesteros M, Tomás-Pejó E. Biotechnological advances in lactic acid production by lactic acid bacteria: lignocellulose as novel substrate. *Biofuels, Bioproducts and Biorefining*. 2018;12(2):290–303. https://doi.org/10.1002/bbb.1852

13. Kim JS, Lee YY, Kim TH. A review on alkaline pretreatment technology for bioconversion of lignocellulosic biomass. *Bioresource Technology.* 2016;199:42–48. https://doi.org/10.1016/j.biortech. 2015.08.0850960-8524

14. Makarova EI, Budaeva VV. Bioconversion of non-food cellulosic biomass. Part 1. *Izvestiya Vuzov. Prikladnaya Khimiya i Biotekhnologiya* = Proceedings of Universities. Applied Chemistry and Biotechnology. 2016;6(2):43–50. (In Russian) https://doi.org/ 10.21285/ 2227-2925-2016-6-2-43-50

15. Bychkov A, Podgorbunskikh E, Lomovsky O, Bychkova E. Current achievement in the mechanically activated processing of plant raw materials. *Biotechnology and Bioengineering*. 2019; 116(5):1231–1244. https://doi.org/10.1002/bit.26925

16. Baig KS, Wu J, Turcotte G. Future prospects of delignification pretreatments for the lignocellulosic materials to produce second generation bioethanol. *International Journal of Energy Research*. 2019;43(1):1411–1427. https://doi.org/10. 1002/er.4292

17. Gismatulina YuA, Budaeva VV. Chemical composition of five *Miscanthus sinensis* harvests and nitric-acid cellulose therefrom. *Industrial Crops and Products.* 2017;109:227–232. https://doi.org/10. 1016/j.indcrop.2017.08.026

18. Kashcheyeva EI, Gismatulina YA, Budaeva VV. Pretreatments of Non-Woody Cellulosic Feedstocks for Bacterial Cellulose Synthesis. *Polymers.* 2019;11(10):1645. https://doi.org/10.3390/ polym11101645

19. Bulletin of Agriculture of the Russian Federation. Available from: http://www.gks.ru/ wps/wcm/ connect/rosstat_main/rosstat/ru/statistics/publications/catalog/doc_1265196018516 [Accessed 15th October 2019]. (In Russian)

20. Chaud LCS, da Silva DDV, de Mattos RT, de Almeida Felipe M das G. Evaluation of oat hull hemicellulosic hydrolysate fermentability employing *Pichia stipites. Brazilian Archives of Biology and Technology*. 2012;55(5):771–777. https://doi.org/10. 1590/S1516-89132012000500017

21. Yadav MP, Hicks KB, Johnston DB, Hotchkiss AT, Chau HK, Hanah K. Production of biobased fiber gums from the waste streams resulting from the commercial processing of corn bran and oat hulls. *Food Hydrocolloids*. 2016;53:125–133. https://doi.org/10.1016/j.foodhyd.2015.02.017

22. Jones MB, Walsh M. *Miscanthus for energy and fibre*. London: Earthscan. 2001. 192 p.

23. Dorogina OV, Vasilyeva OYu, Nuzhdina NS, Buglova LV, Gismatulina YuA, Zhmud EV, et al. Resource potential of some species of the genus *Miscanthus Anderss.* under conditions of continental climate of West Siberian foreststeppe. *Vavilov Journal of Genetics and Breeding.* 2018;22(5):

553–559. https://doi.org/10.18699/VJ18.394

24. Da Costa RMF, Pattathil S, Avci U, Winters A, Hahn MG, Bosch M. Desirable plant cell wall traits for higher-quality miscanthus lignocellulosic biomass. *Biotechnology for Biofuels volume.* 2019;12(1):18 p. https://doi.org/10.1186/s13068-019-1426-7

25. TAPPI T222 om-02. *Acid-insoluble lignin in wood and pulp*. Atlanta: TAPPI Press. 2002.

26. TAPPI T211 om-85. *Ash in wood, pulp, paper, and paperboard*. Atlanta: TAPPI Press. 1985.

27. Hallac BB, Ragauskas AJ. Analyzing cellulose degree of polymerization and its relevancy to cellulosic ethanol. *Biofuels, Bioproducts and Biorefining*. 2011;5(2):215–225 https://doi.org/10.1002/bbb.269

28. Kashcheyeva EI, Budaeva VV. Determination of the reactivity of cellulosic substrates towards enzymatic hydrolysis. *Zavodskaya laboratoriya. Diagnostika materialov* = Industrial Laboratory. Diagnostics of materials. 2018;84(10):5–11. (In Russian) https://doi.org/10.26896/1028-6861-2018-84-10-5-11

29. Miller G.L. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry*. 1959;31(3):426–428. https://doi.org/10.1021/ac60147a030

30. Dotsenko A, Gusakov A, Rozhkova A, Sinitsyna O, Shashkov I, Sinitsyn A. Enzymatic hydrolysis of cellulosic materials using synthetic mixtures of purified cellulases bioengineered at N-glycosylation sites. *3 Biotech*. 2018;8(9):396. https://doi.org/10.1007/s13205-018-1419-4

31. Pavlov IN. A setup for studying the biocatalytic conversion of products from the processing of Nonwood Raw Materials. *Catalysis in Industry*. 2014;6(4):350–360. https://doi.org/10.1134/S2070050414044014X

32. Kashcheyeva EI, Gladysheva EK, Skiba EA, Budaeva VV. A study of properties and enzymatic hydrolysis of bacterial cellulose. *Cellulose.* 2019;26(4):2255–2265. https://doi.org/ 10.1007 / s10570-018-02242-7

33. Skiba EA, Baibakova OV, Budaeva VV, Pavlov IN, Makarova EI, Mironova GF., et al., Bioethanol from Oat Hulls Pretreated by Alkaline Delignification. II. Scaling of Alcoholic Fermentation up to Pilot Process. *Biotekhnologiya* = Biotechnology. 2017;33(3):47–56. (In Russian) https://doi.org/10.21519/0234-2758-2017-33-3-47-56

34. Schläfle S, Tervahartiala T, Senn T, Kölling-Paternoga R. Quantitative and visual analysis of enzymatic lignocellulose degradation. *Biocatalysis and Agricultural Biotechnology*. 2017;11:42–49. https://doi.org/10.1016/j.bcab.2017.06.002

35. Liu L, Sun X, Zhang L, Qing Y, Yan N, Chen J, Wu Y. Obtaining nanofibers from lignocellulosic residues after bioethanol production. *Cellulose.* 2019;26(6):3725–3734. https://doi.org/10.1007/s10570-019-02333-z

36. De Oliveira JP, Bruni GP, el Halal SLM, Bertoldi FC, Dias ARG, da Rosa Zavareze E. Cellulose nanocrystals from rice and oat husks and their application in aerogels for food packaging. *International Journal of Biological Macromolecules*. 2018;124: 175–184. https://doi.org/10.1016/j.ijbiomac.2018.11.205

БИБЛИОГРАФИЧЕСКИЙ СПИСОК

1. Arevalo-Gallegos A., Ahmad Z., Asgher M., Parra-Saldivar R., Iqbal H.M.N. Lignocellulose: A sustainable material to produce value-added products with a zero waste approach – A review // International Journal of Biological Macromolecules. 2017. Vol. 99. P. 308–318. https://doi.org/10.1016/j. ijbiomac. 2017.02.097

2. Chen H., Liu J., Chang X., Chen D., Xue Y., Liu P., et al. A review on the pretreatment of lignocellulose for high-value chemicals // Fuel Processing Technology. 2017. Vol. 160. P. 196–206. https://doi.org/10.1016/j.fuproc.2016.12.007

3. Diaz A.B., Blandino A., Caro I. Value added products from fermentation of sugars derived from agro-food residues // Trends in Food Science and Technology. 2018. Vol. 71. P. 52–64. https://doi.org/ 10. 1016/j.tifs.2017.10.016

4. Liu C.G., Xiao Y., Xia X.X., Zhao X.Q., Peng L., Srinophakun P., et al. Cellulosic ethanol production: Progress, challenges and strategies for solutions // Biotechnology Advances. 2019. Vol. 37. N 3. P. 491–504. https://doi.org/10.1016/j.biotechadv.2019.03.002

5. Kumar A., Gautam A., Khan H., Dutt D. Chapter 9. Organic Acids: Microbial Sources, Production and Applications. In: Microbial Enzymes and Additives for the Food Industry. New York: Nova Science Publishers. 2019.

6. Ха Т.З., Канарская З.А., Канарский А.В., Щербаков А.В., Щербакова Е.Н. Влияние источника углерода на синтез биомассы и экзополисахаридов бактериями *Paenibacillus mucilaginosus* // Известия вузов. Прикладная химия и биотехнология. 2019. Т. 9. N 3. С. 509–518. https://doi.org/10.21285/2227-2925-2019-9-3-509-518

7. Hu F., Ragauskas A. Pretreatment and lignocellulosic chemistry // Bioenergy Research. 2012. N 5. P. 1043–1066. https://doi.org/10.1007/s12155-012-9208-0

8. Demirel F., Germec M., Coban H.B., Turhan I. Optimization of dilute acid pretreatment of barley husk and oat husk and determination of their chemical composition // Cellulose. 2018. Vol. 25, N 11. P. 6377–6393. https://doi.org/10.1007/s10570-018-2022-x

9. Gupta A., Verma J.P. Sustainable bio-ethanol production from agro-residues: A review // Renewable and Sustainable Energy Reviews. 2015. Vol. 41. P. 550–567. https://doi.org/10.1016/j.rser.2014.08.032

10. Cheng Z., Yang R., Liu X., Liu X., Chen H. Green synthesis of bacterial cellulose via acetic acid pre-hydrolysis liquor of agricultural corn stalk used as carbon source // Bioresource Technology.

2017. Vol. 234. P. 8–14. https://doi.org/10.1016/j.biortech.2017.02.131

11. Ong K.L., Li C., Li X., Zhang Y., Xu J., Lin C.S.K. Co-fermentation of glucose and xylose from sugarcane bagasse into succinic acid by *Yarrowia lipolytica* // Biochemical Engineering Journal. 2019. Vol. 148. P. 108–115. https://doi.org/10.1016/j.bej.2019.05.004

12. Cubas-Cano E., González-Fernández C., Ballesteros M., Tomás-Pejó E. Biotechnological advances in lactic acid production by lactic acid bacteria: lignocellulose as novel substrate // Biofuels, Bioproducts and Biorefining. 2018. Vol. 12. N 2. P. 290–303. https://doi.org/10.1002/bbb.1852

13. Kim J.S., Lee Y.Y., Kim T.H. A review on alkaline pretreatment technology for bioconversion of lignocellulosic biomass // Bioresource Technology. 2016. Vol. 199. P. 42–48. https://doi.org/10.1016/j.biortech.2015.08.0850960-8524

14. Макарова Е.И., Будаева В.В. Биоконверсия непищевого целлюлозосодержащего сырья. Часть 1 // Известия вузов. Прикладная химия и биотехнология. 2016. Т. 6. N 2. С. 43–50. https://doi.org/10.21285/2227-2925-2016-6-2-43-50

15. Bychkov A., Podgorbunskikh E., Lomovsky O., Bychkova E. Current achievement in the mechanically activated processing of plant raw materials // Biotechnology and Bioengineering. 2019. Vol. 116. N 5. P. 1231–1244. https://doi.org/10.1002/bit.26925

16. Baig K.S., Wu J., Turcotte G. Future prospects of delignification pretreatments for the lignocellulosic materials to produce second generation bioethanol // International Journal of Energy Research. 2019. Vol. 43. Issue 1. P. 1411–1427. https://doi.org/10.1002/er.4292

17. Gismatulina Yu.A., Budaeva V.V. Chemical composition of five *Miscanthus sinensis* harvests and nitric-acid cellulose therefrom // Industrial Crops and Products. 2017. Vol. 109. P. 227–232. https://doi.org/10.1016/j.indcrop.2017.08.026

18. Kashcheyeva E.I., Gismatulina Y.A., Budaeva V.V. Pretreatments of Non-Woody Cellulosic Feedstocks for Bacterial Cellulose Synthesis // Polymers. 2019. N 11. Issue 10. P. 1645. https://doi.org/10.3390/polym11101645

19. Бюллетени о состоянии сельского хозяйства (электронные версии) [Электронный ресурс]. URL: http://www.gks.ru/wps/wcm/connect/rosstat_main/rosstat/ru/statistics/publications/catalog/doc_1265196018516 (29.08.2019).

20. Chaud L.C.S., da Silva D.D.V., de Mattos R.T., de Almeida Felipe M. das G. Evaluation of oat hull hemicellulosic hydrolysate fermentability employing *Pichia stipites* // Brazilian Archives of Biology and Technology. 2012. Vol. 55. N. 5. P. 771–777. https://doi.org/10.1590/S1516-89132012000500017

21. Yadav M.P., Hicks K.B., Johnston D.B., Hotchkiss A.T., Chau H.K., Hanah K. Production of bio-based fiber gums from the waste streams resulting from the commercial processing of corn bran and oat hulls // Food Hydrocolloids. 2016. Vol. 53. P. 125–133. https://doi.org/10.1016/j.foodhyd.2015.02.017

22. Jones M.B., Walsh M. *Miscanthus* for energy and fibre. London: Earthscan. 2001. 192 p.

23. Dorogina O.V., Vasilyeva O.Yu., Nuzhdina N.S., Buglova L.V., Gismatulina Yu.A., Zhmud E.V., et al. Resource potential of some species of the genus *Miscanthus Anderss.* under conditions of continental climate of West Siberian foreststeppe // Vavilov Journal of Genetics and Breeding. 2018. Vol. 22. N 5. P. 553–559. https://doi.org/10.18699/VJ18.394

24. Da Costa R.M.F., Pattathil S., Avci U., Winters A., Hahn M.G., Bosch M. Desirable plant cell wall traits for higher-quality miscanthus lignocellulosic biomass // Biotechnology for Biofuels volume. 2019. Vol. 12, N 1. 18 p. https://doi.org/10.1186/s13068-019-1426-7

25. TAPPI T222 om-02. Acid-insoluble lignin in wood and pulp. Atlanta: TAPPI Press. 2002.

26. TAPPI T211 om-85. Ash in wood, pulp, paper, and paperboard. Atlanta: TAPPI Press. 1985.

27. Hallac B.B., Ragauskas A.J. Analyzing cellulose degree of polymerization and its relevancy to cellulosic ethanol // Biofuels, Bioproducts and Biorefining. 2011. Vol. 5. Issue 2. P. 215–225 https://doi.org/10.1002/bbb.269

28. Кащеева Е.И., Будаева В.В. Определение реакционной способности к ферментативному гидролизу целлюлозосодержащих субстратов // Заводская лаборатория. Диагностика материалов. 2018. Т. 84. N 10. С. 5–11. https://doi.org/10.26896/1028-6861-2018-84-10-5-11

29. Miller G.L. Use of dinitrosalicylic acid reagent for determination of reducing sugar // Analytical Chemistry. 1959. Vol. 31. N 3. P. 426–428. https://doi.org/10.1021/ac60147a030

30. Dotsenko A., Gusakov A., Rozhkova A., Sinitsyna O., Shashkov I., Sinitsyn A. Enzymatic hydrolysis of cellulosic materials using synthetic mixtures of purified cellulases bioengineered at N-glycosylation sites // 3 Biotech. 2018. Vol. 8. N 9. P. 396. https://doi.org/10.1007/s13205-018-1419-4

31. Pavlov I.N. A setup for studying the biocatalytic conversion of products from the processing of Nonwood Raw Materials // Catalysis in Industry. 2014. Vol. 6. Issue 4. P. 350–360. https://doi.org/10.1134/S207005041404014X

32. Kashcheyeva E.I., Gladysheva E.K., Skiba E.A., Budaeva V.V. A study of properties and enzymatic hydrolysis of bacterial cellulose // Cellulose. 2019. Vol. 26. Issue 4. P. 2255–2265. https://doi.org/ 10.1007/s10570-018-02242-7

33. Скиба Е.А., Байбакова О.В., Будаева В.В., Павлов И.Н., Макарова Е.И., Миронова Г.Ф. [и др.]. Биоэтанол из плодовых оболочек овса, предварительно обработанных методом щелочной делигнификации. II. Масштабирование процесса получения биоэтанола в условиях опытного производства // Биотехнология. 2017.

T. 33. N 3. C. 47–56. https://doi.org/10.21519/0234-2758-2017-33-3-47-56

34. Schläfle S., Tervahartiala T., Senn T., Kölling-Paternoga R. Quantitative and visual analysis of enzymatic lignocellulose degradation // Biocatalysis and Agricultural Biotechnology. 2017. Vol. 11. P. 42–49. https://doi.org/10.1016/j.bcab.2017.06.002

35. Liu L., Sun X., Zhang L., Qing Y., Yan N., Chen J., Wu Y. Obtaining nanofibers from lignocellulosic residues after bioethanol production // Cellulose. 2019. Vol. 26. N 6. P. 3725–3734. https://doi.org/10.1007/s10570-019-02333-z

36. De Oliveira J.P., Bruni G.P., el Halal S.L.M., Bertoldi F.C., Dias A.R.G., da Rosa Zavareze E. Cellulose nanocrystals from rice and oat husks and their application in aerogels for food packaging // International Journal of Biological Macromolecules. 2018. Vol. 124. P. 175–184. https://doi.org/10.1016/j.ijbiomac.2018.11.205

Критерии авторства

Ekaterina I. Kashcheyeva, Galina F. Mironova, Vera V. Budaeva, Hina Khan carried out the experimental work, analyzed the experimental results and prepared the text of the manuscript. Ekaterina I. Kashcheyeva, Galina F. Mironova, Vera V. Budaeva, Hina Khan have equal author's rights and bear equal responsibility for plagiarism.

Contribution

Кащеева Е.И., Миронова Г.Ф., Будаева В.В., Хан Х. выполнили экспериментальную работу, на основании полученных результатов провели обобщение и написали рукопись. Кащеева Е.И., Миронова Г.Ф., Будаева В.В., Хан Х. имеют на статью равные авторские права и несут равную ответственность за плагиат.

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Conflict of interests

The authors declare no conflict of interests regarding the publication of this article.

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