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PHYSICO-CHEMICAL AND MICROBIOLOGICAL CHARACTERIZATION OF STARCH-BASED BIODEGRADABLE FILMS

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Abstract. Research on the manufacturing of experimental biodegradable films (EBF) was carried out in the laboratory. Recipes containing 8, 10, and 15 % of corn and potato starch were analyzed. It was found that the EBF based on potato starch with a 10 % concentration is more plastic and retains its shape well compared to other samples. Microbiological, mechanical, physicochemical, and infrared spectroscopic studies of the EBF and the biodegradable plastics (BP) available on the market in the form of packaging bags that are positioned as biodegradable (BP from “ATB”, BP from “Silpo”, BP from “Roshen”) were performed. The isolation of enrichment soil microbial cultures and their identification by microscopy of permanent mounts were studied. The ability of the isolated microbial cultures to biodegrade starch-based EBFs was experimentally investigated and determined, as well as the peculiarities of biodegradation of starch-based EBFs and BPs, as a result of the activity of microorganisms of different taxonomic groups, were studied.

Keywords: biodegradability, starch-based films, glycerol, electrochemical impedance spectroscopy (EIS), microorganisms.

1. Introduction

Bioplastics produced from renewable biomass are widely used in the market due to their environmental friendliness, natural bioconversion, sustainability, and economic benefits. In addition, the bioconversion products of starch biopolymers in soil can be the source of nutrients for various microorganisms. Therefore, research and improvement of technologies for the production and processing of starch-based biopolymer materials using plasticizers is extremely important. This allows increasing the market volume of bioplastics with different mechanical properties and biodegradability. Scientists around the world are actively working to develop alternative types of polymers that can biodegrade naturally. Some of them are made from potato and corn starch and other plant-based materials[1–9].

Nowadays, European integration of Ukraine and economic development face new challenges in adjusting to the difficult conditions of foreign economic activity, meeting their basic requirements for the introduction of modern scientific developments and technologies for the export and import of goods, capital, investments in safe environmentally friendly goods, ensuring the transition of manufacturing enterprises to zero waste production and rational consumption models[10]. Environmental culture is an important area of modern scientific thought of researchers, government agencies, businesses, and public organizations. An analysis of the current state of global production of environmentally safe plastics indicates an active development of technological processes. Every year, new technologies and mechanisms are being introduced to help save the environment and improve our lives. Ukrainian scientists are working on certified developments of environmentally safe plastic products in accordance with European standards and global practices as part of special projects (biodegradable tableware: Eco Green Plate, BIOTALP, VIOS, Foodscapes, USAID Economic Ukraine, Food BIO Pack projects; biodegradable films made from insect chitin (developed by the Institute of Applied Physics of the National Academy of Sciences of Ukraine), coffee grounds (Rekava), wheat bran, avocado pits (Biofase), starch and biocellulose). Responsible production is envisaged to reduce the negative impact on the environment with the possibility of maximum use of recycled materials in various production processes[11–15].

The definition of “biodegradation” is based on the mechanism of self-healing of the natural ecosystem from

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human waste. Due to the ability to biodegrade under the influence of microorganisms in both anaerobic and aerobic environments, polymers with controlled biodegradability based on natural raw materials have become an alternative to polymers made from natural gas, coal, and oil, which will reduce the environmental burden and carbon dioxide emissions[16–19]. The greatest attention is paid to polylactides, polyhydroxyalkanoates, polycaprolactone, thermoplastic starch, and other biodegradable polymers. Since biopolymers are compatible with human tissues and are well absorbed, they are actively used in medical practice. The range of biodegradable polymeric packaging materials in the food and household chemicals industry is also expanding[20–23].

Electrochemical impedance spectroscopy (EIS) has recently been used as a non-destructive method to measure the functional properties of living organisms[24] and materials, namely their electrical conductivity. Physical changes in biopolymer films affect their electrical characteristics in terms of bioimpedance. In addition, the resistance of such films is directly affected by water percentage and additives[25].

The aim of the research is to experimentally determine the biodegradability of starch-based films and to study their physical and chemical properties during biodegradation. To this end, further goals were to isolate enrichment soil cultures potentially capable of film degradation in the laboratory compared to industrial samples, and to conduct an initial identification of microorganisms using microscopy.

2. Experimental

2.1. Materials

For the production of starch-based biodegradable plastics, corn starch (DSTU 3976-2000) and potato starch (DSTU 4286:2004), glycerol as a plasticizer, and distilled water were used. For the investigation of BP features, enrichment soil microbial cultures isolated from different soil samples were utilized. The studies were conducted with the BP produced in the laboratory, and, for comparison, industrially produced BP from “ATB”, “Silpo”, and Roshen, which are positioned as biodegradable.

2.2. Methods

2.2.1. Preparation of Biodegradable Film

According to the protocol[26], various compositions of the EBF based on corn and potato starch (8, 10, and 15 %) plasticized with glycerol (5 %) were prepared in the laboratory. All components were mixed well in water to improve the penetration of the plasticizer into the starch granules. A transparent, slightly opalescent viscous starch solution was obtained by heating the solution to 75 °C and stirring it continuously with a magnetic stirrer. The films were formed from the solution by casting and drying in a drying oven at 60 °C for 30 min.

2.2.2. Incubation of Soil Samples on Biodegradable Film Samples

The incubation of soil samples on the EBF samples in the medium of nutrient agar was carried out to isolate enrichment soil microbial cultures that are potentially capable of decomposing biodegradable films.

Enrichment soil microbial cultures were obtained as follows: the EBF was applied to the surface of nutrient agar in Petri dishes, after which soil lumps from different econiches were applied to it. The plates were incubated at 37 °C for 14 days.

The colonies of microorganisms that visually caused the decomposition of the EBF were selected, sown on a starch-ammonia medium, and incubated at 37 °C for 7 days.

2.2.3. Seeding of Accumulative Cultures of Microorganisms on Starch-Ammonia Medium

Growth on starch-ammonia medium was carried out to produce enrichment cultures.

Qualitative rapid analysis with Lugol’s solution for starch content in the medium, on which the colonies of the enrichment cultures have grown, was applied to prove their ability to decompose starch.

2.2.4. Microscopy

The light microscopy was used to identify the predominant microorganisms potentially capable of biodegradation of the BP samples under study. The obtained cultures were studied using a BIOLAM light microscope (fuchsin staining, magnification 15×90). The selected samples were sown on starch-ammonia medium and incubated at 37 °C for 7 days. The ability of the cultures to decompose starch was checked by a qualitative reaction of starch with iodine. Lugol’s solution was applied to the surface of a Petri dish, the change in surface color was observed, and the degree of starch decomposition was recorded.

2.2.5. Determination of Mechanical Properties

EBF mechanical properties and its compressive strength were determined using a Kimura model 050/RT-601U tensile tester (Japan, tensile speed range 25–500 mV/cm, step 0.01 mm).

The plastic tensile test was performed at an air temperature of 20 ± 2 °C. The EBF sample for testing was cut into pieces of 10×12 cm, 0.2 cm thick, and inserted into the clamps between the grippers at the initial length of the gauge division l_0 (cm). The number of EBF samples for tensile tests was 3. The methods correspond to standard test methods[27–29].

2.2.6. Study of the Solubility of the EBF sample was conducted at different temperature conditions and times in distilled water[30].

2.2.7. Sterilization of Biopolymer Film

The EBF samples were sterilized in a steam sterilizer GK-20 at a temperature of 121 °C for 30 min under an overpressure of 1 atm to determine the structural changes in the EBF sample during sterilization.

2.2.8. Electrochemical Impedance Spectroscopy was utilized to determine the functional state of the BP, namely its electrical conductivity.

The electric impedance (EI) was measured with a hardware and software module for determining the values of complex resistance based on the AD5933 microchip, which is an integrated converter of measured parameters into a digital code.

2.2.9. Infrared Spectroscopy

FT-IR spectra were recorded on a Spectrum Two FT-IR spectrophotometer (PerkinElmer).

3. Results and Discussion

Three samples of the EBF prepared with different concentrations of potato starch (8, 10, and 15 %) were analyzed. It was found that the sample with a 10 % concentration has greater plasticity and good shape retention compared to the other samples (with 8 % and 15 % concentrations). The corn starch EBF are more moisture resistant and less elastic.

As a result of the incubation studies of soil samples (Fig. 1), two microbial enrichment cultures, white and pigmented, were isolated on samples of the BP from Silpo (1), BP from Roshen (2), the EBF (3), and BP from ATB (4) on nutrient agar. By the method of detecting visual damage, it was experimentally confirmed that the isolated cultures are capable to decompose both the EBF and BP.

Microscopic examination revealed that the microbial enrichment cultures contained microscopic fungi and rod-shaped bacteria, as well as round, filamentous and oval-shaped bacteria (Fig. 2).

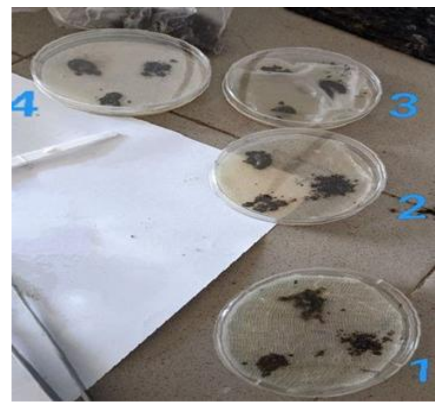

Fig. 1. Samples of different soil samples were applied to agarized nutrient medium on samples: 1 – BP from Silpo; 2 – BP from Roshen; 3 – EBF; 4 – BP from ATB

The commercially available BP and the prepared EBF were placed on agar medium without starch (Fig. 3). It was found that the EBF and BP from ATB biodegraded the fastest, while the biodegradation of the BP from Silpo and Roshen took the longest time. The difference in the biodegradation rates may depend on the admixtures in the tested BP samples. In the EBF, non-toxic glycerol was used as a plasticizer. However, the BP may contain different plasticizers and dyes that are toxic to microorganisms. Moreover, since bags made of BP are used for food packaging, manufacturers may add other antibacterial agents.

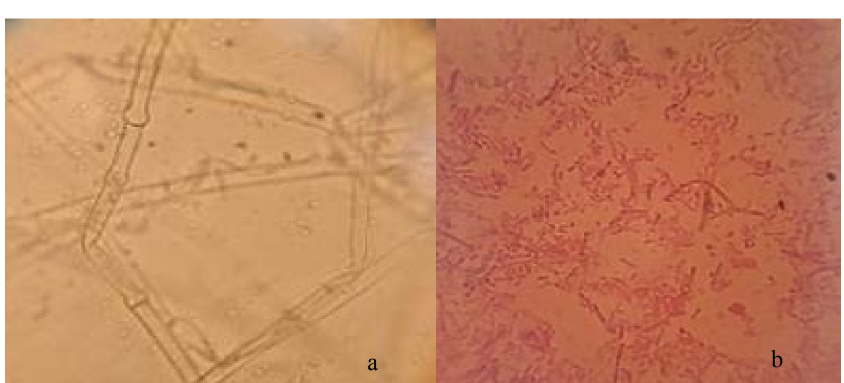

Fig. 2. Results of microscopy of colonies growing on soil samples: a – molds from a soil sample taken from a landfill; b – colonies of rod-shaped bacteria from a sample of white colonies

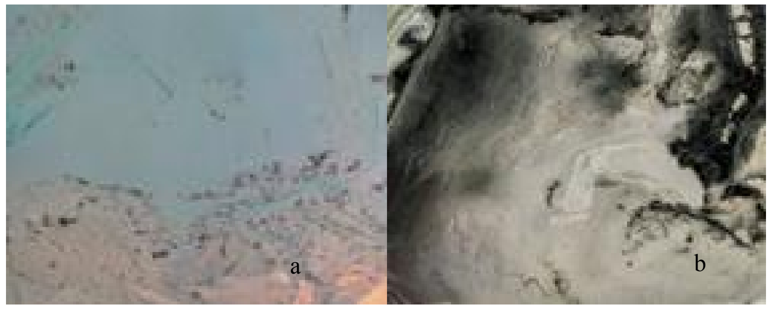

Fig. 3. Decomposition by microorganisms: a – BP; b – EBF

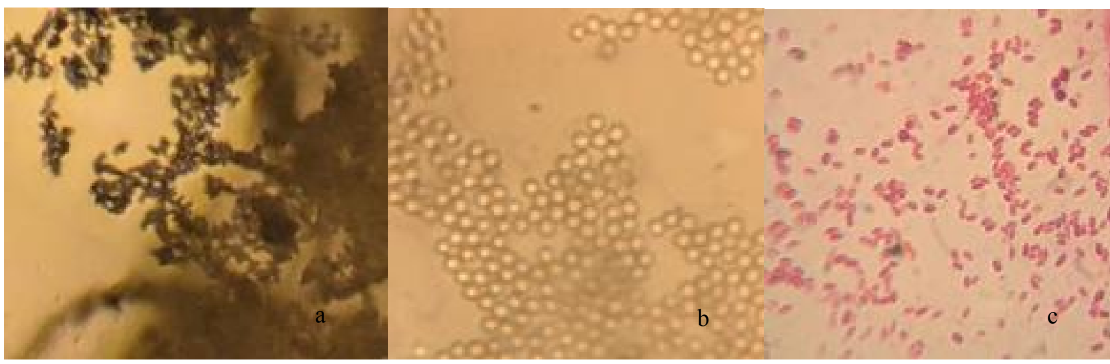

Fig. 4. Micrographs of microorganisms grown on starch-ammonia medium: a – pigmented culture of fungi; b – spores of fungi from pigmented culture; c – oval-shaped and rod-shaped bacteria from white culture

The sowing of microbial enrichment cultures on starch-ammonia medium showed that two types of enrichment cultures were obtained as a result of cultivation on starch-ammonia medium: without color and with black pigment (Fig. 4).

The selected samples were sown on starch-ammonia medium and incubated at 37 °C for 7 days. The ability of the cultures to break down starch was checked by a qualitative reaction of starch with iodine. For this purpose, Lugol's solution was applied to the surface of a Petri dish. The degree of starch decomposition was recorded by the change in surface color.

A qualitative rapid analysis (with Lugol's solution) of the starch content in the medium on which the colonies of the enrichment cultures grew showed that they are able to decompose starch to dextrins and even glucose.

The cultures selected as a result of the previous experiments were sown on samples of polymeric materials (bags): BP from Roshen, BP from Silpo, EBF, and BP from ATB. Jars with liquid starch-free ammonia medium were incubated at 37 °C for 7 days[23]. The source of organic matter was the shredded EBF and BP.

After incubation, the damage on the EBF and BP was visually identified, and fixed fuchsin-stained microsections were made, followed by light microscopy for preliminary identification of the morphology of the isolated microorganisms (Fig. 5).

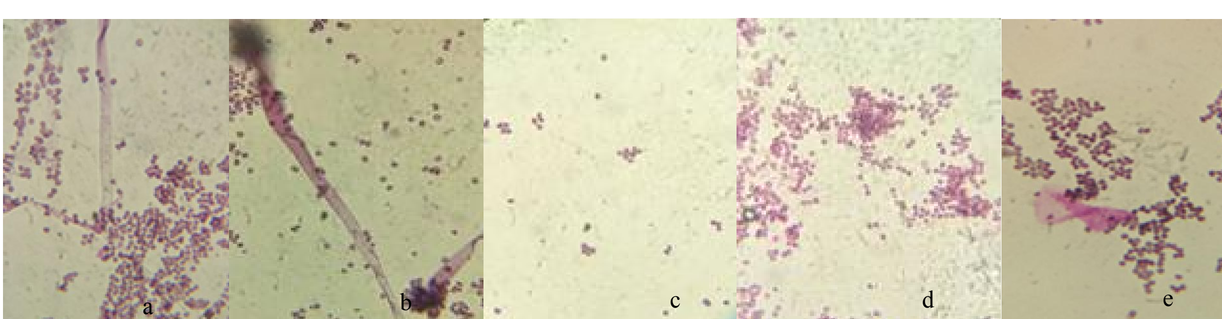

Fig. 5. Micrographs of microorganisms grown on the EBF and BP and agar ammonia medium without starch: a – spores of fungi with hyphae from pigmented culture on the EBF; b – hyphae with sporangia from pigmented culture on the EBF; c – single spores of fungi from white culture on the EBF; d – spores of fungi from pigmented culture; e – spores of fungi from white culture on the BP from Roshen

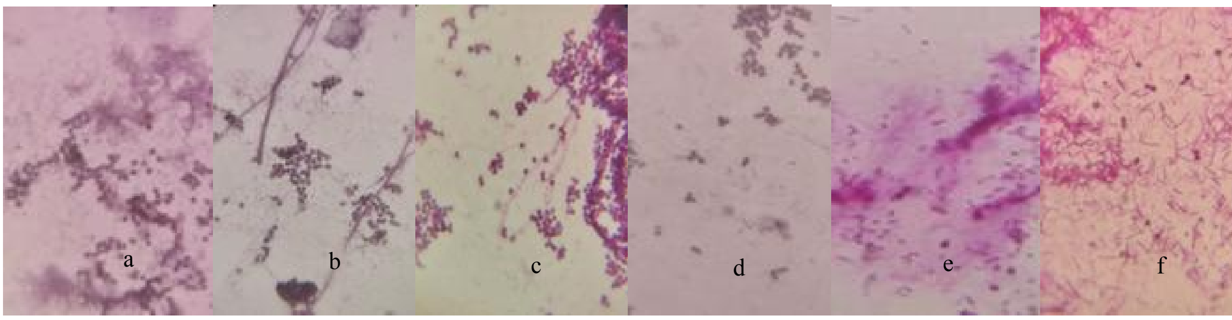

Fig. 6. Micrographs of microorganisms grown on liquid ammonia medium without starch with fragmented samples: a – spores of fungi from pigmented culture on the BP from Silpo; b, c – hyphae with spores of fungi from pigmented (b) and white (c) culture on the BP from ATB; d – spores of fungi from pigmented culture on the BP from Roshen; e, f – round and rod-shaped filamentous bacteria from white (e) and pigmented (f) culture grown on the EBF

Microscopic examination of the samples revealed the following: in the EBF – fungi and bacteria in both colorless and pigmented cultures, in the BP from Roshen – fungal spores and rod-shaped bacteria (Fig. 6).

The results of incubation of the above cultures in a liquid ammonia medium without starch with fragmented BP (Fig. 6) and subsequent microscopy showed that the EBF sample was dominated by oval and rod-shaped bacteria, filamentous bacteria, and bacilli with endospores inside. The other three industrial samples of the BP were dominated by fungi and their spores. The allegedly isolated microbial species belong to the genera *Bacillus, Pseudomonas*, and *Aspergillus*.

The cultures of fungi identified in the BP industrial samples may indicate the presence of antibacterial agents in such products. In the EBF, bacteria are predominantly present because they grow faster than fungi, and the greater development of fungi in the commercial samples is apparently possible due to the presence of substances toxic to bacteria, so fungi have displaced bacteria from the environment through competition for nutrients.

Damage caused by microorganisms to the BP samples No. 3 and No. 4: significant loss of strength, brittleness, and friability were observed (Fig. 7).

To separate the bacterial cultures from the fungal ones, the cultures were inoculated on nutrient agar selective medium for bacteria. In the cultures grown on nutrient agar, filamentous and oval rod-shaped bacteria were identified. Also, a small number of fungi were found growing on the nutrient medium in cultures collected from dishes made of BP. Only bacterial cultures were observed in the EBF. Since bacteria can grow faster on nutrient agar, it can be concluded that only cultures of fungi were present in the BP, so the growth of bacteria was inhibited, obviously, by some additives.

As a result of determining the mechanical properties of the EBF and testing its strength on a Kimura model 050/RT-601U tester, it was found that the EBF could not withstand heavy loads. The sample for tensile testing of the manufactured BP was cut into pieces, 10×12 cm, 0.2 cm thick, and inserted into the clamps of samples 1, 2, and 3 between the grippers at the initial length of the gauge division in three repetitions: 1) $l_0 = 24.4$ cm; 2) $l_0 = 23.7$ cm; 3) $l_0 = 23.7$ cm. We followed the movement of the arrow until the moment of fracture and determined the rupture of the samples, respectively, $l_1 = 25.3$ cm, $l_1 = 24.3$ cm, and $l_1 = 24.3$ cm. The load was chosen so that it created a deformation in the material within 0.2–0.6 mm.

The determined elongation after fracture was 3.1 % (Table).

The physical and chemical properties of the EBF from starch were determined according to various criteria, namely, the study of the solubility of the EBF in distilled water at different temperature conditions, qualitative reactions, the study of changes in the properties of BP under sterilization conditions, the analysis of the functional state of BP using EI, spectral studies of starch and BP based on it.

Table. Properties of the EBF according to various criteria

Properties	Observations
Appearance	Transparent film
The elongation after fracture	3.1 %
Flexural elasticity	Does not deform when bent, elastic
Fragility	Does not deform when broken
Effect of open flame	Combustible
Sterilization	Resists a pressure of 1 atm for 30 min

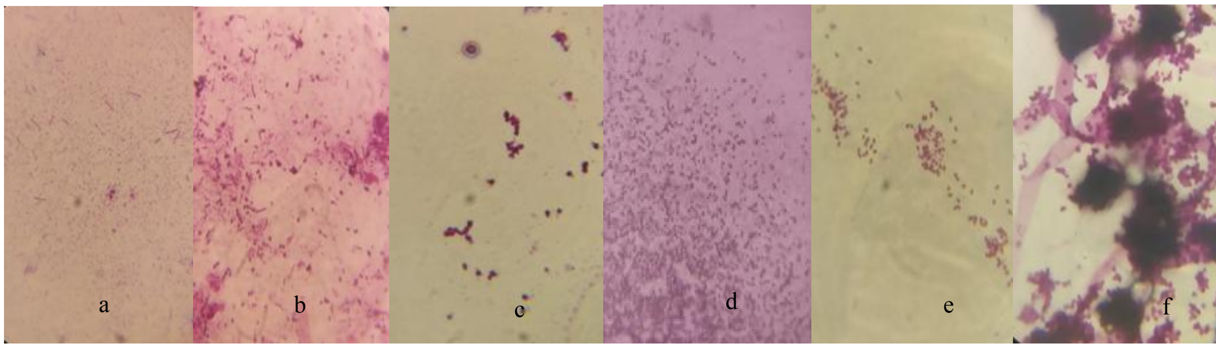

Fig. 7. Micrographs of microorganisms grown on nutrient agar: a – filamentous bacilli from the white culture in the industrial sample of the BP from ATB; b – fungal spores from the pigmented culture of the ATB package; c, d – rod-shaped bacteria from the EBF from the pigmented (d) and white (c) cultures; e, f – spores and hyphae of fungi from the pigmented culture from the industrial sample of the BP from Silpo

Structural changes were not observed in studies on the EBF solubility in tap water at room temperature. Moreover, it was proved that the EBF does not dissolve at a temperature of 100 °C.

Fig. 8 shows the experimental results on the weight change of the EBF immersed in water at room temperature (18 °C) and 37 °C (in a thermostat) for 10 days. The findings indicate an increase in the mass of the EBF from 0.62 g to 0.88 g (42 %) after 5 days of the experiment at room temperature. At 37 °C, after 5 days, an increase in the mass of EBF was observed from 0.76 g to 1.75 g (130 %), which proves better adsorption of water by the biopolymer film plasticized with glycerol. Thus, it can be concluded that the EBF is optimally used at low temperatures, and its biodegradation can occur under conditions inherent in most regions of the climatic zones of Ukraine.

After soaking the EBF in distilled water at 37 °C, a qualitative starch reaction with iodine was performed, where a positive reaction to the starch content in water was observed.

No structural changes were detected as a result of the EBF sterilization at 121 °C and 1 atm, since the EBF does not change its properties during and after sterilization. Therefore, it is possible to expand the scope of its applications, in particular, as a packaging material for medical purposes.

Electrical impedance is a multicomponent indicator that can be used to analyze and predict the functional state of biological tissue and various types of films. We performed 8 measurements of the complex impedance of the EBF at frequencies of 1 kHz, 5 kHz, 10 kHz, 15 kHz, 25 kHz, 50 kHz, 70 kHz, and 100 kHz. The measurements of the EBF immersed in distilled water were carried out for five days. The film samples were removed from the water, dried, and EI measurements were performed.

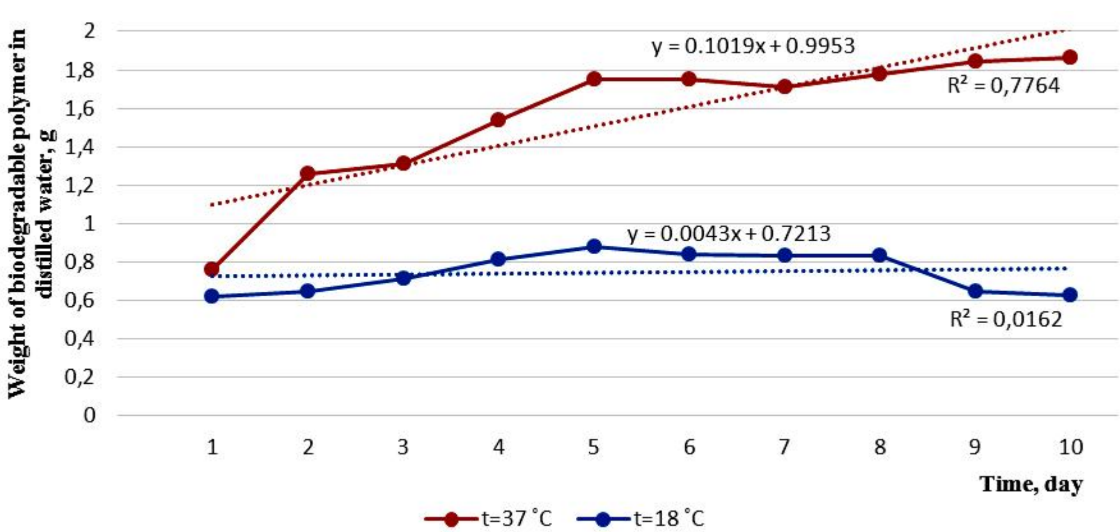

Fig. 8. Dynamics of weight change of the EBF in distilled water at room temperature (18 °C) and 37 °C

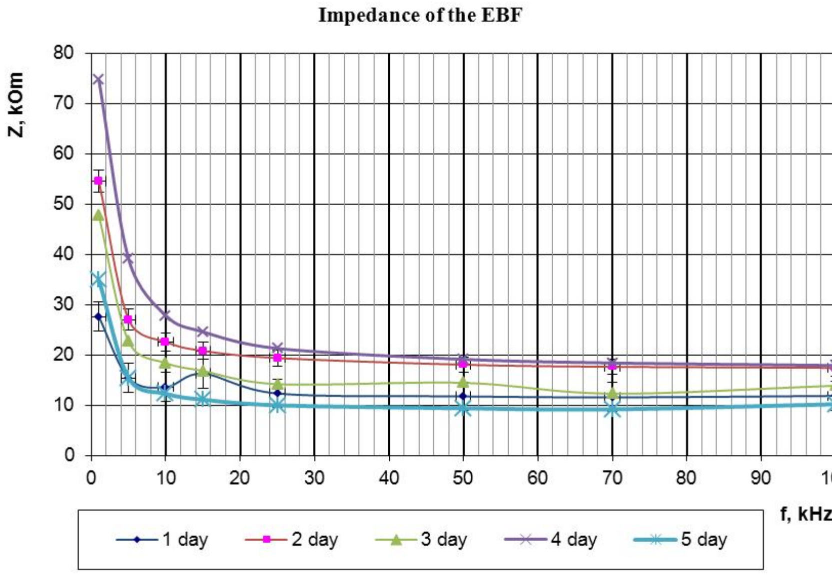

Fig. 9. Electrochemical impedance spectroscopy of the EBF during a five-day soaking in distilled water

EIS studies conducted with the EBF showed that the impedance varies with frequency. EI decreased with increasing frequency (Fig. 9).

In addition, before immersing the EBF in water, its EI was measured. It was found that the EBF did not give EI values at low frequencies, and only at a frequency of 100 kHz its value was 72.2 kΩ. This indicates that the films, at low frequencies, are fairly good insulators. After five days of swelling in water, the resistance of the films at 100 kHz decreased in the range from 53.3 kΩ to 62 kΩ. This indicates that the developed EBFs can absorb water and are good current conductors.

The EBF, the initial starch, and the BP were studied using infrared spectroscopy in the range of 4000–400 cm^{-1} on a FT-IR spectrometer Spectrum Two (PerkinElmer). Fig. 10 shows the spectra of the initial starch (1, blue), the EBF plasticized with glycerol at 70 °C (2, black), and the BP (3, red), superimposed together for comparison.

As can be seen from Fig. 10, there is an ester group in the BP as evidenced by an intensive adsorption band at 1712 cm^{-1} in the spectrum. This is because the BP is produced by the esterification reaction of starch with an acid.

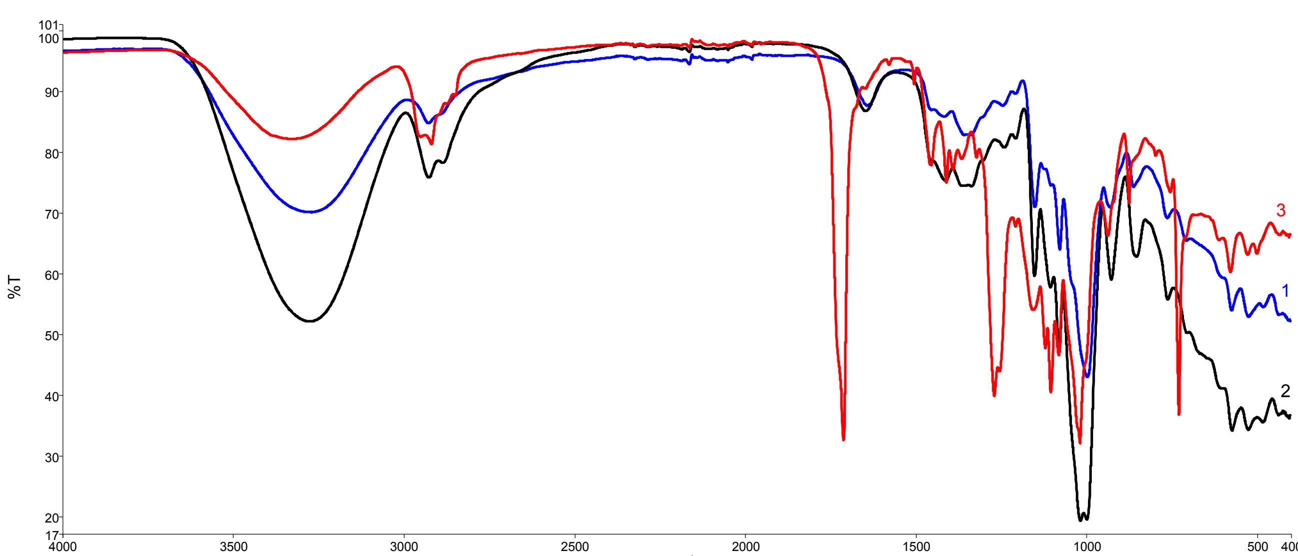

Fig. 10. Infrared spectra of initial starch (1, blue), the EBF (2, black), and the BP (3, red)

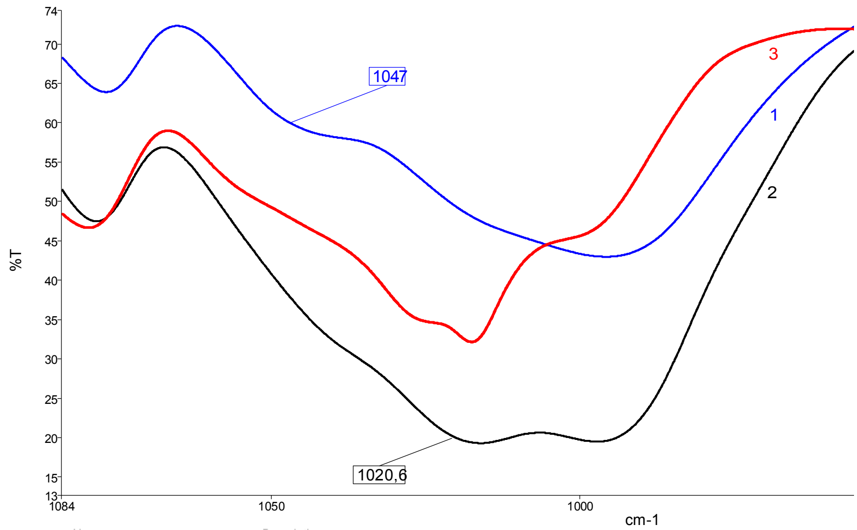

Fig. 11. Infrared spectra in the 1100-900 cm^{-1} range of initial starch (1, blue), the EBF (2, black), and the BP (3, red)

It is known that the absorption band at 1047 cm^{-1} is more intense in the more crystalline starch, and the absorption intensity at 1020 cm^{-1} increases in the more amorphous starch[31]. Such a dependence was observed in our case (Fig. 11), where the initial starch has a shoulder of more intense absorption at 1047 cm^{-1}, and in the EBF, an absorption shoulder at 1020 cm^{-1} appears, which is not present in the initial starch.

4. Conclusions

Experimental biodegradable films (EBFs) based on starch were obtained using glycerol as a plasticizer.

It was found that the EBF can resist sterilization conditions at 1 atm and 121 °C for 30 min.

The EBF sample is better decomposed by microorganisms living in the soil in comparison with industrial biodegradable plastics (BP). This is possible due to the EBF ability to absorb water, making it available for better biodegradation.

The EBF biodegradability was experimentally proven due to the content of only glycerol in the formulation, which is non-toxic to bacteria and fungi. In contrast, the industrial BP, which are positioned as biodegradable, may contain additives toxic for microorganisms. These additives may include synthetic plasticizers, dyes, texture, and strength improvers, as well as specially added antibacterial agents to eliminate food contamination by microorganisms.

The EIS method, which allows studying the electrical properties of starch films, has shown that due to their characteristics, EBFs can be useful in various fields such as food industry, biomedicine, packaging technologies, *etc.*

The EBF formulation used for the preparation of laboratory samples needs to be improved and refined in order to enhance physical and mechanical characteristics compared to the industrial BP samples.

The results obtained are planned to be implemented in further research aimed at developing promising packaging for the food and medical industry.

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ДОСЛІДЖЕННЯ БІОДЕГРАДАБЕЛЬНИХ ПЛІВОК НА ОСНОВІ КРОХМАЛЮ ТА ВИЗНАЧЕННЯ ДЕЯКИХ ЇХНІХ ФІЗИКО-ХІМІЧНИХ ХАРАКТЕРИСТИК

Анотація. *Виконано дослідження із виготовлення у лабораторних умовах експериментальних біодеградабельних плівок (ЕБП) та проаналізовано рецептури з крохмалю кукурудзяного та картопляного з 8, 10 та 15 % вмістом. З'ясовано, що ЕБП на основі картопляного крохмалю з концентрацією 10 % пластичніший і добре зберігає форму, порівняно з іншими зразками. Здійснено мікробіологічні, механічні, фізико-хімічні, ІЧ-спектроскопічні дослідження ЕБП та біодеградабельного пластику (БП), доступного на ринку в формі пакувальних пакетів (БП з "АТБ", БП з "Сільпо", БП з "Roshen"). Виділено нагромаджувальні культури з ґрунту та ідентифіковано їх мікроскопуванням фіксованих мікропрепаратів. Експериментально досліджено і встановлено здатність виділених культур мікроорганізмів до біодеградації ЕБП та опрацьовано специфіку біодеградації ЕБП у результаті діяльності мікроорганізмів різних таксономічних груп.*

Ключові слова: *біодеградабельність, плівки на основі крохмалю, гліцерин, електрохімічна імпедансна спектроскопія (ЕІС), мікроорганізми.*