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Cellulose from Various Parts of Soranovskii *Miscanthus*

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Abstract—The chemical composition of the Soranovskii *Miscanthus* variety harvested in 2011 and 2012 was separately determined in the whole plant, leaves, and stem. In all cases, cellulose was found to prevail in the *Miscanthus* stem, and noncellulosic components (the fat-wax fraction, ash, and lignin), in leaves. Cellulose samples were for the first time obtained from leaves and stems separately by two methods (nitric-acid and combined). The best quality cellulose was derived from stems. For instance, cellulose isolated from stems by the nitric-acid process was better than that from leaves, which was expressed as a higher cellulose content (94.4% versus 91.7%) and degree of polymerization (800 versus 580), as well as low weight fractions of noncellulosic components: ash (0.07% versus 1.01%) and acid-insoluble lignin (0.45% versus 1.51%). The same tendency is observed in celluloses produced by the combined method: Cellulose from stems had better quality than that from leaves; specifically, it had higher degree of polymerization (1040 versus 640) and lower weight fractions of the noncellulosic components: ash (0.14% versus 0.75%), acid-insoluble lignin (0.88% versus 4.12%), and pentosans (6.38% versus 8.53%). It is obvious that cellulose obtained by the nitric-acid method can be suitable for chemical modifications, including nitration. Cellulose from the combined process can be utilized in paper industry.

Keywords: Soranovskii *Miscanthus*, leaf, stem, fat-wax fraction, cellulose, ash content, nitric-acid method, combined method, α-cellulose, residual lignin, degree of polymerization

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INTRODUCTION

It is known that cellulose is one of the most common natural biopolymers synthesized by higher plants, algae, and some types of bacteria. Cellulose is a popular multipurpose compound and raw material for a wide range of new materials, including nanomaterials used in various industries, self-contained paper factories, textile, food, oil, paints, fuel, electronic, pharmaceutical, and medical, due to its valuable properties: high strength, biocompatibility, nontoxicity, biodegradability, and availability (Tkacheva et al., 2013). Historically, large-scale production of cellulose in Russia was traditionally focused on wood. Nevertheless, nonwood sources of cellulose (flax, hemp, reed, straw of grasses) are presented and justified in the modern chemical engineer guide as industrially important raw materials (New Guide..., 2006). The collapse of the former Soviet Union resulted in the loss of cotton plantations which were the source of luxury cotton cellulose and therefore the importance of the development of new sources of nonwood cellulose increased multiply.

Cultivation of plants with high yields of biomass and high cellulose content, grown by traditional methods of agriculture, may be a very promising approach for the implication of new sources of grasses (straw and fruit walls) for which the cost is formed by the delivery of raw materials for cellulose production, the energy crop *Miscanthus sinensis* Andersson requires special attention for the adaptation to climatic conditions, the use of fertilizers and high labor costs in planting, and weeding, but this crop is characterized not only by the possibility of cultivation on soils unsuitable for cultivated crops but also by long-term high yield after a single planting (Bulatkin and Mitenko, 2013). Currently this plant is positioned by foreign researchers as a promising cellulose-containing raw material for the production of cellulose and products of its chemical modification and for the biotechnological production of soluble carbohydrates and biofuels (Somerville et al., 2010). The chemical composition of the *Miscanthus* biomass of different genotypes according to foreign studies, represented mainly by cellulose 40–60% and lignin 10–30% (Jones and Walsh, 2001; Brosse et al., 2012).

The first attempt to evaluate the chemical composition of the leaves and stems of *Miscanthus sinensis* Andersson was undertaken in 1983 by Ukrainian scientists who already considered *Miscanthus* as raw

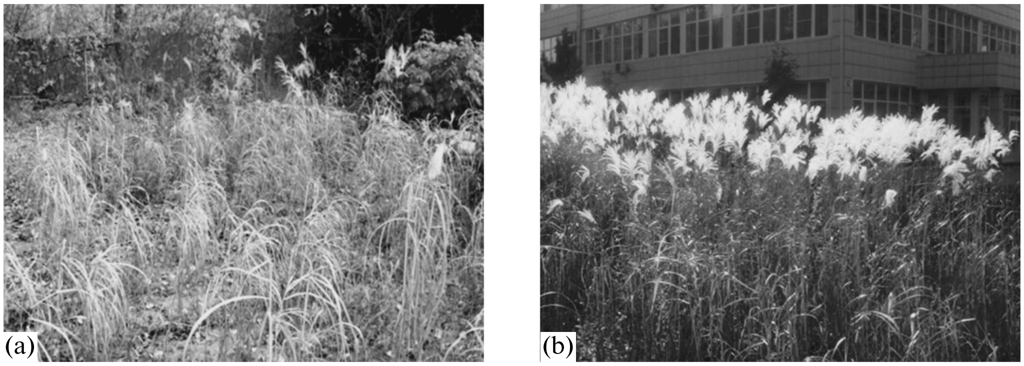

Fig. 1. Plantation of *Miscanthus* in October 2011 (Institute for Problems of Chemical and Energetic Technologies, SB RAS) (a), Plantation of *Miscanthus* in October 2012 (Institute for Problems of Chemical and Energetic Technologies, SB RAS) (b).

material for the paper industry (Krotkevich et al., 1983). P.G. Krotkevich et al. used the four-year shoots of plants supplied by Kiev's Botanical Gardens, Academy of Sciences, Ukraine for the production of cellulose The researchers separated *Miscanthus sinensis* Andersson into morphological parts, determine the chemical composition of these parts and found the differences in the content of cellulose in the stems and leaves: 40.82% and 33.24%, respectively. The authors demonstrated that the samples of cellulose isolated from leaves and stems separately by the soda process, meet the requirements for the production of various types of paper.

The Soranovskii *Miscanthus* variety was produced in 2006 at the Institute of Cytology and Genetics, SB RAS, Russia (*Miscanthus sinensis* Andersson). This variety is characterized by a modified structure of the root system, it forms long shoots with growth buds, rapidly colonizes soil space, and produces a solid and smooth (no mole hills) *Miscanthus* plantation. Since 2013, according to the state register of breeding achievements permitted for use, the Soranovskii *Miscanthus* variety has been a technical culture, a source of cellulosic feedstock suitable for cultivation in all regions of Russia (no. 8854628, http://www.gossort.com/reestr/new_sort.html). Results of investigation of samples of *Miscanthus* grown in the Novosibirsk region on plantations of different ages showed that with the increased age of the plantation, the cellulose content increased and the content of noncellulosic components in *Miscanthus* biomass decreased (Budaeva et al., 2010).

The goal of this study was the determination of the chemical composition of the Soranovskii *Miscanthus* variety harvested in 2011 and 2012 (city Biysk): the composition of the whole plant, leaves and stems separately, the production of samples of cellulose from *Miscanthus* harvested in 2012 by nitrate and the combined methods under the laboratory conditions, and a comparative study of the characteristics of the obtained celluloses.

MATERIALS AND METHODS

The first object of research was the yearling Soranovskii *Miscanthus sinensis* Anderson variety, harvested in 2011, grown on an experimental plot of the Institute for Problems of Chemical and Energetic Technologies, SB RAS, in 2011. Planting material was provided by staff of the Institute for Problems of Chemical and Energetic Technologies, SB RAS and planted in April 2011 on a level plot without plowing the soil in the furrows. The seedlings were not fed, spacing was not stirred, weeding was not done, and irrigation was rare. In October, some plants formed panicle inflorescences, indicating the ripeness of the *Miscanthus*, and the harvest was performed by cutting of all plants. The harvest of *Miscanthus* from in an area of 61 m^2 amounted to 848 plants (Fig. 1a), of which 30 plants were with panicles (3.5% of the total number of plants). The average density of the grown plants was 14 pc./m^2. The mass of the harvest was 2.750 kg, the weight of *Miscanthus* without panicles was 2.500 kg (91%) at 8% moisture. The height of the longest plant was 1.60 m (length of the panicle was 0.28 m, the number of leaves was 9 pcs., the length of a leaf was 0.40–0.55 m).

The second object of research was *Miscanthus* harvested in 2012, grown on the same plot after the first harvest. The growth conditions of *Miscanthus* were similar to the previous year except for the weather conditions, since summer 2012 was abnormally hot. The harvest was done in October 2012. The mass of the harvest at 8% humidity was 12.300 kg (Fig. 1b), 0.202 kg/m^2. The average weight of plant (one shoot) was 0.005 kg. The density of grown shoots was 39 pcs. per 1 m^2. The average length of the stem of ripe *Miscanthus* (with panicle) was 2.00 m. Some plants reached a height of 2.40 m. The average length of panicle was 0.28 ± 0.02 m. The average number of leaves was 9 ± 1 pcs., and the average length of a leaf was 0.60 ± 0.05 m. The average ratio of the weight of the leaf to the weight of the whole plant was 0.539.

Mature plants with the highest height and panicle inflorescences were used for investigation of the chemical composition of *Miscanthus*. The chemical composition of the whole plant and samples of leaves and stems were investigated separately. All *Miscanthus* samples were shredded with scissors. Determination of ash content (based on absolutely dry raw material, a.d.r.m.), the mass fraction (MF) of extractives-fat-wax fraction (FWF) (extractant-dichloromethane, a.d.r.m.), MF of acid-soluble lignin (a.d.r.m.), and MF of cellulose according to Kyurshner's method (a.d.r.m.) was performed according to the standard methods of analysis of plant raw materials (Obolenskaya et al., 1991), MF of pentosans was determined spectrophotometrically (UNICO UV-2804, USA) using Fe-orcinol reagent (orcinol, Panreac, Spain) according to the methods (GOST 10820-75; Obolenskaya et al., 1991). Fatty acid composition of FWF was analysed according to the determination of MF of fatty acids (GOST 30418-96) to their total content in the triglycerides of oils by the gas chromatography mathod using gas laboratory chromatograph with a flame ionization detector and temperature programming Kristallyuks 4000M (Russia, Yoshkar-Ola). Moisture was determined on moisture analyzer MB 23/MB 25 (OHAUS, United States).

Harvest 2012 from two years old plot was used for the production of cellulose. All methods of cellulose isolations are based on the separation of the lignocellulosic matrix into separate polymers, cellulose, hemicellulose, and lignin, followed by dissolution of primarily lignin and secondarily-hemicelluloses in acid liquors. Cellulose during cooking remains in the solid phase; the process is often called delignification, i.e., removal of lignin. Cellulose was produced by nitric-acid and combined methods. The nitric-acid method consists in boiling raw material in a dilute solution of nitric acid at atmospheric pressure, followed by treatment with diluted sodium hydroxide solution (Budaeva et al., 2010). This method was recommended for the isolation of cellulose from the straw of grass by Russian chemist N.I. Nikitin (1962), and it was first used by the authors for *Miscanthus*. During the treatment with nitric acid, in addition to cleavage of the chemical bonds between cellulose, hemicellulose, and lignin, hemicellulose is hydrolyzed with production of soluble xylose and its precursors, easily removed by the following treatment of oxidized nitrolignin by dilute sodium hydroxide. At the recommended parameters of the nitric-acid method (acid concentration, the duration of the process), isolated cellulose is characterized by sufficiently high quality, retains a high degree of polymerization, and does not contain oxidized groups. Increased concentrations or the duration of the process may cause a loss of the target cellulose or lower yield, but nitration of cellulose by a diluted aqueous solution of nitric acid was not described (Nikitin, 1962). The combination method involves the reverse procedure of using diluted solutions of sodium hydroxide and nitric acid (Budaeva et al., 2011).

Analysis of the ash content, MF of residual (acid insoluble) lignin, and MF of α-cellulose was carried out according to standard procedures for intermediates and cellulose (Obolenskaya et al., 1991); MF of pentosans was determined spectrophotometrically (UNICO UV-2804, USA) using Fe-orsionl reagent (orsinol, 10820-75; Obolenskaya et al., 1991), the degree of polymerization (DP) of the cellulose was determined according to the viscosity of the solutions in kadoxen using viscometer IWF-3 with a capillary diameter of 0.92 mm according to the methods (GOST 25438-82; Obolenskaya et al., 1991).

The surface morphology of cellulose fibers was studied by scanning electron microscopy (SEM) using scanning electrochemical microscope SEM JEOLGSM 840 (Japan) after sputtering Pt with the layer of 1–5 nm.

RESULTS AND DISCUSSION

The chemical compositions of the whole plant, leaves, and stems separately of yearling *Miscanthus* harvested in 2011, grown on the plot of the Institute for Problems of Chemical and Energetic Technologies, SB RAS, is presented as a diagram in Fig. 2a.

In the foreign literature, information about the chemical composition of yearling *Miscanthus* is absent. A comparison of these results with the results obtained for yearling *Miscanthus* grown in the Novosibirsk region (Budaeva et al., 2010) revealed that the young plants are characterized by higher values of ash content and MF of acid-insoluble lignin: 6.30% vs. 5.56% and 22.23% vs. 18.46%, respectively. Furthermore, MF of FWF was 5.71% and it was also higher than the data for yearling *Miscanthus*, grown in colder areas: 4.30%.

Analysis of FWF showed the presence of 24 fatty acids, myristic, palmitic, palmitoleic, stearic, oleic, linoleic, linolenic, arachidic, and behenic. These results are consistent with the information about the presence of saturated and unsaturated fatty acids in FWF, extracted with dichloromethane from adult foreign *Miscanthus* (Villaverde et al., 2009).

Comparison of the chemical compositions of the leaf and the stem indicates a higher content of noncellulosic impurities in the leaf: FWF (7.70% vs. 4.30%), ash (11.50% versus 2.96%), and acid-insoluble lignin (23.94 % vs 20.51%). The same pattern was found by P.G. Krotkevich et al. (1983).

The chemical composition of *Miscanthus* harvested in 2012 from the two-year plot (the whole plant, leaf and stem separately) is shown as a diagram in Fig. 2b.

Comparison of these results with the chemical composition of the yearling plant showed a small decrease in MF of FWF (4.78% vs. 5.71%) and a slight increase in MF of cellulose (44.5% vs. 41.7%).

Comparison of the chemical composition of the leaves and stems separately indicates a higher content of noncellulosic impurities in *Miscanthus* leaves: FWF

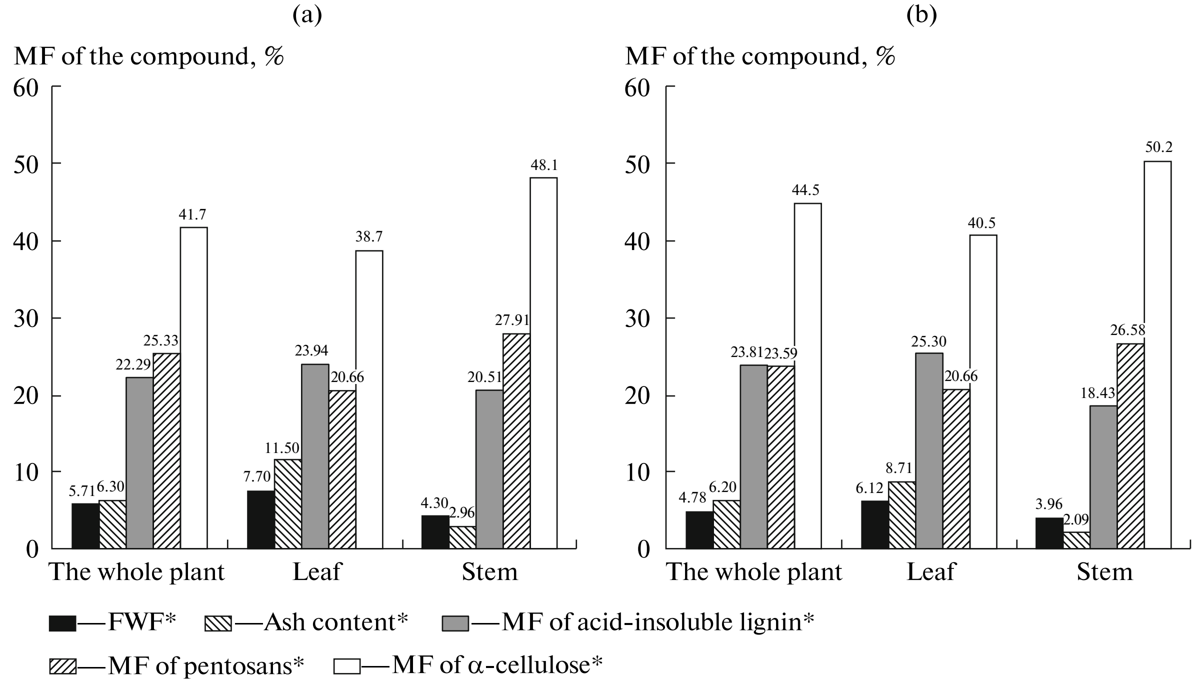

Fig. 2. Mass fraction of fat-wax fraction, ash, acid-insoluble lignin, pentosans, cellulose in yearling *Miscanthus* harvested in 2011 in the whole plant, leaves, and stems separately (a); in *Miscanthus* harvested in 2012 from the two-year-old plantation: in the whole plant, leaves, and stems separately (b).

* On an oven-dry basis.

(6.12% vs. 3.96%), ash (8.71% vs. 2.09%), and acid-insoluble lignin (25.30% vs 18.43%). Similarly to the chemical composition of the 2011 harvest, the gap between MF values of cellulose in the stem and leaf is at the same level (about 10%); MF of pentosans is higher in the stem than in leaves: 26.58% vs. 20.66%. It should be noted that in foreign *Miscanthus* hemicelluloses is presented primarily by pentosans, namely xylan (Jones and Walsh, 2001).

By comparing the *Miscanthus* harvested in 2011 and 2012 and the data in the literature (Krotkevich et al., 1983), we can conclude that the cellulose prevails in the stem, and noncellulosic components (except pentosans) prevail in the leaf. This comparison suggests that the stem is characterized by high cellulose content and a lower content of noncellulosic components in comparison to the leaf regardless of the habitat and the age of the plant. Exceptions are pentosans (noncellulosic components) that predominate in the stem. These patterns were described for the straw of grass in books (Lendel and Morvai, 1978; Sun, 2010), as for the different genotypes of foreign *Miscanthus*, there is no information about the quantitative differences of component composition of leaves and stems, and there are recommendations to use the whole plant for processing (Brosse et al., 2012) without removing the leaf.

The dependence of MF of cellulose on the age of the plot, previously described in the article (Budaeva, 2010), was observed also for these samples of raw material: MF of cellulose in the stem of more mature plants was higher than the corresponding value for the yearling plant (50.2% vs. 48.1%). However, the cellulose content in the *Miscanthus* harvested in 2012 could be even higher were it not for the abnormally hot weather in 2012. The plants were grown in adverse conditions.

Thus, determination of the chemical composition of the Russian *Miscanthus* from one and two year plots revealed the characteristic differences of components in the leaves and stems in favor of the latter and age-related patterns of changes of MF of noncellulosic impurities in the whole plant.

Samples of cellulose from the whole plant, the leaf and stem were obtained separately from two-year-old *Miscanthus*. Characteristics of celluloses obtained from *Miscanthus* harvested in 2012 produced by the nitric-acid method are presented in Table 1 and for the visualization of results in Fig. 3.

The quality of the plant's cellulose is confirmed by the high MF values for α-cellulose-92%, and DP 830 and MF of the sum of the noncellulosic impurities (ash, lignin and pentosans), which was not higher than 3%, which has attracted the attention of researchers to further esterification, including production of esters (cellulose nitrates) (Gismatulina et al., 2012; Yakusheva, 2012).

Table 1. Characteristics of celluloses produced by the nitric-acid method from *Miscanthus* harvested in 2012 for the whole plant, leaves, and stems separately

Raw material	MF of α-cellulose, %*	DP	MF of noncellulosic components*, %		
			ash	lignin	pentosans
The whole plant	91.8 ± 0.5	830	0.62 ± 0.01	1.59 ± 0.05	0.67 ± 0.05
Leaf	91.7 ± 0.5	580	1.01 ± 0.01	1.51 ± 0.05	0.43 ± 0.05
Stem	94.4 ± 0.5	800	0.07 ± 0.01	0.45 ± 0.05	0.40 ± 0.05

* Expressed in terms of a.d.r.m., DP-degree of polymerization.

Table 2. Characteristics of celluloses produced by combined method from *Miscanthus* harvested in 2012 for the whole plant, leaves, and stems separately

Raw material	MF of α-cellulose*	DP	MF of noncellulosic components*, %		
			ash	lignin	pentosans
The whole plant	87.0 ± 0.5	950	0.44 ± 0.01	2.62 ± 0.05	7.10 ± 0.05
Leaf	85.9 ± 0.5	640	0.75 ± 0.01	4.12 ± 0.05	8.53 ± 0.05
Stem	88.5 ± 0.5	1040	0.14 ± 0.01	0.88 ± 0.05	6.38 ± 0.05

The notations are the same as in Table 1.

Comparison of the cellulose obtained from leaves and stems demonstrate that differences in MF values of α-cellulose and DP (92% vs. 94% and 580 against 800) point in favor of the sample of the stem. MF of the sum of noncellulosic impurities in the sample of the stem was one third of the sample of the leaf: 0.95% vs 2.95%; separately for lignin: 0.45% in the cellulose of the stem versus 1.51% for cellulose of the leaf.

Characteristics of celluloses produced by the combined method from *Miscanthus* harvested in 2012 for the whole plant, leaves, and stems separately presented in Table 2 and for the visualization of the results in Fig. 4.

The quality of the cellulose produced by the combined method from the whole plant was lower than that of the cellulose obtained by the nitric-acid method, namely, lower MF of α-cellulose (87% vs. 92%), a higher amount of unwanted noncellulosic impurities (ash, lignin and pentosans), 10% vs. 3%, and DP was at the same level 950 vs. 830, respectively.

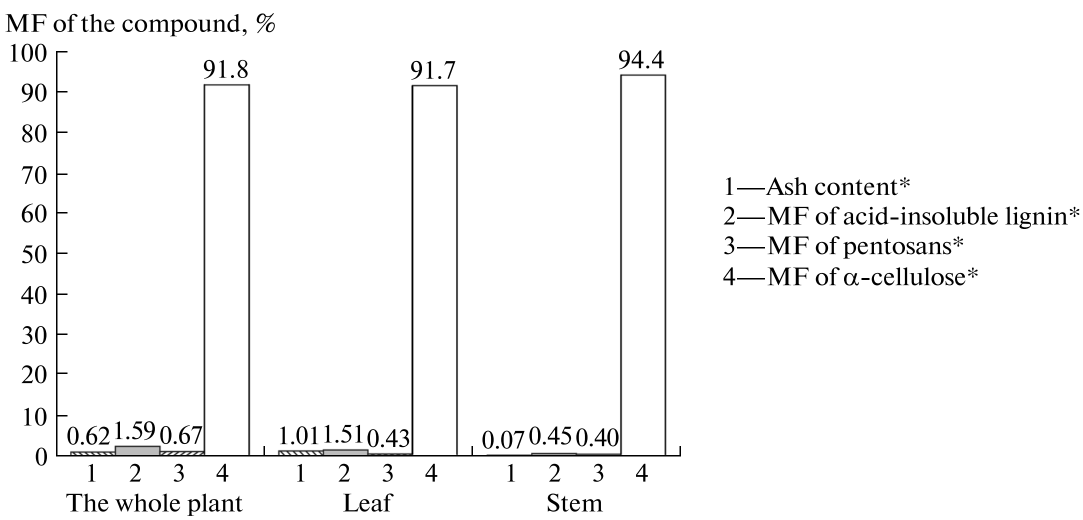

Fig. 3. Characteristics of celluloses produced by nitric-acid method from *Miscanthus* harvested in 2012 for the whole plant, leaves, and stems separately.
* On an oven-dry basis.

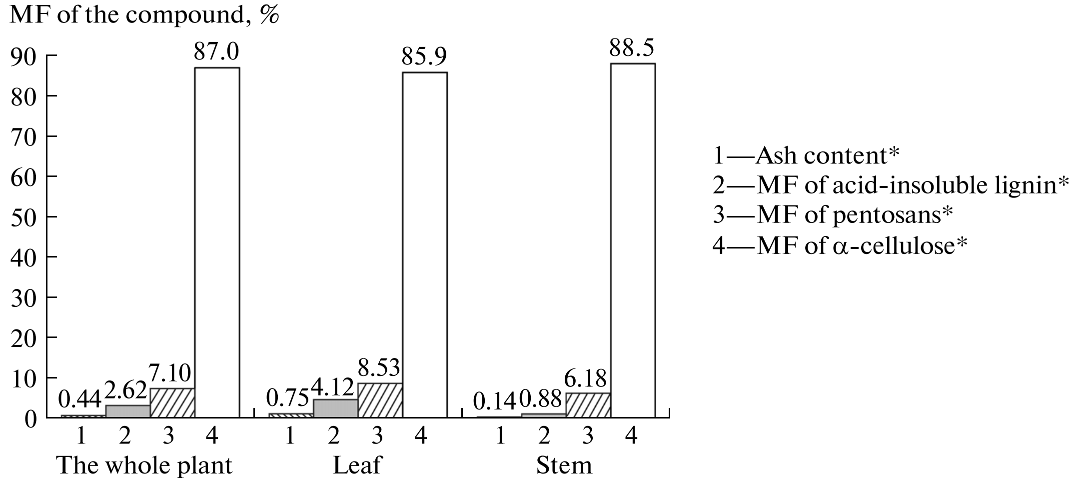

Fig. 4. Characteristics of celluloses produced by combined method from *Miscanthus* harvested in 2012 for the whole plant, leaves, and stems separately.
* On an oven-dry basis.

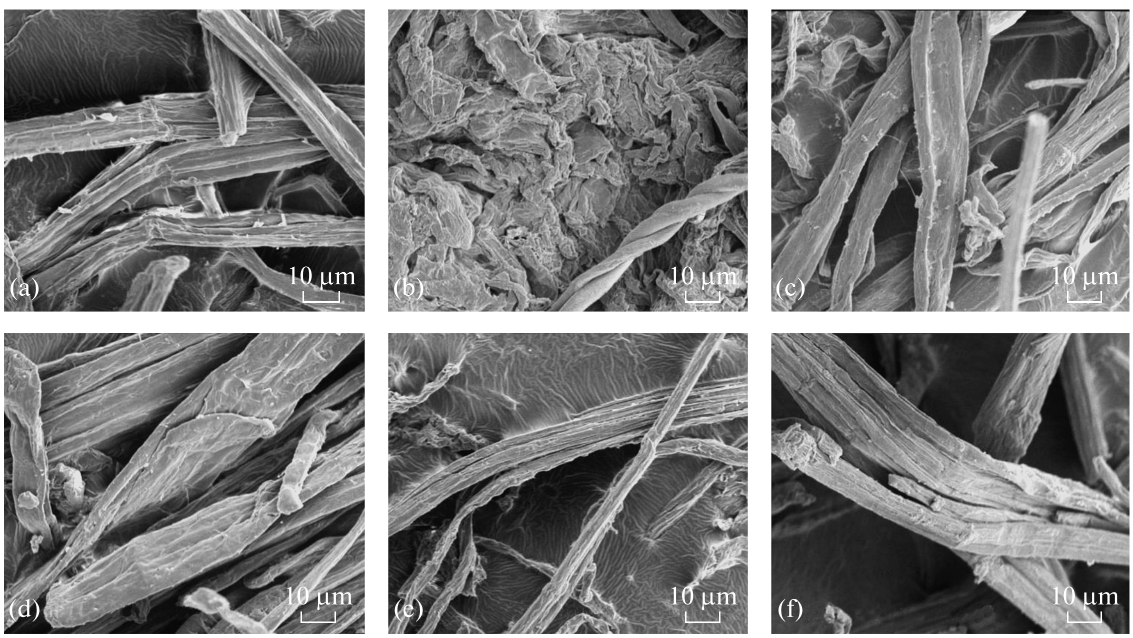

Fig. 5. Cellulose fibers produced from *Miscanthus* from the whole plant, leaf, and stem separately by the nitric-acid method (a, b, c) and combined means (d, e, f), respectively, ×1000.

Similarly to the described nitric-acid method above, cellulose, isolated by the combined method from the stem had a better quality than that from the leaf: MF of α-cellulose was 88.5% vs. 85.9%, the DP was 1040 vs. 640, the ash content was 0.14% vs. 0.75%, the acid-insoluble lignin was 0.88% vs. 4.12%, and MF of pentosans was 6.38% vs. 8.53%.

The obtained results indicate that the harvest with the lowest MF of the leaf is a suitable raw material for the production of cellulose from *Miscanthus*, i.e., grown on the plot with a maximal density of planting, which is achieved in 3–4 years, after this time period, the annual biomass yield is 10–15 t/ha and it remained at this level for 15–20 years (Shumny et al., 2010).

The SEM photos of celluloses obtained from *Miscanthus* by nitric-acid (a, b, c) and the combined (d, e, f) methods are shown in Figure 5. Celluloses obtained by various methods from the whole plant (Figs. 5a, 5d) were not different from each other and were presented by plain, rather long fibers with multiple breaks and ruptures, but on average there was a difference in width of the fibers: 10 nm for nitric-acid cellulose and approximately 40 nm for combined cellulose. Cellulose fiber obtained from the leaf had a very complex

Table 3. Main characteristics of cotton cellulose (GOST 595-79) and cellulose produced from *Miscanthus* by the nitric-acid method

Characteristics	Cellulose from	
	cotton	*Miscanthus*
MF of α-cellulose, %	96.0–98.0	91.7–94.4
Wetting properties, g	140–150	–
Ash content, %	0.2–0.3	0.1–1.0
MF of residue insoluble in sulfuric acid, %	0.3–0.5	–
MF of acid-insoluble lignin, %	–	0.5–1.6
MF of pentosans, %	–	0.4–0.7
DP	1000 (Yakusheva, 2013), 2500–3500 (Shahmina, 2003)	580–800

MF—mass fraction, DP—degree of polymerization, dash—this index is not normalized according to the current regulatory and technical documentation.

shape (Figs. 5b, 5e): nitric-acid cellulose nitrate had long fibers, twisted several times, along with short flat fibers; combined cellulose had very thin long fibers, which retained their shape. The photo (Figs. 5c, 5e) demonstrates that the cellulose of the stem has a classical form of fibers, wherein the nitric-acid cellulose in the photo (Fig. 5c) had fiber in the form of a tube, i.e., with an oval cross section in the sectional and longitudinal hole inside. In general, celluloses produced by the nitric-acid method were smoother (Fig. 5c), and celluloses obtained by the combined method (Fig. 5e) had a rougher texture.

It is known that the standard cellulose is cotton cellulose used for nitration for the production of nitrocellulose, as well as for the production of acetate cellulose and cuprammonium fibers. Some requirements for the quality of cotton cellulose are shown in Table 3 (GOST 595-79; Zhegrov et al., 2011). For cotton cellulose, MF of pentosan is not standardized, but there are recommendations to reduce the content of pentosans in the pulp to a minimal. Dynamic viscosity of cotton cellulose varies in a wide range from 10–20 to 430–850 DP and normalizes the brand of cellulose, but Table 3 shows the degree of polymerization of cotton cellulose (according to Shahmina, 2003; Jakusheva, 2013). The same table shows the main characteristics of the cellulose produced from *Miscanthus* by the nitric-acid method.

The comparison of the experimental cellulose with the standard cellulose shows the advantages of cotton cellulose, but the cellulose from the stem of *Miscanthus* was closed to the cotton cellulose based on ash content, MF of the insoluble residue lignin and MF of pentosans. High values of α-cellulose and DP remained unachievable, which is associated with the nature of the cellulose. For example, it is known that DP of the native cellulose from a foreign *Miscanthus* species is not higher than 1600 (Jones and Walsh, 2001).

In the People's Republic of China, the industrial method for production of cellulose from *Miscanthus saccharifiorus* is kraft pulping: in the mixture of sodium hydroxide with sodium sulfide at a cooking temperature of 165°C with a guarantee of a cellulose product yield of 54%, with residual lignin of 1.15–1.33%. Since 1987, Yueyang Paper Mill produces about 120 t/day of cellulose suitable for offset printing (Jones and Walsh, 2001).

In Russia the sulfate method for production of cellulose from the straw of grass and reed was considered. It is known that this cellulose is characterized by α-cellulose content at the level 75–80%, high content of pentosans of 18–24%, and high ash content; the fibers of straw and reed cellulose are very small; they quickly hydrated during milling, but the fibrillation and longitudinal splitting of these fibers is complicated (Ivanov, 2006). Cellulose produced from *Miscanthus* by the combined method, characterized by MF of α-cellulose of the same order, 85.9–88.5%; MF of pentosans was much lower, 6.4–8.5% and the ash content was very low, 0.14–0.75%. It can be assumed that the paper produced from this cellulose has high rigidity and is good for gluing. Such cellulose must be used in combination with wood cellulose for the production of different types of paper.

CONCLUSIONS

The chemical composition of the Soranovskii *Miscanthus* variety harvested in 2011 and 2012 was determined for whole plants, leaves, and stems separately. We found that in both cases, the cellulose prevails in the stem of *Miscanthus*, and noncellulosic components (fat-wax fraction, ash, lignin) prevails in the leaf.

Samples of cellulose from leaves and stems of *Miscanthus* separately were obtained for the first time by two different methods (nitric-acid and combined). It was established that the most qualitative cellulose is obtained from the stem. Thus, the cellulose obtained from the stem by the nitric-acid method had better quality than that obtained from the leaf; the higher quality was reflected in the high values of the content of α-cellulose (94.4% vs. 91.7%) and the degree of polymerization (800 vs. 580), and low values of the mass fractions of noncellulosic components: ash was 0.07% vs. 1.01% and acid-insoluble lignin was 0.45% vs. 1.51%. The same pattern was observed for celluloses produced by the combined method: cellulose from the stem had a higher quality than that of the leaf: a higher degree of the polymerization value, 1040 vs. 640, and low mass fractions of noncellulosic components: ash came to 0.14% vs. 0.75%, acid-insoluble lignin amounted to 0.88% vs. 4.12%, and pentosans came to 6.38% vs. 8.53%.

It is obvious that the cellulose obtained by the nitric-acid method could be suitable for chemical modification, including nitration; cellulose obtained by the combined method may be applied in the paper industry.

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