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Research Of Cellulases Of Microorganisms Destructing Composite Polymers.

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ABSTRACT

The enzymatic activity and molecular composition of microorganisms present in the soil containing samples of various composite polymers were researched. The presence of microorganisms of the genera Aspergillus, Penicillium, Trichoderma, and Hypocrea in the soil was revealed. The presence of two molecular forms of cellulases, about 63 kDa and about 45-50 kDa was shown in some types of microorganisms.

Keywords: Composite polymers, biological degradation, soil microorganisms, cellulolytic enzymes.

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INTRODUCTION

In the natural environment, almost all polymeric materials are subjected to destruction, however, the rate of destruction for most of them is very small. It depends on the composition of the polymer, the type of plasticizer and filler used in the manufacture of products. Biodegradation of polymers is an extremely complex and time-consuming process, and in order to accelerate and facilitate the decomposition of the substances, they include natural components such as cellulose, starch, amylopectin, amylose, dextrin and others, which are a nutrient medium for bacteria [1]. To make the polymer material more biodegradable, a natural additive is mixed with a synthetic polymer at the micro level. As is known, natural filler can accumulate in less highly ordered areas of the polymer. In addition, the packing density of macromolecules in the boundary layers of the polymer-filler system is about 2 times less than in the rest of the unordered phase of the polymer. Therefore, when the filler is destroyed by microorganisms, the access of fungi to a part of the polymer less durable in relation to biodegradation is facilitated [2].

Biodegradation of synthetic polymers is caused by microorganisms of various systematic groups related to fungi and bacteria. In connection with the above, the aim of the work was to identify the species of micromycetes and their enzymes with high cellulolytic activity involved in the destruction of polymeric materials with the inclusion of rice husks.

MATERIALS AND METHODS

Rice husks (RH) were used as a plant component in the composition of the composite polymer material. The husk had an average particle size of 0.5 mm, contained 40-45% cellulose, 20-25% lignin, 15% hemicellulose. The dosage of vegetable filler was calculated in mass parts (mp) per 100 mp of synthetic polymer. The synthetic components were polypropylene (PP), low-pressure polyethylene (LPPE), synthetic ethylene-propylene triple rubber (EPDR), and ultra high molecular polyethylene (UHMPE). Therewere obtained the following samples of composite materials subjected to biodegradation in soil: PP + 10 mp RH + 5,10, 30,50,70 mp EPDR, PP + 10 mp RH + 5,10, 30,50,70 mp SMPE, LPPE + 5,10,15,30,50 mp RH, 40% LPPE + 60% PP + 10 mp RH, 20% LPPE + 80%PP + 10 mp RH, 10% LPPE + 90% PP + 10 mp RH, 0,5% LPPE + 99,5%PP + 10 mp RH.

As a medium for the destruction of materials used specially prepared soil, which consisted of 2 parts of sand, 2 parts of humus, 1 part of horse manure and 4 parts of black soil. The soil was placed in boxes, the thickness of the layer was 30±5 cm. Soil moisture was maintained of not less than 60%, the pH of the soil used was 6.2. Boxes with soil were stored in laboratory conditions at room temperature and natural light. The test method consisted in immersing the polymer samples vertically into the ground with subsequent exposure for 5 months.

Microbiological methods

One of the main groups of microorganisms involved in the decomposition of complex organic compounds is soil microscopic fungi. Imperfect microscopic fungi (genera Aspergillus, Penicillium, Trichoderma, Cladosporium, Fusarium) are among the most active plastic destroyers causing various damages and their destruction [3]. Since the polymers we studied contained plant waste, cellulolytic micromycetes were also taking an active part in their destruction. In aerobic conditions, a significant role belongs to fungi, of which the most active biodestructors are mushrooms of genera Aspergillus, Chaetomium, Trichoderma, Fusarium, etc. [4]

To assess the microbiological activity of the soil and to identify its properties, allowing to accelerate the destruction of polymeric materials, cellulolytic micromycetes were isolated on the Czapek medium with carboxymethyl cellulose (CMC) as a carbon source. Further, selected species was determined by morphological parameters using a key to species [5-10] and cultured in pure culture on the liquid Czapek medium. Species names of fungi were specified on the updated lists of published species in the "Index fungorum" database. The activity and molecular composition of cellulases were determined in the culture fluid.

Measurement of cellulase activity

The enzymatic activity of cellulases was determined by hydrolysis of CMC immobilized in polyacrylamide gel [11]. Samples of the culture liquid were kept in contact with the gel for 20 minutes, and then the gel was stained with a solution of Congo red. Areas with hydrolyzed CMC were painted weaker. The image of the gel was processed in a software, determining the total RGB values of the color of the gel areas. These values in the model experiment were in linear dependence on the activity of cellulase, so the obtained values were used as optical units of enzymatic activity.

Zymography of cellulases

Sample preparation consisted of following steps. The buffer for samples containing Tris-glycine electrode buffer, glycerin (20%), SDS (0.1%) and β-mercaptoethanol (0.1%) was added to the equal volume of the enzyme solution. The samples were heated for 20 minutes at 70 °C.

The electrophoresis was performed in 6% PAAG with 0.1% SDS and 0.1% soluble CMC, pH 8.8, at 4 °C and 11 V/cm. After that, the gel was incubated for 1 hour in a phosphate buffer, pH 6, at room temperature on shaker. Gel was stained with 0.015% Congo red.

RESULTS AND DISCUSSION

Species composition of microorganisms

No cellulolytic fungi were found in the sample containing low-pressure polyethylene and polypropylene.

From the soil with the sample of PP + 10 mp RH + 30 mp SMPE, there were isolated Aspergillus niger and Aspergillus bertholletius, at increasing the part of SMPE up to 50 mp there were allocated Penicillium lanoso-griseum, Penicillium spinulosum and Hypocrea minutispora.

From the soil with the sample of PP + 10 mp RH + 5 mp EPDM there were isolated Aspergillus fumifatus, Aspergillus orizae, Trichoderma rossium, at increasing the part of EPDM up to 10 mp there were allocated Penicillium lanoso-griseum and Hypocrea minutispora, but fell out Asp. orizae. In the soil with a polymer containing 30 mp EPDM, there were only discovered 2 species of micromycetes, Penicillium lanoso-griseum and Hypocrea minutispora.

In the study of the qualitative composition of cellulolytic micromycetes, in soil samples containing LPPE with 15 mass parts of rice husks, only two species were identified. The abundance of Aspergillus fumigatus was 90%, and Trichoderma rossicum - 10%. In soil samples with LPPE and 5, 10, 30 and 50 mass parts of rice husk growth of cellulolytic micromycetes was not detected.

From the studied species of fungi, Trichoderma rossicum is considered the most famous destroyers of substrates that contain cellulose [12].

Enzymatic activity of microorganisms cellulases

Using the culture fluid of microorganisms there was obtained an image of a gel with distinct differences of the individual samples according to the degree of hydrolysis of the substrate and the intensity of the color. The activity of each sample of the culture fluid was determined five times. Enzymatic activity was expressed in optical units of enzymatic activity calculated as the difference between the mean values of the color of the sites corresponding to individual samples and control sites (wells with a sterile medium).

The obtained results show that the cultures of Aspergillus fumigatus, Aspergillus glaucus and Aspergillus oryzae species had the greatest activity among the studied microorganisms (Fig. 1).

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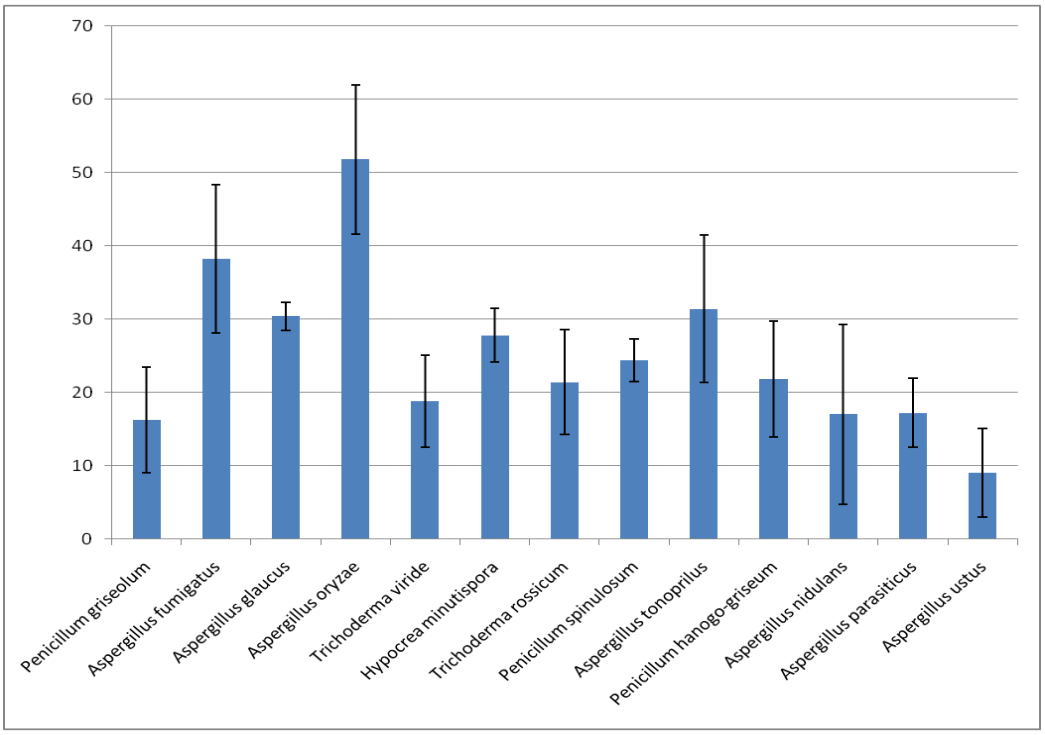

Figure 1: Cellulolytic activity in the culture fluid of microorganisms. On the vertical axis-optical units of enzymatic activity. The 95% confidence interval is shown as the error value.

Samples of culture liquids with the highest value of cellulolytic activity were further analyzed by electrophoresis in an immobilized substrate (zymography) to determine the molecular composition of cellulases.

Molecular composition of cellulose

The presence of three enzymes differing in molecular weight (2 forms with a molecular weight of about 60-70 kDa and one form with a much larger molecular weight) was revealed in the culture liquid of microorganisms (Fig. 2).

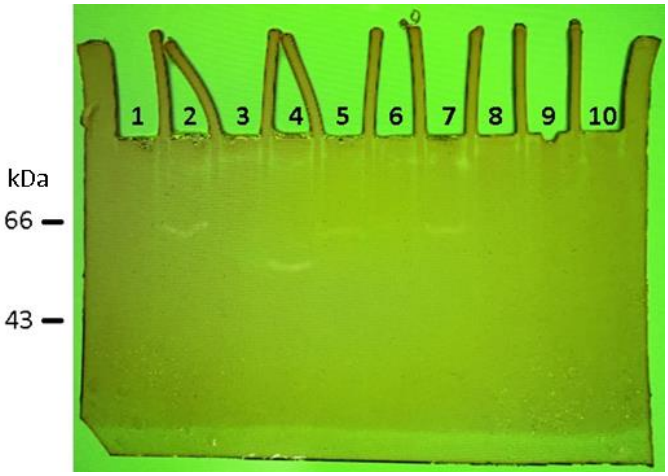

Figure 2:. Zymogram of cellulases of culture liquid of microorganisms. Colored with Congo red. 1 – Penicillum griseolum, 2 – Aspergillus fumigatus, 3 – Aspergillus glaucus, 4 – Aspergillus oryzae, 5 – Trichoderma viride, 6 – Hypocrea minutispora, 7 – Trichoderma rossicum, 8 – Penicillum spinulosum, 9 – Aspergillus tonoprilus, 10 – markers (not detected with Congo red coloring).

Analysis of information in NCBI and PDB showed that various taxonomic groups, in particular Aspergillus, have the cellulase molecule containing 569 aminoacids, with molecular weight of about 63 kDa (high MW form), and the cellulase containing 400-450 aminoacids, with a molecular weight of about 45 - 50 kDa (low MW form). Also for hydrolases, in particular, cellulases, the ability to form dimers and tetramers is shown; apparently, the proteins present at the top of most tracks on the electrophoregram are dimers and tetramers or enzymes aggregates.

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