Features of processing the biomass of genus Miscanthus plants into carbohydrate-containing substrates for biotechnology

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Abstract. The article presents the characteristics of the chemical composition of the biomass of genus Miscanthus plants and the features of its use for the production of carbohydrate-containing substrates for biotechnology. It is shown that the Chinese mycanthus "Ferner Osten" is not characterized by a high content of lipidic and protein compounds. The herbaceous plant is rich in cellulose, lignin and fiber. To achieve the maximum conversion of the miscantus biomass, a preliminary chemical treatment with dilute sulfuric acid was performed. The optimal temperature for pretreatment of miscanthus biomass with 1% sulfuric acid solution is 130 °C with a process duration of 30 minutes. With an increase in temperature exposure, the yield of cellulose decreases markedly. Thus, when the temperature rises to 140°C and the process lasts 30 minutes, the pulp yield decreases by 31.38 %. With an increase in the duration of chemical treatment from 30 to 60 minutes, the pulp yield decreases from 79.07% to 61.08%. The residues of oligosaccharides and lignin were determined in the chemical hydrolysates of Miscanthus sinensis "Ferner Osten". Among monosaccharides, glucose and xylose predominate, 40.5 and 24.4% of the dry matter, respectively. To increase the yield of sugars, it is planned to carry out two-stage processing.

1. Introduction

Plant biomass, which is a renewable source of raw materials and has such a valuable compound as cellulose in its composition, is an inexhaustible source for obtaining a wide range of materials with new target properties [1].

The herbaceous plant of the genus Miscanthus contains up to 70% of polysaccharides, such as cellulose and hemicellulose, gives high yields of biomass and, with appropriate processing, can become a promising raw material for obtaining substrates for the cultivation of microorganisms. Currently, numerous studies are conducted to study the possibility of using miscanthus in the manufacturing of products for various types of industry [2].

The greatest interest for research is represented by Miscanthus giganteus (giant miscanthus), Miscanthus sinensis (chinese miscanthus) and Miscanthus sacchariflorus (sugar-sacchariflorus miscanthus), which are the most widespread in terms of the number of agricultural areas occupied and the volume of their use both in Russia and abroad [3].

The appearance of the plant is shown in Figure 1.
The plant of the Miscanthus genus is a lignocellulose raw material that is difficult to process, therefore, its effective use requires the introduction of a preliminary processing stage into the technological process. Processing is necessary to separate the main components. Pretreatment is a key step for the subsequent steps of enzymatic hydrolysis and fermentation to maximize the production of the desired products [4].

The purpose of this research is to select the technological parameters of the preliminary chemical treatment of Miscanthus biomass for the subsequent production of carbohydrate-containing substrates for biotechnology.

2. Materials and methods
Miscanthus sinensis "Ferner Osten", harvest 2019, was selected as object of research. Perennial herbaceous plant, from 0.8 to 2 m high, sometimes up to 3.5 m. The yield is 10.1-15.4 t/ha.

To determine the mass fraction of ash in the plant biomass, a standard method was used in accordance with GOST R 56881-2016.

The mass fraction of moisture was determined according to GOST R 56889-2016.

The mass fraction of fiber, crude fat and protein was evaluated according to the requirements of GOST 32040-2012 "Feed, mixed feed, feed raw materials. Method for determining the content of crude protein, crude fiber, crude fat and moisture using near-infrared spectroscopy."

The mass fraction of lignin was estimated according to GOST 11960-79 "Semi-finished fibrous products and raw materials from annual plants for pulp and paper production (Method for lignin determination)".

To determine the mass fraction of cellulose, GOST 16932-93 (ISO 638-78) "Cellulose. Determination of dry matter content".

The mass fraction of reducing sugars was determined by spectrophotometric method at a wavelength of 530 nm using a reagent of 3,5-dinitrosalicylic acid in terms of glucose.

3. Results and discussion
At the first stage of the research, the chemical composition of non-wood lignocellulosic raw materials - Miscanthus sinensis "Ferner Osten" was studied. Mature plants were selected for the analysis.

At the same time, plants characterized by a higher height and containing the maximum number of inflorescences were selected. The results of studying the chemical composition of samples of whole Miscanthus sinensis "Ferner Osten" are shown in Figure 2.
The analysis of the results of studying the chemical composition of the whole plant of Miscanthus sinensis, presented in Figure 1, indicates that the moisture content is no more than 9.0 %, which does not contradict the literature data. It is shown that the Chinese mycanthus is not characterized by a high content of lipidic and protein compounds. The herbaceous plant is rich in cellulose, lignin and fiber. It is known that hemicellulose covers the cellulose microfibrilles in plant cell wall protects cellulose from enzymatic action. Removal of hemicellulose increases the enzymatic hydrolysis of cellulose [5, 6].

Hemicellulose can be hydrolyzed by hemicellulase, but pretreatment, for example, with dilute acid, which can remove the hemicellulose, is more preferable, so that there is no need to use additional enzyme preparations [7, 8, 9].

For the implementation of the maximum conversion of Miscanthus biomass preliminary mechanical processing of the dried biomass was performed on the chopper. After that, the chemical treatment process was carried out, varying such parameters as the duration of the process and the temperature.

A 1% solution of sulfuric acid was used as the reaction agent. To do this, 10 g of dry crushed biomass of the herbaceous plant Miscanthus sinensis "Ferner Osten" were placed in a round-bottomed two-necked flask equipped with a magnetic stirrer and a thermometer.

Water was added to the biomass in a ratio of 1:10 and 4 ml of sulfuric acid was added with stirring. Results of selection of technological parameters of chemical treatment of Miscanthus sinensis "Ferner Osten" biomass in with 1% sulfuric acid are presented in table 1.

Table 1. Results of selection of technological parameters of chemical treatment of Chinese miscanthus "Ferner Osten" biomass with 1% sulfuric acid.

| Process duration, min | Pulp yield, %, at the appropriate temperature, °C |
|----------------------|--------------------------------------------------|
|                      | 100                     | 110                     | 120                     | 130                     | 140                     |
| 10                   | 3.02±0.05               | 16.07±0.45              | 38.07±0.84              | 48.33±1.15              | 21.22±0.65              |
| 20                   | 3.09±0.05               | 19.05±0.54              | 41.11±0.93              | 53.08±1.29              | 27.07±0.83              |
| 30                   | 4.06±0.05               | 24.13±0.69              | 55.04±1.35              | 79.07±4.14              | 47.69±1.15              |
| 40                   | 4.13±0.05               | 27.08±0.78              | 63.33±1.60              | 76.05±2.61              | 40.17±1.23              |
The analysis of the results presented in Table 1 shows that the optimal temperature for pretreatment of miscanthus biomass with 1% sulfuric acid solution is 130 °C with a process duration of 30 minutes. With an increase in temperature exposure, the yield of cellulose decreases markedly. Thus, when the temperature rises to 140°C and the process lasts 30 minutes, the pulp yield decreases by 31.38 %. With an increase in the duration of chemical treatment from 30 to 60 minutes, the pulp yield decreases from 79.07% to 61.08%.

Using the MALDI-TOF spectrometry technique, the residues of oligosaccharides and lignin were determined in the hydrolysates of the Miscanthus sinensis "Ferner Osten". Analysis of the water fraction of the reaction mixture after the removal of cellulose showed the presence of low-molecular fragments of hydroxy-cinnamic alcohols and phenoxycoumaronic acids, which are part of lignin.

When processing lignin-cellulosic plant biomass with low-concentration acid solutions, the lignin structure changes, but this does not lead to delignification of the raw material.

The results of the study of the fractional composition of oligosaccharides in the acid hydrolysate of Miscanthus sinensis "Ferner Osten" are presented in table 2.

### Table 2. Results of the study of the chemical composition of the Miscanthus sinensis "Ferner Osten" hydrolysate after cellulose removal.

| Indicator       | Name     | % (of dry matter) |
|-----------------|----------|-------------------|
| Monosaccharides | Arabinose | 2.45±0.12         |
|                 | Xylose   | 24.4±1.22         |
|                 | Galactose| 0.5±0.03          |
|                 | Glucose  | 40.5±2.02         |
|                 | Uronic acids | 2.1±0.1    |

The results of the experiments show that as a result of Miscanthus sinensis "Ferner Osten" biomass pretreatment with 1% solution of sulfuric acid in the hydrolysate, monosaccharides accumulate, among which glucose and xylose predominate.

### 4. Conclusions

In this way, on the basis of the conducted studies, the parameters of the preliminary chemical treatment of Miscanthus sinensis "Ferner Osten" biomass with 1% solution of sulfuric acid were selected: temperature 130°C, duration 30 minutes. The pulp yield is 79.07%.

The advantage of this type of treatment is a significant increase in the reactivity to enzymatic hydrolysis of the substrate due to the removal of a significant amount of hemicelluloses with an increase of cellulose mass fraction in the substrate, an increase of available surface area, and changes in the structure of lignin. However, in the process of hemicelluloses chemical hydrolysis, there is an accumulation of by-products, which can cause the formation of inhibitory compounds.

In this regard, in further studies related to the processing of the biomass of genus miscanthus plants into carbohydrate-containing substrates for biotechnology, it is planned to use a two-stage pretreatment. The first stage is performed for the hydrolysis of hemicellulose and the second stage, in which the solid material from the first stage is again pre-treated under more stringent conditions. This approach allows to obtain higher yields of sugar than the one-stage pre-treatment, as well as to improve the lignin dissolution.
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