ANNOTATION

to the thesis for the degree of Doctor of Philosophy (PhD) on specialty 6D070100 - Biotechnology

of Makhatov Zhaksylyk Baumanuly

Development of technology for enzymatic depolymerization of polysaccharides of wheat straw for obtaining the glucose and sorbitol

General description of the thesis work. The dissertation is devoted to the development of an effective enzymatic depolymerization of wheat straw with carbohydrase of filamentous fungi for the purpose of producing glucose, with the subsequent production of sorbitol.

Relevance of the research topic. In Kazakhstan, among agricultural crops in terms of the volume of cultivation, wheat is the leader. For example, in 2018, the wheat harvest amounted to 32 million tons, in 2019 - about 33 million tons, and waste (wheat straw and bran) amounted to more than 12-15 million tons / year. Annual renewability, low cost, and a large volume of these wastes dictate the need to develop biotechnology for their conversion based on the fermentolysis of cellulose, the main structural component. The wide variety of enzymes provides great opportunities for the effective conversion of this pesticide raw material into biologically valuable substances, sugar and other products.

Despite the fact that by now there are many approaches to the processing of wheat straw, most of them remain unrequired. Part of the straw is used for feeding livestock and as a support for animals, the rest of it is burnt or burnt on the fields. Although straw is extremely promising for processing as a feedstock for various types of production.

In this regard, it is of particular interest to search for enzymatic technical depolymerization of polysaccharides in wheat straw.

Consequently, the development of a complex technology for the processing of wheat straw cellulose to glucose, then to copbit, will not only allow to improve the ecological situation, but also to produce raw material and additional crops for various industries.

Purpose and objectives of the research

The purpose of the work is the development of an effective biotechnology for the de-polymerization of wheat straw polysaxapids for the production of glucose and copbit by means of enzyme hydrolysis.

To achieve the desired goal, it was necessary to solve the following tasks:

- to carry out the scanning of advanced cellulase-producing stocks and with the help of the microbiological process on the basis of the results of microbiological studies;
- to select the optimal composition of the nutritive medium and conditions for the cultivation of stamps in order to obtain a balanced ferment complex;
- to develop effective methods of selection and purification of the complex of hydrolytic enzymes;

- to develop the technology of obtaining a high-efficiency multi-enzyme composition for deep and step-by-step depolymerization of wheat straw;

- to develop the conditions and technology of enzyme processing for the de-polymerization of polysaxapids of wheat straw with the aim of generating a maximum output of glucose and glucose.

Objects of research. Microbial sources of pectinases were mycelial folds of the genus Aspergillus, obtained in the laboratory of the Department of Biotechnology, M.Auezov from the soils of the South-Kazakhstanskaya region, Penicillium and Trichoderma, obtained in the laboratory of microbial enzymes of the Institute of Microbiology of the Academy of Sciences of Uzbekistan. The source of cellulolytic enzymes was the consortium of microorganisms *«Cellozyme G20x»*, obtained as a result of the joint deep cultivation of the *A. awamori F-RKM719* strain. Tr.viride 121, commercially available products: *Cellic CTec (Novozum, Denmark), Accellerase 1000 and Accellerase DUET*.

Wheat straw obtained from the Steklovidnaya-24 variety, zoned and cultivated in the Turkestan region, served as an object for enzymatic hydrolysis.

Scientific novelty of the research

Development of biotechnology for the production of a new highly active enzyme preparation *«Cellozyme G20x»* - a source of enzymes of cellulase, xylanase, β-glucanase, pectnase action, for the effective de-polymerization of polycaxbitol salt with copolymer salt and copolymer.

In the process of research, a concoptium of microorganisms-producers of cellulolytic enzymes - *Tr. viride 121* and *A. awamori F-RKM 0719*, which ensures an increase in cellulolytic activity, as well as the activity of the accompanying enzymes. The optimal composition of the nutrient medium and the optimal cultivation conditions for micromycetes *Tr. viride 121* and *A. awamori F-RKM 0719*. In order to increase the catalytic activity and stability of the enzymatic complex of the consortium of micromycetes *Tr. viride 121* and *A. awamori F-RKM 0719* methods of isolation and purification were developed, as a result of which the enzyme preparation *«Cellozyme G20x»* with a high degree of purification was obtained.

Practical and theoretical value of the work. In the result of the use of a concoptium of microorganisms-producers of cellulolytic enzymes - *Tr. viride 121* shtamm and *A. awamori F-RKM 0719* razpabotana and ekcpepimentalno obocnovana effective texnologiya polucheniya and used fepmentnogo ppepapata *«Cellozyme G20x»* (microbial consortium) providing optimalnye papametpy fepmentativnoy obpabotki policaxapidov colomy pshenitsy pozvolyuschaya and receive up to 2,2% glucose and sorbitol. Teopeticheckaya znachimoct paboty zaklyuchaetcya in tom chto ona pacshipyaet ppedctavlenie o biotexnologicheckix ocnovax polucheniya and ppimeneniya vycokoeffektivnyx tsellyuloliticheckix fepmentnyx ppepapatov *«Cellozyme G20x»* (a consortium of microorganisms), the manufacture of polysaccharides from agricultural production of vegetable wastes.

The results of research work implemented in the biotechnological production of LLP "Ana-zher", a specialized enterprise for the production of biologically active products in Kazakhsatan.

The development is protected by a utility model patent No. 3429 "Method for producing glucose from wheat straw."

The main provisions of the thesis are used in the educational process in the discipline "Biotechnology of microorganisms".

Conceptual positions and results of research can be used in the preparation of special curriculum for doctoral students, undergraduates, bachelors in the biotechnological field, as well as in the development of scientific textbooks, books educational-methodological aids.

The main provisions of the dissertation submitted for defense

- screening of advanced cellulase-producing stems and a concoptium of microorganisms - producers of cellulitic enzymes - *Tr. viride 121* and *A. awamori F-RKM 0719*, which ensures an increase in cellulolytic activity, as well as the activity of the accompanying enzymes;

- cultivation conditions, the composition of the nutrient medium and technologies that provide the physiological needs of producers and the maximum formation of a balanced enzyme complex, using various food waste as components of the nutrient medium;

- the technology of radiation and the characteristics of the complex enzyme preparation *«Cellozyme G20x»* (consortium of microorganisms).

- optimal parameters of enzymatic treatment of wheat straw polysaccharides, technology of using the enzyme preparation *«Cellozyme G20x»* (consortium of microorganisms) and a comparative assessment of the effectiveness of new enzyme preparations on the effectiveness of depolymerization of wheat straw.

Conclusions:

1. An effective biotechnology for the enzymatic de-polymerization of wheat straw polysaxapids has been developed based on the use of a new enzymatic preparation *«Cellozyme G20x»* (a consortium of microorganisms), which provides the yield of glucose and sorbitol from the feedstock to 2.2%.

2. As a result of screening, out of 46 strains of fungi, 24 cultures were selected, using the methods of stepwise selection on selective nutritional media, advanced cultures of *A. awamori F-RKM 0719* and *Tr. viride 121*. Cultural-morphological and biochemical characteristics for the synthesis of a complex of cellulolytic enzymes, including five representatives of carbohydrases, have been studied.

3. A concoptium of microorganisms-producers of cellulolytic enzymes - *Tr. viride 121* and *A. awamori F-RKM 0719* strains. Optimal conditions and composition of the nutrient medium for co-cultivation of *Tr. viride 121* and *A. awamori F-RKM 0719*, which were introduced sequentially, which ensured an increase in catalytic activity within 30% - 70% due to their synergistic effect.

4. As a result, with a high degree of purification and isolation of fermental solution of the concoptium *Tr. viride 121* and *A. awamori F-RKM 0719*, a new enzyme preparation *«Cellozyme G20x»* (consortium of microorganisms) was obtained. The physicochemical properties of the preparation *«Cellozyme G20x»* at temperatures from 30^0C to 70^0C of enzymatic hydrolysis of wheat straw were studied and the optimal

parameters of action and the stability of the preparation were set at 50C at a temperature of fermous.

5. High purity β-1,4-endoglucanase was obtained from the complex preparation *«Cellozyme G20x»* by gel-column chromatography on Sephadex. The presence of three forms of endoglucanase in the composition of *«Cellozyme G20x»*, the molecular weights of which are in the range of 35-36 kDa, was established by EF on 7.5 PAGE.

6. Comparative evaluation of enzyme preparations for the hydrolysis of microcrystalline cellulose (MCC), carboxymethyl cellulose (CMC), xylan, and also the pectin showed that the calculated maxima *Tr.viride 121* and *A. awamori F-RKM 0719* is suitable for similar applications Genencor International PS AOZ143-1.1EN Optiflow RC 2.0 and PS A03197-1.0EN Acellerase CB100 based on activities and values of specific activity.

7. In an experimental production test, the enzyme preparation *«Cellozyme G20x»* showed the efficiency in bioconversion of wheat straw with a high yield of glucose and sorbitol up to 2.2%.

Publication of research results. 15 scientific works were published on the topic of the dissertation, of which 2 article in a journal included in the international database Scopus, 3 articles in publications recommended by the Committee for Control in Education and Science of the Ministry of Education and Science of the Republic of Kazakhstan, 9 theses in international conferences, 1 patent for a utility model.

Structure and volume of dissertation. The dissertation consists of an introduction, a review of domestic and foreign scientific, technical and patent literature, an experimental part, the sources used, conclusions and applications, includes 121 pages, 37 figures, 24 tables. The list of literature consists of 161 sources.

The dissertation was carried out within the framework of the implementation of the financially funded in 2015 - 2017 grant MES RK "Development of an innovative technology for deep processing of carbonaceous raw materials and waste in order to obtain xylitol and copbita by means of combined chemical and enzymatic hydrolytic hydrolysis and hydrogenation.