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Combined use of Bacteria and Enzymes in the Degradation of Polymer Materials

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ABSTRACT:
The development of the plastics industry, pro-ecological directives and environmental problems related to the decomposition of biodegradable plastics create necessity to develop a way to properly manage waste from materials that should not be deposited in the environment.
A bioproduct has been developed to be used for accelerating the biodegradation of polymer materials. The bioproduct includes carefully selected microorganisms and enzymes. It has been observed that metabolic activity of selected bacteria increases significantly in the presence of plastic material, causing degradative changes. In addition, enzymes were used that also stimulate the decomposition of polymer materials. Impact of bacteria and enzymes on polymer biodegradation was analyzed using different methods: BOD, mass loss, SEM-EDX and FTIR-ATR. Also the effect on plant growth was examined. Five bacterial strains were selected as the active ingredients of the bioproduct, including two bacterial strains from the designated collection and three isolated from environment. Four hydrolytic enzymes have been selected that have the potential to accelerate the decomposition of polymer materials.

Keywords: bacteria, enzymes, biodegradation, polymer materials

1. Introduction

For many years, efforts have been made to reduce the use of fossil fuels, which are the raw material, among others, for the production of conventional polymer materials. The growing interest in biodegradable plastics should have a positive impact on the environment from an ecological point of view (Lassoued et al., 2021). Meanwhile, it turns out that they pose a serious problem after use. Low consumer awareness or unclear labeling on packaging causes them to end up in plastic waste containers, which makes recycling conventional materials more difficult (Ayilara et al., 2020). Research shows that biodegradable plastics deposited in compost according to their intended purpose do not decompose within the expected time.

In laboratory tests, materials obtain compostability certificates and are carried out in controlled conditions, different from real ones. Meanwhile, composting in the environment depends on many factors (Nemet et al., 2021; Unmar & Mohee, 2008). The pace of processes is influenced by, among others: large temperature fluctuations during

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the day, humidity depending on the weather and the irrigation system used, degree of aeration, chemical and biological composition of the compost (Ho et al., 2022).

Researchers raise the problem of improving the quality of compost produced from organic waste in industrial composting plants. Emerging plastic waste, including biodegradable ones, complicates the composting process (Luyt & Malik, 2019). One of the solutions is the preliminary treatment of organic waste using appropriate devices (Alessi et al., 2020). However, large amounts of plastic waste often cause damage to these devices during the process. Moreover, their use involves additional costs. Meanwhile, industrial composting plants are often part of larger waste processing plants that are obliged to recultivate former landfills (Xu et al., 2020). For this purpose, produced compost is used. In addition to using it for their own purpose, many companies strive to certify their own product and commercially sell mature compost as a substrate for growing plants. However, this requires guaranteeing high and repeatable quality.

The solution is to develop a product whose composition is adapted to accelerate the decomposition of polymeric materials in compost. There are products on the market that generally accelerate the decomposition of organic matter, but their effectiveness in the degradation of plastics has not been proven. Biological products may contain two types of active agents: microorganisms or enzymes.

Previous studies have identified bacteria that have the potential to accelerate the decomposition of polylactide (PLA) foils (Janczak et al., 2023). Based on the ISO 846 standard bacterial strains that intensively grew on the foil after 28 days of contact, were considered potentially capable of degrading polymer materials. According to the assumption of the methodology included in the standard, microorganisms intensively growing on the surface of polymer materials can use the polymer as a carbon source. The individual effectiveness of each of the 5 selected bacterial strains was confirmed in quantitative analyses. The presented research verified their effectiveness in combination with selected hydrolytic enzymes.

The aim of the presented research, in addition to confirming the effectiveness of the selected bacterial strains, is to determine the interaction between enzymes and the bacterial mixture, as well as to verify their synergistic effect on the degradation of PLA.

2. Material and methods

2.1 Polymer material

The tests were carried out on samples of PLA foil made from granules (Ingeo TM Biopolymer, 2003D, Nature Works® LLC, USA). The foil was extruded using a single-screw lab extruder Plasti-Corder PLV 151 (Brabender, Germany). Before the process, the material was dried at 80 °C for 8 hours. The film was extruded at the temperature of the heating zones and extruder head of 190 °C ± 5 °C. A foil with a thickness of ok. 0.09 mm was obtained.

2.2 Microorganisms

Five bacterial strains were used. They came from the national collection (2 strains) and own collection of environmental strains (3 strains) (Table 1).

Table 1: Bacterial strains and their origin.

No.	Strain	Origin
1.	Unidentified (KJ-D-3)	Own collection, polymer waste from landfill
2.	Unidentified (KJ-LIN-2-26)	
3.	Unidentified (KJ-LIN-7-32)	
4.	*Bacillus subtilis* (PCM 2224)	National collection, Polish Collection of Microorganisms (registered in the World Collection of Microorganisms - WFCC under number 106)
5.	*Bacillus cereus* (PCM 2018)	

2.3 Enzymes

Hydrolytic enzymes were used, which, when used individually, caused changes in the structure of the foil indicating degradation (unpublished data). Proteinase K from *Tritirachium album* solution in Tris/HCl buffer (Sigma Aldrich, USA) was selected. It is the most popular enzyme used in the degradation of PLA. In addition, food enzyme products were used: bromelain, papain and nattokinase (GymBeam, Germany).

2.4 BOD

The biochemical oxygen demand (BOD) of a mixture of bacterial strains in the presence of PLA was checked to assess the possibility of their combined use for PLA degradation. This is a conventional indicator determining the amount of oxygen (mg O_2/dm^3) that is necessary for the oxidation of organic compounds, in this case polymeric materials, by aerobic microorganisms. Microorganisms consume oxygen and produce carbon dioxide, which is absorbed by NaOH. The reduction of oxygen causes the pressure in the reactor to drop (Yamashita et al. 2022).

Measurements were carried out for 14 days using the OXI-TOP Control (WTW, Poland). According to the previous methodology (Janczak et al. 2023), the research was carried out in reactors with a volume of 500 ml, each containing 200 ml of liquid medium, the gas volume was 300 ml. Fragments of PLA foil measuring 20 mm × 20 mm were used in the tests. Before being placed in the reactors, the foil was weighed and sterilized with 70% ethanol.

Mineral medium prepared in accordance with the international standard (ISO 846) was used as the medium. A sterile physiological 0,85% NaCl solution was used to prepare a bacterial suspension, i.e., a twice-diluted inoculum with a solution turbidity of 2 McF. Suspensions of single bacterial strains were mixed in equal volumes and added 500 µl/reactor.

Additionally, the same method was used to check how the presence of individual enzymes would affect the effectiveness of bacterial strains. Enzymatic solutions were prepared containing 0.2 mg of enzyme/100 ml of sterile demineralized water. 1 ml of such a solution was added to the selected reactors.

BOD tests were repeated three times. The results were read continuously, every 90 minutes and were shown as an average BOD a day.

2.5 Foil analysis

After completing the BOD measurements, the foil samples were removed from the reactors. The samples were cleaned by rinsing with sterile demineralized water, dried in sterile Petri dishes containing a disk of filter paper and left under sterile conditions in a

desiccator for 24 h. The samples were subjected to the following analyses: mass loss, microscopic analysis and infrared spectroscopy.

Mass loss was determined in accordance with the international standard (ISO 846) based on initial and final mass measurements performed on an MSI05DU analytical balance (Mettler Toledo, Switzerland). The results were recorded with an accuracy of 0.001 g.

Microscopic analysis was performed using a scanning electron microscope - SEM (Hitachi SU8010, Japan) at ×1000 magnification and 2 kV voltage. Additionally, in order to determine the change in O_2 and C content (% by mass) in the analyzed samples, an analysis of the oxygen to carbon ratio (O/C) was carried out. For this purpose, elemental analysis of the surface of foil samples was performed using energy dispersive X-ray analysis (EDX) using the SEM-EDX (Thermo Scientific Ultra Dry, USA). The EDX spectrum of the sample surface allows for a semi-quantitative analysis of the elemental composition to a depth of approximately 1 μm (Hockaday et al. 2007). During EDX analysis, the preparations were exposed to a voltage of 20 kV and a current of 15 μA. The surface elemental analysis lasted 30 seconds at ×100 magnification and a working distance of 15 mm.

Infrared spectroscopy (FTIR) with reflectance (ATR) was used to determine the carbonyl index. FTIR spectra were recorded in the range of 400-4000 cm^{-1} using a FTIR Cary 630 (Agilent, USA) equipped with a diamond crystal (spectral resolution < 2 cm^{-1}). The focus was on the region of the carbonyl group at 1746 cm^{-1}, which is susceptible to biodegradation processes. The carbonyl index was calculated in relation to the asymmetric vibrations of the CH_3 group at 1450 cm^{-1} (Puszczykowska et al. 2022).

2.6 Phototoxicity

Previous studies (Janczak et al. 2023) confirmed the lack of phytotoxicity or the effect of stimulating the germination and growth of spring rapeseed (*Brassica napus L.*) after the use of selected bacterial strains. In the presented research, the phytotoxicity of enzymes was checked based on the OECD/OCDE 208/2006. The number of germinated seeds (%) and the size of hypocotyls (mm) were determined after 5 days of incubation at 26 °C with a photoperiod of 16 h/8 h. The tests were performed using 60 seeds for each variant.

3. Results

3.1 BOD

Previous studies (Janczak et al. 2023) confirmed the lack of phytotoxicity or the effect of stimulating the germination and growth of spring rapeseed (*Brassica napus L.*) after the use of selected bacterial strains. In the presented research, the phytotoxicity of enzymes was checked based on the OECD/OCDE 208/2006. The number of germinated seeds (%) and the size of hypocotyls (mm) were determined after 5 days of incubation at 26 °C with a photoperiod of 16 h/8 h. The tests were performed using 60 seeds for each variant.

BOD was determined for a mixture of selected bacteria in the presence of enzymes: proteinase K (Pro), bromelain (Bro), papain (Pap) and nattokinase (Nat). The control was a bacterial suspension without enzymes. The tests were carried out on variants

with and without PLA foil (Fig. 1). The device records results from 0.1 mg/L to 400 mg/L. Higher values were defined as > 400 mg/L (Fig. 1).

The variants without PLA showed higher BOD in the presence of each of the enzymes used in the first four days. For comparison, on the fourth day BOD for the control variant (Ctr - bacterial suspension without enzymes) was 5.6 mg/L, papain (Pap) > 400 mg/L, bromelain (Bro) 250 mg/L, nattokinase (Nat) 205 mg/L and proteinase K (Pro) 14.1 mg/L. In the presence of nattokinase (Nat), despite the initial increase in BOD, after 5 days BOD values were found below 0.1 mg/L and the measurement was terminated. While in the control variant (Ctr) the values were recorded for one day longer, after which the BOD value also dropped below the detection limit. In the presence of proteinase K (Pro), the measurement lasted until day 9. In the presence of bromelain (Bro) and papain (Pap), measurements were made until the end of incubation (14 days), and for papain (Pap) from days 3 to 14 the BOD value was > 400 mg/L (Fig. 1a).

The presence of the foil allowed BOD measurement in the control variant (Ctr) to be one day longer (6 days) compared to the variant without foil. Measurement of the nattokinase (Nat) variant was completed on the same day. The highest values were obtained in the presence of papain (Pap), although they were slightly lower than in the variants without foil. BOD values in the presence of papain (Pap) from day 5 remained at the level of approximately 400 mg/L until the end of incubation. Bromelain (Bro) with PLA influenced the increase in BOD until the 6th day, then the BOD values were lower than in the variant without PLA, and on the 9th day the measurement was stopped due to the BOD value below the detection limit. In the presence of PLA, it was possible to record results for the proteinase K (Pro) variant for longer, even though the values in the initial days were at a comparable, relatively low level, up to approximately 20 mg/L (Fig. 1b).

(a)

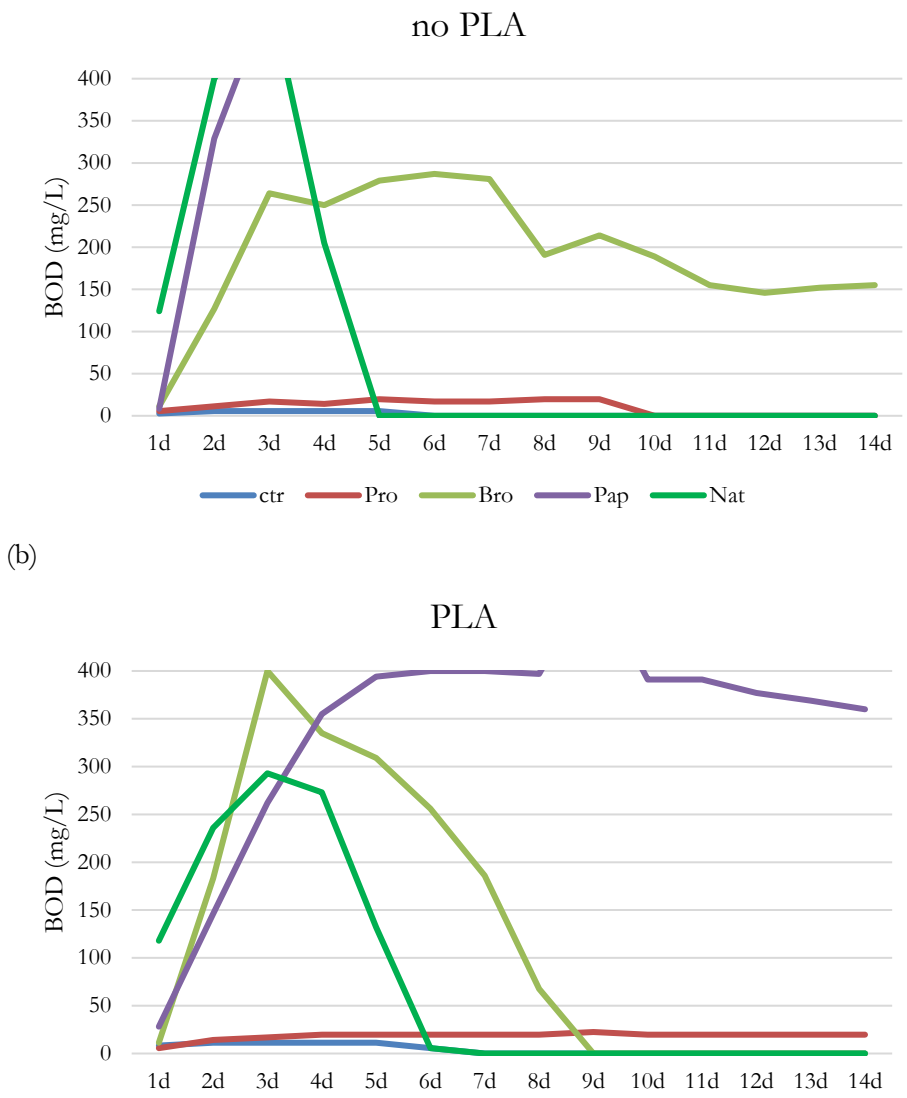


Fig. 1. BOD: (a) variants without PLA; (b) variants with PLA. Signs: x axis – day of incubation; Ctr – bacterial suspension without enzymes; Pro – proteinase K; Bro – bromelain, Pap – papain; Nat – nattokinase.

3.2 Foil analysis

In order to assess the effectiveness of the enzymes used in the degradation, PLA samples were removed and the samples were cleaned as stated in the methodology description, and foil analyzes were performed. The results were compared to the values for samples incubated in the presence of bacterial strains (Ctr). The research aimed to

assess the usefulness of selected enzymes as factors supporting biodegradation caused by previously selected bacteria.

3.2.1 Mass loss

After 14 days, a PLA mass loss of up to approx. 1% was found for each of the variants used, except for the papain (Pap) variant, for which the mass loss was greater and amounted to over 36% (Fig. 2).

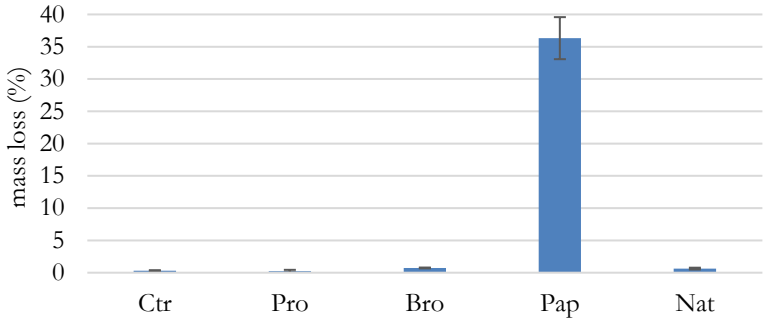


Fig. 2. Mass loss of PLA film. Signs as in Fig. 1.

3.2.2 SEM-EDX

A microscopic analysis of changes in the surface structure of each sample was carried out. On the control sample (Ctr - bacterial suspension without enzymes), evenly distributed bacterial cells and accompanying surface changes were observed, visible in the photo as clear spots around the places of attachment of bacterial cells. Comparable changes were observed after incubation with proteinase K. Similar changes were observed in the presence of papain (Pap) and nattokinase (Nat). The difference was that the bacterial cells were grouped, and individual cells were less numerous. Additionally, structures indicating the formation of a biofilm were observed. Film cracks were observed on the sample incubated with nattokinase (Nat). In the sample incubated with bromelain (Bro), structures indicating the biofilm being formed were most numerous and evenly distributed over the entire surface of the foil (Fig. 3).

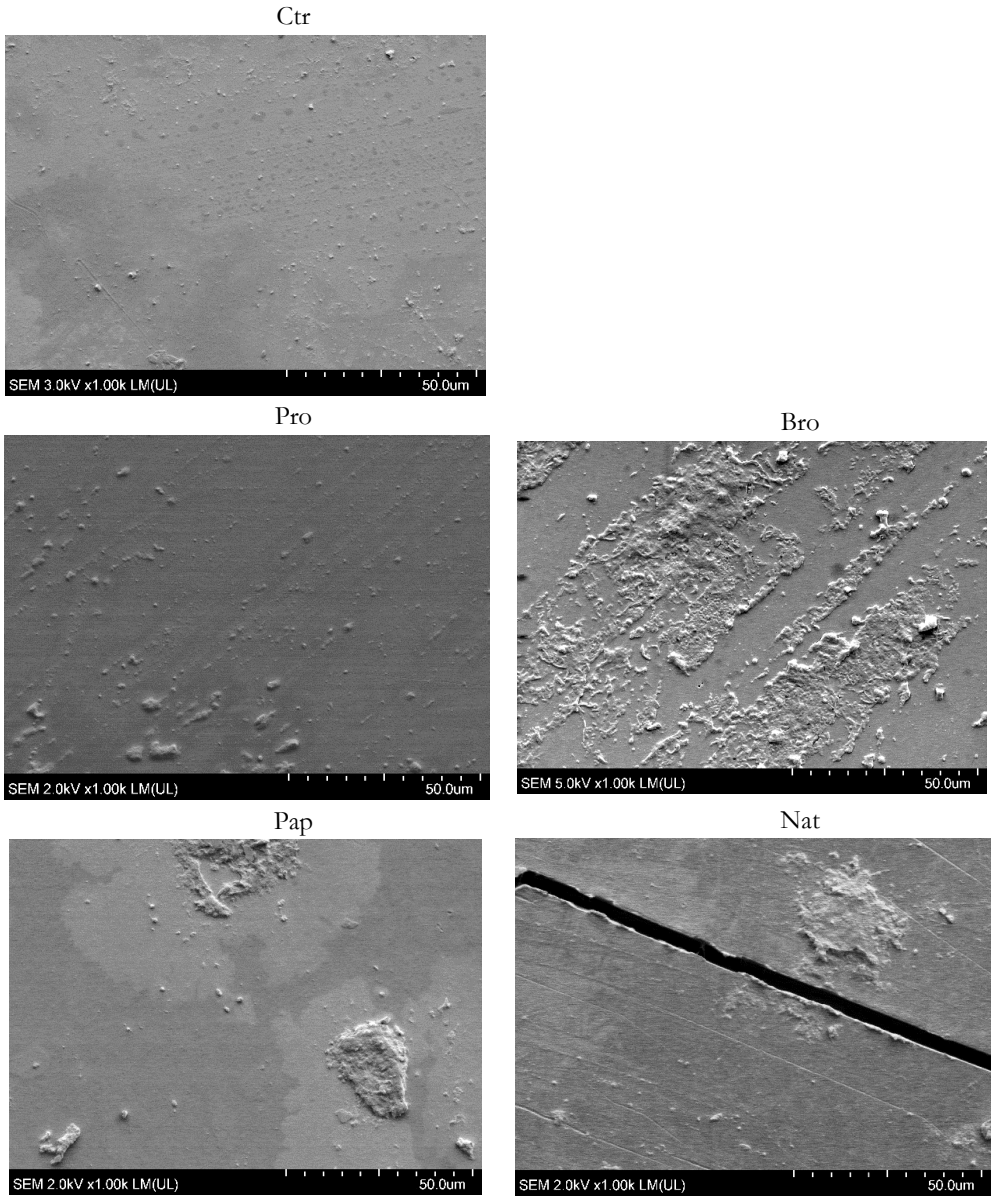


Fig. 3. SEM analysis of PLA film. Signs as in Fig. 1.

Next, the O/C ratio was analyzed. In each variant, including the control (Ctr), an increase in this parameter was observed, indicating progressive degradation processes. The presence of each enzyme stimulated this effect. The greatest increase in the O/C ratio (from 1.6 to 2.6) was observed after adding papain (Pap) to the medium. For PLA incubated with other enzymes, the O/C ratio ranged from 2.3 to 2.5 (Fig. 4).

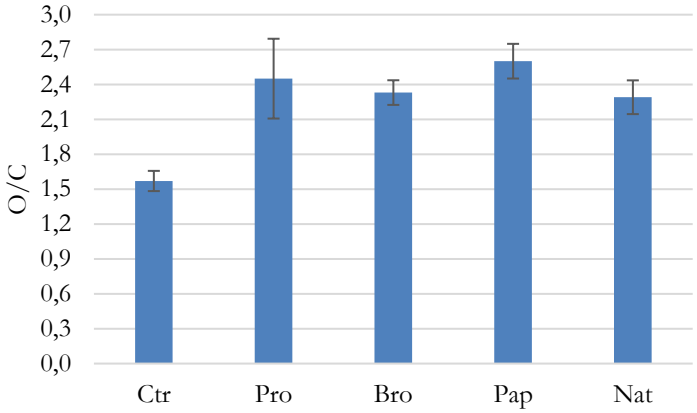


Fig. 4. O/C ratio of PLA film. Signs as in Fig. 1.

3.2.3 FTIR

The carbonyl index was determined based on the results of FTIR-ATR analyses. This parameter decreases during progressive degradation. In most variants, the presence of enzymes did not affect changes in the carbonyl index compared to the control (variant with bacteria without enzymes). The carbonyl index ranged from 6.2 in the papain variant (Pap) to 6.7 in the proteinase K variant (Pro). A significant decrease in the value was noted after incubation in the presence of nattokinase (Nat). The value of the carbonyl index in this variant was 5.6 (Fig. 5).

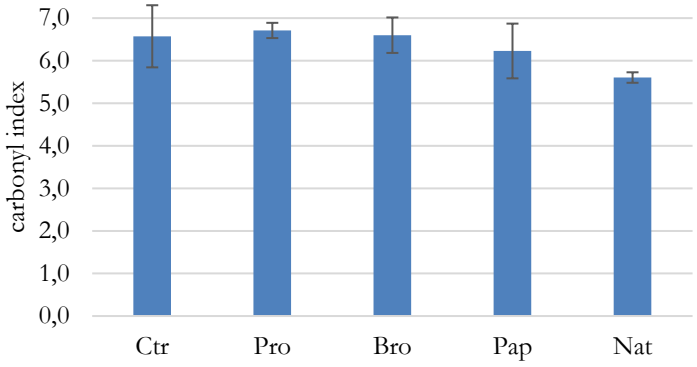


Fig. 5. Carbonyl index of PLA film. Signs as in Fig. 1.

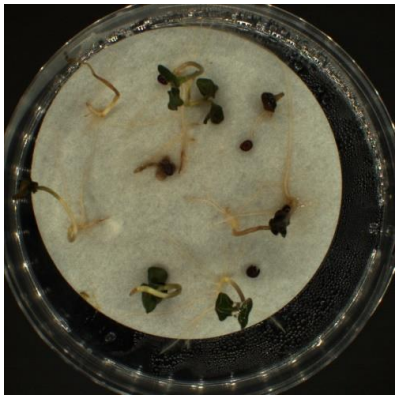
3.3 Phytotoxicity

To confirm the possibility of using the enzymes used to accelerate the degradation of PLA, their phytotoxicity towards spring rapeseed seeds was checked. In the presence of bacteria without enzymes, 48 out of 60 seeds germinated. Similar results were recorded

in the presence of proteinase K (Pro) (46 seeds germinated) and nattokinase (Nat) (48 seeds germinated). After breeding with bromelain (Bro) and papain (Pap), a larger number of germinated seeds was found: 54 and 56 seeds, respectively. (Fig. 6).

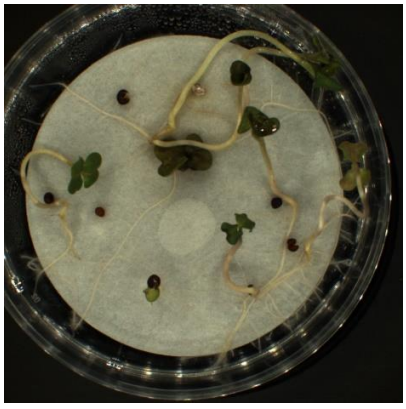
For control plants (Ctr), the average hypocotyl size was 12.89 mm. The result was influenced by the large standard deviation between the results. Similar values were obtained for plants growing in the presence of bromelain (Bro) and papain (Pap). In the presence of proteinase K (Pro) and nattokinase (Nat), hypocotyls were longer and had an average of 18.33 mm and 16.86 mm, respectively (Fig. 6).

Ctr



S: 48/60
H: 12.89 ± 7.753

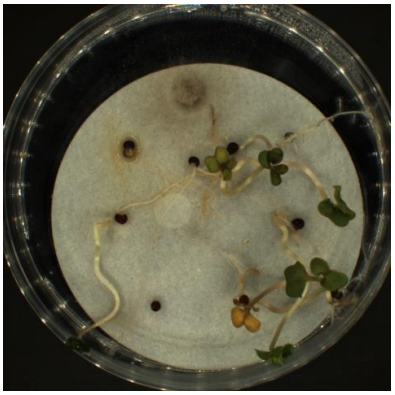
Pro



S: 46/60
H: 18.33 ± 6.831

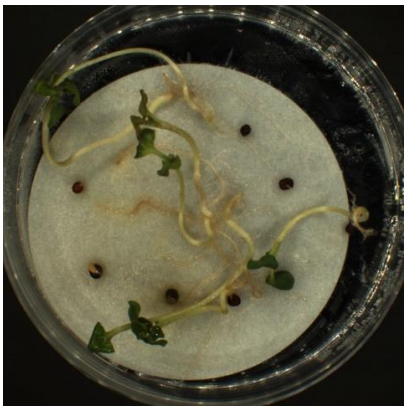
Pap

Bro

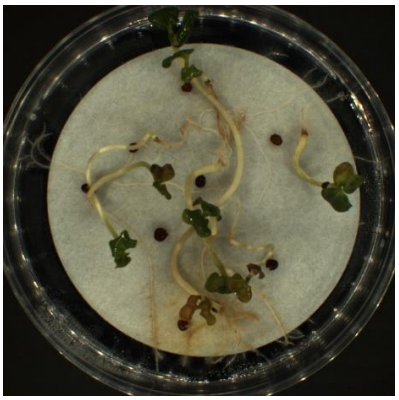


S: 54/60
H: 12.22 ± 3.632

Nat



S: 56/60
H: 12.00 ± 6.000



S: 48/60
H: 16.86 ± 4.581

Fig. 6. Phytotoxicity of enzymes towards spring rapeseed. Signs: S – number of germinated seeds (max. 60 pieces); H – hypocotyl size (mm); others as in Fig. 1.

4. Discussion

The aim of the research was to determine whether the presence of selected hydrolytic enzymes can support bacteria in the degradation of polymer materials, using the example of PLA. It is a material that is both the most popular among biodegradable materials and the most difficult to decompose (Xu et al. 2022). The research was conducted using a mixture of bacteria selected in previous studies, which showed the potential to accelerate PLA degradation and lack of phytotoxicity (Janczak et al. 2023). During the analysis of the results, the focus was primarily on variants in which a stronger effect was obtained than in the presence of bacteria alone, which have the potential to accelerate the degradation of PLA.

The selection of strains for testing began several years ago (Janczak et al. 2018, Janczak et al. 2020). The selection was based, among others, on the method included in the ISO 846 standard, which assumes that polymers are susceptible to degradation if the microorganisms listed in the standard show growth on their surface under minimal conditions when the polymer material is their only source of carbon. It was deduced that by isolating microflora from anthropogenically degraded areas exposed to contact with contaminants, including microplastics, using a minimal culture medium for incubation in the presence of the most difficult-to-decompose biodegradable material, we will select microorganisms that effectively influence the PLA decomposition process. Further studies of the observation of biodeterioration of polymeric materials confirmed the hypothesis. In the previously selected bacterial strains, the activity of producing hydrolytic enzymes from the protease group was found. For the current studies, new strains of bacteria were obtained which also demonstrated hydrolytic activity, and its potential was increased by the addition of other proteases: proteinase K, bromelain, papain and nattokinase. Studies have shown that the combination of these two factors: bacteria and enzymes, has a positive effect on accelerating the degradation processes of PLA.

In the variant without enzymes, containing only a mixture of bacteria, lower BOD was observed than in the variants with enzymes, which proves the positive effect of enzymes on the microorganisms used. Also, the presence of PLA made the BOD measurement possible one day longer due to the greater metabolic activity of the bacteria present. The effectiveness of BOD measurement in the selection of microorganisms has been demonstrated in previous studies by our own and other authors (Richert et al. 2021, Swiontek Brzezinska et al. 2024). The method was used by Wang et al. (2022) examining the metabolic activity of microorganisms in order to assess their usefulness in the degradation of polymer contaminants from seawater. The researchers in this case, similarly to our research, hypothesized that the greatest potential in this aspect would be demonstrated by bacterial strains from environments exposed to contact with stress factors in the form of pollution. Also, the analysis of PLA samples after the test showed degradation changes in the control variant, without enzymes, which confirms that the selection was correctly carried out in the earlier stage of the research. Noticing significant differences between the variants used in just 14 days proves the special potential of the selected active factors. Further research should focus on verifying the effectiveness of the selected agents on other types of polymer materials. However, demonstrating its effectiveness against PLA is a very important aspect, as research indicates that it is a material that is more difficult to degrade than other natural materials. Nevertheless, demonstrating the effectiveness against PLA is a very important aspect, as research indicates that it is a material that is more difficult to degrade than other natural materials such as PCL, PHB (De Falco et al. 2021, Engler et al. 2023). The complexity of the bacterial mixture used most likely resulted in significant deviations in plant measurements during the phytotoxicity test, even though each strain used individually was not toxic to the same plant (Janczak et al. 2023).

In the presence of proteinase K, which is the most popular enzyme used in the degradation of PLA, degradation changes were found, but they were not as spectacular as in the other cases (Seok et al 2022, Urinov et al. 2022). However, it did not have a toxic effect on the strains used, especially when PLA was additionally present in the medium. Probably the enzyme facilitating the degradation of the polymer material, including proteinase K, made it easier for the bacteria to absorb carbon from the polymer with less energy associated with the production of their own enzymes. This can also be proven by bacterial cells evenly attached to the PLA surface, observed during microscopic analysis. The potential of microorganisms and enzymes in the degradation of polymer materials, including petroleum products, was noticed by Mohanan et al. (2020). In their review, they paid attention primarily to the synergism between the strains used together and their diverse enzymatic activity. They pointed out the potential of using mixtures of microorganisms, thus confirming the results of studies by other researchers showing that each of the microorganisms, characterized by different hydrolytic activity, initiates degradation processes in different parts of the polymer chain (Nair et al. 2016). Surprisingly, the presence of proteinase K additionally stimulates the plants to grow. However, there are no literature reports on the effect of this enzyme on rapeseed plants or other species. Although it has been proven that proteases have a beneficial effect on plants, e.g., by increasing tolerance to salinity or organic solvents (Verma et al. 2016, Srivastava et al. 2017).

There are few reports on the use of other enzymes originating from food products. In the study by Nguyen et al. (2014) it was shown that proteinase K has a stronger effect than papain in limiting the formation of biofilm of the pathogenic *Listeria sp.* and has a stimulating effect on other microorganisms. In the present research, papain showed the highest effectiveness, in the presence of which the highest BOD was observed, oscillating at the upper measurement limits until the end of incubation. Papain was the only enzyme used to cause a 36% weight loss, while in the remaining variants this value was approximately 1%. In this variant, the highest increase in the O/C ratio was also observed, from 1.6 for the control variant with a mixture of bacteria, to 2.6. In the presence of papain, more rapeseed seeds germinated, although it did not stimulate the growth of hypocotyls. So far, there are no reports on the use of this enzyme in the degradation of polymer materials. The mechanisms of effectiveness can be found in the structure of this enzyme. Papain belongs to the group of proteases that cause enzymatic degradation of polyesters by hydrolysis (Lim et al. 2005, Banerjee et al. 2014). The use of papain may be a cheaper alternative for the commercial production of plastics composting accelerators compared to the relatively expensive proteinase K. However, to discover more effective enzymatic solutions for waste management it is necessary to test a wider spectrum of polymeric materials.

Bromelain is commonly used protease, among others, in the meat industry (Kumar & Pandey 2018). Studies have shown its positive effect on microorganisms and plants. In the presence of this enzyme, a significantly higher BOD was recorded (from 5.6 mg/L to approx. 250 mg/L), and structures indicating the forming of biofilm were observed during microscopic evaluation. No such significant impact on the degradation changes of the materials themselves was found, however, a beneficial effect on hydrolytically active microorganisms may, indirectly, also have a positive impact on the degradation at a later stage. Bromelain had a positive effect on the germination of rapeseed, although, similarly to papain, it did not result in a greater increase in the length of the hypocotyl.

The last enzyme used was nattokinase, which is an enzyme produced by strains of the *Bacillus* genus, which in our previous studies showed the highest effectiveness in the degradation of, among others, PLA, as well as a positive effect on plants grown for biomass, including miscanthus (Dąbrowska et al. 2021). The study showed a positive effect of nattokinase on the increase in BOD, although the presence of PLA in this case did not extend the measurement possibilities over time. This enzyme, in turn, was the only one used to significantly reduce the carbonyl index, which is an indicator of advanced degradation processes (Celik et al. 2023). In turn, the presence of nattokinase, similarly to proteinase K, had a stimulating effect on the growth of rapeseed hypocotyls.

In addition to biotic factors, the rate of biodegradation is also influenced by abiotic factors such as temperature fluctuations, humidity and oxygen content (aeration). The composting phase is determined based on composting temperature measurements. The optimal temperature for the growth of microorganisms is 25-40 °C in the mesophilic phase and above 45 °C in the thermophilic phase (Jainet et al. 2019). However, too high a temperature (above 65 °C) causes protein denaturation and the destruction of microflora (Ho et al. 2022). Enzymes are also partly composed of protein. However, once the optimal temperature is reached, higher temperatures cause rapid degradation of the enzyme and

irreversible loss of activity (Zhang et al. 2018). Our research demonstrated the activity of mesophilic bacterial strains, which means that their greatest activity in real composting conditions will take place before the thermophilic phase, in which, after the destruction of microflora in the event of overheating, the next process will be the thermal degradation of polymer materials. Thermo degradation is considered to be the basic mechanism of degradation of aliphatic polyesters such as PLA (Chrysafi et al. 2021, Kervran et al. 2022). This process, similarly to other types of degradation, is faster for materials with smaller fragments (larger active surface), lower molecular weight, etc. Thanks to the use of enzymes and bacteria selected during research, it indirectly increases the efficiency of degradation in the thermophilic phase. These degradative changes, which would only take place in the thermophilic phase, are initiated in the mesophilic phase.

Water is a solvent for nutrients. If the humidity level is lower than 30%, the metabolism of microorganisms is limited. However, an increase in humidity above 70%, without ensuring dynamic cultivation conditions, and thus aeration, will result in a change of conditions to anaerobic and slow down the composting process, additionally creating conditions for the development of microorganisms causing rotting and an unpleasant, undesirable smell of compost (Turan et al. 2008). Hence, in our research conducted in a liquid medium, dynamic shaking was used during incubation. When composting under environmental conditions, it is essential to monitor the substrate moisture.

Aeration is considered to be a key element in accelerating the composting of polymeric materials, as oxygen serves as an electron acceptor for the biodegradation of organic matter and influences the nitrogen conversion (Ma et al. 2021, Zhao et al. 2022, Ho et al. 2022). Further study of variable abiotic factors under actual environmental conditions is essential and may provide insights into optimizing conditions for faster degradation.

The obtained results will be used to develop a product that accelerates the composting of selected polymer materials. It can be assumed that due to the high activity of hydrolytic strains, the product will also accelerate the decomposition of the basic organic matter subjected to composting. The developed product will undoubtedly contribute to the implementation of activities aimed at sustainable development by ensuring effective degradation of PLA as an alternative to conventional, petroleum-derived polymer materials.

5. Conclusion

The research conducted showed that each of the enzymes used has the potential to be used together with selected bacterial strains to accelerate the degradation of PLA. The use of two active agents, bacteria and enzymes, can stimulate degradation directly or indirectly. The application potential of the enzymes used is increased by the fact that none of the enzymes used showed phytotoxicity. Proteinase K, although it did not have a significant impact on the degradation processes of PLA, stimulated the metabolism of microorganisms and had a beneficial effect on plants. Papain was the only one that showed multidirectional effects, i.e., it contributed most to the progress of PLA degradation and had the most beneficial effect on the metabolism of microorganisms and seed germination. Although bromelain did not show such spectacular results in the degradation of PLA, it

had a beneficial effect on microorganisms and plants. In turn, nattokinase was the only one that caused a significant decrease in the carbonyl index of PLA, which indicates the degradation of carbonyl bonds and advanced deterioration of the material.

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