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ABSTRACT

This article describes the technology of organic recycling of polylactide/halloysite biocomposites using the sugar-protein condensation theory. For this purpose, polymer biocomposites were produced with a polylactic acid structure and reinforced in the form of halloysite nanoparticles by 1; 2.5; and 5% by mass. A new method of decomposition of the produced biocomposites was developed. For this purpose, the composting process uses complex sugars in the form of beet molasses. This action is based on Stevenson's theory of protein-sugar condensation. Thus, the validity of this theory was confirmed, as research showed that this modification significantly influences the acceleration of the composting process of the produced biomaterials. For each phase of the process, the parameters of accelerated composting were defined by determining the temperature, degree of humidity, and quantitative scale of acidity and alkalinity. The degree of decomposition of biocomposites was assessed based on microbiological tests, hardness, weight loss, viscosity-average molecular weight tests, and structure assessment using macro and microscopic examinations (SEM). Based on the microbial tests, it was shown that composting also seems to be an alternative method of infectious waste disposal in the case of using biocomposites for products, e.g., medical products.

1. Introduction

Polymer composite materials can be highly beneficial in many aspects of engineering technology (Czarnecka-Komorowska and Mencel, 2014; Kaczor et al., 2021; Leluk et al., 2020; Pappu et al., 2019; Piesowicz et al., 2016; Rydzkowski, 2011; Rydzkowski et al., 2020; Tomporowski et al., 2018; Zielińska et al., 2021). However, they can also pose a significant threat to the natural environment (Beluns et al., 2021; Platnieks et al., 2021; Ashvinder K. Rana et al., 2021; Ashvinder Kumar Rana et al., 2021). Lau et al. identified the main problems associated with polymer composites (Lau et al., 2018). Specific fiber-reinforced polymer composites, e.g., carbon, were too strong and challenging to disintegrate and degrade. The cost of producing polymeric materials is sometimes too high (Daminabo et al., 2020; Singha and Thakur, 2008, 2009a). In contrast, the recycling of composite materials is occasionally difficult due to their long decomposition time (Pimenta and Pinho, 2011) and to toxins released during the degradation and/or destruction process (Krawiec et al., 2020a, 2020b; Thakur et al., 2018). The degradation process can occur naturally, such as under the influence of temperature, sunlight, chemicals, or seawater (Singha and Thakur, 2009a; Thakur et al., 2011, 2013). For these reasons, contamination from composite waste is very harmful to the environment and worries many researchers (Li et al., 2016; Yang et al., 2012) who are trying to solve the problem of their disposal. Therefore, due to the increasingly restrictive environmental standards, using "environmentally friendly" materials is essential (Ates et al., 2020). Polymers derived from

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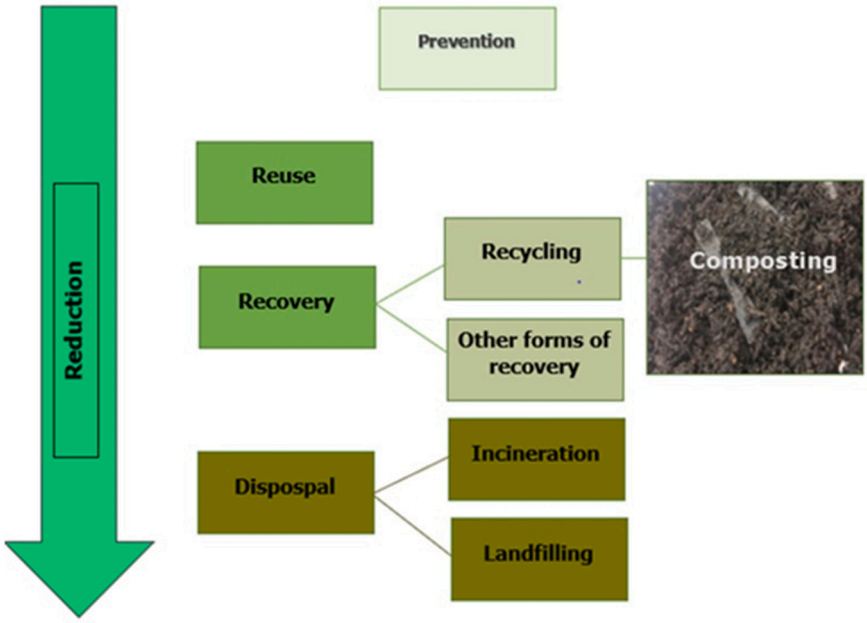

Fig. 1. Composting as a solution to the problem of waste from polymers and polymer composites (own elaboration based on (Sadeghi and Mahsa, 2015)).

renewable sources are currently a promising alternative to traditional so-called petrochemical polymers because they belong to the group of biodegradable materials, and by limiting the extraction of mine deposits, they reduce the formation of pollutants, including greenhouse gases (Auras et al., 2004; Garlotta, 2001; Liu et al., 2013; Singha and Thakur, 2009a; Uppal et al., 2022) and thus comply with the current laws and regulations regarding environmental protection (Singha and Thakur, 2009b; Singha and Thakur, 2008). One such polymer is polylactide (PLA) which can be modified with halloysite (nanotubes) (HNT) nanofillers (Czarnecka-Komorowska et al., 2021; Dubey et al., 2017). Due to the relatively low price and natural availability, HNT is an attractive and user-friendly polymer filler that can replace the more expensive carbon nanotubes in high-performance and multifunctional polymer nanocomposites. Halloysite belongs to the silicate minerals, so due to its natural origin, it is treated as an environmentally friendly nanofiller. HNT nanotubes are widely used to produce ceramics, cement, and fertilizer products as animal feed additives.

Polylactide-halloysite materials can also be used to produce disposables (e.g., mouthpieces, handles, device tips, drug applicators) and disposable packaging and other products for, e.g., biomedical applications. Due to the direct contact of these products with microorganisms living in the human body, they can become contaminated with potentially pathogenic organisms on the skin or mucous membranes and thereby constitute the so-called infectious waste. A bacterial biofilm will then form on the surface of the biocomposite, consisting of structured bacterial cells that exhibit phenotypic characteristics different from those found in the planktonic form (Otto, 2018). Therefore, research on the safety assessment of storage or processing of these biocomposites is necessary. The advantage of creating composites with a matrix of halloysite-reinforced polylactide, which is a ceramic, is their recycling which can be problematic in the case of other composites (Navarro et al., 2018; Oliveux et al., 2015). The selection of the appropriate polymer waste recycling technology depends on many factors, including the type and structure of the polymer, its source of origin, etc. Any process of recycling waste of natural or synthetic origin involves changes in the chemical structure of macromolecules, which results in their degradation and, consequently, the deterioration of physicochemical properties (reduction of elongation and temporary strength, an increase of stiffness, or loss of gloss and change of color of polymeric materials) (Chen and Jakes, 2001). The effect of structural changes in polymer macromolecules is chain breakage, reducing the average molecular weight. In the case of organic polymers, i.e., PLA, environmental factors such as water, radiation, environmental pollutants, and microorganisms strongly influence their degradation process, which promotes the organic recycling process (Kliem et al., 2020). One of the ways to recycle biopolymers and biocomposites is by composting (Sadeghi and Mahsa, 2015). It is well known that this is a controlled process of waste degradation, under aerobic conditions, with the participation of microorganisms, which is compost (Kaplan, 1998). Compostable polymers subject to the process of organic recycling are a valuable form of polymer waste management (Sadeghi and Mahsa, 2015) (Fig. 1). In the form of compost, the obtained product is a microbiologically clean material with favorable properties used to fertilize the soil.

Compost can be used to enrich all types of soils and arrange urban green areas, e.g., as a substrate for lawns and reclamation of the post-industrial regions (EKO DOLINA, 2021). The processing of biodegradable plastics (such as PLA or PLA nanocomposite/halloysite) by composting is currently considered an appropriate form of material recovery in many parts of the world. It is assumed that about 70% of organic substances undergo mineralization processes, while only 30% are transformed into humus compounds (Szewczyk, 2016). The soil composition is shaped with nitrogen compounds and microorganisms, e.g., bacteria of the *Bacillus* genus (Deb et al., 2013). Among the processes of transformation of organic matter, it is decomposition that most often occurs.

On the other hand, composting is defined as (Szewczyk, 2016) mostly decomposition. For this decomposition to occur, the correct chemical composition of the components and their specific mass fraction, together with the mass fraction of appropriate microorganisms, and proper temperature and humidity, must be ensured. Decomposition can be anaerobic and aerobic with complex organic compounds (proteins, fats, and carbohydrates). Two parallel biochemical processes take place, i.e., mineralization and humification. This paper decided to use the composting process to decompose the produced composites using the sugar-protein condensation theory (Stevenson, 2015). In this theory, Stevenson states that sugars and amino acids, formed as products of the metabolism of soil microorganisms, undergo non-enzymatic polymerization to form nitrogenous polymers of brown color. As a result of the reaction of sugar with a nitrogen compound, e.g., an amino acid, N-glucosamine is formed. This then converts to N-aminodeoxyketosis.

Table 1
Quantitative composition of biocomposites and sample markings in C1 technology 1 (C1) and 2nd technology (C2).

Symbol	Composting	Matrix (PLA)	Reinforcing (HNT)
Polylactide (PLA)	C1 and C2	100 wt%	0 wt%
PLA/1 HNT/C1	C1 technology	99 wt%	1 wt%
PLA/2.5 HNT/C1		97.5 wt%	2.5 wt%
PLA/5 HNT/C1		95 wt%	5 wt%
PLA/1 HNT/C2	C2 technology		
PLA/2.5 HNT/C2			
PLA/5 HNT/C2			

This compound may fragment to form 3-carbon chains of aldehydes and ketones such as acetol, diacetyl, and dehydrate to form furfurals. The compounds obtained in this way are highly reactive, and in the presence of nitrogen compounds, they quickly polymerize into complex nitrogen systems (Stevenson, 1994). This theory's weakness is that the reactions mentioned above are prolonged at soil temperatures.

Similar transformations occur during the dehydration (reduction of the amount of water) of food products. This is due to the effect of high-temperature sugar on biomaterials. Protein glycation begins at changing temperatures, i.e., the non-enzymatic attachment of simple six-carbon sugars, mainly glucose, to the free amino groups of collagen and elastin. As a result, advanced glycation products are formed. Advanced Glycation End-Products (AGEs) are irreversibly bound to proteins and damage them. AGEs arise from the effects of sugar, protein, and/or fat-containing products at elevated temperatures. The higher the temperature and the longer the exposure time, the more advanced glycation products are formed. The glycation process involves developing advanced glycation products (AGEs) during a non-enzymatic reaction between reducing sugars and proteins, lipids, or nucleic acids, leading to rapid destruction. Therefore, it is proposed to add sugar to the composting process in beet molasses, which contains large amounts of nitrogen compounds. It is a cheap energy substance, biodegradable, and easily absorbed by the soil. It is planned to assess its impact on the rate of compost formation from biodegradable materials, especially polylactide (PLA), together with halloysite nanotubes (HNT).

The authors of the study found that beet molasses can act as a catalyst (a kind of carbohydrate booster) for accelerating the decomposition of cyclodextrin (derived from maize containing starch and found in polylactide). This concerns dextrins in particular, formed as a result of enzymatic or mineral acid-induced hydrolysis of starch (Nadia et al., 2021). Dextrins are formed as a result of the decomposition of starch and other polysaccharides, when α-1,4-glycosidic bonds connecting glucose repeat units break due to the action of, e.g., α-Amylase. All dextrins are relatively easily available because, after being absorbed by organisms (e.g., soil bacteria), they undergo decomposition similarly to polysaccharides. Dextrins are readily dissolved in water, which is essential for the composting process. It is also well known that the components of sugars (present in, e.g., molasses) perform a key role in processes such as the differentiation, division, survival, and migration of microbial cells. It is these processes that serve as a basis for the formation of increasingly complicated structures that contribute to the emergence of complex multicellular organisms, which accelerate the decomposition of biopolymers. Molasses and its constituents (carbohydrates) can thus serve as a kind of "medium" which accelerates the composting process.

It has been known for a long time that peptidoglycans are elements of the cellular walls of bacteria and fungi. Recent research indicates that sugars play an important role in the transmission of signals between the environment and the cell as well as between cells (Korgaonkar and Whiteley, 2011; Naseem et al., 2012; Naseem and Konopka, 2015). When bacteria grow, their cellular wall undergoes reconstruction and N-acetylglucosamine molecules are released into the environment. These molecules have a varied influence on bacteria, stimulating them to increase or decrease the production of virulence factors (the process involves the activity of penetration and damage to the tissues), and in the case of soil bacteria, these molecules stimulate them to reproduce and release products of microbial metabolism. Another important factor is the high content of nitrogen in molasses. Green plants and bacteria living in the soil environment absorb nitrogen mostly from its compounds present in the soil. These compounds originate from the mineralization/decomposition of organic matter and from nitrogen fertilizers (Ramm et al., 2020). Bacteria are absolutely essential to this process. They are responsible for the reduction of atmospheric nitrogen to ammonia nitrogen, which is available to plants. This process is carried out by the rhizobacteria/mycorrhizal bacteria living in symbiosis with legume plants, and free-living bacteria present in the soil, mainly from the Azotobacter Species. It is a vitally important process as there is no other way of introducing nitrogen into the cycle of living matter and thus also into the decomposition of compost. No other organisms possess the nitrogenase enzyme and are, therefore, unable to assimilate molecular nitrogen. Plants that do not live in symbiosis with nitrogen-fixing bacteria absorb nitrogen in the form of nitrate and ammonium ions. These ions are present in water and soil as a result of putrefaction processes as well as the action of free-living nitrogen-fixing bacteria. Because of this, the addition of nitrogen present in molasses accelerates putrefaction processes and bacterial growth.

2. Experiment

2.1. Materials

The biocomposites were manufactured using a polylactide by NatureWorks (Minneapolis, Minnesota, USA) under the trade name Ingeo™ Biopolymer 3260HP as the matrix material (NatureWorks, 2020). The polylactide was characterized by a semicrystalline structure with MFR= 65 g/10 min (210 °C; 2.16 kg), tensile strength ca. 63 MPa, tensile elongation of 3%, and molded linear shrinkage of approx. 0.3%. Halloysite (nanotubes) in the form of powder by Sigma-Aldrich (Sigma-Aldrich, 2020) was used as the nanofiller. This nanofiller has the following properties: size d×L: 30–70 nm × 1–3 μm, the pore size of 1.26–1.34 ml/g, refractive index of halloysite: n20/D 1.54, density of 2.53 g/cm^3, and the surface of the nanotube: 64 m^2/g.

2.2. Processing of biocomposites (PLA/HNT)

The biocomposites production process consisted of two stages. In the first stage, the surface of the halloysite was modified using a natural polymer in the form of gelatin (protein biopolymer). Natural gelatin is a mixture of proteins and peptides obtained by the acid method involving partial decomposition of collagen with acids was used to HNT surface modification. It consists of the repeating units of amino acids, such as glycine, proline and hydroxyproline. The gelatin was characterized by binding strength (Bloom value) of 50–300, viscosity of 15–70 mPa·s and average molecular weight of 150.000 g/mol. The modification was carried out in the mixing process using an ultrasonic agitator in demineralized water, witha mass ratio of gelatin to halloysite of 1:2. The process was carried out at a frequency of 250 kHz and a temperature of 80 °C for 3 h. After completion of the reaction, the water was evaporated, and the dry product was ground to a powder in a ball mill. Next, the natural polymer-modified halloysite by 1; 2.5; and 5% by mass were introduced into the polylactide by melt extrusion using an extruder of the Laborextruder LSM30 type with L/D 22.9, Leistriz (Nürnberg, Germany). The nanopowder was pre-dispersed in the polymer support (masterbatch), which facilitated the production of a composite with good dispersion of the filler in the polymer matrix. Before extrusion, the PLA granules were dried under vacuum at 70 °C for 12 h. The extrusion temperature was 200 °C, the screw speed was 50 rpm, and the extrusion capacity was 1.5 kg/h. The resulting extrudates were air-dried and granulated. Then, using an injection molding machine by Dr. Boy GmbH & Co (Neustadt-Fernthal, Germany), standardized samples

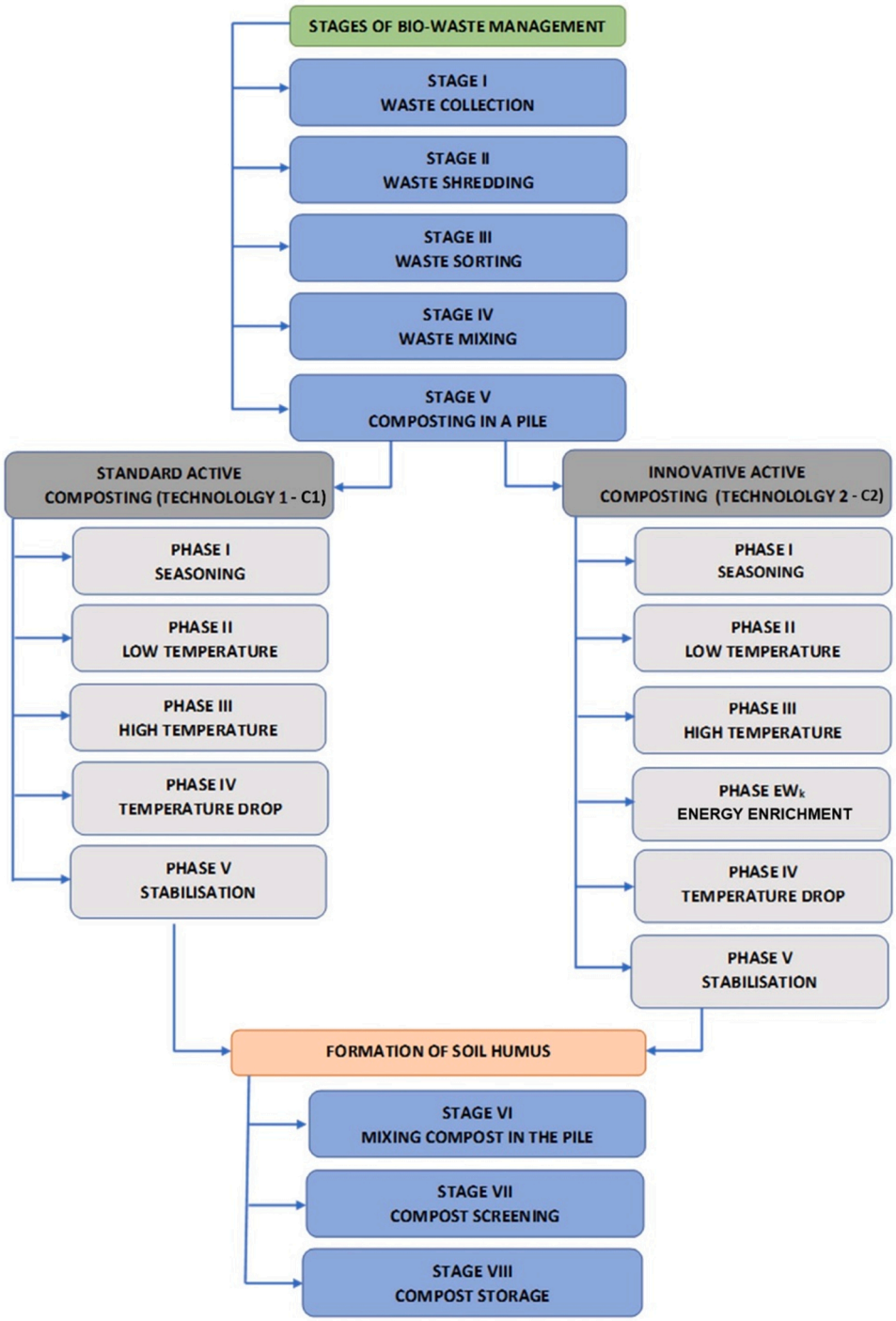

Fig. 2. The composting scheme in the industrial pile (C1 technology) and composting with the proprietary use of complex sugars to confirm the theory of sugar-protein condensation (C2 technology with the help of complex sugars).

(2 mm × 4 mm × 80 mm) were produced from the granules. The injection process parameters were as follows melt temperature of 200 °C, feed temperature of 175 °C, nozzle temperature of 190 °C, injection pressure about 50 MPa, clamping pressure about 40 MPa, and mold temperature of 120 °C. Table 1 shows the composition of the biocomposites and the designations of the samples.

2.3. Scenario of composting processes

The study of the decomposition of biocomposites over time was carried out in laboratory conditions simulating composting in an industrial pile (Rhim et al., 2013; Siracusa et al., 2008). The conditions in the pile cause interactions between individual stimuli resulting in material degradation, which allows for testing composite degradation by the abbreviated degradation method. The paper presents two scenarios of biocomposite recycling with the use of simulation recycling technology; the so-called classic, by composting in an industrial pile (Technology 1, designation C1), and modified recycling technology, i.e., composting in an industrial heap with the proprietary use of complex sugars to confirm the theory of sugar-protein condensation (Technology 2, using complex sugars, designation C2). The composting algorithm in the industrial heap (C1 technology) and composting with the

Table 2
Average parameters of the composting process (C1 technology).

Parameters	C1 technology				
	Phase 1	Phase 2	Phase 3	Phase 4	Phase 5
Temperature, °C	34	48	65	54	45
Humidity, %	60	55	51	54	55
Ph, -	6.7	6.7	6.7	6.7	6.7
Time, days	2	6	11	43	28

Table 3
Average parameters of the composting process (C2 technology).

Parameters	C2 technology					
	Phase 1	Phase 2	Phase 3	Phase Ewk	Phase 4	Phase 5
Temperature, °C	34	49	64	80	66	45
Humidity, %	60	54	49	43	49	55
Ph, -	6.7	6.7	6.7	7.1	6.7	6.7
Time, days	2	6	17	23	24	18

proprietary use of complex sugars to confirm the theory of sugar-protein condensation (C2 technology with complex sugars) are shown in Fig. 2.

2.3.1. Composting in a pile (C1 technology)

The composting process was carried out in a substrate consisting of 30% leaves, 20% wood chips, 30% grass, 10% soil, and 10% compost. After mixing the ingredients, the compost substrate was seasoned. The purpose of the seasoning was to select the appropriate moisture content of the components of the modified compost. Then, the composite samples were placed in a laboratory incubator, in glass vessels, in order to maintain constant parameters of the surroundings of the piles. Throughout the composting process, the temperature and the amount of water required to re-moisten the heap is controlled or regulated depending on the temperature and humidity measurements with a frequency of a minimum 6 h. The C1 composting process was performed following the parameters presented in Table 2. Each sample was placed in a separate of 500 ml vessel, so that the pile covered here by a minimum of 5 cm on each side. The pile mass during composting is shifted at least once every seven days for forty-two days, and then at least once every fourteen days. The composting effects was checked for 30, 60, and 90 days (Polish Committee for Standardization, 2002). Exemplary parameters of the composting process are described in the papers (Grabowski, 2015; Jędrczak and Haziak, 2005; Krysztoforski, 2011; Pilarski and Pilarska, 2009; Saveyn and Eder, 2014; Von Wistinghausen et al., 1999). The most effective composting process is maintaining the temperature of 54 °C with 55% moisture content and substrate pH 6–7.5, and the nitrogen to carbon ratio is 25:1–35:1 (Grabowski, 2015; Jędrczak and Haziak, 2005; Krysztoforski, 2011; Pilarski and Pilarska, 2009; Saveyn and Eder, 2014; Von Wistinghausen et al., 1999).

During the C1 composting process, mainly in the first month, a significant increase in temperature was observed (by approx. 20 °C) in piles, up to a temperature of 72 °C. The composting process consisted of several stages: waste collection, grinding, sorting, stirring, and active composting in apile, where four phases were distinguished:

1. Phase 1, the so-called seasoning phase (stabilization of the moisture content of the mixed compost components),
2. Phase 2, the so-called low-temperature phase (the phenomenon of hydrolysis and oxidation),
3. Phase 3, the so-called high-temperature phase (breakdown of proteins and complex carbohydrates),
4. Phase 4, the so-called phase of temperature decrease (decomposition of substances and reduction of the volume of the pile),
5. Phase 5 is the so-called stabilization phase (homogeneous soil humus formed, and the pile is mixed, screened, and stored) of compost.

2.3.2. Composting in a pile with the use of complex sugars (C2 technology)

The composting process was carried out (as was the case in composting technology 1) in a substrate consisting of 30% leaves, 20% wood chips, 30% grass, 10% soil, and 10% compost, in the stage of energy enrichment, beet molasses by CANISIUS was additionally added to the substrate in a proportion of 10% to the water used to water the samples. The theory of weight distribution dictated the addition of beet molasses by sugars (Stevenson, 2015), which assumes that sugars and amino acids, formed as products of soil microorganism metabolism, undergo non-enzymatic polymerization to form nitrogenous polymers, creating N-glucosamine. However, this compound is highly reactive, and in the presence of nitrogen compounds (found in sugars, e.g., beet molasses), it quickly polymerizes into complex nitrogen forms. Therefore, the study's authors prove that complex sugars can be used for the biodegradation (recycling) of PLA/HNT biocomposites. The defined stages of composting in the described technology are analogous to those in Technology 1. The only difference is that in the "active pile composting" stage, after phase 3, an additional phase defined as the energy enrichment phase (Ewk) was introduced. This is a proprietary solution, the so-called energy enrichment phase, which adds beet molasses to the compost. The content of basic ingredients in the beet molasses was as follows: water - 20%, dry substance - 80%, including: sucrose - 50% and other compounds - 30%, including: reducing sugars - 0.3%, raffinose - 0.5%, carbonate ash - 8.6%, nitrogen-free acids - 4.5%, amino acids - 5.5%, nitrogen compounds (total) - 1.7%, and others. A mechanical laboratory agitator mixed beet molasses with the compost at 23 °C. During the stage defined as active composting, significant fluctuations in the pile's temperature were observed. In the energy enrichment phase, the temperature reached as much as 87 °C, caused by the addition of complex sugars and, as a consequence, an exothermic reaction between the compost components. As a result of the exothermic reaction, there was also a thermal degradation of the polylactide matrix of the biocomposite.

The C2 composting process was carried out following the parameters presented in Table 3. The composting effects was checked for 30, 60, and 90 days.

3. Characterization

3.1. Hardness

Five samples from each material group were tested, and the results were averaged. According to the PN-EN ISO, the hardness of biocomposites PLA/HNT was measured using a Shore D hardness tester (Sauter HBD 100 GmbH, Balingen, Germany) according to the PN-EN ISO 868:2005 (Polish Committee for Standardization, 2005).

3.2. Measurement of the loss of mass over time using the gravimetric method

The weight loss in the tested materials was measured using the gravimetric weight method. The samples were tested before the composting process and 30, 60, and 90 days after the composting process. Three samples from each material group were tested, and the results were averaged. The loss in the mass (m) was calculated from the formula (1):

$$m = \frac{m_0 - m_1}{m_0} \cdot 100\% \quad (1)$$

where: m_0 - initial sample weight (before composting) [g], m_1 - weight of samples after the process (after composting) [g].

Fig. 3. Biocomposites in the suspension of microorganisms.

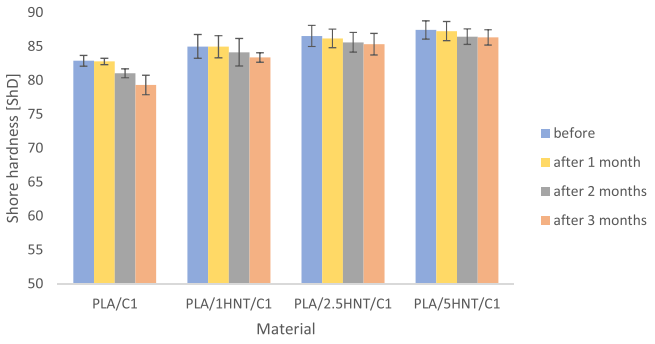

Fig. 4. Shore hardness for PLA and its biocomposites before and after the C1 composting process (Czarnecka-Komorowska et al., 2021).

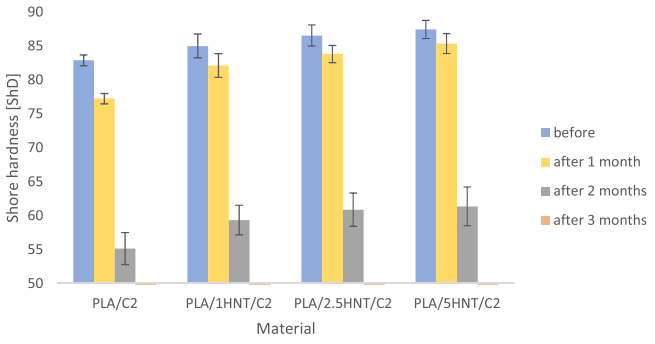

Fig. 5. Shore hardness for PLA and its biocomposites before and after the C2 composting process.

3.3. Viscosity average molecular weight measurement

To determine the intrinsic viscosity by the viscometric method, weights of polymeric materials were prepared, weighing from 0.2475 g to 0.2525 g, and placed in clean and dry 50 ml flasks. The samples were then poured with 25 ml of solvent chloroform. The next step consisted of thermostating the samples in an ultrasonic bath until the material was completely dissolved. The water bath temperature was kept in the range of 30–40 °C. Flasks with the solution and clean solvent were thermostated to a temperature of 35 °C for 20–30 min in the water bath in which the measurement was made. After this time, the flasks were filled with solvent to a level of 50 ml. The nanoparticles were then filtered off. The marking conditions were solvent chloroform, polymer solution concentration: 0.5 g/dl, measurement temperature: 35 °C. In practice,

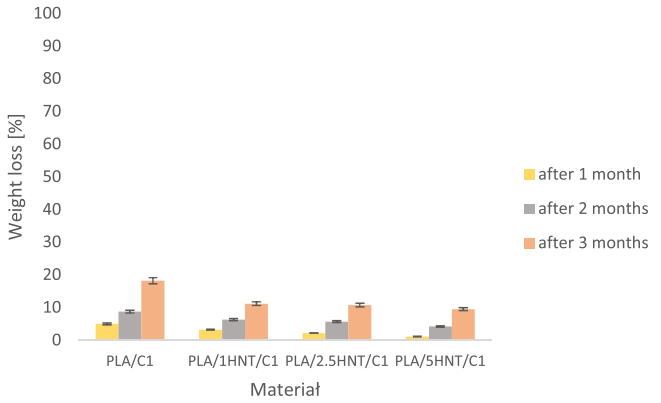

Fig. 6. Weight loss over time for PLA and its biocomposites after the composting process (30, 60, 90 days) in C1 technology.

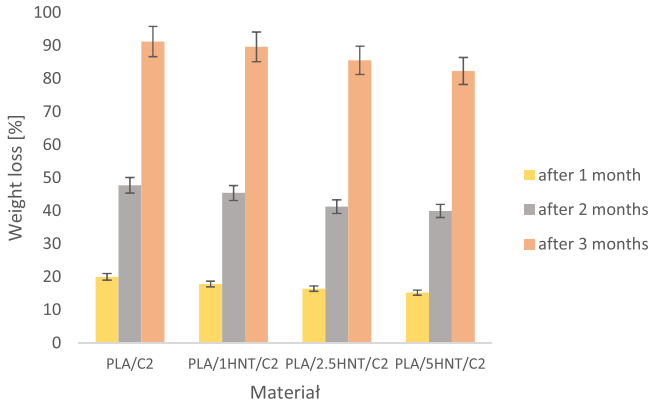

Fig. 7. Weight loss over time, for PLA and its biocomposites after the composting process (30, 60, 90 days) in C2 technology.

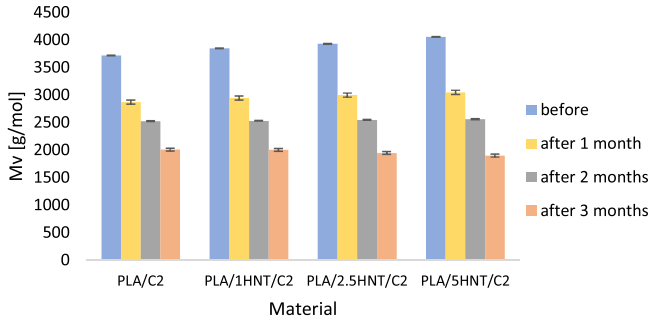

Fig. 8. Comparison of the average molecular weight PLA and its biocomposites before and after composting – C1 technology.

the intrinsic viscosity is often measured to estimate the molecular weight (Wojnowska-Baryła et al., 2020). Relative viscosities of PLA/HNT biocomposites before and after the composting process were performed in chloroform and at 35 ± 0.5 °C using an Ubbelohde capillary viscometer (Instrument Co., Ltd., China). The values of the intrinsic viscosity [η] can be derived from the following Eqs. (2) and (3) (Błędzki et al., 1981):

$$\eta_r = \frac{t_s}{t_0}, \quad \eta_{sp} = \eta_r - 1 \tag{2}$$

where t_s and t_0 are the efflux times of the PLA/HNT solution and the solvent [s], respectively, η_r is the relative viscosity, and η_{sp} is the incremental viscosity.

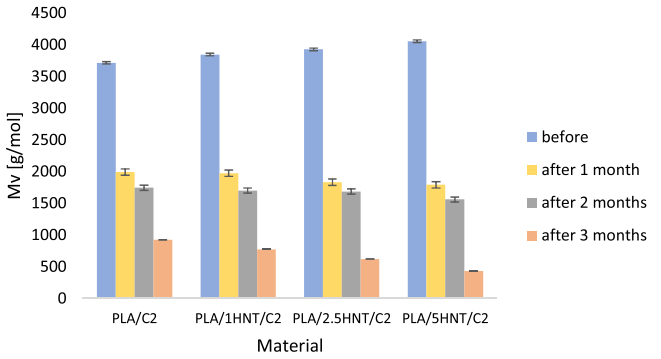

Fig. 9. Comparison of the average molecular weight of PLA and its biocomposites before and after composting – C2 technology with the use of complex sugars.

The intrinsic viscosity [η] values can be derived/calculated accordnig to the following equation (Eq. 3) Salomona – Ciut (Solomon and Gotesman, 1967):

$$[\eta] = \frac{\sqrt{2}}{c\sqrt{\eta_{sp} - ln\eta_r}} \tag{3}$$

where η_r is the relative viscosity, η_{sp} is the incremental viscosity, and c is the solution concentration (g/dl), c= 2 m, and m is the sample mass in g.

The intrinsic viscosity of a polymer in a given solvent increases with the polymer's molar mass. This relation is the base for the viscometric method to assess the molar mass of a polymer from the equation of Mark–Houwink (Eq. 4) (Ahmed and Varshney, 2011):

$$[\eta] = K(\overline{M}_v)^a \tag{4}$$

where $(\overline{M}_v)$ is the mean molecular weight, and K and a are the viscometric constants, which vary in function of the nature of the solvent, temperature, and chemical structure of the polymer (Czarnecka-Komorowska et al., 2020; Kumar et al., 2010; Ryzhov and Fenselau, 2001). The exponent a takes values between 0.5 and 0.8 for flexible chain polymers. Thus, log-log plots of [η] against molecular weight have the intercepted log (K) and slope a. The K and a value for PLA were found to be 1.25×10^{-4} and 0.65, respectively (Kasaai, 2007).

3.4. Macroscopic examination

During the macroscopic examination, the morphology of the surface of biocomposites was analyzed with the naked eye or at a magnification of about 50x, e.g., with a magnifying glass,by the standard (PN-EN 1321:2000, ISO17639) (Polish Committee for Standardization, 2013). Due to the applied high magnification, it was possible to assess the surface structure, e.g., color, separations, smoothness (topography), cracks, and other structure discontinuities.

3.5. Evaluation of the microstructure through a scanning electron microscope (SEM) and a light microscope

After the composting process, the surface morphology of PLA composites with the addition of halloysite was analyzed using a scanning electron microscope (Mira 3, TESCAN, Brno, Czech Republic) with high-resolution imaging. The microstructure analysis was performed for samples previously coated with a thin layer of carbon powder (about 20 nm). The surface morphology of the PLA/HNT was investigated using the secondary electron (SE) signal of an accelerating voltage equal to

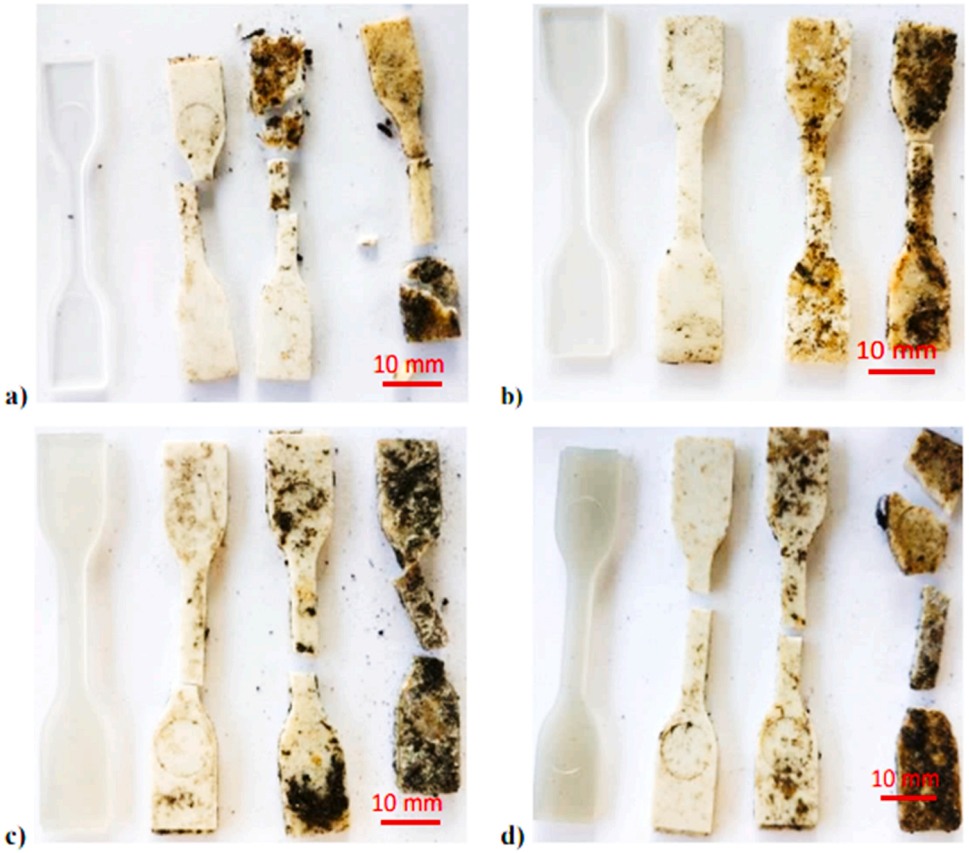

Fig. 10. Macroscopic view of biocomposites before and after 30, 60, and 90 days of composting using the C1 technology (Czarnecka-Komorowska et al., 2021). PLA (a); PLA/1HNT/C1 (b); PLA/2.5 HNT/C1 (c); PLA/5HNT/C1 (d).

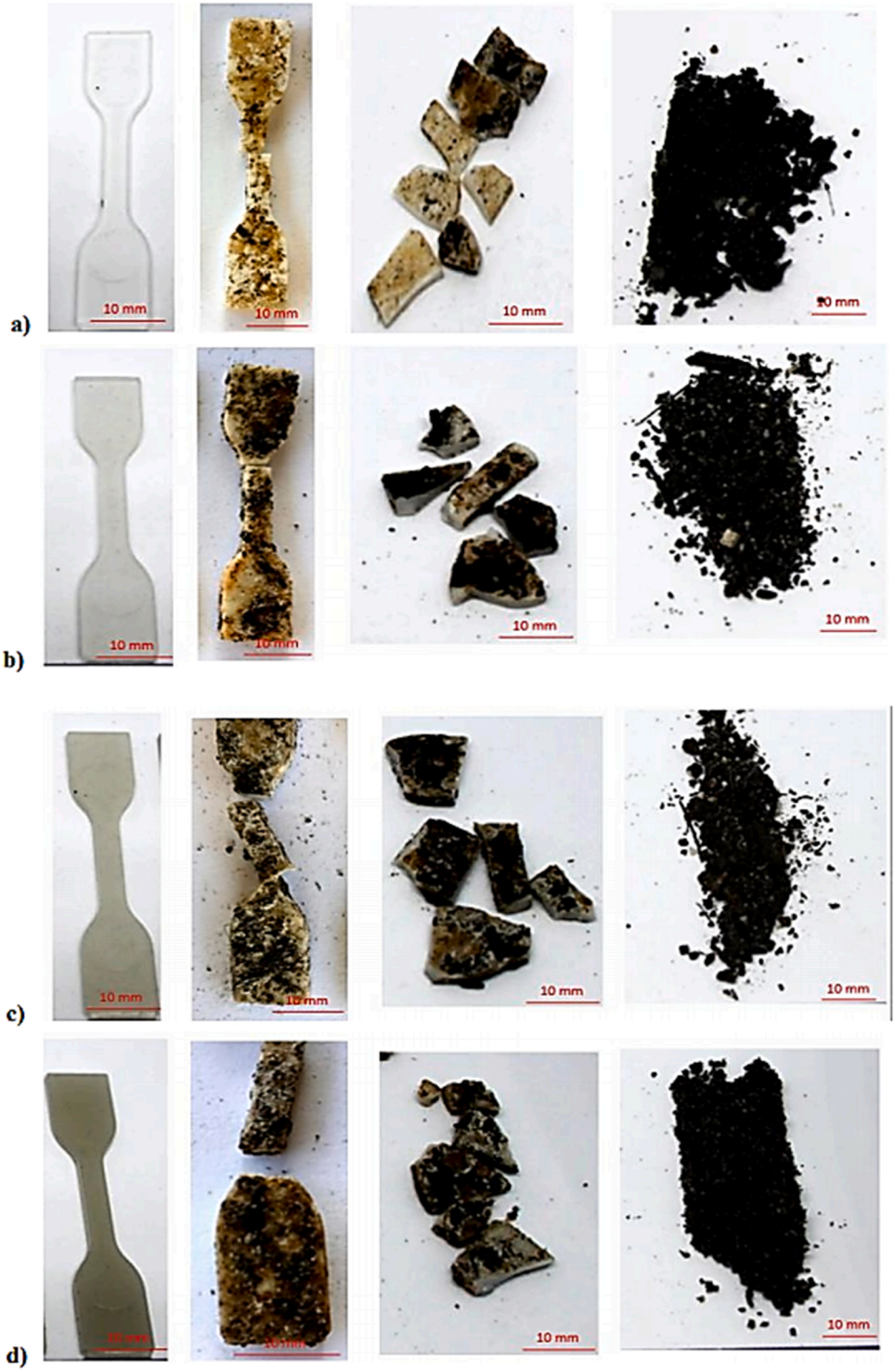

Fig. 11. Macroscopic view of biocomposites before and after 30, 60, and 90 days of composting using the C2 technology. PLA (a); PLA/1HNT/C2 (b); PLA/2.5 HNT/C2 (c); PLA/5HNT/C2 (d).

12 kV. A different range of magnifications was used to better visualize the analyzed structures.

The microscopic evaluation of the bacteria was made based on the Gram method of staining microorganisms, commonly used (Kunicki-Goldfinger, 1998) in microbiology, using biological light microscopy. The examination was performed using a biological microscope OLYMPUS CX21LED. Gram staining is used to classify microorganisms into Gram-positive and Gram-negative, depending on the structure of the bacterial cell wall. It also allows assessment of the shape and size of cells. The speed of making the preparations is an invaluable help in the microbiological laboratory (Szewczyk, 2019).

3.6. Microbiological examination

The microbiological examination aimed to contaminate PLA/HNT biocomposites with potentially pathogenic microorganisms for humans, capable of producing a bacterial biofilm.

The use of reference strains with precise microbiological

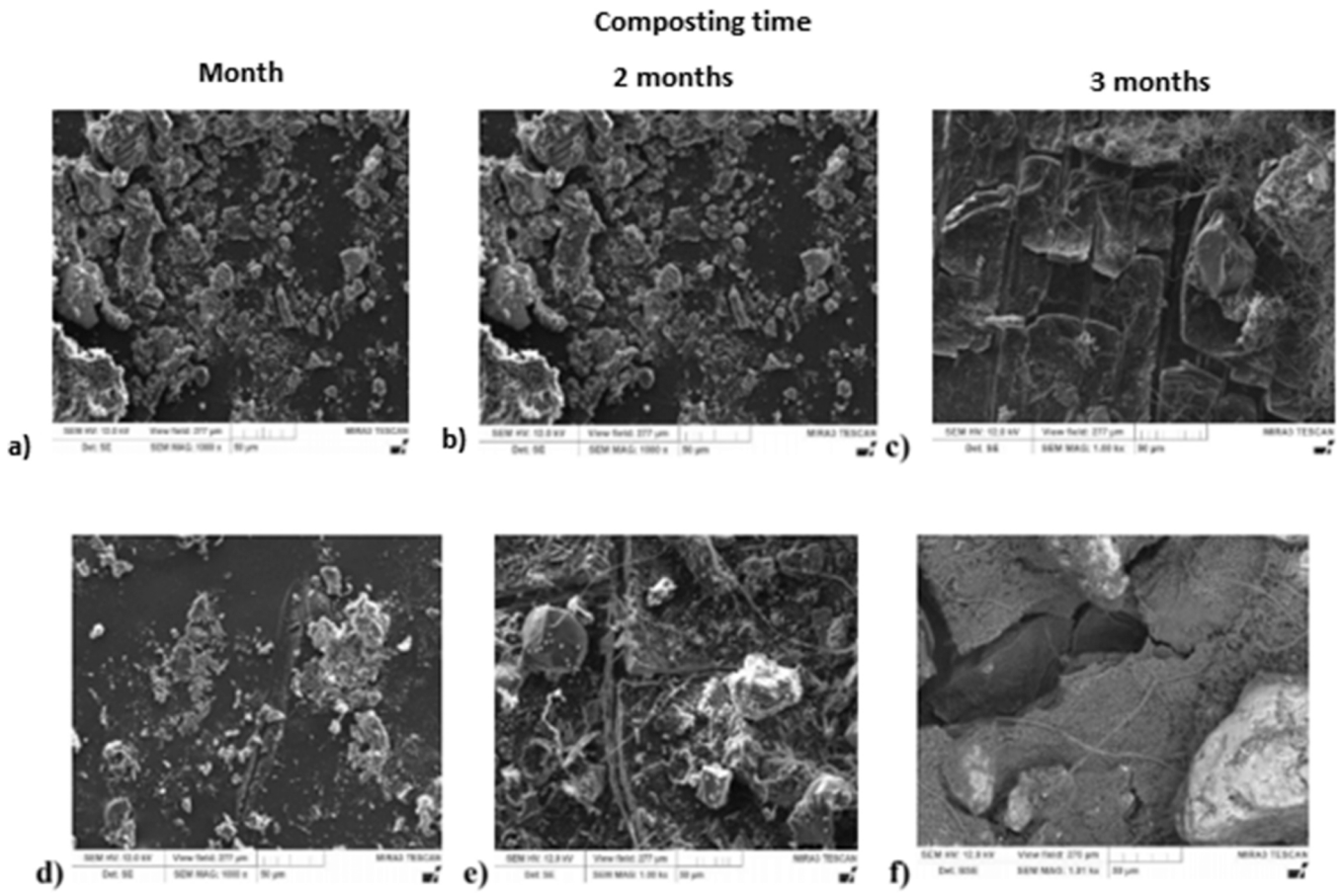

Fig. 12. SEM micrographs of the structure of the biocomposites before and after composting in technology C1: PLA (a), PLA/1HNT/C1 (b), and PLA/5HNT/C1 (c) (Czarnecka-Komorowska et al., 2021).

characteristics (ATCC, 2021) allows comparison of the results of microbial tests obtained after the composting process. It will enable determining the environmental safety of this process.

To contaminate the biocomposite, suspensions of the reference strains, i.e., *Staphylococcus aureus, Candida albicans,* and *Acinetobacter baumanii* (Moosavy et al., 2008), were introduced. The density of the suspensions was the same (0.5 McF) and was based on the MacFarland scale (McF) used in microbiology to determine the density of bacteria (Mysłowska, 2016). PLA/HNT material samples with dimensions of 10 mm × 5 mm x 1 mm were used for the tests.

The biocomposites were introduced into the prepared suspensions of microorganisms. Each suspension contained one reference strain. The materials prepared in this way were incubated at 37 °C for 24 h (Fig. 3).

After incubation, the tested composites were transferred to a composter. After the composting period (for each composting phase) in the C1 and C2 technology, the material samples were submitted for microbiological testing to identify bacteria preserved on the biocomposite. Swabs were taken from the samples and placed on a Petri slide using a sheep blood agar medium for testing. The use of microbiological media allows for microorganisms' cultivation, initial differentiation, and qualification for detailed diagnostic tests. Agar medium is non-selective and is most often used as a medium for the growth of aerobic and anaerobic bacteria, Gram-positive, Gram-negative, and fungi (Gil-Setas et al., 2003). The material was shown on a 5% sheep blood agar medium from bioMerieux Polska Sp. z o.o. (Poland, Warsaw) (Mikołajczyk et al., 2016), and incubated in an oxygen atmosphere at 35 °C for 48 h.

Biochemical tests were used for the microorganisms' phenotypic studies. The final identification was carried out by mass spectrometry MALDI - TOF MS (Matrix-Assisted Laser Description/Ionization Time-of-Flight Mass Spectrometry) (Zenobi and Knochenmuss, 1998). This technique is a quick method of detecting and identifying microorganisms, especially useful in detecting species not belonging to the most frequently isolated and identified pathogenic species. Using mass spectrometry, the MALDI-TOF MS technique assesses microorganisms based on automatic analysis of their protein profile (molecular protein fingerprint) and its comparison with spectrophotometric reference standards from the database (Ryzhov and Fenselau, 2001).

4. Results and discussion

Hardness test results, weight loss, viscosity average molecular weight, and macroscopic and microscopic studies after the composting process, carried out for two scenarios in the C1 and C2 technology, allowed assessing the efficiency of both approaches.

4.1. Analysis of the hardness of biocomposites after the composting process

Figs. 4 and 5 show the Shore hardness results for PLA/HNT biocomposites and pure PLA determined before and during the C1 and C2 technology composting process after 30, 60, and 90 days.

The results show (Figs. 4 and 5) that in the initial stage of composting, i.e., after 30 days of organic recycling in the C1 technology, there was a slight reduction in hardness for all composites and unmodified PLA. Fig. 4 shows that in both the case of the PLA matrix and its biocomposites with 5% HNT content after 90 days of composting, a comparable hardness decrease of about 4.0% was noted.

Similarly, in the case of C2 technology, as shown in Fig. 5, a reduction in hardness was observed for all tested materials. Fig. 5 shows that after 30 days for PLA, the hardness decreased only by about 6%, and for

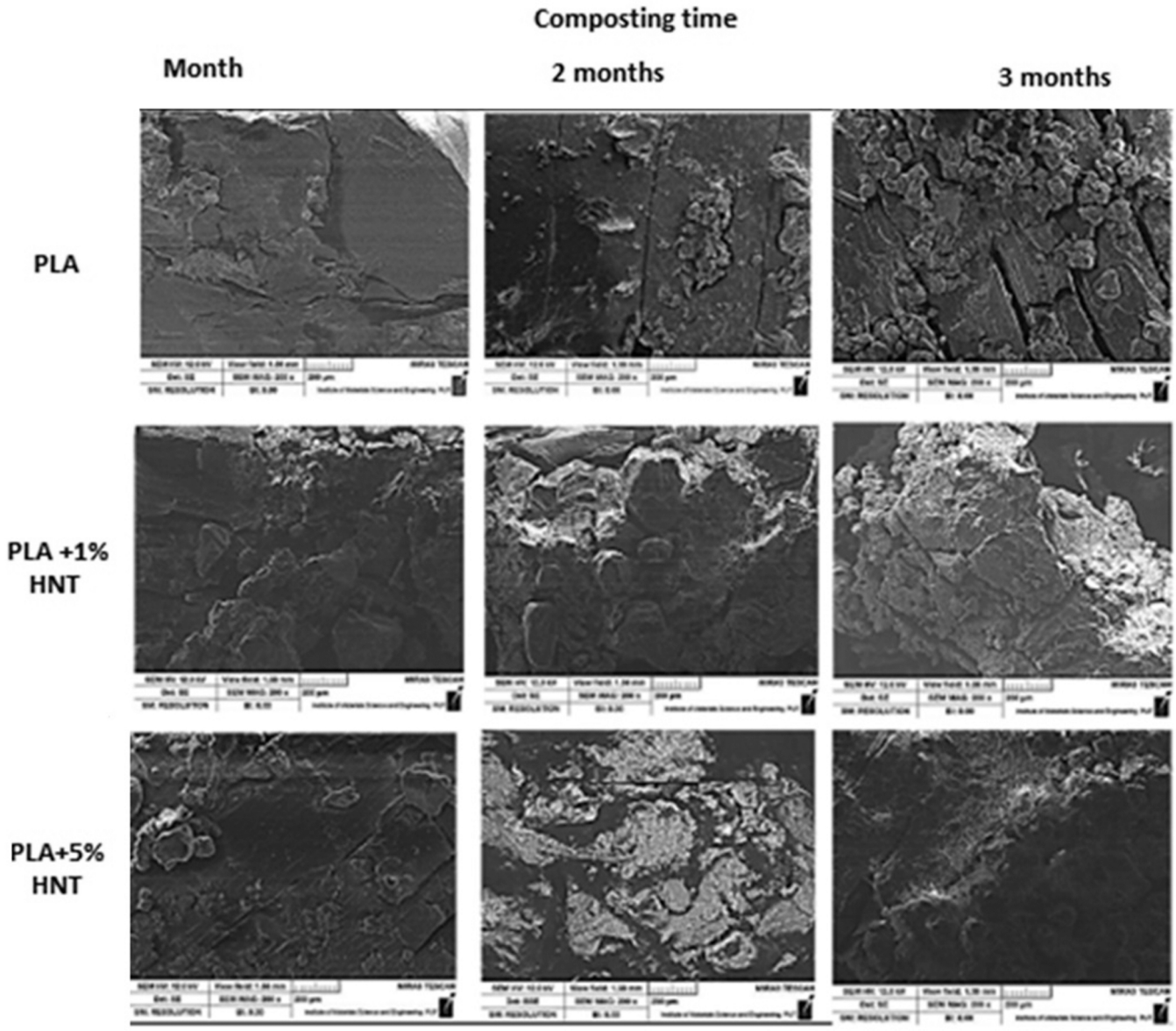

Fig. 13. SEM micrographs of the structure of the biocomposites before and after composting in technology C1: PLA (a), PLA/1HNT/C2 (b), and PLA/5HNT/C2 (c).

PLA with 1% HNT content, by about 3%. However, after 60 days of composting, the hardness for PLA and PLA/5 wt% HNT decreased significantly by about 30%. It was observed that the biomaterials, after 3 months of composting in C2 technology with molasses decomposed into a powder, which proves a significant degradation of the biocomposites. Changes in hardness prove that the addition of HNT affects the mechanical properties. However, it delays the decomposition process of PLA/HNT biocomposites about the PLA matrix, favors the decomposition of the PLA matrix, which is further confirmed by the results of the viscosity average molecular weight (Czarnecka-Komorowska et al., 2020).

4.2. Analysis of the weight loss of biocomposites after the composting process

The assessment of the weight loss over time for PLA and its biocomposites in the composting process with the C1 and C2 technology carried out after 30, 60, and 90 days is shown in Figs. 6–7.

Results of weight loss measurement (Figs. 6 and 7) revealed that all biocomposites showed an incubation time of 30 days. There was a proportionally slight decrease in weight from 1% to 5% for the C1 and 15–20% for the C2 technology. The most significant weight loss for both technologies is shown by pure matrix material, confirmed by this (Kumar et al., 2010; Kümmerer, 2007). For technology 1, the rate of decay increased after 1 month. The decomposition of the PLA was directly related to the hydrolytic degradation of ester groups; therefore, it is essential to control the composting parameters, i.e., temperature and humidity. After 2 months of composting, the pure PLA's most significant degradation was also shown; however, the weight loss increased to 6% for the PLA/1HNT biocomposite. After 3 months of composting, all C1 technology composites showed about 10% weight loss. For technology 2, similarly to technology 1, an increase in decomposition rate was observed after 30 days, but the rate of weight loss is much faster for this technology. After 2 months of composting, the composites achieve a weight loss of 40%, and after 3 months, about 80%. Based on the conducted research, it can be concluded that the participation of HNT causes a delay in the degradation process, which is manifested by a lower weight loss after the same period as compared to the pure PLA since the halloysite did not degrade due to its inorganic nature.

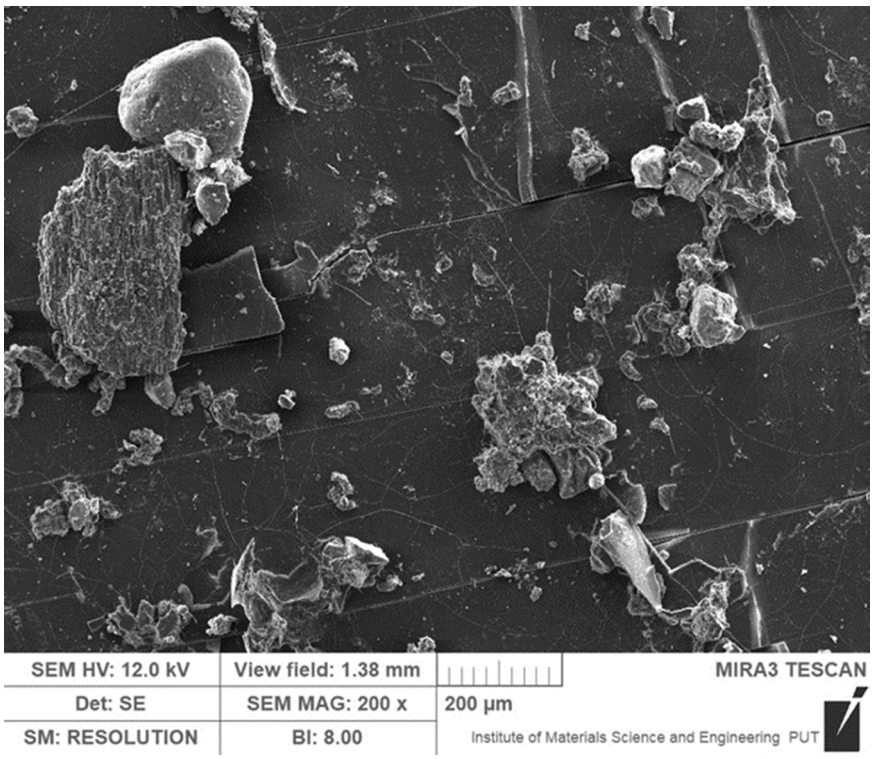

Fig. 14. SEM micrograph of the morphology of PLA/5/HNT biocomposite after 30 days composting in C2 technology.

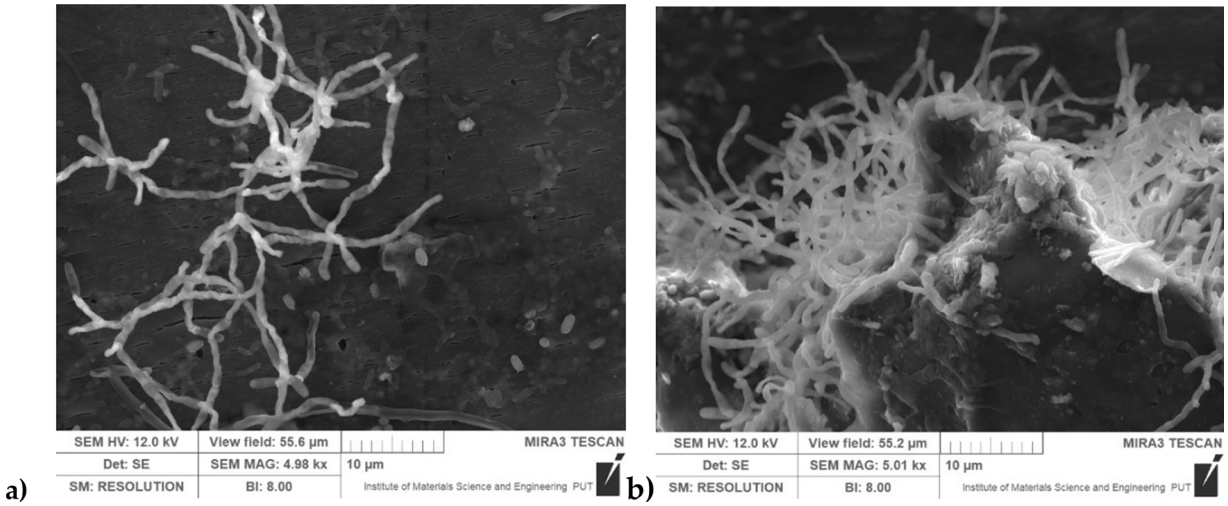

Fig. 15. Examples of soil flora in the material after composting: a) C1 technology, b) C2 technology.

4.3. Analysis of viscosity average molecular weight of biocomposites after the composting process

Figs. 8–9 show the results of the viscosity-average molecular weight tests (see chapter 3) of composted biocomposites during 1, 2, and 3 months. Average molecular weight degradation results for pure PLA matrix and PLA/HNT biocomposites before and after the composting process: recycling technology 1 (Fig. 8) and recycling technology 2 (Fig. 9) with the use of complex sugars.

By analyzing the results of the viscosity-average molecular weight presented in Fig. 8 regarding the C1 recycling technology, it can be observed that all PLA/HNT composites are degraded as a result of composting. However, none of these composite materials was completely degraded. On the other hand, in the C2 technology, the composites underwent almost complete decomposition after 90 days (Fig. 9). The results obtained for both technologies allow describing the degradation process in conditions similar to composting in an industrial pile. In the case of all composites, in the first stage of composting, the process responsible for the systematic reduction of the average molecular weight of the tested samples is the process of polylactide hydrolysis, with the resulting low molecular weight oligomers penetrating the environment. In the second stage, they are bioassimilated by the microorganisms present there. In the initial phase of degradation of composites, water penetrated PLA, leading to hydrolytic cleavage of PLA ester bonds and a rapid decrease in the average molecular weight. The observed average molecular weight allowed for the formation of crystalline structures among the short chains of PLA. Therefore, the crystallinity of PLA increased immediately in the first stage, which is

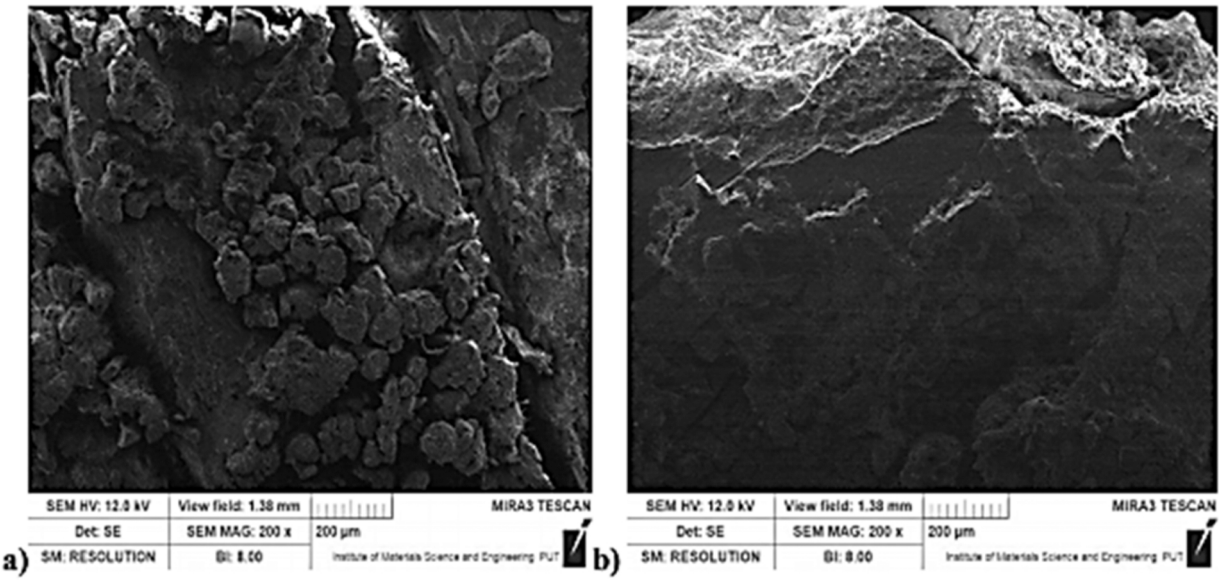

Fig. 16. SEM image of the morphology PLA/1HNT/C2 after 60 days (a), and after 90 days (b) of composting in technology C2 (Kostecka, 2021).

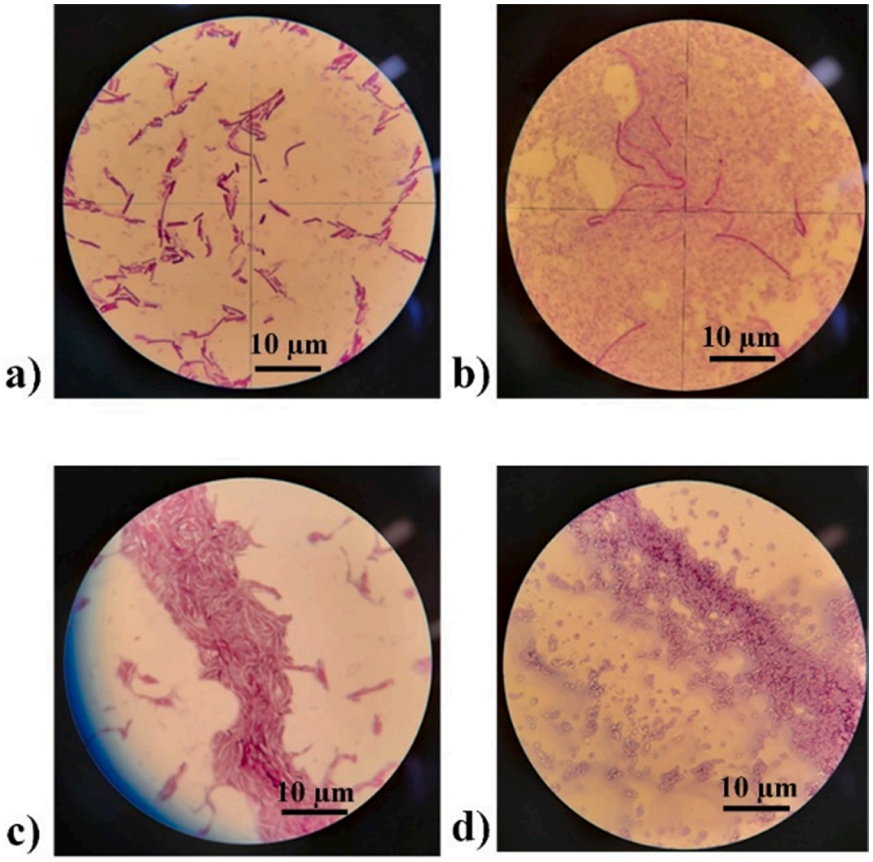

Fig. 17. Differentiated bacterial cells collected from grown colonies on a sheep blood agar medium (natural light microscope, area 100x). Post-composted.

manifested in the turbidity of the sample. It was observed that the addition of halloysite helps to increase the rate of decomposition of the polymer matrix (Figs. 8–9). As a result of composting for 1 month, a decrease in viscous average molecular weight was observed by about 23% for pure PLA and about 25% for a biocomposite containing 5% by weight of HNT, for the C1 technology, and about 46% for pure PLA and about 89% for a biocomposite containing 5% by weight HNT for technology C2. As a result, the most significant decrease in the average molecular weight was found for the sample containing 5% by weight of HNT, composted within 90 days in the C2 technology. This demonstrates an increase in the efficiency of the process due to adding beet molasses to the composting process.

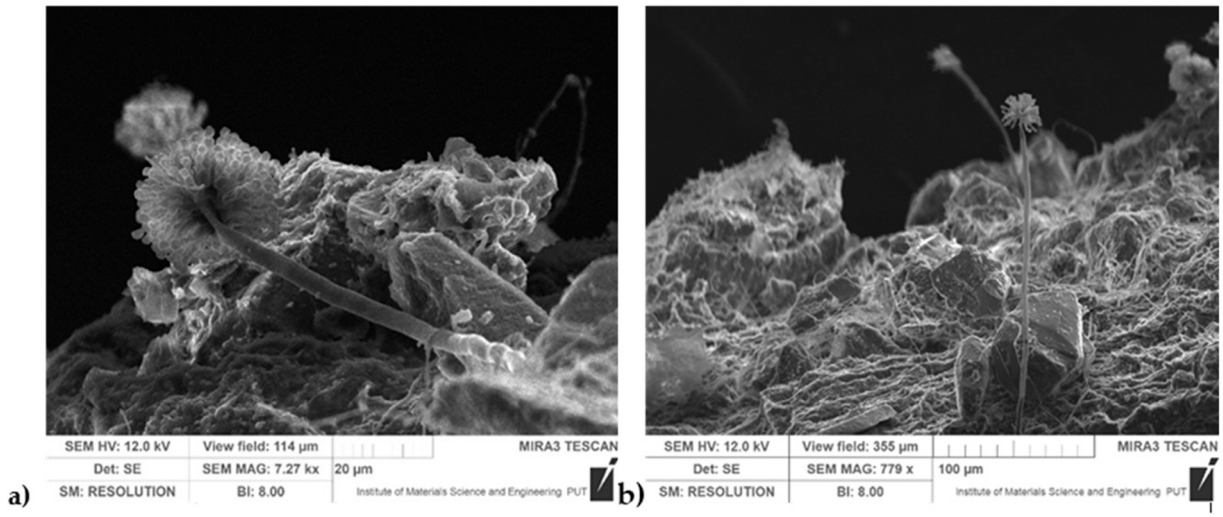

Fig. 18. SEM image of the separation of soil bacterial flora on the agar substrate on biocomposites with different HNT content after composting: a) 2.5 HNT, b) 5HNT.

4.4. Macroscopic analysis of the structure of biocomposites after composting

Figs. 10 and 11 compare the morphology of pure PLA samples and their biocomposites with the addition of HNT before and after the three-month composting process in the C1 and C2 technology. As shown below, all samples of both PLA and its composites underwent significant hydrolytic degradation, which is manifested by partial or complete degradation of the biomaterials under the proposed composting conditions (Hoüglund et al., 2012). Fig. 10 a-d shows the change in materials' surface appearance during the composting process for technology C1 and Fig. 11a-d for technology C2. It was observed that all materials, regardless of the technology, turned cloudy after 1 month of composting, which is related to the hydrolytic degradation and increased brittleness of the materials. In the other degradation process, yellowing and organic sediment deposition, most likely a metabolic product, could be observed on the surface of the samples. At the same time, it is worth noting that the higher the content of HNT filler in the biocomposite, the greater the susceptibility to sediment formation.

4.5. Analysis of the microstructure of PLA/HNT biocomposites before and after the composting process using the SEM technique

To assess the degree of biodegradation, the structure of the pure PLA and its PLA biocomposites of various HNT content was observed using the SEM technique. Figs. 12–13 show the surface of the biocomposites before and after 90 days of composting for pure PLA and PLA+ 5% HNT, respectively. Fig. 14 also shows the effect of composting time (1, 2, 3 months) on the surface morphology of the biocomposites with 1% and 5% HNT content.

Fig. 12 shows apparent differences in the surface morphology of the biocomposites depending on the content of the nanotubes in PLA, composted using the C1 technology. In the case of composites with 5% HNT content, a significantly higher increase in the material's brittleness is visible compared to the biocomposite with a lower amount of HNT. Moreover, it was found that the composting time significantly influences the decomposition process of the biocomposites; after 3 months, there was a significant disintegration of the sample surface, especially in the case of PLA/5HNT composite (Fig. 12 f).

Fig. 13 shows SEM images illustrating the effects of C2 composting. There are apparent differences in the surface morphology of the composites depending on the nanotube content in PLA. It was observed that with time (1, 2, 3 months), the PLA matrix undergoes brittle fracture, which is evidenced by transverse cracks in the microstructure. On the other hand, in the case of biocomposites with 1% and 5% HNT content, you can see accumulated lumpy areas loosely connected with the material substrate. This may indicate visible grouped halloysite nanofillers, cracks, tearing off (separating) from the biocomposite structure (Fig. 14), which proves the destruction of the material as a result of composting with the addition of complex sugars (especially the lack of consistency between the phases and the destruction of the PLA matrix). Significant surface changes for the composite become apparent over time. After 3 months of composting, the tested biocomposites, by C2 technology, are characterized by delamination and numerous cracks, which have the character of brittle fractures.

In the composted samples, a specific type of structure was also identified, which was defined as the characteristic soil flora (Fig. 15).

The presented research on the microstructure clearly shows that the composite materials degraded in the biocomposting process (Figs. 12–14). The biodegradation initially manifests as an increase in the fragility of the material, then in the form of transverse cracks, delamination visible on the sample surface, and separation of the surface layer from the sample substrate (Fig. 14). In the case of biocomposites, the formation of crack propagation in the presence of nanofiller agglomerates or HNT nanotubes was observed (Figs. 12–13), which promotes faster destruction of the material by disturbing the biocomposite substrate and creates a greater possibility of the placement/deposition of bacteria, microorganisms contained in the soil, or other factors (e.g., beet molasses addition) affecting the decomposition of the biocomposite. In C2 technology, the formation of lumps was observed, and about C1 technology (Fig. 16 a), the accumulation of delamination and brittle interface fractures. It is especially characteristic for clusters of nanofillers until the fine microstructure in the form of a powder is obtained, proving that the material reacts (Fig. 16 b). The obtained SEM images show the destructive effect of complex sugars on the structure of polylactide/halloysite biocomposites, resulting in cracking and, consequently, the disintegration of the microstructure polylactic acid (PLA) and its PLA/HNT biocomposites.

4.6. Results of microbiological tests with microscopic evaluation

After the composting process, microbiological examination of the biocomposites showed that no microorganisms used to contaminate the biocomposite were found in the tested samples. Only microorganisms were observed (Fig. 17) from the gram-positive species that are harmless to humans. *Staphylococcus aureus*, *Candida albicans* fungi, and

Acinetobacter baumannii gram-negative non-fermenting rods are replaced during composting with soil flora.

The soil flora assessed based on the MALDI-TOF method showed the presence of microorganisms of the type *Paeniglutamicibacter* (*Glutamicibacter*), also known as *Arthrobacter,*which are species gram-positive bacteria, belonging to the class of *Actinomycetes*, which play an invaluable role in the environment, including by decomposing organic matter in the soil (Busse, 2016). There was also a presence of other typical soil bacteria such as *Bacillus sp.*, *Bacillus cereus*, and*Bacillus muralis* (He et al., 2013).

Bacteria of the *Bacillus spp* (Fajardo-Cavazos et al., 2016) genus are ubiquitous in the environment. They are found in soil, freshwater, and food (especially perishable food). These bacteria can produce spore forms - endospores, thanks to which they can survive in unfavorable conditions (such as increased temperature). Species of the *Bacillus* genus are characterized by a high growth rate, an efficient system of synthesis, and the secretion of extracellular proteins allowing the synthesis of up to 20–25 g l-1 of the product (Deb et al., 2013). Moreover, most of them are safe species for humans and animals (they have GRAS status - Generally Recognized As Safe) (Pietraszek and Walczak, 2014). The research estimated that half of the resulting enzyme preparations are obtained with the participation of *Bacillus* bacteria, including proteases, amylases, pullulanase, and isomerases, with approx — 25% of thesebeing α-amylases (Szczęsna-Antczak and Trzmiel, 2008). However, the species of bacteria *Cibacterpaeniglut*sis an environmental bacterium commonly found in soil, including compost piles (Assandri et al., 2020).

Fig. 17 shows an example of bacterial growth on a 5% sheep blood agar from swabs collected from biocomposites after the composting process after 48 h of incubation.

The resulting separations from the soil bacterial flora on the agar substrate on biocomposites after the composting process are confirmed by SEM microscopic photos (Fig. 18).

Composting conditions significantly impact the tested bacteria with soil flora, mainly due to the temperature difference. Because the species of bacteria used in the study belong to the group of mesophilic bacteria that grow in the temperature range 30–45 °C, their survival throughout the composting process was impossible. In their place, soil bacteria settled on the surface of the material.

The temperature during composting in phase 1 (Tables 1 and 2) in both C1 and C2 technologies was 34 °C, the temperature in phase 2 stabilized to the level of 48 °C, i.e., in both cases, it was within the optimal range for the development of mesophilic bacteria (Starzyk et al., 2015), which form at the beginning of the composting process. After a specific time in the pile, in phases 3 and 4, corresponding to the technology C1 and phases 3 and 4 in technology C2, a significant increase in temperature occurred (max. 72 °C and max. 87 °C) in technology C1 and C2, respectively, which proves the initiation of the thermophilic phase of composting. In this phase, a decrease in the number of mesophilic bacteria is observed. Their number increased again in phase 5 in the technology C1 and C2, i.e., in the last phase of composting, when the temperature drops 45 °C. This is confirmed by Wang and Fang, who claim that the mesophilic microflora grows after the thermophilic phase at the cooling stage of the compost pile (Wong and Fang, 2000). The results of microbiological tests correlate with the proposed theory of the decomposition of PLA/HNT biocomposites by also composting with the participation of simple sugars.

After the composting process, conducting microbiological tests consisting of identifying microorganisms on contaminated samples of biodegradable PLA/HNT material, remaining after the composting process, showed that the bacterial flora potentially pathogenic for humans has been replaced by the characteristic soil flora. Identified, typical strains of soil bacteria present in the compost of plant origin are not pathogenic for humans and the environment. In the context of the possibility of using biodegradable materials to produce single-use products and the promising preliminary results of microbiological tests, it should be emphasized that these studies should be continued to establish the most effective use of the influence of microorganisms on the biodegradation of materials.

5. Conclusions

Based on the conducted investigations, it was found that the composting process of biocomposites can be accelerated based on the theory of protein-sugar condensation, which is documented by structural and physicochemical studies.

As a result of the interaction of complex sugars (beet molasses), the biocomposite was decomposed with an estimated value of about 90%, which is confirmed by macro and microscopic studies and the viscosity average molecular weight, hardness, and weight loss. The use of biopolymers as an alternative to composites based on petrochemical polymers, rapidly degraded with the addition of complex sugars, will accelerate composting in industrial composting plants and reduce the cost of the process.

Polylactide/halloysite biocomposites (PLA/HNT) seem to be environmentally friendly, for which decomposition by composting is possible, which is confirmed by the conducted tests, especially microbiological.

Typical soil flora was found in the microbial tests of biocomposites made after composting in a pile.

Research shows that in the case of post-consumer waste based on biocomposites, the composting process may be an alternative to other methods of waste disposal.

CRediT authorship contribution statement

Dorota Czarnecka-Komorowska: Conceptualization, Investigation, Writing – review & editing. **Małgorzata Tomasik:** Writing – review & editing. **Vijay Kumar Thakur**: Supervision, Writing – review & editing. **Ewelina Kostecka:** Conceptualization, Investigation, Writing – review & editing. **Tomasz Rydzkowski:** Investigation, Writing – review & editing. **Katarzyna Gawdzińska**: Conceptualization, Investigation, Supervision, Writing – review & editing. **Jaromir Mysłowski** Writing – review & editing. **Katarzyna Bryll:** Investigation, Supervision, Writing. **Joanna Jursa-Kulesza:** Investigation, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare no conflict of interest.

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