JOURNAL OF CRITICAL REVIEWS

ISSN- 2394-5125VOL 7, ISSUE 15, 2020

# APPLICATIONS OF A COMPOSITE ENZYME PREPARATION FOR STRIPPING COTTON SEEDS

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Received: 16 March 2020 Revised and Accepted: 17 June 2020

**Abstract**
The article presents the results of research on enzymatic hydrolysis fibrous parts of cotton (cotton seed lint). A fairly high concentration of semen with cellulase enzyme peparate is shown. The paper also found that the ability to hydrolyze the cotton seed pod can serve as a criterion for evaluating the quality of seeds, the degree of their contamination and suitability for sowing. This suggests the possibility of developing new promising methods for evaluating the quality of seeds in seed production.
**Keywords:**Stripping cotton seeds, enzymatic hydrolysis, endogluconase, cellobiohydrolase, exoglucosidase

## 1. Introduction

Applications of a composite enzyme preparation for Stripping cotton seeds.
1.Existing methods of mechanical and aerodynamic Stripping of cotton seeds make it possible to get up to 200 thousand tons of feed per year. The idea arose to develop a new method of enzymatic hydrolysis Stripping of cotton seeds, in which the product was glucose.

## 2. Enzymatic Stripping of cotton seeds.

Studies conducted on the enzymatic hydrolysis of the fibrous residue after mechanical Stripping of seeds have shown that delint has a higher reactivity compared to lint and cotton fiber . In this regard, the possibility of conducting enzymatic hydrolysis of the fibrous part of seeds without preliminary removal of the fiber was tested. Recently, the processes of bioconversion of renewable lignocellulose raw materials into various products (alcohols, organic acids, amino acids, etc.) have reached an industrial scale [1, 2]. the Main component of such raw materials is cellulose, its content in the source material can reach 40-50% or higher [3].The stage of enzymatic hydrolysis of cellulose to glucose in these processes is the key and most time-consuming. For effective hydrolysis of cellulose, it is necessary to have a balanced composition of the cellulase complex, including endoglucanases (EG) and cellobiohydrolases (CBG), which cleave the polymer substrate to cellobiose and other oligosaccharides, as well as □-glucosidases (BG), which catalyze the hydrolysis of oligosaccharides to glucose [4]. Currently, the search for new, more active cellulases remains an urgent task. Intensive research is also underway to increase the specific activity of enzymes and improve their other properties using protein engineering methods [5-7]. To optimize the composition of the cellulase complex, approaches based on creating model mixtures of purified enzymes and testing their hydrolytic ability in relation to various cellulose - containing substrates are often used [7-9].

## 3. Materials and methods

For industrial enzymatic hydrolysis of cellulose-containing raw materials, cellulose complexes of enzymes from a number of bacteria and fungi are usually used. The enzymes of the cellulase complex include endo-β-1,4 glucanase exocellobiohydrolase and β-glucasidase. Recently, cellulase complexes of enzymes are increasingly used in 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: celloviridine Gzx, pectofoedine Gzx and pectinase 500.
Cellulose preparation from Trichoderma viride-celloviridine GZH produced by the Volga biochemical plant with an activity of 83 units / g. for C activity, 65 units / g. for endoglucanase and 3000 units / g. for cellobiase
Pectinase preparation Aspergillus foetidus-pectofoetidine GZH produced by the Volga biochemical plant with pectolytic activity of 90 units/g. and 37 units/g .for cellobiase.
Pectinase preparation from Aspergillus foetidus "Pectinase" 500 produced by the Moscow experimental industrial plant of enzyme preparations, with pectolytic activity of 800 units/g and cellobiase activity of 3200 units / g (actual data).
Methods for determining total cellulase activity can be divided into two groups. The first category includes tests using pure forms of cellulose as a substrate. This large group of methods includes methods for determining the activity of cellulases by hydrolysis of filter paper( FPA), carboxymethylcellulose (so-called CMC-azic activity), cotton (so-called C1-activity), microcrystalline cellulose (avicelase activity), colored cellulose, amorphous cellulose.
In recent years, the most commonly used method for determining cellulase activity is the hydrolysis of filter paper, which is recommended by the IUPAC international Commission on biotechnology as the main standard test for cellulase activity.

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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 substrate, as well as the method of pre-processing of raw materials, the presence of a multi-enzyme complex and other enzymes (ligninases, hemicelluloses, proteinases, pectinases, etc.), which are usually not detected when determining the activity of enzyme preparations, when using standard cellulase tests.
Therefore, the use of the latest methods for determining the cellulose activity may be uninformative in terms of the activity of the complex to a specific material. In this case, the only reliable method is to test the enzyme preparation using the substrate that is the target for subsequent enzymatic saccharification.
Common to both groups of methods is a method for determining activity, which consists in registering soluble hydrolysis products: glucose, cellobiose, total reducing sugars, colored soluble products). Existing methods of qualitative and quantitative determination of reducing sugars (BC) and glucose allow determining the activity of the entire cellulase complex using various soluble and insoluble substrates.
We conducted a comparative determination of the activity of three poly-enzyme preparations in relation to cotton fiber of cotton grade f-108, which was determined by the formation of glucose and reducing sugars using the Shomodi – Nelson method . As a unit of activity, the amount of the enzyme was taken, which leads to the formation of 1 μm of BC in 1 min from the corresponding substrate.

For the selection of optimal parameters of the hydrolysis experiments were carried out on hydrolysis at different pH and temperature.

The initial rate of enzymatic hydrolysis depends significantly on the degree of adsorption of enzymes on the substrate.
In some cases, the initial reaction rate can be increased by adding surfactants( surfactants), which increase the wettability of cellulose, and consequently its bioavailability for enzymes. We conducted a study of the initial rate of the enzymatic reaction for glucose output when adding different concentrations of TRITON x-100 surfactants.
Thus, in this section were determined: the optimal ratio of 3:1 composition of enzyme preparations of celloveridine GZH and pectofoetidine GZH, optimal pH=5.0 and temperature 55□C, 0.5% concentration of Triton X-100.
These results were used to study the features of enzymatic hydrolysis of the fibrous part of the cotton seed (seed down).

**4. Finding**

Important factors affecting the effectiveness of enzymatic hydrolysis of cellulose are the qualitative and quantitative composition of the cellulase complex, as well as the kinetic parameters of the active components.
According to modern concepts, the composition of the cellulase complex may include endogluconase, cellobiohydrolase, exoglucosidase and cellobiase, and the component composition and activity of individual components vary significantly depending on the type and conditions of cultivation of microorganisms-producers of the cellulase complex.
In this regard, the first task. which is usually solved when developing the method of enzymatic hydrolysis of this cellulose raw material – this is the choice of the most active composition of enzyme preparations. Therefore, we first selected the composition of enzymes for hydrolysis of cotton seed linings. the results are shown in table 1.
As can be seen from table 1, complete Stripping of seeds is achieved in 3 to 6 hours. In this case, glucose is formed from the fluff with an output of 3 – 8 g/l.

| Composition of the enzyme preparation | Relation целл/пектоф | Time of exposure, hours | The initial velocity of denudation | Glucose output , г/л | Naked seeds | non-stripped seeds |
|---|---|---|---|---|---|---|
| the cellovyridin the pectofoetidin | 1:1 | 4 | 0,176 | 0,704 | 19 | 31 |
| the cellovyridin the pectofoetidin | 2:1 | 3 | 0,349 | 1,056 | 30 | 20 |
| the cellovyridin the pectofoetidin | 3:1 | 4 | 0,521 | 2,156 | 41 | 9 |
| the cellovyridin the pectofoetidin | 4:1 | 3 | 0,831 | 2,920 | 50 | - |
| the cellovyridin the pectofoetidin | 5:1 | 4 | 0,656 | 2,420 | 39 | 11 |
| the cellovyridin the pectofoetidin | 6:1 | 4 | 0,315 | 1,298 | 44 | 6 |
| the cellovyridin the pectofoetidin | 1:2 | 4 | 0,239 | 1,078 | 3 | 47 |

ISSN- 2394-5125VOL 7, ISSUE 15, 2020

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|---|---|---|---|---|---|---|
| the cellovyridin the pectofoetidin | 1:3 | 4 | 0,441 | 1,051 | 11 | 39 |
| the cellovyridin the pectofoetidin | 1:4 | 6 | 0,170 | 1,029 | 16 | 24 |
| the cellovyridin the pectofoetidin | 3:2 | 3 | 0,623 | 2,820 | 50 | - |

TABLE 1: Effect of the ratio of celloviridine GZH and pectofoetidine GZH on the duration of Stripping of cotton seeds.

E= 50 ед/г, E = 3%, S = 50 шт, t = 45°

The most effective were two enzyme preparations celloviridine GZH and pectofoetidine GZH in a ratio of 3:2. This composition for 3 hours completely exposed the seeds at the output of glucose 2.8 g/l.

An increase in the duration of exposure is an unfavorable factor for the sowing qualities of seeds, since they swell and lose their biological properties. In this regard, it was necessary to choose the conditions under which the exposure would occur in a minimum time. This can be achieved by increasing the concentration of the enzyme preparation.

As can be seen from table 2 the concentration of the enzyme preparation can be increased to 3% by reducing the time of enzymatic hydrolysis to 3 hours. Further increase in the concentration of the enzyme does not make sense, since it does not reduce the time of complete Stripping of seeds.

As you know, the main satellites of cellulose are lignin, pentosans, fat-waxy substances and a number of others that determine the availability of enzyme and water molecules for cellulose molecules. To increase the availability of cellulose is usually used surface active agents (surfactants). We also used this technique to increase the availability of pre - treated pubescent surfactant seeds for an hour. Triton X-100 was used as a surfactant.

Table 3 presents data on the effect of surfactant concentration on wetting and duration of seed Stripping. The table shows that surfactants at a concentration of 0.1% practically do not affect the Stripping of seeds, but an increase in the concentration of surfactants to 0.5% leads to better Stripping in 0.5 hours. Reducing the processing time by 0.5% Right to 0.5 hours also gave a positive result. A further increase in the concentration of surfactants did not affect the reduction of seed Stripping time. Therefore, all subsequent experiments were performed with 0.5% surfactant for 1 hour.

| № | The concentration of enzymes | The processing time for 0.5%surfactant | The time of exposing a seed, watch | Glucose output g / l | naked seeds | not naked seeds |
|---|---|---|---|---|---|---|
| 1 | 0,1 | 1 hour | 8 | 0,11 | 48 | 2 |
| 2 | 0,25 | 1 hour | 6 | 0,352 | 42 | 8 |
| 3 | 0,5 | 1 hour | 7 | 0,638 | 50 | - |
| 4 | 0,75 | 1 hour | 5 | 0,506 | 42 | 8 |
| 5 | 1,0 | 1 hour | 5 | 0,704 | 41 | 9 |
| 6 | 1,25 | 1 hour | 5 | 1,034 | 44 | 6 |
| 7 | 1,5 | 1 hour | 5 | 1,54 | 45 | 5 |
| 8 | 2,0 | 1 hour | 3 | 0,902 | 33 | 17 |
| 9 | 2,5 | 1 hour | 3 | 2,2 | 49 | 1 |
| 10 | 3,0 | 1 hour | 3 | 3,04 | 50 | - |
| 11 | 3,5 | 1 hour | 3 | 2,89 | 50 | - |

TABLE 2: Processing time of cotton seeds depending on the concentration of the enzyme preparation.
E= 50 ед/г, E = 3%, S = 50 шт, t = 45°

| № | the concentration of the surfactant% | Swelling time, hours | Time of exposure | naked seeds | not naked seeds |
|---|---|---|---|---|---|
| 1 | 0,1 | 1,00 | 3,0 | 11 | 39 |
| 2 | 0,5 | 1,00 | 0,5 | 50 | - |
| 3 | 0,5 | 0,50 | 1,0 | 50 | - |
| 4 | 1,0 | 0,50 | 1,0 | 50 | - |
| 5 | 1,0 | 1,00 | 0,5 | 50 | - |
| 6 | 1,5 | 1,00 | 1,0 | 50 | - |
| 7 | 1,5 | 0,50 | 2,0 | 50 | - |

ISSN- 2394-5125VOL 7, ISSUE 15, 2020

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|---|---|---|---|---|---|
| 8 | 2,0 | 1,00 | 1,0 | 50 | - |
| 9 | 2,0 | 0,25 | 3,0 | 45 | 5 |

TABLE 3: The effect of the concentration of surface-active agents (surfactants) on the duration of exposing seedsE= 50 ед/г, E = 3%, S = 50 шт, t = 45°

**5. Conclusion**

An enzymatic method for Stripping cotton seeds is proposed for the first time. It is shown that treatment of pubescent cotton seeds in a mixture of celloviridine GZH and pectofoetidine GZH in a ratio of 3:2, in a total concentration of 3%, when mixed, leads to complete denudation of seeds in 2 hours. At the same time, the seeding quality of the seeds was not violated.

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