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The effect of temperature on the biodegradation of different types of packaging materials under test conditions

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Abstract: The development of civilization contributed to the exponential growth in the production of plastics. Policy of the, so-called, "European Green Deal" places particular emphasis on reducing the use of plastics through various mechanisms, including their reuse, recycling and, in particular, the development of new biodegradable and compostable plastics. In order to check if plastics are suitable for biodegradability and compostability they must undergo a series of tests in accordance with applicable standards. The biodegradation test procedures are very general and allow for the use of different temperatures of the biodegradation process in the test. The aim of the research was to evaluate the influence of temperature on the biodegradation process of selected packaging materials. The obtained results show a significant influence of the temperature of the biodegradation process of all 3 tested types of packaging materials: oxy-biodegradable, corn starch and paper. Statistically significant differences in the biodegradation rate of the tested packaging materials were demonstrated in as low as 40°C, despite the low intensity of the process. As the process temperature increased to 45 and 50°C, a statistically significant increase in CO_2 productions was recorded. CO_2 is produced by the degradation of polymers and is an indicator for this process. At 50°C, the highest decomposition rate, resulting in the highest CO_2 production, was recorded in the case of corn starch films. Oxy-biodegradable material showed worst degradation potential what excludes it from composting processes.

Introduction

The increase in consumption of goods is related to the increase in man-made waste, which directly threatens the natural environment. In recent years, it has been observed that the contamination with plastics and various packaging materials is particularly dangerous for the land, seas and oceans. Changes in the aquatic and terrestrial ecosystems due to anthropogenic impacts such as plastic pollution can have a dramatic global impact.

In July 2021, the, so-called, Single-Use Plastic Directive, which introduces, inter alia, a ban on the marketing of ten single-use products whose alternatives are safer for the environment, will enter into force. In addition to European regulations, each EU member state determines its internal environmental policies.

Due to the fact that the problem of plastics remains unresolved, various ways to reduce their impact on the environment are being considered. One of such methods is the production of plastics and packaging materials that are biodegradable (process can take 2–3 years) and at the same time their decomposition products are not toxic to the environment. In addition, the production of bioplastics and biomaterials often requires lower energy inputs, and thus the emission of greenhouse gases (GHG) to the atmosphere is reduced. Such bioplastics contain natural polymers or salt mixture that biodegrade much faster than traditional plastics (Yashchuk et al 2012). The main biomaterials are cellulose, chitin and chitosans, PLA (polylactic acid), PHA (polyhydroxyalkanoates), thermoplastic starch or PBS (polybutylene succinate). Products based on corn starch are of particular interest due to high yield and share in crop mass (Herniou-Julien et al. 2019, Ivankovic et al. 2017, Wróblewska-Krepsztul et al. 2018).

Bioplastics, i.e., polymers of biological origin, biodegradable or compostable or both, play a small but significant role in the global market for thermoplastics. Currently, bioplastics account for less than 1% of the world's thermoplastic production capacity. Polymers that are wholly or partially derived from renewable raw materials, including bio-polyethylene and bio-polyethylene terephthalate (PET), account for 58% of the production capacity. The rest are biodegradable or compostable polymers. Many compostable polymers, including polylactic acid and starch/copolyester compounds, in addition to being biodegradable, are of biological origin (Wróblewska-Krepsztul et al. 2018).

Due to their use and intention to avoid a negative impact on the environment, biomaterials must be biodegradable and compostable at the same time, and the compost produced in the process must not be toxic to plants. (Herniou-Julien et al. 2019). Most importantly, full biodegradability (biodegradable plastic is "a plastic that degrades because of the action of naturally occurring microorganisms such as bacteria, fungi, and algae", and a compostable plastic is "a plastic that undergoes degradation by biological processes during composting to yield carbon dioxide, water, inorganic compounds, and biomass at a rate consistent with other known compostable materials and leaves no visually distinguishable or toxic residues"), is necessary according to ASTM and ISO standards, otherwise serious health and environmental consequences may occur in effect of micro and nanoplastic spread process and migration in food chain because after fragmentation, the plastic still exists in the environment (Abdelmoez et al. 2021). On the other hand, the production of 1 kg of bioplastics requires 1–2 kg of maize or 5–10 kg of potatoes, which may result in deforestation for the cultivation of plants used in the production of these materials. To avoid this process, it is possible to produce maize or potatoes on degraded/contaminated areas which are not suitable for food production (Ivankovic et al. 2017).

At first glance, it is impossible to distinguish biodegradable and/or compostable plastics from non-biodegradable and non-compostable plastics. Therefore, it becomes necessary to properly label packaging materials that meet the requirements of the standards for biodegradation and/or compostability of the material. The guidelines contained in the standards do not provide reference test methodologies based on specific analytical or instrumental methods, but only indicate the proposed detection methods, e.g., CO_2 or proposed test conditions, e.g., process temperature. The standards indicate the recommended temperatures for the biodegradation process, but at the same time lower or higher temperatures, depending on the technical capabilities of the laboratories can be used. However, it is important that the method is previously validated for other process temperatures.

Only a few commercial bioplastics are completely degradable in the environment. Preferred end-of-life options for plastics include industrial composting or recycling. Unfortunately, due to the lack of composting infrastructure and low recycling rates, plastics (such as conventional plastics) often end up in landfills or incinerators. Moreover, packing material with prodegradant additive d2w® is recognized as compostable and a lot of bio-waste can be collected in cities waste bins. Unfortunately, biodegradation is not the same as compostability, but these terms are often used interchangeably. Not every biodegradable product is compostable. However, each compostable plastic should be biodegradable. Moreover, packing material with prodegradant additive d2w® is recognized as compostable and a lot of bio-waste can be collected in cities waste bins. The possibility of composting packaging materials can be assessed using soil burial degradability test according to SR EN ISO 846/2000, and it takes a long time (Vasile et al. 2018).

As mentioned above, the standards for biodegradation tests, e.g., EN 13432:2002 and EN 14995:2009, describe the test procedures in a very general manner. It is permissible to measure CO_2 released in the decomposition process, as well as classical titration, weight, and instrumental methods based on respirometric or manometric measurements. Although these standards indicate the temperature of the biodegradation process to be carried out at 58°C, nonetheless, they also indicate that this temperature may be higher or lower, after experimental confirmation, that it does not affect the course of the biodegradation process itself. Therefore, the research was undertaken to investigate the influence of temperature on the biodegradation process conducted under aerobic conditions with the use of a respiratory activity test kit. The studies based on carbon dioxide release give much more reliable data on biodegradability than those based on, for example, weight loss measurements of samples or photos showing bacterial colonies on the surface of the tested materials (McLauchlin et al. 2012). The aim of the research was to determine the influence of lower temperatures than recommended or usually known as mesophilic conditions, in the initial stage of the aerobic biodegradation process on the degradation of biodegradable polymers.

Materials and methods

Three different polymers (Fig. 1) used in packaging materials were tested: d_2w® oxy-biodegradable film (Oxy test), corn starch film (Corn test), and a paper bag (Pap test). These materials were selected for comparison of easy degradable material (paper), hard degradable LDPE with d_2w and corn starch material with compostability certificate. In final effect we have one biodegradable material (with d_2w® additives – exact composition of sample 3 has not been reported), and two compostable materials (corn starch and craft paper) made of two natural polymers: corn starch (corn) or cellulose (Pap). All used packaging materials are commercial and easy available on the market. Corn material was marked with a compostability certificate (OK COMPOST INDUSTRIAL and OK COMPOST HOME) from TUV-AUSTRIA.

The tests were carried out on the basis of European standards: EN ISO 14855-1; EN 13432; EN 14995. The tested materials were shredded in accordance with the standard into pieces close to 2×2 cm dimensions. The compost intended for incubation met the requirements of the standards and contained approx. 50% moisture and over 15% VOC (volatile organic compounds) calculated on a dry matter basis. The pH in the aqueous solution (1 part of compost to 5 parts of deionized water) ranged from 7.00 to 9.00. In addition, the compost released from 50 to 150 mg CO_2 per 1 g of VOC during the first 10 days of testing. This compost was mixed with tested materials in the proportion of 6:1 (Fig. 2), ensuring the homogenization and oxygenation of the compost beforehand (Maria et al. 2010).

Three series of tests were carried out consecutively for the temperatures of 40, 45 and 50°C, and each stage of the tests lasted 21 days in order to characterize the initial phase of the biodegradation process.

The tests were carried out using the manometric method with the OxiTop system in 2.5 dm^3 glass containers (Fig. 3).

The prepared sample, moistened to about 50%, was placed in a container and then closed with an adapter with a measuring head. The CO_2 concentration was read automatically every 28 minutes. The pressure inside the container and the temperature were monitored each day. When the oxygen content dropped below 10%, the containers were aired. The samples were treated in the same way in each of the 3 measurement series. The samples were analysed in 3 replications each time. The same compost, which was a component of each sample with the tested polymers, was used as a blank test. Based on the pressure difference, the released CO_2 was calculated using an algorithm that took into account the mass and moisture of the sample.

The following parameters were determined in the tested samples: pH, total organic carbon (TOC), volatile organic compounds (VOC), and water content; also elemental analysis (CHNS) was performed. Reaction (pH) was determined according to the standards PN-EN ISO 10523:2012 PN-EN 12457-4:2006, TOC was determined according to the standard PN-EN 13137:2004 Method B, VOC was determined as loss on ignition LOI according to the standard PN-EN 15169:2011+Ap1:2012, a water content was determined according to the standard PN-EN 15934:2013-02, Total Carbon, Total Nitrogen and Total Hydrogen were determined according to the standard PN-EN 15407:2011, and Total Sulphur was determined according to the standard PN-EN 15408:2011.

Fig. 1. Tested materials 1–1a. Oxy, 2–2a. Corn, 3–3a. Pap before and after shredding (photo: Biernacki, M.)

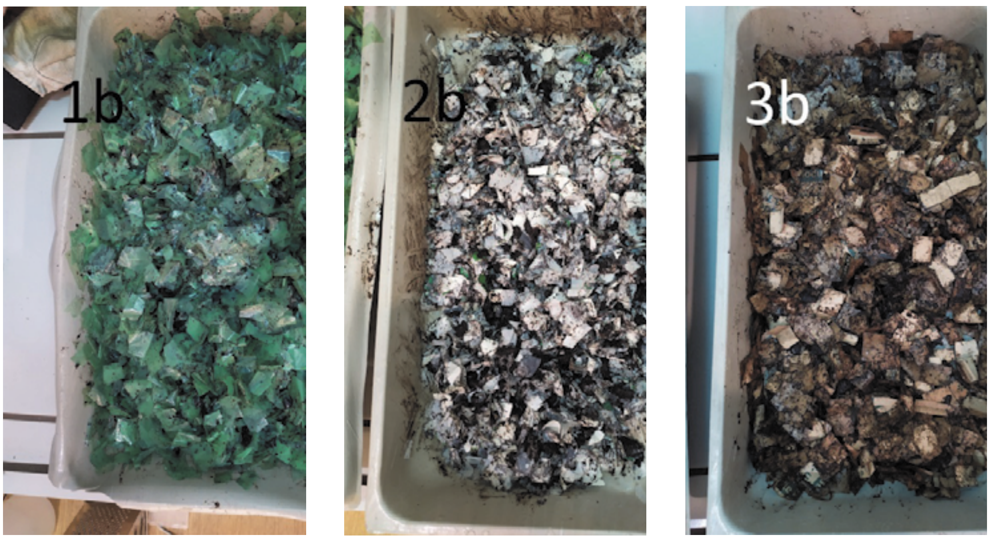

Fig. 2. Compost mixed with Oxy, Corn and Pap samples in the proportion 6:1 (photo: Biernacki. M.)

From the cumulated amount of released CO_2, the degree of biodegradation *Pp* was calculated from the formula:

$$Pp = \left(\frac{(CO2)P-(CO2)B}{ThCO2}\right) * 100\% ,$$

where:
P_p – practical carbon dioxide released,
$(CO_2)P$ – carbon dioxide released by the sample in mg per 1 g LOI,
$(CO_2)B$ – carbon dioxide released by the blank sample in mg per 1 g LOI (Maria et al. 2010).

Statistical analysis

Determinations of CO_2 emissions were performed in 3 independent repetitions for each sample. The obtained results of CO_2 emission tests of the tested materials were subjected to statistical analysis using the Statistica 13.1 program (StatSoft Polska, StatSoft, Inc. USA). In order to assess the differences between the studied samples, the obtained results were tested with the non-parametric Kruskal-Wallis test, with the adopted significance level ($\alpha = 0.05$) for N = 4332 and with the SS test. Statistically significant differences were calculated for $p = 0.05$.

Results and discussion

The composition of the tested materials (Tab. 1) was diversified, and the high content of carbon and hydrogen distinguishes Oxy from other biodegradable materials.

In fact, the Oxy sample is polyethylene (LDPE) with a biodegradable additive in front of Corn and Pap samples which are made of natural, full compostable polymers. Finally, in the case of Oxy material, microplastic particles remain in the environment and easily migrate through the food chain (Markowicz & Szymańska-Pulikowska 2019, Arefian et al. 2020). The elemental composition of the compost (Tab. 2) as the blank sample as well as the mixtures of compost and tested polymers shows a similar C/N ratio of all samples, which indicated that the composting tendency was maintained. The addition of compost not only provides the microorganisms that are necessary for biodegradation process, but also is a source of nutrients, which significantly improve the living conditions and development of microflora. The production of CO_2 in the biodegradation test of the Corn sample is almost double that of the other samples at the same time, indicating that it biodegrades faster.

The rate of the decomposition process carried out by microorganisms depends on moisture, reaction, access to nutrients and oxygen, temperature value, and the nature of the material itself, including its hydrophilicity, where the research of various materials has been widely described in the literature (Ghorpade et al. 2001, Maria et al. 2010). The compounds most susceptible to degradation are polymers of natural origin such as lignin and cellulose which creates a good possibility for moisture penetration into material (Spiridon et al. 2019). In the case of traditional plastics (as well as bioplastics, e.g., polymers with the addition of corn starch), the fragmentation

Fig. 3. Sample in glass container (left), and OxiTop system in thermostatic cabinet (right) (photo: Biernacki. M.)

Table 1. Characteristics of tested packaging materials

	Oxy	Corn	Pap
Material type	d_2w®	corn starch	craft paper
pH	10.3	6.1	7.1
C [%]	80.9	54.5	39.2
H [%]	14.8	6.86	5.8
N [%]	<0.05	<0.05	<0.05
S [%]	<0.03	0.031	0.053
Grammage [g/m^2]	76.1	134.8	74.7

of the material is also of additional importance (Abioye et al. 2019, Markowicz & Szymańska-Pulikowska 2019). Low temperature (below 10°C) decomposition is also effective due to the experiment with corn starch foil and total decomposition in compost conditions in 14–21 days and in 15–29 days in soil environment. Similar results were obtained in room temperature with different paper types, where the highest decomposition took only 10 days (over 95% degradation in the case of no ink paper). (Ahmed et al. 2018; Domka et al. 2009). Although the first period of biodegradation is the most intense, during the test carried out at 40°C (Fig. 4), the decomposition of samples was slow for all tested materials as in Gomez and Michel research (2013). The experiments with bioplastics carried out at room temperature gave similar results, although on the 30th day of the experiment the beginning of biofoil fragmentation was observed (Luchese et al. 2018). The colonies of mesophilic microorganisms which predominate in the compost after the hot composting phase ended are not able to carry out the process at such a high temperature with adequate efficiency, similarly to the thermophilic ones, for which the temperature is too low and their multiplication takes time. However, despite the small amount of CO_2 production, statistically significant differences were noted between the tested samples, except for the Oxy and Pap samples, which indicates a similar rate of degradation of both materials. Paper perceived as particularly susceptible to decomposition under composting conditions showed no advantage over other packaging.

At the temperature of 40°C, the highest recorded carbon dioxide production (Corn test) did not exceed 9 mg CO_2/g, but degradation process was started.

During the test carried out at 45°C, the degradation of samples was faster (Fig. 5), and there were statistically significant differences between all tested samples. The process was more intense in each container. It is very interesting due to results which indicate that oxy-degradable materials are not suitable for composting process (Markowicz et al.2018). On test day 10, the occurrence of a characteristic odour in the Corn sample containers was noted. In this case, the activity of actinomycetes, whose cellulolytic enzymes are effective at higher temperatures, is likely to be observed (Du et al. 2008). The samples degraded to a greater extent (Fig. 6). The Pap samples partially decomposed, while the Oxy and Corn samples remained unchanged, only twisting.

The highest CO_2 production on day 21 (for the Compost sample) exceeded 14 mgCO_2/g.

As predicted, the highest efficiency of decomposition was recorded in the tests carried out at 50°C (Fig. 6). In the first days of the experiment, the tested materials swelled, which can be explained not only by the binding of water, but also by the intensive development of compost microorganisms.

Table 2. Characteristics of compost and mixtures prepared for biodegradation tests

Parameter	Compost	Compost:Oxy 6:1	Compost:Corn 6:1	Compost:Pap 6:1
pH	8.0	8.3	7.6	7.2
Moisture [%]	40.5	44.6	45.8	43.7
C [%]	10.67	13.72	11.22	11.88
H [%]	1.11	1.58	2.55	1.32
N [%]	0.62	0.54	0.44	0.58
S [%]	0.062	0.072	0.090	0.087
C/N	17.2	25.4	25.5	20.5
LOI [%]	17.78	23.57	37.26	22.44
$ThCO_2$ [gCO_2/g]	4.162	5.628	9.614	5.430

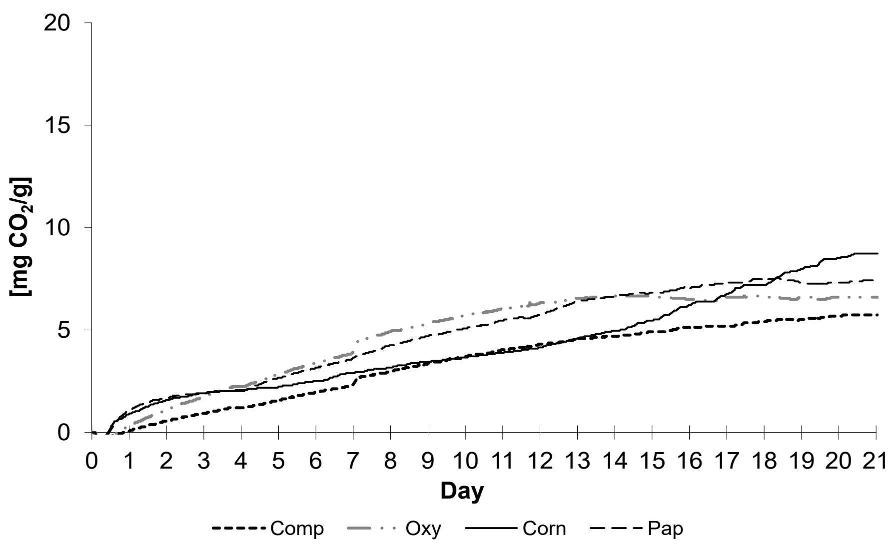

Fig. 4. Production of CO_2 during decomposition of tested materials at 40°C

A characteristic odour, which disappeared after 7 days, was noted in the Corn sample containers. Despite the observed changes, only in this case there were no statistical differences between the Comp and Corn trials. All the containers required ventilation after 2 days from the start of the test, subsequent ventilation depended on the intensity of the process. The most active degradation process was recorded in the case of Corn samples and they required frequent airing. On the 3rd day of the experiment, signs of degradation were noticed on the Pap samples, the pieces began to twist and turn grey. The Oxy samples only turned from bright green to brownish green at the end of the test and a reduction in sample volume was noted. The Corn samples also decreased in volume, while the Pap samples crumbled (degraded) and the remaining pieces crumbled to dust when rubbed with the fingers. At this temperature, statistically significant differences were noted for all tested materials, which indicates a different enzymatic activity of microorganisms.

The lack of increase in CO_2 production, shown in the form of horizontal sections in the graph, is due to the airing of the containers and a temporary slowdown in the decomposition process. A significant degree (70% on day 21) of LDPE film decomposition with 50% starch content was also recorded at lower temperatures during the experiment in soil (Abioye et al. 2019).

The progress of biodegradation can also be observed visually, comparing the appearance of the samples after the experiment conducted at different temperatures (Fig. 7). Decomposition (or only fragmentation) of material strongly depends on the climate factors and material thickness or surface which can be colonized with microflora. In the case of high (2–3 mm) thickness of PLA material, decomposition after 1 and 3 month in conditions simulating composting reach only 5 and 18% respectively (Czarnecka-Komorowska et al. 2021).

The thermophilic process conditions (55°C) accelerate the decomposition rate as a result of the high enzymatic efficiency and degradation of easily degradable polymers such as bacterial polyhydroxybutyrate PHB takes place within 10 days. However, in the case of poly (butylene adipato-Co--terephthalate) PBAT, considered to be readily biodegradable, the samples were not decomposed at this temperature even

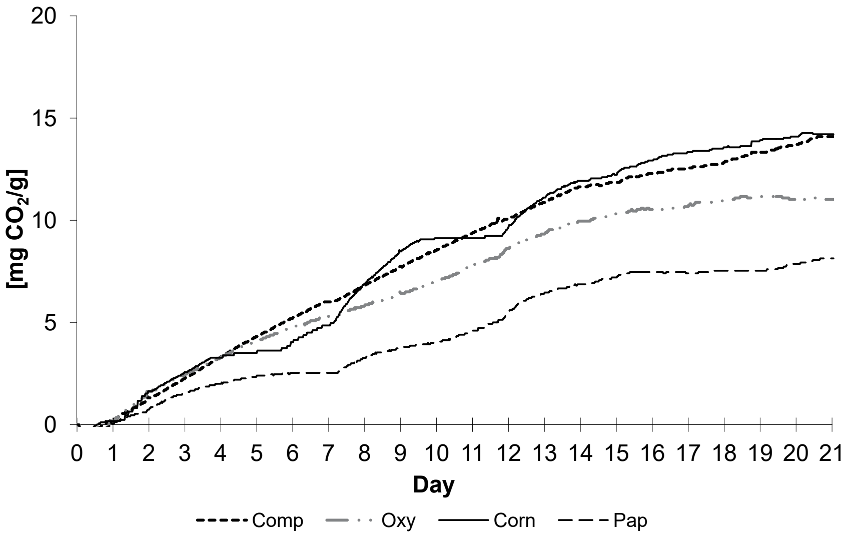

Fig. 5. Production of CO_2 during the decomposition of tested materials at 45°C

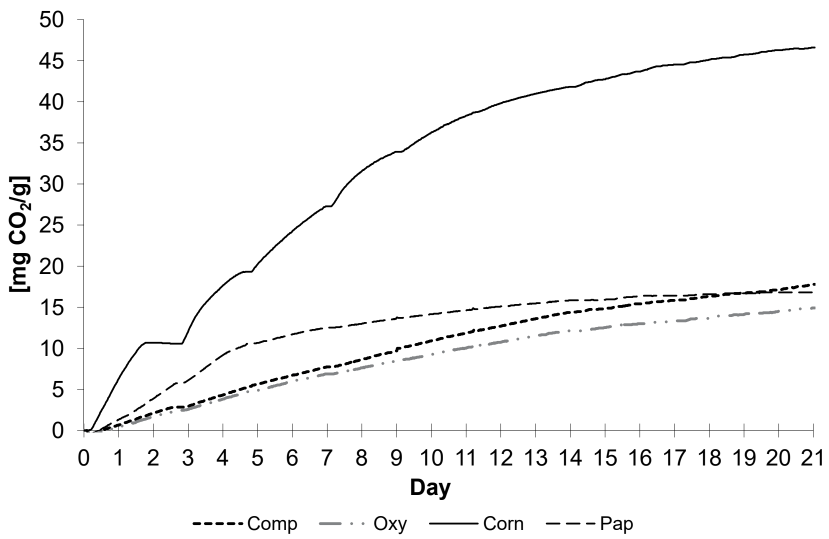

Fig. 6. Production of CO_2 during the decomposition of the tested materials at the temperature of 50°C

after 40 days of incubation (Popa et al. 2011, Tabasi & Ajji 2015). Hydrophilic properties are the most important due to better substrate accessibility for cellulases and other enzymes produced by microorganisms (Ahmed et al. 2018). An important parameter is also fragmentation, which generates a larger contact surface at the beginning of the experiment, in contrast to the progressive fragmentation being a result of the action of ligninolytic enzymes secreted by microorganisms (Arefian et al. 2020). The results of the statistical SS test from the experiment carried out here show that the process temperature corresponds in 29% to the amount of CO_2 released. Thus, temperature is only a component of the net degradation rate. Other factors such as the contact surface, the hydrophobicity of the material, products formed during decomposition or the availability of nutrients also play an important role (Seruga et al. 2019).

The obtained results of tests carried out at the temperatures of 40, 45 and 50°C indicate a quick, except for the lowest

Fig. 7. Changes in the appearance of Oxy (1c–e), Corn (2c–e) and Pap (3c–e) tests after tests at 40 (A), 45 (B) and 50°C (C) (photo: Biernacki M.)

temperature, but very diverse biodegradation of the tested materials (Fig. 8). The test carried out at 40°C showed a slow rate of biodegradation for all packaging materials, even for paper which is sometimes used as control sample (Adamcova et al. 2016).

However, tests of the Oxy, Corn and Pap materials showed a relatively quick start of the process – after several hours the emission of CO_2 begins to increase. Considering the influence of temperature, no significant differences were noted only in the case of paper that degraded at 40 and 45°C. The data show the greatest differences in CO_2 production between 45 and 50 degrees for the Corn and Pap samples. Comparing the median value between these samples, it can be seen that it increased disproportionately greater than in the Comp and Oxy samples. This indicates a significant acceleration of the process, which ultimately allows for a faster biodegradation of the material. The results also show that the process rate is similar among the Comp and Oxy samples, while the Corn and Pap samples degradation rate is not a linear function of temperature. It confirms thesis that oxy-biodegradable materials have low potential for degradation in short time, so are not suitable for composting (Markowicz et al. 2018). Some authors use paper samples as blank in degradation tests, but a real potential of paper degradation speed was shown only in 50°C.

Carbon dioxide production per 1g LOI for individual temperatures was diversified (Tab. 3). It was assumed that the addition of packaging material would result in an increased amount of CO_2 released during the process.

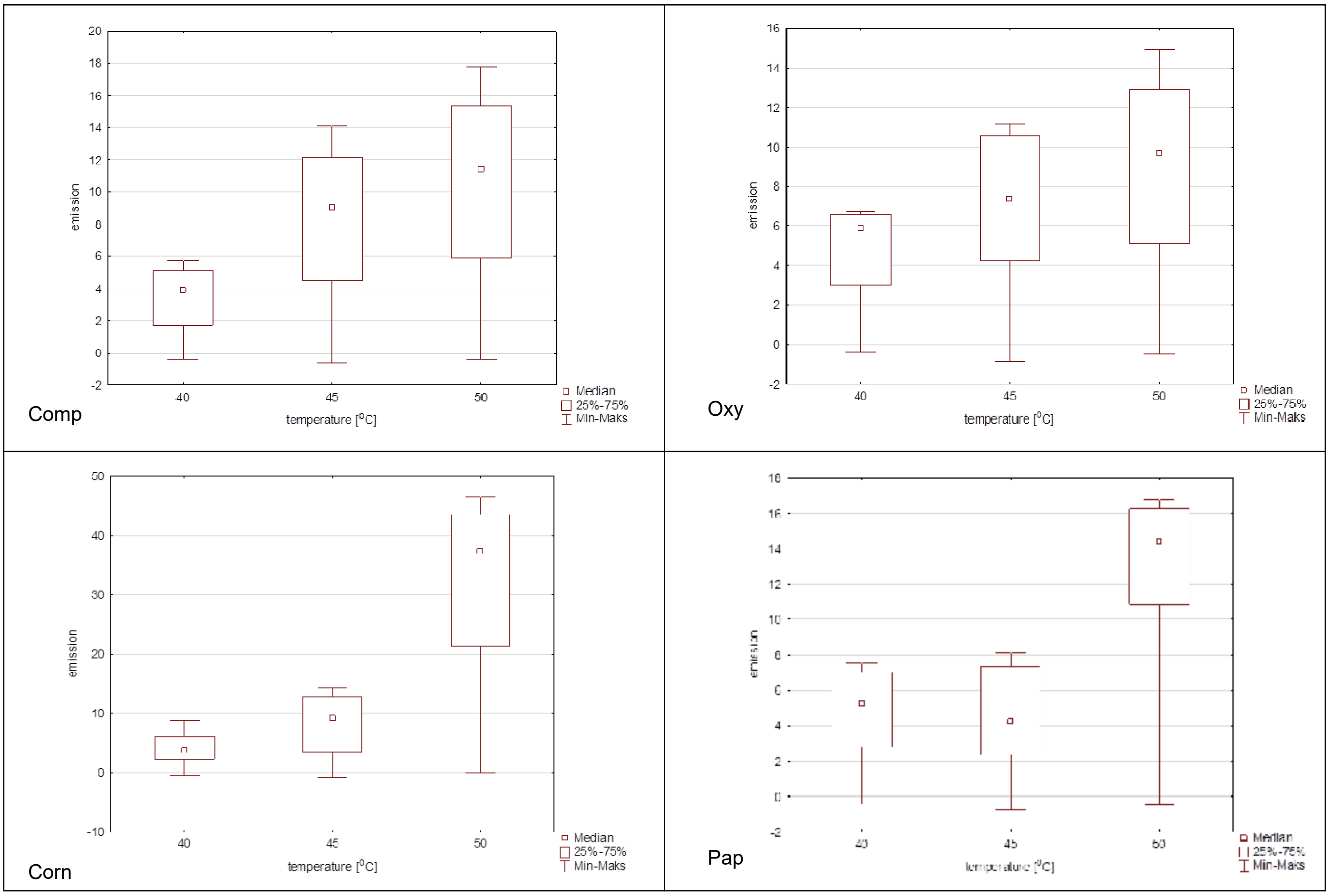

Fig. 8. Comparison of the distribution of CO_2 production results (min-max, and median) from the tested materials and compost

Tab. 3. Carbon dioxide production (mg CO_2 per 1 g LOI) at different temperatures

Type of material	Number of days	40°C	45°C	50°C
Comp	10 days	19.4	48.2	61.5
	21 days	39.8	79.2	100.0
Oxy	10 days	24.3	29.7	39.3
	21 days	28.0	46.7	63.3
Corn	10 days	9.8	21.1	97.3
	21 days	23.4	33.9	125.1
Pap	10 days	22.6	18.1	63.1
	21 days	33.1	36.2	74.9

The large contact surface of the compost and samples, i.e., the polymeric material constituting a nutrient medium for microorganisms, did not increase the amount of CO_2 produced. However, in almost all tests, inhibition of the biodegradation process was noted instead of stimulation, which indicates the difficulties in adaptation of microorganism colonies in such a short time.

The calculated correlation coefficients (Tab. 4) of the biodegradation rate of the compost and the tested samples indicate the best fit of the Oxy curves, which indicates a slow decomposition process of this material. The distribution of oxy-biodegradable materials depends mainly on the additive used by the manufacturer in order to obtain the material's degradability. Microplastic particles formed in the vicinity of the cells of microorganisms that degrade the material may also be an inhibitor of the process (Gorokhova et al. 2020).

Biodegradable packaging is perceived as environmentally friendly. However, only biodegradable and at the same time compostable materials which are non-toxic for plants are considered safe for the environment. Only a thorough analysis of the possibility of oxygen decomposition in reasonable time can confirm their applicability in practice without adversely affecting the process parameters in the composting plant (Youssuf & Al-Sayed 2019).

Conclusion

The aim of the study was to determine the effect of temperatures in the initial stage of the process of aerobic biodegradation of polymers marked by producers as biodegradable or compostable, which are used for the production of packaging materials, on the amount of CO_2 released. In the experiment lasting 21 days, statistically significant differences were shown in the biodegradation rate expressed as CO_2 produced during the degradation of individual materials at all test temperatures.

The highest respiratory activity of microorganisms was recorded for the Corn test at the temperature of 50°C. The CO_2 production was even higher than in the Pap tests, which may mean that these samples are more biodegradable. The Oxy and Comp tests reacted with a proportional increase in CO_2 production in relation to the temperature used, while in the case of Corn and Pap tests, a leap in the activity of thermophilic microflora was noted between the temperatures of 45°C and 50°C. Finally, the temperatures of 45°C and 50°C showed good decomposition speed but 40° was not sufficient for microbial activity. Paper samples were the most susceptible for decomposition.

The test results show the influence of temperature on the biodegradation process. The standards recommend testing at a temperature of around 58°C, but a lower temperature is acceptable. The obtained results show that it is possible to use lower temperatures in biodegradation tests. However, tests at lower temperatures must be validated so that they can be used for testing biodegradable materials for compliance with standards.

It should also be remembered that the biodegradation test itself does not give information about whether the material is safe for the environment. In order to determine the environmental impact, compostability and ecotoxicity tests (compost quality) should also be performed.

Tabele 4. Correlation coefficients of the rate of biodegradation

	Oxy	Corn	Pap
40°C	0.9739	0.9380	0.9960
45°C	0.9969	0.9941	0.9880
50°C	0.9995	0.9873	0.9427

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