Dilute sulfurous acid pretreatment and subsequent enzymatic hydrolysis of corn cobs

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Abstract. The conditions (solid to liquid ratio, acid concentration, temperature, process duration) were selected for the effective high-temperature pretreatment of corn cobs with dilute sulfurous acid. The activity of the enzymatic complexes «CellicCTec2» and «CellicHTec2» of the company «Novozymes» in the process of corn cobs hydrolysis was studied. The maximum yield of reducing sugars was 45 % of absolutely dry substance at a concentration of the enzymatic preparation «CellicCTec2» 3 % (of corn cobs absolutely dry substance) and solid to liquid ratio 1:30. The total amount of extracted reducing substances is 66 % of the theoretical yield of carbohydrates in the corn cobs.

1. Introduction

Obtaining industrially important chemicals based on the chemical and microbiological transformation of secondary resources into food, feed, and technical products are one of the areas of modern biotechnology. A significant source of environmental pollution is waste plant materials, most of which are in the form of durable polymers such as cellulose, hemicellulose, and lignin. In addition, many types of agricultural waste contain little protein, vitamins, and minerals, so they are not used as feed in their pure form. Approximately 204 million dry metric tons of corn residues are returned to the ground as a waste by-product has enormous potential in use [1]. The corn market is growing rapidly every year. Corn cob is an important byproduct of the sweet corn processing industry and is available in sufficient quantity as 33.5 to 44.6 million metric tons alone in the USA, more than 10 million tons in China, 25 million tons in Russia [2]. Corn cob is a prominent agro-industrial waste which has a maximum content of pentosans (33–40 %) and a minimum content of lignin (15–17 %). Corn cob is a byproduct of starch and canning production, which creates favorable conditions for its processing. The output of the cob is 25–35 % of the cob mass.

Currently, several dozens of ways to convert plant raw materials have been developed. One of the effective methods is high-temperature hydrolysis with dilute mineral acids [3–5]. The preprocessing regimes and reactor design play a crucial role in obtaining the optimal amount of reducing sugars and the minimum number of inhibitors. Since the agro-industrial wastes have different physicochemical and structural characteristics, it is advisable to select individual methods of pretreatment based on the properties and characteristics of a particular type of raw material.

The aim of the work is to study the effect of various conditions of chemical and enzymatic hydrolysis of corn cobs on the yield of reducing sugars.
The study of these issues will increase the efficiency of the complex processing of cellulose-containing raw materials and reduce the environmental impact.

2. Materials and methods

Plant raw material was subjected to grinding, fractionation using a set of sieves to a particle size of not more than 1.5 mm and was dried at a constant temperature.

Corn cobs were pretreated in a laboratory setup [3, 4], which provides an opportunity to study the kinetics of high-temperature hydrolysis processes. The weight of the raw material load was 2.5 g. The duration of hydrolysis ranged from 3 to 30 minutes. Sulfurous acid was used as a hydrolyzing agent, which has relatively high catalytic activity and fewer problems associated with recovery and neutralization of excess acid in hydrolyzates. The acid concentration and temperatures were varied within 0–2.5 % by weight and 190–250 °C respectively.

The content of monosaccharides (arabinose, xylose, glucose, galactose, rhamnose) was performed using the HPLC method on a column «CarboPacPA-1» (4x250 mm, «Dionex», USA) using a pulsed amperometric PAD detector («Dionex»). The error in the implementation of experiments during chromatography was less than 10 % relative. The content of reducing substances (PB) in hydrolysates and fermentolizates was determined by the Bertrand copper-reduction method. The determination error was ± 0.53 % mass.

The enzyme preparation «CellicCTec2» was used as a biocatalyst during enzymatic hydrolysis. Major characteristics: cellulase CAS 9012-54-8 and xylanase CAS 37278-89-0; density – 1.15 g/ml; optimum temperature is 45–50 °C; optimum pH is 5.0–5.5; activity is 115.6 FPU/ml.

Enzymatic hydrolysis of solid residues was carried out in Erlenmeyer flasks with the addition of 40 μg of 1 % solution of tetracycline in 70 % ethanol per 50 ml of the reaction medium on an Elpan-357 rotary shaker (Poland) (rotation frequency of 150 rpm).

The concentration of the enzyme complex was 0.05 g of enzyme per 1 g of absolutely dry raw material. The process proceeded in sodium citrate buffer with a variation in pH from 3.0 to 6.0, a solid to liquid ratio of 1:30, and a temperature of 35.0–60.0 ± 2 °C. To prevent the growth of microorganisms, 40 μl of a 1 % solution of tetracycline in 70 % ethanol was added to each flask. The sampling interval for determining glucose ranged from 4 to 12 hours, the duration of hydrolysis was 120 hours. The pH control in the reaction medium of hydrolysis during the process was carried out. The use of citrate buffer made it possible to maintain the pH of the medium in the range of 4.6–5.1. The pH of enzymatic hydrolysates was determined using a Multitest ionomer IPL-513 pH meter.

3. Results and discussion

Chemical hydrolysis makes it possible to obtain simple sugars, which are used as the main carbon source for microbiological synthesis. Various factors may influence the efficiency and rate of hydrolysis with dilute mineral acids. A decrease in the solid to liquid ratio can lead to an increase in the concentration of sugars, savings in the hydrolyzing agent, and a decrease in the number of reagents for its further neutralization. On the other hand, at low solid to liquid ratio, due to a decrease in the degree of impregnation of biomass, a slowdown of the processing speed is possible. A study of the kinetics of pretreatment of corn cobs at solid to liquid ratio 1:3.5 and 1:5.8, at 200 °C and acid concentration of 2.5 % by mass. showed that changing the solid to liquid ratio does not have a significant effect on the speed of the process. It is advisable to use low values of the solid to liquid ratio, which contributes to obtaining higher concentrations of sugars.

Further studies of the pretreatment processes were carried out with a solid to liquid ratio of 1:3.5. Depolymerization of easily hydrolyzable polysaccharides to oligo- and monosaccharides can also occur in the absence of mineral acid during hydrothermal treatment of plant biomass. Autohydrolysis is the most environmentally friendly and reasonably efficient process among existing hydrolysis technologies [6]. In this case, the catalyst is organic acids, which are formed from acetyl and methoxyl groups contained in the composition of hemicelluloses and pectin raw materials. The study of the hydrolysing agent concentration effect on the efficiency of the pretreatment of corn cobs was carried
out at 200 °C and the duration of the process from 3 to 30 minutes. Kinetic dependences of the reducing substances yield in the concentration range of sulfuric acid 0–2.5 % of the mass. are shown in figure 1, 2.

According to the obtained data, pretreatment of the corn cobs in the presence of dilute sulfuric acid doubles the yield of total sugars and reduces the optimal duration of the process. The influence of temperature on the yield of reducing substances was investigated with temperature varying from 180 °C to 250 °C with an interval of 10 °C. Figure 3 shows the results obtained in the pretreatment of the corn cob from 3 to 12 minutes at sulfuric acid concentrations of 2.5 % by weight. An increase in temperature helps to reduce the pretreatment duration which is necessary to achieve the maximum yield of reducing substances. On the other hand, at high temperatures, the decomposition of the monosaccharides to furfural or hydroxymethylfurfural, which significantly reduces the concentration of sugar in liquid hydrolysates. In a series of experiments, the maximum conversion of raw materials exceeds 20 % of absolutely dry substance (table 1). Figure 4 presents the data on the conversion of raw materials obtained with various options of pretreatment (scale factor for the concentration of sulfur dioxide is 10).

![Figure 1](image1.png)  ![Figure 2](image2.png)

**Figure 1.** Kinetics of the reducing substances concentration depending on the concentration of the hydrolysing agent (reducing substances, % by mass).

**Figure 2.** Kinetics of the reducing substances concentration depending on the concentration of the hydrolysing agent (reducing substances, % of absolutely dry substance).

| Table 1. Optimal conditions for the production of reducing sugars in the liquid fraction of hydrolysates. |
|---------------------------------------------------------------|
| **Temperature, °C** | **Sulfuric acid concentration, % mass.** | **Hydrolysis duration, min** | **Corn cobs conversion, % of absolutely dry substance** |
|---------------------|------------------------------------------|----------------------------|-----------------------------------------------------|
| 190                 | 2.5                                      | 10                        | 20.9                                                |
| 200                 | 1.77                                     | 10                        | 20.3                                                |
| 200                 | 2.5                                      | 10                        | 20.2                                                |
| 220                 | 1.18                                     | 10                        | 20.2                                                |
| 240                 | 1.18                                     | 7                         | 23.8                                                |
Figure 3. The kinetics of the reducing substances yield at various temperatures of the pretreatment at 2.5 % mass. sulfuric acid concentration.

Figure 4. Conversion of corn cobs under various pretreatment conditions.

An analysis of the pretreatment conditions with a high yield of reducing substances shows that with increasing temperature there is a tendency to a decrease in the concentration of sulfuric acid. Processing the solid fraction remained after hydrolysis with water made it possible to additionally extract from 4.2 % to 5.6 % of reducing substances (of absolutely dry substance of raw materials). Thus, the pretreatment of corn cobs with dilute sulfuric acid at high temperatures is quite effective, but does not provide a complete conversion of the raw materials.

To determine the depth of pentosans and cellulose depolymerization, the monosaccharide composition of the obtained hydrolysates was studied by HPLC. Figure 5 presents the results of hydrolysates HPLC and the corresponding concentrations of reducing substances in the liquid fraction of the hydrolysate (concentration of H2SO3 1.18 % mass.; 200 °C). It was found that xylose, arabinose and glucose are predominating in the hydrolysis products, as well as traces of galactose and rhamnose. Kinetic curves indicate a quick conversion of pentosans. The cellulose-containing residue obtained after the formation of pentoses is an environmental problem, but at the same time, it can serve as a promising substrate for further enzymatic hydrolysis to obtain simple sugars. The study of enzymatic hydrolysis was carried out for corn cobs pretreated with 2.5 % of the mass. sulfuric acid at 200 °C for 10 minutes, and for corn cobs didn’t subject to pretreatment. As biocatalysts, the enzymatic complexes «CellicCTec2» and «CellicHTec2» of the «Novozymes» company were used. According to the manufacturer, the optimal conditions for the operation of the «CellicCTec2» and «CellicHTec2» enzyme complexes are 45–50 °С, pH 5–5.5 [7]. Investigation of the influence of pH on the degree of conversion of corn cobs by the «CellicCTec2» enzyme complex was carried out for the conditions: pH 3–7; 50 °C; duration 48 hours. As shown in figure 6, the optimum action of the complex lies within the limits specified by the manufacturer. Further studies of enzymatic hydrolysis were carried out at a temperature of 50 °C, pH 5.
The effect of the «CellicCTec2» dosage 1–6 % (of raw material absolutely dry substance) on the yield of the reducing substances (% wt.) is shown in figure 7, 8.

![Figure 7](image7.png)  ![Figure 8](image8.png)

**Figure 7.** The effect of «CellicCTec2» dosage and solid to the liquid ratio on the concentration of the reducing substance 1:30.  **Figure 8.** The effect of «CellicCTec2» dosage and solid to the liquid ratio on the concentration of the reducing substance 1:20.

The content of reducing substances in hydrolysates obtained using the «CellicHTec2» complex, as well as a mixture of the «CellicHTec2» and «CellicCTec2» is shown in figure 9. For comparison, the results of corn cobs not subjected to pretreatment hydrolysis are also presented. According to the data obtained, pretreatment with sulfuric acid has a significant effect on the degree of raw materials conversion during subsequent enzymatic hydrolysis, since it allows the removal of hemicelluloses, thereby increasing the available surface area of cellulose. Pretreatment (2.5 % wt. H2SO3; 200 °C; during 10 min.) allows to increasing the concentration of reducing substances in the fermentolizate using the «CellicCTec2» complex in 2.1 times, compared with the same data for corn cobs hydrolysis without pretreatment.

![Figure 9](image9.png)

**Figure 9.** The effect of enzyme complex «CellicHTec2» dosage (solid to liquid ratio 1:30).

The highest concentration of reducing substances in the hydrolysates is observed at 96 hours of hydrolysis at dosages of the enzyme complex of 3 and 6 % of the absolutely dry substance of raw materials. Since the differences in the values of the reducing substances, in this case are insignificant (1.9 and 2.2 % of the mass, respectively), it is possible to take the optimum concentration of 3 % of corn cob absolutely dry substance for the «CellicCTec2».

The low concentration of reducing substances when using the «CellicHTec2» complex is due to the fact that it has a pronounced xylanase activity, and most of the xylans were removed during the
preliminary treatment of corn cobs with sulfurous acid. The highest concentration of reducing substances was observed when using two enzyme complexes and amounted to 2.24 % mass. The decrease in the solid to liquid ratio during the enzymatic hydrolysis, as expected, leads to an increase in the concentration of reducing substances in the hydrolysates, but the degree of conversion of the corn cobs decreases, as shown in figure 10. The enzymes of cellobiohydrolase and endoglucanase in the process of hydrolysis are inhibited by the products of cellulose hydrolysis (glucose and cellobiose) [8].

Thus, a high concentration of glucose and cellobiose with a lower solid to liquid ratio causes a decrease in the activity of cellulolytic enzymes. Figure 11 shows the total yield of reducing substances during the complex treatment of corn cobs. The two-stage treatment of corn cobs, including pretreatment with sulfuric acid and enzymatic hydrolysis, made it possible to extract 45.0 % of reducing substances (% absolutely dry substance) in total, 20.2 % of them through pretreatment, 4.2 % – with additional processing of the solid fraction with water, 20.1 % – with enzymatic hydrolysis.

**Figure 10.** Influence of solid to liquid ratio on the reducing substances yield during enzymatic hydrolysis with pre-treatment («CellicCTec2», 3 % of corn cob absolutely dry substance).

**Figure 11.** Complete processing of corn cobs.

4. Conclusion
The optimal parameters of high-temperature pretreatment of corn cobs with sulfurous acid (2.5 % wt. H2SO3; 200 °C; during 10 min., solid to liquid ratio of 1:3.5) and subsequent enzymatic hydrolysis were determined. The maximum yield of reducing sugars was 45 % of absolutely dry substance at a concentration of the enzymatic preparation «CellicCTec2» 3 % (of corn cobs absolutely dry substance) and solid to liquid ratio 1:30. The total amount of extracted reducing substances is 66 % of the theoretical yield of carbohydrates in the corn cobs. According to the experimental data, hydrolysis of corn cobs with sulfurous acid and subsequent enzymatic hydrolysis can be recommended for obtaining of individual monosaccharides, as well as nutritional media. Such approach gives economic benefits in recycling of corn.

Acknowledgments
We would like to express our gratitude to the «Novozymes» company for kindly provided enzyme preparation «CellicCTec2» and «CellicHTec2» for this investigation.

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