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ENZYMATIC PRODUCTION OF GLUCOSE SYRUPS FROM CELLULOSE-CONTAINING PLANT WASTES

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A method has been developed for obtaining glucose syrups from cellulose-containing plant wastes under the action of a complex of cellulases from a hybrid strain of fungus. Purified glucose syrups containing 19-20 mg/ml of reducing sugars have been obtained.

The organization of the industrial production of saccharine substances, especially glucose, is a serious problem of Uzbekistan's food industry. At the present time, great attention is being devoted to the use of highly productive strains that have been specially chosen, selectively bred, or sometimes even constructed by gene-cell engineering which effect the supersynthesis of enzymes — i.e., produce them in amounts far exceeding the needs of the cells themselves.

The search for active cellulase producers and the development of biotechnological processes for the enzymatic hydrolysis of cellulose-containing biological materials are promising in this connection [1]. Apparently, the only route to a marked increase in the production of glucose and other sugars is their enzymatic formation from cellulose-containing wastes. Our task was to obtain glucose syrups from various cellulose-containing wastes using cellulolytic enzymes from a hybrid strain of fungus.

By fusing protoplasts of two strains of fungus of the *Aspergillus* genus (*A. terreus* and *A. ustus*), using the cell engineering method, we have obtained hybrids producing a more active cellulase [2]. The optimum conditions for the cultivation of the hybrid in a fermenter are: temperature 40°C, pH of the nutrient medium 5.5, with aeration by the passage of 1 liter of sterile air through 1 liter of medium per minute, stirring at the rate of 300-320 rpm, and the use as seed material of 2-3% of a 5-10-day culture grown under deep conditions.

The biosynthesis of cellulase by the fungus took place in the logarithmic phase of the growth of the population and reached a maximum by the beginning of the stationary phase. The maximum specific growth rate was 0.61 h^{-1}. In the stationary phase, the cellulose activity of the fungal culture liquid did not change appreciably, but it fell gradually during the dying-off phase.

As is known, cellulase is a complex enzyme and exhibits a number of cellulolytic activities according to the substrate upon which it acts. Our study has showed that the enzyme preparation of the hybrid strain contained a complete complex of cellulases and xylanases. The compositions of the cellulases were the same in the enzyme preparation and in the culture liquid (Table 1). The far-reaching hydrolysis of a cellulose substrate to glucose is apparently explained not only by a high activity, but also by the presence of a complete set of cellulases and an active xylanase. Thanks to the active xylanase, an intensive action is exerted on maize cobs, which are rich in xylan.

It must be mentioned that, because of the inhibition of activity by organic solvents, the activities of the individual enzymes of the complex from the culture liquid were twice as great, and their specific activity calculated to 1 mg of protein 10 times as great, as the activities of the enzyme preparation obtained by precipitation with ethanol.

We carried out the enzymatic saccharification of several types of the most readily available cellulose-containing raw material: cottonplant leaves, guza-paya [cottonplant stems], and rice straw and husks, and also wheat straw and maize cobs.

The best results in saccharification by the enzymatic method were obtained on the complex preliminary treatment of the cellulose-containing wastes by a mechanical method, with steam, and with alkali. This led to a 2- to 3-fold rise in the degree of enzymatic saccharification (Table 2). When the conditions were varied, the characteristics of saccharification on a

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TABLE 1. Activities of Individual Components of the Cellulase Complex of the Hybrid

Component	Culture liquid		The G10X preparation	
	total activity, units/ml c.l.	specific activity, units/mg protein	total activity, units/ml c.l.	specific activity, units/mg protein
C_1-enzyme	5.6	64.5	2.6	18.2
C_x-enzyme Na-CMC-ase	21.6	712.8	10.1	392.4
Cellobiase	7.5	247.5	5.2	58.3
Xylanase	78.2	250.2	8.1	56.7

TABLE 2. Saccharification Capacity of the Hybrid as a Function of the Pretreatment of the Cellulose-Containing Raw Material

Substrate	Reducing sugars, mg/ml hydrolysates		
	steaming	treatment with alkali	treatment with acid
Guza-paya	2.2	3.7	3.32
Cottonplant leaves	5.4	6.7	4.2
Rice husks	2.7	4.7	4.0
Rice straw	5.62	7.4	6.1
Maize cobs	8.5	9.1	6.4
Reeds	8.0	9.1	6.1
Wheat straw	3.9	7.6	4.2

TABLE 3. Characteristics of the Glucose Syrups Obtained by the Enzymatic Hydrolysis of Maize Cobs in Various Stages of Purification

Index	Syrup		
	initial	passed through the cation exchange resin	passed through the anion-exchange resin
Amount of sugars, mg/ml	19.4	19.7	20.2
Color	Dark brown	Light brown	Light yellow
Amount of dry matter, %	30.0	24.6	23.8
Amount of protein, %	0.07	–	–
Ash content, %	1.34	0.75	0.25
pH	4.1	2.45	5.1
Color index, arb. units	2.5	0.56	0.084
Effect of bleaching, %	–	75.3	96.4

column reactor and with stirring were preserved, in the main. The maize cobs and wheat straw underwent the best saccharification (3-4 mg/ml of reducing sugars), while the efficacy of saccharification in the column reactor was 4-6 orders of magnitude [sic] higher than in a reactor with periodic stirring.

On chromatograms of the hydrolysates obtained in the column reactor the most intensely colored spots corresponded to glucose. The maize cob hydrolysates contained galactose, xylose, and arabinose, in addition to glucose, while in the case of hydrolysis with stirring arabinose was absent.

To purify the hydrolysates, we developed a scheme including stagewise passage through activated carbon, a cation-exchange resin (KU-10), and an anion-exchange resin (AN-31). As a result we obtained clear solutions containing various amounts of reducing sugars (19-20 mg/ml).

The main indices (color, ash content, and amounts of protein and dry matter) of the glucose syrups in various stages of purification are given in Table 3.

The purified hydrolysates contained no proteins or pigments and the dry matter content had fallen by 6.2% and the ash content by a factor of 5.3. In the final account, the effect of bleaching the solution reached 96.4%.

Thus, an enzymatic method of obtaining glucose syrups promising for use in several sectors of the medical and food industries has been developed.

EXPERIMENTAL

The hybrid strain was grown under the conditions described in [2].

Cellulase activity was determined in a filtrate of the culture liquid obtained on the growth of the hybrid in Mandels' medium [3] on a shaking machine (230-250 rpm) at 38-40°C for 100-120 h.

The hybrid cellulase preparation was obtained from the culture liquid by precipitation with ethanol in a ratio of 1:3. The cellulolytic activity of the initial culture liquid was 5.6 units/ml, and the yield of preparation from 1 liter of culture liquid was 2.8 g. Cellulase activities were determined on cotton fiber (the C_1 enzyme) and on Na-CMC (the C_x enzyme), cellobiase activity on a 0.05% solution of cellobiose, and xylanase activity on a 1% solution of xylan, from the formation of reducing sugars determined by the Somodgyi–Nelson method [4, 5].

As the unit of cellulase activity we took the amount of enzyme by the action of which on the substrate 1 mg of glucose was formed under the conditions of the experiment in 1 h.

The raw material, mechanically ground in a ball mill (particle size 0.25 mm), was treated with superheated steam at an elevated pressure (1 atm.) in an autoclave in a 1% solution of NaOH or H_2SO_4 at a liquor ratio of 1:10 for 20 min. Saccharifying capacity was studied by acting on the treated cellulose-containing material (liquor ratio 1:20) at 50°C, pH 4.5 in a column reactor or in a stirring reactor for 3-6 h.

Samples (4 g) of mechanically treated plant wastes were charged into a thermostated column (1.5 × 3.5 cm) through the jacket of which was passed hot water to maintain a temperature of 50°C — the optimum for saccharification. The culture liquid with an activity of 1.8-2.0 units/ml that had been acidified to pH 5.5 with acetic acid was passed through the column with the cellulose-containing substrate, and the amount of reducing substances in the hydrolysates was analyzed every 60 min. In a control, distilled water was used in place of the culture liquid.

In the stirring variant, hydrolysis was conducted in 250-ml Erlenmeyer flasks. Each was charged with 2 g of comminuted plant wastes and then 10 ml of a 1% solution of NaOH was added, and the mixture was sterilized and was acidified to pH 5.5 with 2-3 drops of concentrated acetic acid. For hydrolysis we used 30 ml of culture liquid (with cellulase activity), and the incubation mixture was kept at 50°C with constant stirring in a shaking machine for 3 h.

The amounts of sugars in the hydrolysates were determined by the Somodgyi–Nelson method and by paper chromatography [6]. For calculating specific activities, the amounts of protein in the culture liquids and enzyme preparations were determined by the Lowry method [7].

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