CELLULOSE CHEMISTRY AND TECHNOLOGY

MECHANICAL PRETREATMENT OF CORN STRAW IN A CENTRIFUGAL ROLLER MILL

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Processes taking place during multiple mechanical treatment of corn straw in a semi-industrial centrifugal roller mill are illustrated. The data showing the effect of morphology, specific surface area, degree of cellulose crystallinity on the reactivity of the pretreated material are presented. A 2.9-fold increase in the reactivity of lignocellulose and a 2-fold increase in the yield of low-molecular carbohydrates during subsequent enzymatic hydrolysis of activated material were achieved. A series of proposals concerning the modifications of existing centrifugal roller mills and the development of new ones intended for processing plant raw material have been made.

Keywords: centrifugal roller mill, mechanical pretreatment, lignocellulose materials, enzymatic hydrolysis

INTRODUCTION

Obtaining the components of biofuel from renewable lignocellulose raw material is an important and urgent problem for industry.[1-3] Suitable kinds of lignocellulose raw material were selected for the majority of regions in the world,[1] including lignocellulose of plants growing in a specific region, unclaimed agricultural wastes[4-5] and urban refuse.[6] The efficiency of the introduction of various kinds of biofuel (for example, bioethanol, biobutanol, biogas) was demonstrated.[7,8] Methods of lignocellulose processing into the components of biofuel and chemicals are under investigation.[2,9,10]

Up to the present, the most thoroughly studied processes are those used to obtain bioethanol, a promising component of biofuel and raw material for further chemical processing.[3] In this case, the key stage is the enzymatic hydrolysis of polymeric carbohydrates of lignocellulose into low-molecular products that can be assimilated by ethanol-producing microorganisms. At the present stage, the major problem is the comparatively low reactivity of cellulose. The reactivity may be enhanced with the help of many methods: mechanical treatment,[11-13] treatment with ultrasound,[14] supercritical liquids,[15] chemical reagents[16-19] (oxidizers, solvents) etc.[10,20,21]

From the viewpoint of ecological safety and simplicity of operations, the most promising approaches to cellulose activation are the mechanical methods, as these methods do not produce wastes and do not contaminate the environment.[22] Mechanochemical pretreatment excludes liquid-phase stages (dissolution, extraction, evaporation) and allows obtaining products in a solid form, thus simplifying the processing technology and storage.

The major obstacle for the development of mechanical methods of lignocellulose pretreatment is the difficult scaling from laboratory developments to industrial equipment.[23,24] The major part of works have been carried out on the laboratory scale and aimed at the fine grinding of the raw material,[11,12,25,26] achievement of the maximal possible reactivity of cellulose,[27,28] which is often not advantageous due to the high energy consumption.[26,29] In addition, there are no clearly formulated requirements for the design of the devices for mechanical treatment of lignocellulose raw material; the structure of this raw material is complex due to the multilevel hierarchy: plant – plant tissues – cells – cell walls – cell wall polymers.[30]

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The goal of the present work was to carry out an experimental study of the processes taking place during the step-by-step treatment of corn straw in a semi-industrial centrifugal roller mill. Generalizing the information obtained in the study, it was necessary to formulate a number of requirements for the development of new centrifugal roller mills and the modification of the existing ones.

EXPERIMENTAL

The materials used in the study were: corn straw *Zea mais* (total stalk, origin: Novosibirsk Region, Russia, humidity 5%), CelloLux-A enzymatic complex (Sibbiofarm Corporation, Berdsk, Russia). The activity profile of the complex is: xylanase – 8000 UA/g, cellulase – 2000 UA/g, β-1.3-glucanase – up to 1500 UA/g, glucoamylase – 20 UA/g.[31]

The chemical composition of corn straw, determined according to the published procedure, included the following biopolymers: 24.7 (± 0.7) % hemicellulose, 38.6 (± 1.1) % cellulose and 17.8 (± 0.3) % lignin.[32]

Preliminary grinding of the plant raw material to particles with a size around 1 mm was carried out with a 8255 Nossen disintegrator (Germany) with the disc rotation frequency of 3500 r.p.m. and with energy consumption of no more than 30 kWh/t

Microscopic examination of sample morphology was carried out with a Hitachi TM-1000 electron scanning microscope (Japan) after preliminary deposition of a platinum layer on the sample surface.

Cellulose crystallinity degree was determined on the basis of X-ray diffraction from powdered samples, using a Bruker D8 Advance diffractometer (Germany), crystallinity index was calculated using the generally accepted Segal's method.

Particle size was determined by means of Dynamic Light Scattering (DLS) with the help of a Microsizer 201 instrument (St. Petersburg, Russia).

Specific surface area was measured by means of the thermal desorption of nitrogen (calculation according to the BET equation), using a Sorbtomer instrument (Novosibirsk, Russia).

Step-by-step treatment of corn straw

A weighed portion of corn straw, which had been previously ground by disintegrator was treated mechanically in a semi-industrial continuous centrifugal roller mill TM-3 type (Fig. 1) (Novits com., Novosibirsk, Russia). After the treatment, a small part of the product was taken for examination, and the residue was treated mechanically once more.

The mill design involves a stator and a rotor. The rollers at the axes of the rotor are pressed to the stator due to the centrifugal force during the rotation of the rotor and exert mechanical action on the material between them. Varying the eccentricity of the rollers, one may treat the raw material in the attrition-shear mode (without eccentricity) or in the shock-and-shear mode (for rollers with displaced centre). The intensity of the shock action is unambiguously determined by the eccentricity value and the mass of rollers; they can be made of different materials. We used steel rollers without eccentricity. The rate of material treatment was 50 kg/h with an energy consumption of 300-340 kWh/t. The temperature of the material under treatment was controlled by water cooling of the stator and did not exceed 70 °C.

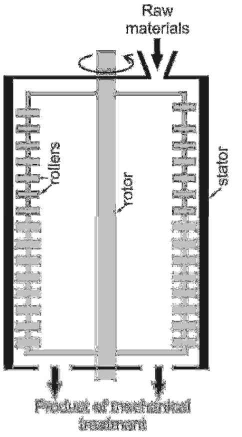


Figure 1: Design of TM-3 type centrifugal roller mill

Determination of the reactivity of the products of mechanical treatment

To determine the reactivity of initial and mechanically activated corn straw, we carried out its enzymatic hydrolysis under the conditions of lack of enzymes, and then determined the sum of reducing carbohydrates (calculated for glucose).

For this purpose, we added 4.0 ml of the solution of enzymes with the concentration of 1.0 mg/ml to the weighed portions of 100 mg of initial or mechanically treated corn straw. The suspension was kept at a temperature of 50 ^oC for 1 h, then heated for 15 minutes at 95 ^oC for complete inactivation of the enzymes, cooled to the room temperature, and centrifuged to separate the non-dissolved lignocellulose residue (10 minutes at the frequency of 7000 r.p.m.). The supernatant was used to determine the total amount of reducing carbohydrates.

Determination of the total amount of reducing carbohydrates

The total amount of reducing carbohydrates was determined using the standard procedure, involving the reduction of potassium ferricyanide $K_3[Fe(CN)_6]$. For this purpose, to 1.0 ml of carbohydrate solutions with concentrations of 30 to 150 mg/l, we added 3.0 ml of 0.06% $K_3[Fe(CN)_6]$ solution, mixed and kept at 100 ^oC for 10 minutes. After cooling, the solutions were examined by means of photometry at the wavelength of 420 nm with distilled water as a blank. Glucose solutions with concentrations of 30.0-150 mg/l were used to plot the calibration curves.

RESULTS AND DISCUSSION

A number of factors are known to be responsible for the reactivity of lignocellulose materials: chemical composition (to a higher extent, the concentration of lignin, which inhibits the enzymes), specific surface area, crystallinity degree and the degree of cellulose polymerization. In the case of mechanical treatment of the lignocellulose material with constant chemical composition, its reactivity is first of all determined by the type and intensity of mechanical action in a grinding or activating device. The mechanical action produces changes in the surface area of powder products, of the crystallinity degree and of other factors to a greater or lesser extent, which leads to an increase or a decrease in the reactivity of the material under treatment.

Morphology of initial raw material and resulting products

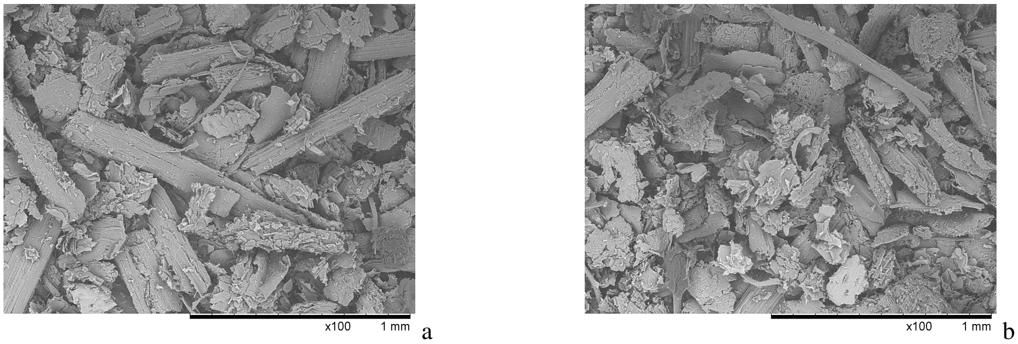
The morphology of product particles is an important parameter, allowing for a qualitative evaluation of the efficiency of grinding lignocellulose materials. The micrographs of the initial biomass (ground preliminarily in the disc disintegrator) and the biomass after step-by-step treatment in the centrifugal roller mill are presented in Figs. 2-3. One can see (Fig. 2) that the initial material is composed of particles of 0.3-1.0 mm in size. The particles have a clear morphology, which is characteristic of the initial corn tissues. Several types of particles, corresponding to different tissues in the plant, can be distinguished.

The particle size of the product after single treatment in the centrifugal roller mill decreases substantially (the average particle size determined by means of LDS is equal to 43 µm), while the morphology of the particles changes dramatically. It becomes impossible to differentiate the particles on the basis of their origin from definite tissues; all the particles look like pressed or plastically expanded composites. The crushing type of action, which is evident for the design of the mill (Fig. 1), is also evidenced by the discovery of a large number of aggregates in the sample (Fig. 3a). These aggregates have a dense layered structure formed by compressing a large number of smaller-sized particles. A typical size of the aggregates is 30-150 µm; when suspended in water, they decompose liberating particles of sizes within the range shown below in Fig. 4.

Subsequent mechanical treatment (Fig. 3b) causes further grinding of the lignocellulose material and, which is the most important, almost complete destruction of the aggregates. According to LDS, the average particle size is 25 µm. After triple and fourfold treatment, no aggregates are detected in the product; the average particle size is about 16 µm in both cases (Fig. 3c); the product looks homogeneous. The morphology of the products is very similar.

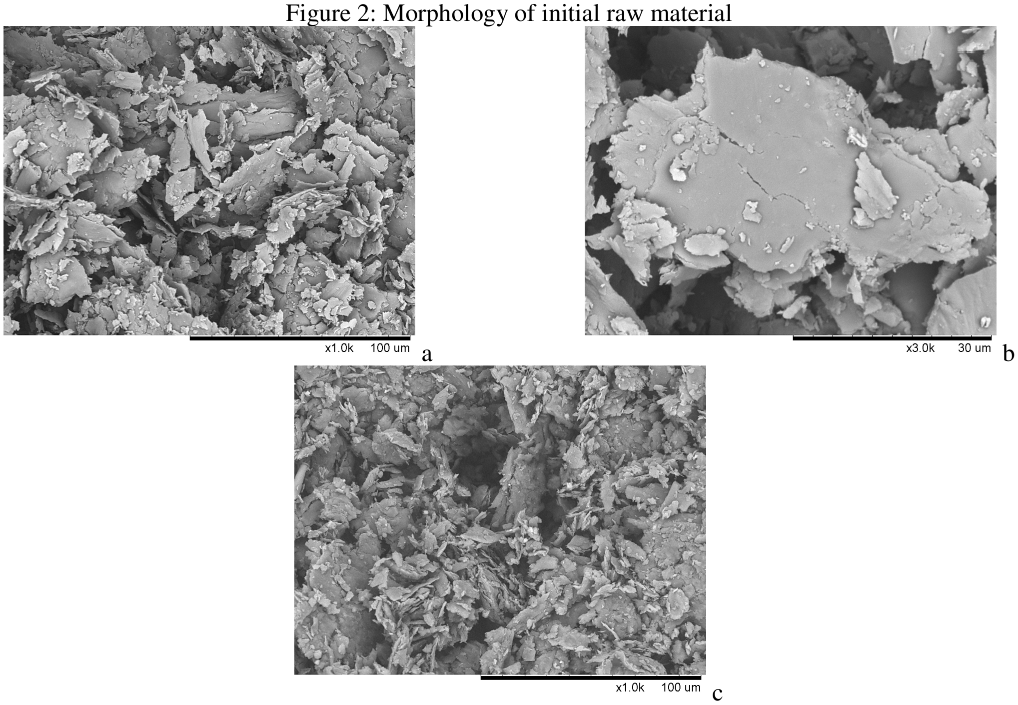
It may be assumed that the addition of a definite number of rollers with eccentricity to the set of milling bodies will contribute to destroying the aggregates due to the shock action, to prevent their formation and to achieve more efficient grinding of the raw material.

For a more detailed study of the grinding process, we separated and analyzed the fractions with particle size less than 150 µm. The particle size distributions are presented in Fig. 4. Several intervals can be distinguished in the curves: up to 7 µm; 7-10 µm; 12-16 µm; 20-30 µm; 30-75 µm; and larger than 75 µm. Previously, while studying the ultrastructure of lignocellulose materials, we demonstrated that corn straw in its perpendicular cross section consists of several cell types.[32] At the periphery of fibres, the cell diameters are within the ranges 6-10 µm, 10-18 µm, 20-30 µm, 30-75 µm, while the diameters of the cells in the centre of fibres are within the range from 75 µm to 150-180 µm.



a b

Figure 2: Morphology of initial raw material



a b c

Figure 3: Morphology of the products of one (a), two (b) and three (c) cycles of mechanical treatment

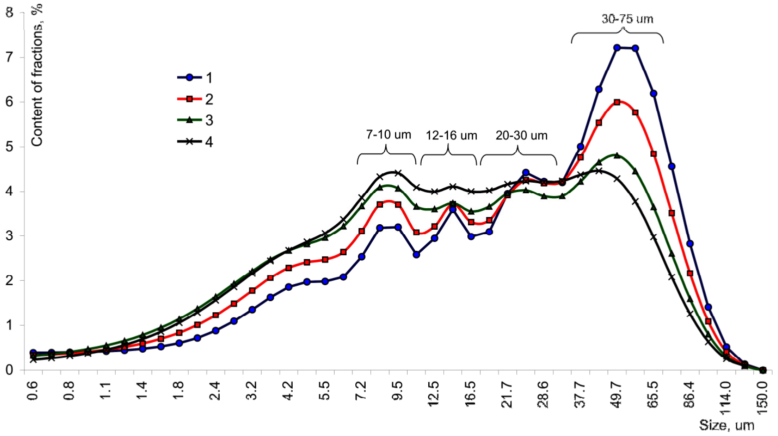


Figure 4: Curves of the grain size distribution of the products of one (1), two (2), three (3) and four (4) cycles of mechanical treatment

The data obtained from Fig. 4 confirm the assumption that the lignocellulose materials with high lignin content are destroyed through layering along cell walls.[32] In addition, it may be assumed that the cells with the diameter larger than 30 μm are extremely unstable under mechanical action due to insufficiently thick cell wall, get destroyed during grinding and contribute into the fractions with smaller particle sizes. In turn, the cells with smaller diameter are relatively stable to mechanical action.

Determination of the parameters responsible for the reactivity of lignocellulose

A consequence of material grinding is the increase in the specific surface area. In our case, this parameter is especially important, because the enzymatic hydrolysis of lignocellulose is a heterogeneous process.[33] At first, endo-glucanases get sorbed on the surface of polymeric carbohydrates and catalyze depolymerization; only after that, oligomeric carbohydrates react with exo-glucanases in solution. As can be sees from the data shown in Table 1, the stepwise mechanical activation leads to a substantial increase in specific surface area of the material; in this situation, the major part of the area is the area of pores 1.7-300 nm in diameter.

According to the notions of the kinetics of heterogeneous processes, the surface area is responsible first of all for the hydrolysis rate at the initial moment of the process, when mainly the reactive amorphous regions of carbohydrates react. To provide complete hydrolysis, it is necessary to achieve an as complete amorphization of the cellulose crystalline regions as possible.

As a result of the treatment in the centrifugal roller mill, the index of cellulose crystallinity decreases from 64% for initial corn straw to 31% for the material after fourfold activation (Fig. 5), which should cause a substantial increase in the transformation degree.

Table 1
Dependence of specific surface area on the number of mechanical treatment cycles

Sample	Specific surface area, m^2/g	Specific volume of pores 1.7-300 nm in diameter, m^2/g
Initial corn straw	0.5	0.2
1 cycle of mechanical treatment	1.4	1.0
2 cycles of mechanical treatment	1.8	1.2
3 cycles of mechanical treatment	2.1	1.5
4 cycles of mechanical treatment	2.4	1.7

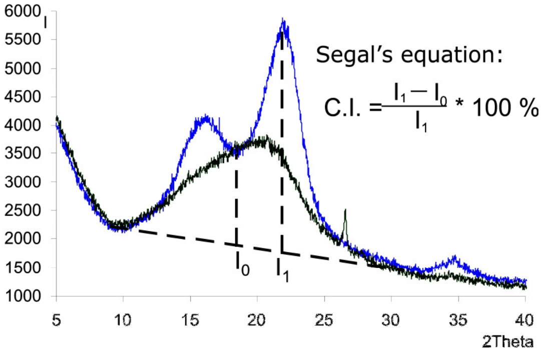


Figure 5: Diffraction of X-rays from the sample of initial and mechanically activated (4 cycles) corn straw (C.I. – crystallinity index)

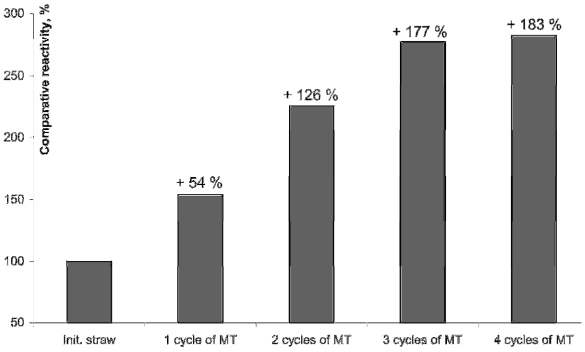


Figure 6: Dependence of the relative reactivity of corn straw on the number of mechanical treatment cycles

Changes of the reactivity of the material during mechanical treatment

Increased reactivity can be predicted for the materials after multiple mechanical treatments in a centrifugal roller mill. For instance, an increase in the specific surface area almost by a factor of 5 must cause a substantial increase in the rate of hydrolysis at the initial time interval. A decrease in the average particle size to 15 μm and amorphization of a half of the cellulose crystalline regions, in turn, must increase the total yield of the reaction.

The diagram showing relative changes of the reactivity of the materials under investigation is presented in Fig. 6 (to remind, we understand reactivity as the rate of enzymatic hydrolysis at the initial moment of time). One can see that the reactivity increases most efficiently during the first three cycles of mechanical treatment. Further grinding does not cause substantial activation. Hence, a requirement for the design of centrifugal roller mills arises. For each type of lignocellulose raw material, the size of the grinding zone is to be chosen so that the product with the highest reactivity could be obtained. For example, in our case it is necessary to increase the length of the grinding zone approximately by a factor of three, keeping constant the other dimensions.

The effect of cellulose amorphization from 64 to 31% was confirmed experimentally by a twofold increase in the yield of carbohydrates from complete enzymatic hydrolysis. Thus, the hydrolysis of initial corn straw allows for transforming 23% of the total amount of polymer carbohydrates into the low-molecular form. The time within which the reaction reaches a steady level is equal to 8 hours. At the same time, corn straw after three and four cycles of treatment within 10 hours provides a yield of soluble carbohydrates of 46.3 and 47.2%, respectively.

The residue after enzymatic hydrolysis, consisting in the crystalline regions of cellulose and lignin, can be subjected to repeated mechanical and enzymatic treatment or may be utilized as ecologically safe powder fuel.

Thus, the use of mechanical treatment and the subsequent enzymatic hydrolysis allow obtaining low-molecular carbohydrates from corn straw. These carbohydrates are suitable for further biotechnological processing into biofuel components and chemicals. The residue of the technological process is ecologically safe solid fuel that can be included into the industrial cycle, to increase the economic indexes due to lignocellulose processing and replacement of nonrenewable kinds of powder fuel in power engineering.[34]

CONCLUSION

Processes that occur during multiple mechanical treatment of corn straw in the centrifugal roller mill were studied. The obtained data illustrate the changes in the morphology of the powdered product, the effect of specific surface area and cellulose crystallinity degree on the reactivity of the treated material. At the first stage of mechanical treatment, the formation of dense aggregates consisting of ground particles is observed. With an increase in treatment time, the aggregates get destroyed into constituent particles.

It was established that the cells of corn straw with diameters larger than 30 μm are unstable under mechanical action and are destroyed into smaller fragments. An increase in surface area by a factor of 4.8 causes an increase in the reactivity of the lignocellulose material by a factor of 2.8 (the maximal value achieved), while a decrease in cellulose crystallinity degree from 64 to 31% increases the yield of low-molecular carbohydrates approximately by a factor of 2.

The use of mechanical treatment and the subsequent enzymatic hydrolysis allow obtaining low-molecular carbohydrates from corn straw. The residue of the technological process is ecologically safe solid fuel that can be included into the industrial cycle, to increase the economic indexes due to lignocellulose processing and replacement of nonrenewable kinds of powder fuel in power engineering.

Some proposals were put forward concerning the modification of existing centrifugal roller mills and the development of new ones for processing raw plant material. It is necessary to alternate the shock action with shear action, which will prevent the formation of too large aggregates. In order to achieve an economically important balance between the energy consumed for treatment and an increase in the reactivity as a result of the treatment, it is necessary to choose the size of the grinding zone for each type of the raw lignocellulose material.

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REFERENCES

[1] D. L. Klass in “Encyclopedia of Energy”, edited by Cutler J. Cleveland, Elsevier Inc., 2004, vol. 1, p. 193.

[2] R. E. H. Sims, W. Mabee, J. N. Saddler, M. Taylor, *Bioresource Technol.*, **101**, 1570 (2010).

[3] O. Konur, *Biomass. Bioenerg.*, **47**, 504 (2012).

[4] Z. Tan, S. Liu, N. Bliss, L. L. Tieszen, *Biomass. Bioenerg.*, **47**, 372 (2012).

[5] M. Barz, M. K. Delivand, *J. Sust. Energ. Environ.*, **Special issues**, 21 (2011).

[6] A. S. M. Hussin, S. R. A. Collins, Z. Merali, M. L. Parker, A. Elliston *et al.*, *Biomass. Bioenerg.*, **51**, 17 (2013).

[7] A. L. Borrion, M. C. McManus, G. P. Hammond, *Biomass. Bioenerg.*, **47**, 9 (2012).

[8] C. E. Wyman, *Ann. Rev. Energ. Environ.*, **24**, 189 (1999).

[9] D. Chiaramonti, A. M. Rizzo, M. Prussi, S. Tedeschi, *Biomass Conv. Bioref.*, **1**, 9 (2011).

[10] R. P. Chandra, S. M. Ewanick, P. A. Chung, K. Au-Yeung, L. Del Rio *et al.*, *Biotechnol. Lett.*, **31**, 1217 (2009).

[11] J. Hilgert, N. Meine, R. Rinaldi, F. Schuth, *Energ. Environ. Sci.*, **6**, 92 (2013).

[12] Z. Lin, H. Huang, H. Zhang, L. Zhang, L. Yan, *et al.*, *Appl. Biochem. Biotechnol.*, **162**, 1872 (2010).

[13] E. G. Shapolova, A. L. Bychkov, O. I. Lomovsky, *J. Int. Sci. Publ.: Mater. Meth. Technol.*, **6**, 196 (2012).

[14] M. W. Easson, B. Condon, B. S. Dien, L. Iten, R. Slocek *et al.*, *Appl. Biochem. Biotechnol.*, **165**, 1322 (2011).

[15] G. Muratov, C. Kim, *Biotechnol. Bioprogress. Eng.*, **7**, 85 (2002).

[16] C. Martin, A. B. Thomsen, *J. Chem. Technol. Biotechnol.*, **82**, 174 (2007).

[17] C. I. Ishizawa, T. Jeoh, W. S. Adney, M. E. Himmel, D. K. Johnson *et al.*, *Cellulose*, **16**, 677 (2009).

[18] R. Sharma, V. Palled, R. R. Sharma-Shivappa, J. Osborne, *Appl. Biochem. Biotechnol.*, **169**, 761 (2013).

[19] R. Sindhu, P. Binod, K. U. Janu, R. K. Sukumaran A. Pandey, *World J. Microb. Biot.*, **28**, 473 (2012).

[20] G. Brodeur, E. Yau, K. Badal, J. Collier *et al.*, *Enzyme Res.*, **2011**, 1 (2011), doi:10.4061/2011/787532

[21] P. Kumar, D. M. Barrett, M. J. Delwiche, P. Stroeve, *Ind. Eng. Chem. Res.*, **48**, 3713 (2009).

[22] O. I. Lomovsky, I. O. Lomovsky, in “Enhancing Extraction Processes in the Food Industry”, edited by N. Lebovka, Taylor & Francis Group, Boca Raton, London, New York, 2011, pp. 61-398.

[23] F. Masarin, P. C. Pavan, M. P. Vicentim, P. B. Souza-Cruz, C. Loguercio-Leite *et al.*, *Holzforschung*, **63**, 259 (2009).

[24] M. Akhtar, M. J. Lentz, E. G. Horn, *Resour. Conser. Recyc.*, **28**, 241 (2000).

[25] M. A. Millett, M. J. Effland, D. F. Caulfield, in “Hydrolysis of Cellulose: Mechanisms of Enzymatic and Acid Catalysis”, edited by R. D. Brown, American Chemical Society, 1979, pp. 71-89, doi: 10.1021/ba-1979-0181.

[26] G. G. D. Silva, M. Couturier, J.-G. Berrin, A. Buleon, X. Rouau, *Bioresource Technol.*, **103**, 192 (2012).

[27] O. V. Alekseeva, O. V. Rozhkova, A. N. Prusov, *Fibre Chem.*, **29**, 103 (1997).

[28] A. N. Prusov, K. N. Zheleznov, O. V. Alekseeva, V. A. Padokhin, O. V. Rozhkova, *Colloid. J.*, **64**, 601 (2002).

[29] L. Kokko, H. Tolvanen, K. Hamalainen, R. Raiko, *Biomass. Bioenerg.*, **42**, 219 (2012).

[30] P. H. Raven, R. F. Evert, S. E. Eichhorn, “Biology of Plants”, New York, Worth Publishers, 1986, pp. 775.

[31] A. L. Bychkov, O. I. Lomovskii, *Russ. J. Appl. Chem.*, **83**, 1106 (2010).

[32] A. L. Bychkov, E. I. Ryabchikova, K. G. Korolev, O. I. Lomovsky, *Biomass. Bioenerg.*, **47**, 260 (2012).

[33] A. P. Sinitsyn, A. V. Gusakov, V. M. Chernoglazov, “Bioconversion of Lignocellulosic Materials”, Moscow, Moscow University Press, 1995, pp. 220.

[34] O. Lomovsky, A. Bychkov, I. Lomovsky, V. Logvinenko, A. Burdukov, *Thermal Sci.*, (2014) (In Press), doi:10.2298/TSCI130820167L.