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# Mechanical Activation of the Process of Enzymatic Saccharification of the Carbohydrates of Rice Husk

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## Abstract

Effect of the mechanical activation of rice husk on the progress of subsequent enzymatic hydrolysis was studied. It was established that mechanical treatment promotes an increase in specific surface area, destruction of the silica shell coating lignocellulose, and substantial amorphization of the crystal regions of cellulose. As a result, the degree of conversion of polymer carbohydrates into soluble saccharides increases practically by a factor of 13. So, mechanoenzymatic treatment of rice husk allows one to obtain carbohydrate-containing intermediate product that may be used for further microbiological processing.

**Key words:** mechanoenzymatic hydrolysis, rice husk, biofuel, carbohydrates

## INTRODUCTION

Many papers dealing with the structure and properties of rice husks were published during the recent decades [1–4]. It is known that rice husks are distinguished by high strength characteristics, chemical stability, low feeding power, low volume weight, high ash value. These properties are due to the high content of the inorganic component – silicon dioxide; its fraction can reach 20 %. In addition, rice husks contain cellulose 34–43 %, hemicellulose 5–37 %, and lignin 19–47 % [5]. Due to this fact, rice husks can be considered as a source of carbohydrates that can be used for microbiological preparation of biofuel, first of all bioethanol and biogas – the most intensively developing directions in alternative power engineering and green chemistry of recent years.

However, carbohydrates of initial rice husks are characterized by low conversion into soluble saccharides and therefore by low feeding power for the producers of biofuel components. This is connected with the fact that along with complicated supramolecular structure of the lignocarbohydrate complex of cell walls, rice husk particles are coated with a continuous silica membrane hindering direct enzymatic hydrolysis and preventing efficient pretreatment processes known in chemical technology that would be able to enhance the reactivity of polymeric carbohydrates.

In view of the high chemical stability due to the structure, rather rigid treatment procedures, mainly hydrolytic ones, are applied at present to process rice husks. For example, the effect of acid and alkaline hydrolysis on the destruction of rice husk structure (degradation of hemicellulose, lignin and partially silicon dioxide) and liberation of cellulose was illustrated in [6–9]. To increase the solubility of rice husk components, it is proposed to use preliminary carboxylation followed by fine grinding [10]. Modification of surface by carboxylation prevents aggregation of particles in the raw material because this treatment renders hydrophilic properties to the surface.

Disadvantages of the majority of proposed solutions are connected with preliminary treatment of rice husks in the liquid phase, complicated and multistage processes, the use of aggressive or dangerous reagents (concentrated alkalis, acids, organic solvents *etc.*), high pressure and temperature. Various reactions of saccharide degradation and lignin condensation leading to the formation of side products are possible under these conditions. In addition, the use of hydrolyzed rice husks as food or fodder supplements requires the necessary purity of the products because they can contain residual toxic reagents.

The goal of the present work was experimental investigation of the effect of mechanical activation on enzymatic hydrolysis of rice husks. Investigation of the enzymatic hydrolysis of non-food cellulose substrates which include rice husks, and the effect of mechanical activation on this process is important because the use of enzymes provides ecological safety of the utilization of wastes from plant raw material processing into products required in national economy.

## MATERIALS AND METHODS

### *Reagents and materials*

We used $D(+)$-glucose (99 %, Acros Organics), cellulolytic enzymatic preparation; potassium (III) ferricyanide of kh. ch. reagent grade (State standard GOST 4206–75), acetate buffer with pH 4.7, rice husks (Krasnodar Territory, Russia).

Cellulolytic preparation Celloviridin (Cellolux 2000 trade mark, manufactured at Sibbiofarm plant, Berdsk City, Russia) is a mixture of enzymes formed during drying the cultural liquid of *Trichoderma viride* fungi. The preparation is characterized by the hydrolase activity (activity units/g): xylanase 8000, cellulase 2000, β-glucanase up to 1500, glucoamilase 20.

### *Mechanical activation of rice husks*

Treatment was carried out in a roller mill RM-20 and a planetary centrifugal mill AGO-2 equipped with water cooling. The conditions of mechanical treatment in AGO-2: the frequency of reactor rotation 630 $min^{-1}$, acceleration of milling bodies 20$g$, time of mechanical activation 60–600 s. In the case of RM-20 mill, the frequency of rotation was 1000 $min^{-1}$, time of residence of the material under treatment in the zone of mechanical action was 40–60 s.

### *Enzymatic hydrolysis*

A weighted portion of initial or mechanically activated rise husks was suspended in the buffer solution with pH 4.7 and hydromodulus 5000. Then enzymatic complex Celloviridin was added in the amount of 2 % with respect to initial husks, and incubation at 50 °C under automatic agitation at the frequency of 120 $min^{-1}$ was performed. Each hour samples were taken to determine the amount of reducing carbohydrates.

### *Determination of the total amount of reducing carbohydrates*

To 1.0 mL of the solutions of carbohydrates with the concentration 30 to 150 mg/L, we added 3.0 mL of 0.06 % solution of $K_3[Fe(CN)_6]$, mixed and stored at 100 °C for 10 min. After cooling, the solutions were examined with a photometer at the wavelength of 420 nm. Standard solutions of glucose with the concentrations 30.0–150 mg/L were used to plot the calibration curves.

## RESULTS AND DISCUSSION

It is known that silicon in rice husks and in some plants is incorporated into hydrated amorphous silica or opal. Silica is concentrated near the external surfaces of plant tissues where it gets condensed with the formation of cellulose-silica shell [2]. This dense and rigid shell hinders both mechanical and chemical action on deeper lying tissues. In addition, cellulose microfibrillas in the plant material are immersed into the amorphous lignin-hemicellulose matrix. This ultrastructure of lignocellulose components promotes the stability of plant materiasl to high mechanical load. In this connection, the major task of mechanical treatment is removal of the silica membrane, lignocellulose grinding, amorphization of the crystal regions of cellulose, an increase in specific surface area of the raw material to a level suitable for interaction with enzymes.

The optimal conditions of the mechanochemical extraction of silicon dioxide from the plant material matrix were determined. For this purpose, initial rice husks were treated in mechanical activators with different types of action. It was established that grinding proceeds in the best way in the activators involving wear and crushing type of mechanical action which is realized in roller mills. In less efficient shock-and-shear mode of treatment characteristic of the mills of planetary type, no substantial changes of the tissues of this plant material occur within 1–10 min, the silica shell is conserved and prevents enzyme sorption on carbohydrate surface.

During the treatment of rice husks in RM-20 roller mill. the morphology of plant tissues gets substantially damaged (Fig. 1): particle size decreases substantially, silica membrane is removed, the particles of different tissues in the initial biomass get homogenated. The access of enzyme molecules to substrate surface appears to be simplified as a result of the observed processes, which must lead to higher rates of enzymatic hydrolysis.

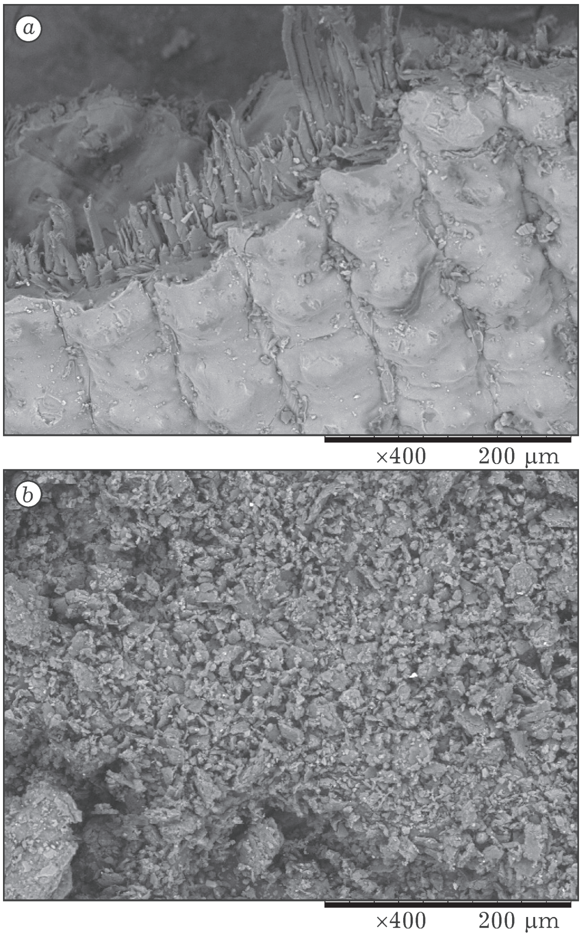


Fig. 1. Micrographs of the samples of initial (*a*) and mechanically activated (*b*) rice husks.

The results of experiments on the enzymatic hydrolysis of initial and activated husks confirmed our assumption. One can see in the data shown in Fig. 2 that the initial rate of enzymatic hydrolysis increases several times as a result of mechanical activation, and this may point to an increase in the surface suitable for enzyme sorption. We also observed an increase in the total yield of carbohydrates, which is the evidence of the transition of a substantial part of polymer carbohydrates into the form suitable for hydrolysis.

For separate interpretation of processes that take place during enzymatic hydrolysis, initial and hydrolyzed samples were studied with the help of electron microscopy (contribution from tissue morphology), X-ray diffraction (contribution from the crystal structure of cellulose) and thermal desorption of argon (contribution from specific surface area).

According to the data of electron microscopy (Fig. 3), mechanically activated sample gets hydrolyzed to a higher extent than initial plant raw material. The micrographs of initial rice husks show only slight changes of the surface coated with silica shell. Only small areas with

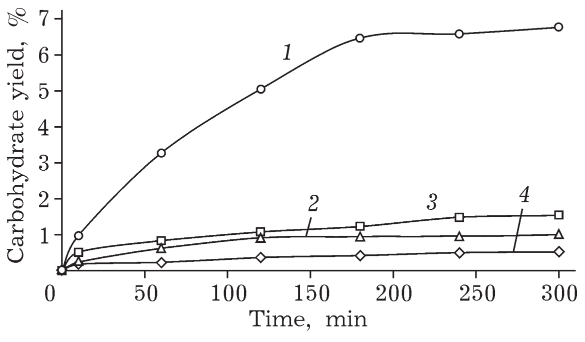


Fig. 2. Yield of water-soluble carbohydrates: *1* – water extraction of initial rice husks (without the addition of enzymes), *2* – enzymatic hydrolysis of initial rice husks, *3* – water extraction of husks after mechanical activation, *4* – enzymatic hydrolysis of rice husks after mechanical activation.

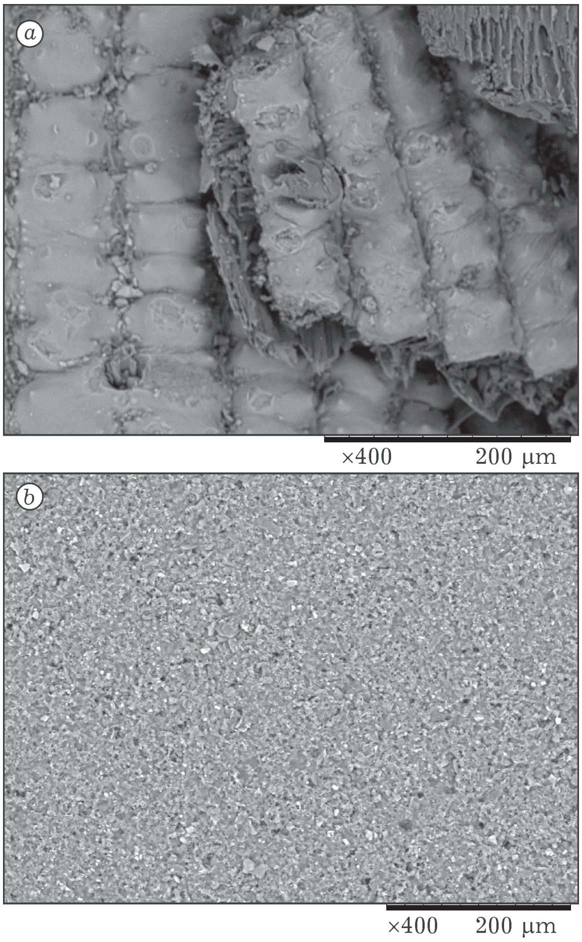


Fig. 3. Micrographs of the samples of initial (*a*) and mechanically activated (*b*) rice husks after enzymatic hydrolysis.

destroyed regions are observed, small hydrolyzed particles of biomass are accumulated in tissue pits, however, silicon dioxide layer still protects the major part of lignocellulose from external action. At the same time, hydrolyzed particles of activated husks decrease in size substantially, their structure changes, they become smoother and more rounded, which is the evidence of enzymatic hydrolysis proceeding uniformly over the whole surface.

To determine the contribution from the surface into an increase in the reactivity of the raw material, we measured the specific surface are using the method of thermal desorption of argon. One can see in the data presented in Table 1 that the specific surface area increase as a result of mechanical activation from 0.43 to 2.26 $m^2/g$. It was shown in [11, 12] that among other processes taking place during mechanical activation, an increase in surface area makes the largest contribution into an increase in the initial hydrolysis rates.

In addition, it is known [12] that the total yield of soluble carbohydrates is to a higher extent affected by the ratio of crystal and amorphous fractions of cellulose in the material. This parameter can be estimated using Segal method [13] relying on the data on X-ray diffraction.

It was shown (see Table 1) that mechanical activation of rice husks leads to substantial amorphization of cellulose promoting an increase in total conversion degree because enzymatic preparations hydrolyze amorphous nonstructured substrates easier than crystal ones.

It is necessary to note that the index of crystallinity of the samples increases after hydrolysis. First, this may be connected with the fact that hydrolysis of cellulose proceeds mainly in amorphous regions. As a consequence, the ratio of the crystal part of amorphous part increases as hydrolysis proceeds. Second, an increase in crystallinity degree of cellulose may be caused by the known effect of amorphous cellulose recrystallization back into the crystal form during wetting with water. However, as in our case the substantial part of amorphous cellulose is hydrolyzed and cannot participate in recrystallization, an increase in crystallinity index is mostly due to the dissolution of amorphous parts rather than cellulose recrystallization.

So, we described the processes that take part during mechanical activation of rice husks and

TABLE 1

Crystallinity index and specific surface are for rice husks treated under different conditions

| Rice husk samples | Crystallinity index, % | Specific surface, $m^2/g$ |
|---|---|---|
| Initial | 36.6 | 0.43 |
| After enzymatic hydrolysis | 39.1 | 0.67 |
| Mechanically activated | 12.8 | 2.26 |
| After enzymatic hydrolysis | 22.7 | 2.38 |

subsequent enzymatic hydrolysis. It can be concluded that mechanical activation leads to an increase in the reactivity of polymer carbohydrates incorporated into the raw material, due to disordering of the structure of tissues and removal of screening silica membrane, a decrease in particle size and an increase in surface area, as well as amorphization of the crystal regions of cellulose. Enzymatic hydrolysis of activated rice husks allows one to hydrolyze 6.7 % of carbohydrates (of the total amass of raw material) during 5 h. For comparison, initial rice husks are practically not hydrolyzed with enzymes, and the yield of carbohydrates under the same conditions is about 0.5 %.

## CONCLUSIONS

1. For efficient mechanical activation of rice husks, the most suitable grinding devices are those with wear and crushing type of action (for example, roller mills). Mechanical treatment in the devices of this kind allows one to enhance the reactivity of the raw material.

2. In addition to a decrease in particle size and removal of silica membrane, mechanical activation allows one to increase the available surface area of substrate substantially and to decrease the degree of cellulose crystallinity. These effects bring noticeable contributions into an increase in the rates of hydrolysis and total yield of soluble carbohydrates.

3. The semi-product obtained as a result of mechanical activation and enzymatic hydrolysis is suitable for further microbiological processing into bioethanol and biogas.

4. The application of mechanoenzymatic hydrolysis allows one to increase substantially the yield of water-soluble compounds and decreases the screening effect of silica membrane, so the nutritional value of rice husks increases. Thus, hydrolyzed rice husks can be considered as a cheap component of fodder for cattle and poultry breeding.

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