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INVESTIGATION OF HYDROLYTIC ACTIVITY OF ENZYME PRODUCTS DURING HYDROLYSIS OF CELLULOSE IN COTTON FIBER

Soatov Askarali Mengdovulovich
Tashkent State Technical University
Assistant of the Department of Biotechnology
Email address: asqarsoatov@gmail.com
Tel: 91 234 99 44

Nazarov Kamolzhon Karimovich
Tashkent State Technical University
BFN душ. Head of the Department of Biotechnology,
Email address: nazarovkomoljon@gmail.com
Tel: 91 165 96 00

Annotation
The cellulase complex of a number of bacterial and fungal enzymes is usually used for the enzymatic hydrolysis of cellulose-containing raw materials on an industrial scale. Currently, cellulase enzyme complexes are increasingly used in the textile, pulp and paper, food, feed, and other industries. In our studies, we studied the possibility of splitting cellulose (in the release of cotton fiber cellulose using three types of enzyme complexes.

Keywords: cellulose, cellulose, celloviridin, pectofotedin, pectinase.

Introduction
Currently, complex cellulose enzymes are increasingly used in the textile, pulp and paper, food and other industries. The activity of industrial enzyme preparations is one of the important criteria for comparing the effectiveness of enzyme preparations for the bioconversion of cellulose-containing raw materials. The fact is that each enzyme preparation obtained by exposure to a number of specific substrates and the final product of cellobiose bioconversion with the formation of glucose has a specific activity [1].
In our studies, we studied the possibility of converting three enzyme preparations for the enzymatic hydrolysis of cotton cellulose into different amounts of the same substrate: celloviridin GZx, pectofoedin GZx, and pectinase 500.

Literature Review
Recently, the processes of bioconversion of renewable lignocellulosic raw materials into various products (alcohols, organic acids, amino acids, etc.) have reached an

industrial scale [2, 3]. The main component of such raw materials is cellulose; its content in the initial material can reach 40–50% and higher [4]. The stage of enzymatic hydrolysis of cellulose to glucose in these processes is the key and most laborious. Effective hydrolysis of cellulose requires the presence of a cellulase complex balanced in composition, including endoglucanases (EG) and cellobiohydrolases (CBH), which cleave the polymer substrate to cellobiose and other oligosaccharides, as well as □-glucosidase (BG), catalyzing the hydrolysis of oligosaccharides to glucose [5]. At present, the search for new, more active cellulases remains an urgent task. Intensive developments are also underway to increase the specific activity of enzymes and improve their other properties by protein engineering methods [6–7]. To optimize the composition of the cellulase complex, approaches based on the creation of model mixtures of purified enzymes and testing of their hydrolytic ability with respect to various cellulose-containing substrates are often used [7–10].

Important features of cellulase enzyme preparations are their different activity with respect to different substrates (cellulose samples), the specificity of the enzyme complex component, and the ability to have a hydrolytic effect during the bioconversion of wastes containing various types of cellulose. raw materials.

Various types of raw materials containing cellulose are usually determined by "several factors", i.e. a certain concentration of the substrate and enzyme preparation, ambient temperature, composition and pH level, and the time of the hydrolysis process. To determine the optimal level of these factors, the effect of different pH-environment, temperature and quantitative ratio of the enzyme preparation on the implementation of cellulose hydrolysis as a result of the action of the cellulose enzyme complex on a sample of cotton fiber cellulose (substrate) was studied.

Research Methodology

In this work, the following technical enzyme preparations were used for the enzymatic saccharification of cellulose-containing materials.

Cellulase preparation from *Trichoderma viride* - celloviridia GZx produced by the Privozhsky Biochemical Plant with an activity of 83 U/g. according to C activity (passport data), 65 units / g. by endoglucanase and units/g. for cellobiase (actual data).

Pectinase preparation *Aspergillus foetidus* - pectofoedia GZx produced by the Privozhsky Biochemical Plant with *pectolytic* activity of 90 units/g, PcC/g (passport data) and 37 units/g. for cellobiase (actual data).

Pectinase preparation from *Aspergillus foetidus* "*Pectinase*" 500 produced by the Moscow Pilot Plant of Enzyme Preparations, with pectolytic activity of 800 U/g (passport data) and cellobiase activity of 3200 U/g (actual data).

Using glucose-peroxidase (determination of the amount of formation of glucose during enzymatic hydrolysis of cellulose) and Shomogyi-Nelson (determination of the amount of formation of reducing sugars during enzymatic hydrolysis of cellulose) methods, the activity of three polyenzymatic preparations of cellulose in relation to samples of cotton fiber of the F-108 variety was studied to determine the amount of glucose and reducing sugars resulting from the bioconversion of cellulose [11].

Analysis and Results

For the industrial enzymatic hydrolysis of cellulose-containing raw materials, cellulase complexes of enzymes from a number of bacteria and fungi are usually used [12]. Cellulase complex enzymes include endo-β-1,4 glucanases (EC 3.2.1.4), exocellobiohydrolases (EC 3.2.1.91), and β-glucasidases (EC 3.2.1.21) [12; 13]. Recently, cellulase complexes of enzymes have been increasingly used in the textile, pulp and paper, food, and other industries.

In our work, we studied the possibility of using three commercial enzymatic complexes for the enzymatic hydrolysis of cotton cellulose: celloviridin G3x, pectofoedin G3x, and pectinase 500.

Cellulase preparation from *Trichoderma viride* - celloviridin GZx produced by the Privozhsky biochemical plant with an activity of 83 U/g. according to C activity, 65 units/g. by endoglucanase and 3000 units/g. by cellobiase.

Pectinase preparation *Aspergillus foetidus* - pectofoedin GZh produced by the Privozhsky biochemical plant with pectolytic activity of 90 units/g. and 37 units / gr. by cellobiase.

Pectinase preparation from *Aspergillus foetidus* "Pectinase" 500 produced by the Moscow Experimental Plant of Enzyme Preparations, with a pectolytic activity of 800 U/g and a cellobiase activity of 3200 U/g (actual data).

To determine the activity of cellulases, there are practically no generally accepted methods that are standard in terms of conditions and method of calculation. For each specific substrate, its own methodology is selected and modified, based on certain general principles. In this regard, the first task of our work was the choice of a method for determining the activity of cellulase enzyme complexes in the hydrolysis of cotton fibers.

Methods for determining the total cellulase activity can be divided into two groups. The first includes tests using pure forms of cellulose as a substrate. This numerous group of methods includes methods for determining the activity of cellulases by the hydrolysis of filter paper (FPA), carboxymethyl cellulose (the so-called CMC-ase activity), cotton (the so-called C1-activity), microcrystalline cellulose (avicelase activity), colored cellulose, amorphous cellulose. In recent years, as the main method for determining cellulase activity, the method of assessment by hydrolysis of filter paper, which is recommended by the International IUPAC Commission on Biotechnology as the main standard test for cellulase activity, has been most often used.

The second group includes methods for determining the activity of cellulases in relation to certain types of cellulose-containing raw materials: cellolignin, straw, sawdust, cotton waste, waste paper, etc. In this case, a significant role in cellulase preparations is played by the presence of non-cellulose impurities in the composition of the substrate, as well as the method of pretreatment of raw materials, the presence in the composition of the multienzyme complex and other enzymes (ligninases, hemicelluloses, proteinases, pectinases, etc.), which usually do not are detected by determining the activity of enzyme preparations using standard cellulase tests. Therefore, the use of the latest methods for determining cellulase activity may be of little information in terms of the activity of the complex to a particular material. In this case, the only reliable method is to test the enzyme preparation using the substrate that is the target for the subsequent enzymatic saccharification.

Common to both groups of methods is the method for determining activity, which consists in the registration of soluble products of hydrolysis: glucose, cellobiose, total reducing sugars, colored soluble products). Existing methods for the qualitative and quantitative determination of reducing sugars (RS) and glucose make it possible to determine the activity of the entire cellulase complex using various soluble and insoluble substrates.

In mass testing of enzyme preparations or varieties of cellulose-containing raw materials, activity is usually determined by a “single point”, i.e. at certain concentrations of the substrate and enzyme preparation, temperature, composition and pH of the medium, the time of hydrolysis. To establish these optimal characteristics, a preliminary study of hydrolysis by this enzyme complex on this substrate is carried out. In addition, when determining the activity of an enzyme preparation using insoluble cellulose, such as cotton fiber, it is necessary to take into account the possible non-linear dependence of the initial rate of cellulose hydrolysis on the concentration of the substrate.

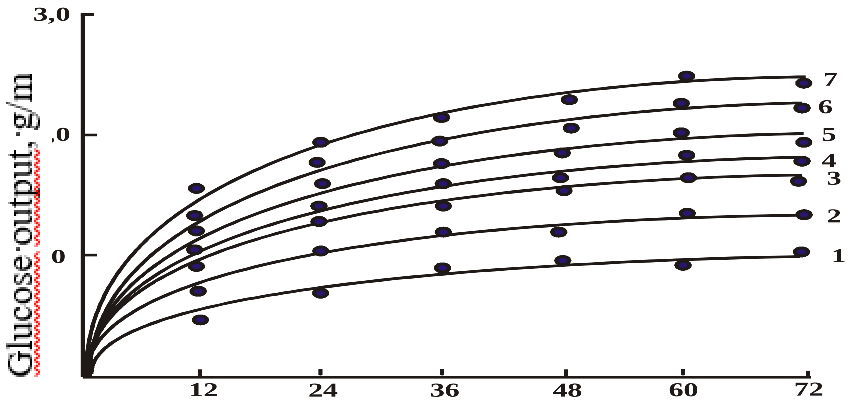
Figures 1 and 2 show the results of these experiments, from which it can be seen that the mixture of celloveridin G3x and pectofoetidin G3x in a ratio of 3:1 turned out to be the most active. We used this mixture in all our further experiments.

To select the optimal hydrolysis parameters, hydrolysis experiments were carried out at various pH and temperature. As can be seen from fig. 3. hydrolysis proceeded most rapidly at medium pH = 5.0 and at a temperature of 55–60 °C.

The initial rate of enzymatic hydrolysis significantly depends on the degree of adsorption of enzymes on the substrate. In some cases, the initial reaction rate can be increased by adding surface-active substances (surfactants), which increase the wettability of cellulose, and, accordingly, its bioavailability for enzymes. We conducted a study of the initial rate of the enzymatic reaction in terms of the yield of glucose with the addition of various concentrations of the surfactant Triton X-100. The results are shown in Figure 4. It can be seen from the figure that 0.5% Triton X-100 increases the initial rate of enzymatic hydrolysis most effectively. Higher concentrations of Triton X-100 have no effect.

Thus, in this section, the following were determined: the optimal 3:1 ratio of the composition of celloveridin G3x and pectofoetidin G3x enzyme preparations, optimal pH=5.0 and temperature 55°C, 0.5% concentration of Triton X-100.

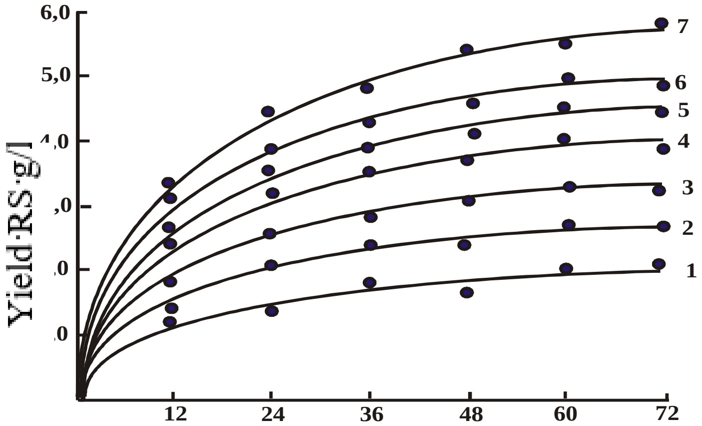
These results were used to study the characteristics of the enzymatic hydrolysis of fibers from various cotton lines.



Hydrolysis time, hours

Fig. 1. Glucose yield (g/l) during the enzymatic hydrolysis of cotton fibers of the F-108 variety using various enzyme preparations: celloviridin G3x, pectofoedin G3x and pectinase 500 in various ratios.

1 - pectinase 500, 2 - pectofoetidin G3x, 3 - celloveridin G3x,
4 - celloveridin GZx and pectofoetidin GZx in the ratio 1:1.,
5 - celloveridin GZx and pectofoetidin GZx in a ratio of 2: 1.,
6 - celloveridin GZx and pectofoetidin GZx in a ratio of 4: 1.,
7 - celloveridin G3x and pectofoetidin G3x in a ratio of 3:1.



Hydrolysis time, hours

Fig. 2. The yield of reducing sugars (RS) (g/l) during the enzymatic hydrolysis of cotton fibers of the F-108 variety using various enzyme preparations: celloviridin G3x, pectofoedin G3x and pectinase 500 in various ratios.

1 - pectinase 500, 2 - pectofoetidin G3x, 3 - celloveridin G3x,
4 - celloveridin GZx and pectofoetidin GZx in the ratio 1:1.,
5 - celloveridin GZx and pectofoetidin GZx in a ratio of 2: 1.,
6 - celloveridin GZx and pectofoetidin GZx in a ratio of 4: 1.,
7 - celloveridin G3x and pectofoetidin G3x in a ratio of 3:1.

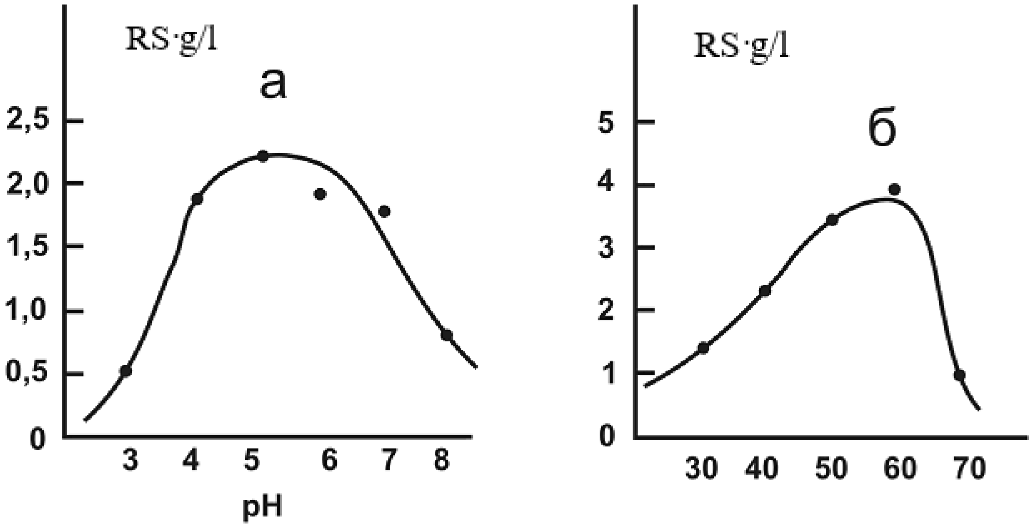


Fig. 3. Dependence of cellulase activity on the pH of the medium (a) and temperature (b).

A mixture of celloveridin G3x and pectofoetidin G3x enzymes in a ratio of 3:1. The substrate is cotton fiber grade F-108. Hydrolysis time 12 hours.

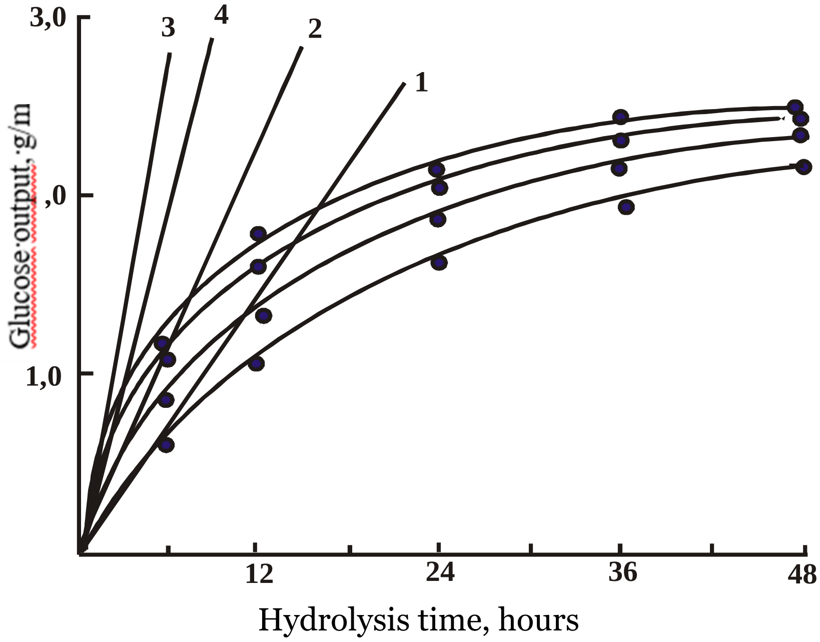


Fig 4. Effect of surfactant concentration on the initial rate of hydrolysis

The rate of hydrolysis of cotton fiber grade F-108 with a mixture of celloviridin G3x and pectofoedin G3x enzymes in a ratio of 3:1 in the absence of surfactants (1), in the presence of 0.1% Triton X-100 (2), in the presence of 0.5% Triton X- 100 and in the presence of 1.0% Triton X-100.

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