THERMAL, MECHANICAL AND BIODEGRADATION STUDIES OF BIOFILLER BASED POLY-3-HYDROXYBUTYRATE BIOCOMPOSITES

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Abstract

Biodegradable poly-3-hydroxybutyrate [P(3HB)] and natural materials (fillers) − clay, peat, and birch wood powder − were used to prepare powdered blends and, then, pellets and granules. Pellets were produced by cold pressing of polymer and filler powders; granules were produced from the powders wetted with ethanol. Properties of initial P(3HB) and fillers and blends thereof were studied using IR spectroscopy, DSC, X-ray analysis, and electron microscopy. No chemical bonds between the components were revealed: the blends were physical mixtures. The degree of crystallinity of the blends was lower than that of the initial polymer, suggesting different crystallization kinetics of the blends. Introduction of increasing amounts of the fillers into the polymer progressively decreased mechanical strength of the pellets, as confirmed by the decrease in Young's modulus. The study of degradation of the blends in soil showed that the mass loss of the blends over 35 days of incubation in soil varied between 30 and 50% of the initial mass of the products, depending on the type of the filler.

Keywords: P(3HB), fillers, wood flour, clay, peat, polymer blends, physicochemical properties, degradation in soil

1. Introduction

The concept of sustainable development is the basic idea of the 21st century. New methods of economic management should decrease the rates of consumption of nonrenewable fossil resources, preserving them for future generations, enable more effective use of energy resources, and introduce new, functional, and environmentally friendly recyclable materials in industrial production. Annual production of synthetic plastics has exceeded 320 million tons, and they largely accumulate in landfills, occupying fertile arable lands. Plastic wastes ruin municipal sewage and drainage systems. Up to 10% of the annual plastic production leaks into the Global Ocean [1,2]. Therefore, development of new environmentally friendly materials, which will be able to be completely degraded in the environment without releasing toxic products, joining the global cycles, is among the priorities for critical technologies of the 21st century at the international level.

Industrial ecology and green chemistry are tools for creating new ecofriendly materials including those manufactured from renewable sources [3]. Hybrids based on synthetic polyolefins and natural materials have been increasingly used in various applications. Polymers synthesized by living systems, the so-called biopolymers, have attracted considerable attention, too. Among various natural materials, a special place is occupied by polyhydroxyalkanoates (PHAs) − microbial polymers that can be used in different areas, primarily in medicine. PHAs

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are degraded to harmless products (CO_2 and H_2O); they are biocompatible, mechanically strong, thermoplastic, capable of blending with other materials, and processable by conventional methods from different phase states [4-9].

Recently, degradable materials have been used to construct ecofriendly new-generation formulations to protect crops and reduce the risk of uncontrolled spreading of pesticides and accumulation thereof in the biosphere [10]. PHAs have been studied as candidates for this application. Suave et al. [11] described P(3HB) microparticles loaded with the insecticide malathion. Hydroxybutyrate-co-hydroxyvalerate copolymers were used as carriers for the herbicides atrazine and ametryn [12-14]. A series of studies described P(3HB) microparticles, granules, films, and pellets loaded with the herbicide metribuzin and the fungicide tebuconazole; release kinetics of the active ingredients and degradation of the polymer matrix were investigated in soil microecosystems [15-17]. The efficacy of the formulations was evaluated in laboratory wheat ecosystems contaminated with weeds and infected with fusarium pathogens [18-19].

However, the high cost hinders the use of PHAs in technical applications such as agriculture (films and pots for greenhouses, carriers for fertilizers and agrochemicals); production of packages, containers, and household goods; construction industry; etc., which require very large amounts of material. PHA cost can be reduced by upgrading production processes and by using cheaper substrates. Another approach is to use PHA blends with cheaper materials: this can both reduce the cost of the material and modify the properties of the polymers. Most of the available studies, however, describe blends of degradable poly-3-hydroxybutyrate with the soluble companion polymers – polylactide and polyglycolide, polycaprolactone, polyaniline, etc., which are mainly intended for producing medical devices and preparations in the form of films, nonwoven membranes, and microparticles for drug delivery.

There are few published data on production and properties of PHA blends with solid phase fillers. Some relatively recent studies investigated natural materials as fillers for modifying properties of PHAs. They described results of filling polyhydroxyalkanoates with clay and its derivatives [20-23], plant fibers [24,25], lignin and holocellulose from a lignocellulosic biowaste [26], and wood chips and powder [27-29]. As those studies describe different methods of production and investigation of PHA-based blends, it is difficult to compare them and draw any conclusion about the consistent effects of the fillers on the properties of these polymers.

The purpose of this study was to produce PHB biocomposites using clay, peat and wood flour as biofillers and to study its thermal, mechanical and morphological properties.

2. Experimental
2.1. Materials

Samples of P(3HB) were synthesized by using the *Cupriavidus eutrophus* B10646 wild-type strain, at the Institute of Biophysics SB RAS. Cells were batch-cultured under strictly aseptic conditions, following the previously developed technology [30-31]. Polymer was extracted from cells with chloroform, and the extracts were precipitated using hexane. The extracted polymers were re-dissolved and precipitated again 3-4 times to prepare homogeneous specimens. The composition of polymers was analyzed with a GC-MS (6890/5975C, Agilent Technologies, U.S.).

Three natural materials were used as fillers: peat, wood flour and clay. High-moor peat "Agrobalt-N", state registration 0428-06-209-139-0-0-01, was produced by OOO "Akademiya tsvetovodstva", Russia). Wood flour was produced by grinding wood of birch (*Betula pendula* Roth) using an MD 250-85 woodworking machine ("StankoPremyer" Russia). Then it was dried at 60°C for 120 h until it reached constant weight, and 0.5 mm mesh was used to separate the particle size fraction. Clay was taken from the mine "Kuznetsovskoye", the Krasnoyarskii Krai, Russia. Its composition: loss on ignition – 6.92 %; SiO_2 – 60.1 %; Al_2O_3 – 19.17 %; Fe_2O_3 – 6.72 %; CaO – 2.02 %; MgO –2.12 %; SO_3 – 0.65 %; Na_2O – 0.88 %; K_2O – 1.45 %.

2.2. Preparation of blends of P(3HB) and natural materials and fabrication of pellets and granules

The polymer and fillers were pulverized by impact and shearing action in ultra-centrifugal mill ZM 200 (Retsch, Germany). To achieve high fineness of polymer grinding, the material and the mill housing with the grinding tools were preliminarily cooled at −80°C for about 30 min in an Innova U101 freezer (NEW BRUNSWICK SCIENTIFIC, U.S.). Grinding was performed using a sieve with 2-mm holes at a rotor speed of 18000 rpm. Fillers were not precooled before grinding; a sieve with 2-mm holes was used, and the grinding was performed at 6000 rpm. Fractionation of the polymer and filler powders was carried out using vibratory sieve shaker AS 200 control (Retsch, Germany). Fractioning time was 10 min, amplitude 1.5 mm; a 100 g sample was used. Fractions of the polymer and filler powders of 200 μm and less were selected and used.

Then, polymer powder was mixed with different filler powders in benchtop planetary mixer SpeedMixer DAC 250 SP (Hauschild Eng., Germany); the blend time was 1 min, and the speed was 1000 rpm. The blends contained different amounts of the fillers: 10, 30, and 50%.

The homogenized blends were used to produce pellets and granules. Pellets were prepared by cold pressing at 36 bar using a laboratory semiautomatic press (Minipress, Minsk, Belarus). Granules were prepared by mixing wet polymer paste (40%) in ethanol in planetary mixer SpeedMixer DAC 250 SP (Hauschild Eng., Germany), with the mixing time 1 min at 1000 rpm, and granulating in screw granulator Fimar EAC (Italy) with a 6 mm nozzle. The granules were dried in a fume hood at room temperature for 24 h.

Poly-3-hydroxybutyrate, peat, clay, and wood powders, powdered P(3HB)/filler blends, and granules and pellets were examined by using state-of-the-art physicochemical methods.

2.3. Experimental techniques

SEM microscopy

The surface microstructure of pellets and granules prepared from blends was analyzed using scanning electron microscopy (a TM-3000 Hitachi microscope with the QUANTAX 70 program, Japan). Prior to microscopy, the samples were sputter coated with platinum (at 10 mA, for 40 s), with an Emitech K575XD Turbo Sputter Coater (Quorum Technologies Limited, U.K.). Elemental analysis of the samples was done using a scanning electron microscope equipped with a system for X-ray spectroscopy (a TM-3000 Hitachi microscope with QUANTAX 70 software).

FTIR spectroscopy

All initial materials and blends were analyzed using FTIR spectroscopy. IR spectra were taken in the 400-4000 cm^{-1} range using a "NICOLET 6700" FT-IR spectrometer (Thermo Scientific, U.S.) and a Smart Orbit accessory, by the attenuated total reflection (ATR) technique.

Differential scanning calorimetry (DSC)

Thermal analysis of the initial materials and blends was performed using a DSC-1 differential scanning calorimeter (METTLER TOLEDO, Switzerland). Powdered samples (4.0 ±0.2 mg each) were placed into the aluminum crucible and compressed prior to measurement. Every sample was measured at least 3 times. The specimens were heated at a rate of 5 °C/min to 200 °C, then cooled to -20 °C, held for 20 minutes and re-heated to 320 °C. Glass transition temperature (*Tg*), crystallization temperature (*Tc*), melting point (*Tmelt*), and thermal degradation temperature (*Tdegr*) were determined from peaks in thermograms using the "StarE" software.

X-ray diffraction (XRD)

X-Ray structure analysis and determination of crystallinity of initial materials and blends were performed employing a D8 ADVANCE X-Ray powder diffractometer equipped with a

VANTEC fast linear detector, using CuKa radiation (Bruker, AXS, Germany). The samples had a circular shape because during measurement the sample spins in a direction perpendicular to the surface. The scan step was 0.016°, measurement time in each step 114 s, and scanning range from 2° to 60° (from 48° to 60° there only was a uniformly decreasing background); the registered parameter was intensity of X-rays scattered by the sample; $55°/0.016°=3438$ times. The degree of crystallinity was calculated as a ratio of the total area of crystalline peaks to the total area of the radiograph (the crystalline + amorphous components). Measurement accuracy: point measurement accuracy ± 0.4 PPS, with the lowest intensity 1.5 PPS and the highest intensity 32 PPS; the error in determination of the degree of crystallinity, which was calculated based on multiple measurements, was 2% or less.

Mechanical analysis

Mechanical properties of the pellets and granules were investigated using an electromechanical tensile testing machine Instron 5565 (U.K.). At least five samples were tested for each blend type. Measurements were conducted at ambient temperature. The samples had a cylindrical shape 6 mm in diameter with and height of 4 mm. The speed of the crosshead was 1 mm/min at ambient temperature. Young's modulus (E, MPa) was automatically calculated by the Instron software (Bluehill 2, Elancourt, France). To obtain Young's modulus, the software calculated the slope of each stress-strain curve in its elastic deformation region. Measurement error did not exceed 10%.

Moisture absorption was investigated gravimetrically in dynamic mode. The amounts of water absorbed by pellets and granules were measured 3, 5, and 24 h after contact with water.

Degradation of samples in soil

Pellets and granules were weighed, placed into nylon mesh bags, and buried in the field soil (200 g in 250-cm^3 containers) at a depth of 2 cm. They were incubated in soil for 35 days at a temperature of 25 °C and soil moisture content of 50%. The agro-transformed soil was collected at field laboratory "Minderlinskoye" of the Krasnoyarsk State Agrarian University (forest-steppe, 56°N, 92°E, the Krasnoyarsk Territory, Siberia, Russia). The soil was chernozem with heavy loam particle-size composition, density of between 0.80 and 1.24 g/cm^3, pH 7.3, with high contents of ammonium nitrogen (35 mg/kg) and nitrate nitrogen (9.2 mg/kg). The soil contained 280 mg/kg of phosphorus and 250 mg/kg of potassium.

Every 7 days, the specimens (3 in a bag) were removed from the soil to determine their mass loss – the parameter indicating biodegradation of pellets and granules.

2.5. Statistics

Statistical analysis of the results was performed by conventional methods, using the standard software package of Microsoft Excel. Arithmetic means and standard deviations were found. The statistical significance of results was determined using Student's test (significance level: $P \leq 0.05$). Statistical analysis of surface properties of the samples was performed by using embedded methods of the DSA-4 software.

3. Results and discussion
3.1. Characterization of P(3HB) and initial natural materials

Poly-3-hydroxybutyrate and natural materials (clay, peat, and birch wood flour) with considerably different properties (Table 1) were used to produce P(3HB)/filler blends: fine powder and paste prepared with ethanol (40% moisture content).

Table 1.- Physicochemical properties of initial materials and blends

Sample	C_x,%	$T_{melt.}$, °C	$T_{cryst.}$, °C	$T_{degr.}$ °C	Melting enthalpy, (J/g)
Initial materials:					
P(3HB)	75	176	108	287	89.3
clay	53			200*	
peat	9			130*	
wood flour	26			220*	
Blends (P(3HB)/filler = 70/30):					
P(3HB)/clay:					
90:10	72	177	110	286	52.4
90:30	60	175	110	283	38.5
90:50	56	176	109	276	29.8
P(3HB)/peat:					
90:10	60	174	107	288	64.1
90:30	48	176	108	292	59.7
90:50	42	177	110	290	48.7
P(3HB)/wood flour:					
90:10	65	178	107	279	66.9
90:30	47	175	106	273	51.3
90:50	44	171	104	274	45.4

(*) - start of $T_{degr.}$

P(3HB) is a highly crystalline material, with the prevailing crystalline phase, and its degree of crystallinity (C_x) is usually above 60-65%; the C_x of the P(3HB) sample used in this study is 75%. X-ray diffraction pattern of P(3HB) is shown in Figure 1a. Diffraction peaks in 2Θ = 13.4, 16.8, 20, 22.2 and 25.5° have a similar pattern when compared with previous crystallographic data for this material [Ikejima, Inoue, 2000; Araujo et al., 2011].

X-ray diffraction patterns of the fillers differed from the diffraction pattern of P(3HB). Wood flour showed distinct diffraction peaks at 2θ = 14.2, 19.6, and 25.5°, in contrast to the amorphized peat. X-ray examination of clay revealed numerous narrow diffraction peaks, and the most distinct one was at 23.5°. Clay had the highest C_x (53%). In wood flour and, especially, peat, amorphous phase prevailed, and their degrees of crystallinity were low: 26 and 9%, respectively.
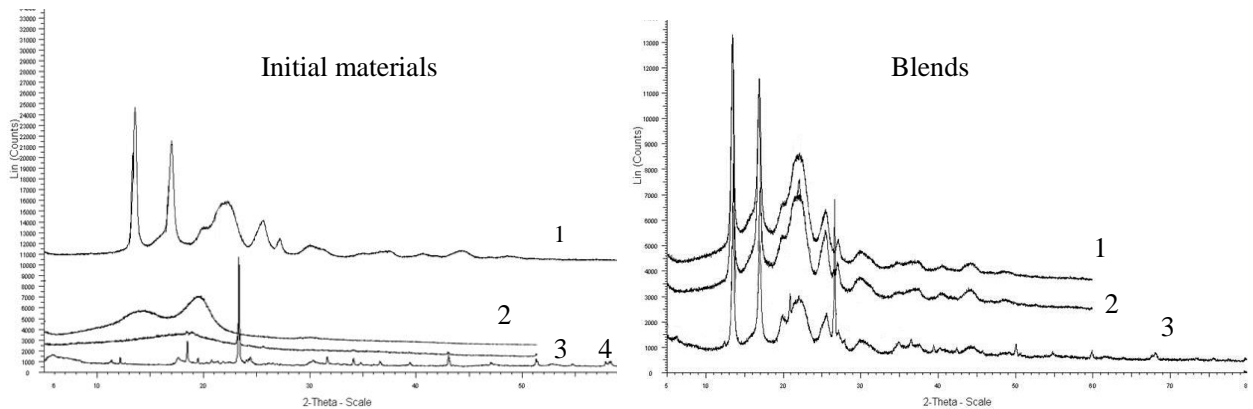

Fig. 1. X-ray diffraction patterns of initial materials: 1 – P(3HB); 2 – wood flour; 3 - peat; 4 – clay; and blends (P(3HB)/filler = 70/30): 1 – P(3HB)/wood flour; 2 – P(3HB)/peat; 3 – P(3HB)/clay

Thermal properties of the polymer and fillers are different too (Fig. 2 a). P(3HB) is a thermoplastic material, with a considerable difference between melting point (176°C) and thermal decomposition temperature (287°C). Its peak of thermal decomposition is in the …??°C range, and the onset of the process is at 280°C. P(3HB) crystallization temperature is 108°C and melting enthalpy is 89.3 J/g. By contrast, none of the fillers is a thermoplastic material. Initial heating of these materials (and blends) gives a small peak at a temperature between 70 and 130°C, which is associated with evaporation of moisture and volatile organic compounds (denoted by arrows in Fig. 2). After moisture evaporation, thermal decomposition of peat begins at 130°C and thermal decomposition of wood flour at 220°C. For clay, at a temperature between 60 and 200°C, moisture evaporates and decomposition and evaporation of volatile organic compounds begin (the process is endothermal); however, at temperatures between 200 and 400°C, decomposition is accompanied by heat release.
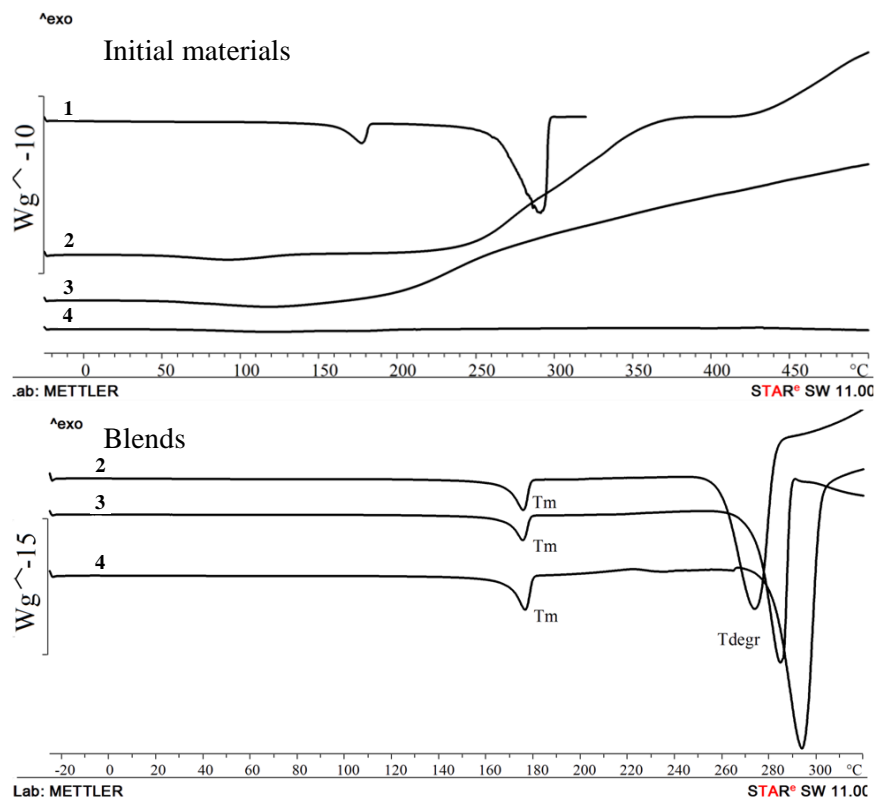

Fig. 2. DSC of initial materials: 1 – P(3HB); 2 – wood flour; 3 – peat; 4 – clay; and blends (P(3HB)/filler = 70/30): 2 – P(3HB)/wood flour; 3 – P(3HB)/peat; 4 – P(3HB)/clay

Possible structural differences between the blends were detected using IR spectroscopy, which is employed to study the structures of various macromolecules. Variations in the intensity of the bands in the low-frequency range are indicators of both the crystalline to amorphous phase ratio in the blend and the type of interactions of the components in the blend.

FT-IR spectra of P(3HB) and the three fillers were taken in the 400-4000 cm^{-1} range (Fig. 3). The spectra show not only the groups of carbon compounds (C=O, C-OH, CH$_2$, CH$_3$) contained in all initial materials but also the groups and compounds characteristic of individual components. These are CH, CH2, CH3, C-OH, and COOH groups for P(3HB), S-S-, C=C, C=N, and N − H groups for peat, Si-O-Si(AL) and Fe-O in α-Fe$_2$O$_3$ for clay, and -C-O-;-C-O-C- for birch wood flour.
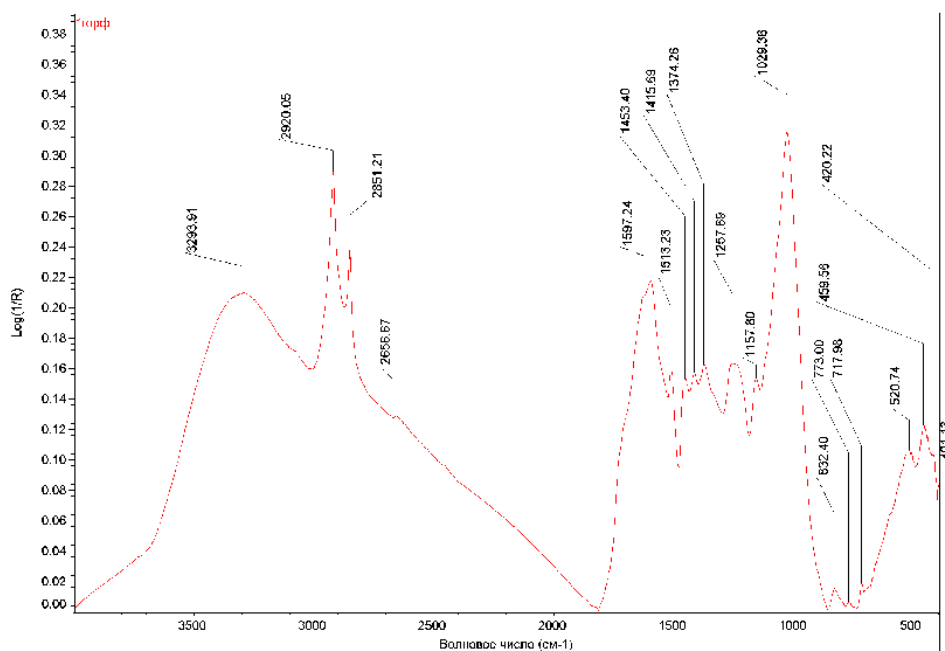
Fig. 3. FT-IR spectra of P(3HB) and initial materials: clay, wood flour, and peat

The IR absorption spectra of P(3HB) (Fig. 3 a) contain absorption bands corresponding to vibrations of the main structural components of polymers except the absorption bands of vibrations of the terminal C-OH and COOH groups. The bands of the ordered optical densities (crystalline phase) are in the 1228 cm^{-1} range while the bands of the amorphous phase are shifted to 1182 cm^{-1}. This is consistent with the data reported in other studies [32,33]. The spectra show distinct absorption bands of asymmetric stretching of CH$_3$- and CH$_2$- groups (2978 and 2994 cm^{-1}); symmetric stretching of CH- and CH$_2$ groups (2994 and 2934 cm^{-1}); stretching of conjugated (1687 cm^{-1}) and unconjugated (1720 cm^{-1}) carbonyl groups C=O; skeletal CH vibrations (599 cm^{-1}); and CH bending (622 cm^{-1}). PHAs are hydrophobic compounds, in which molecules of water interact with the hydrogen atom of the methyl group or with the oxygen atom of the carboxyl group through hydrogen bonding. The narrow bands at 3436 cm^{-1} are typical of the hydrogen bonded OH group. The 1687 cm^{-1} band is characteristic of hydrogen vibration in the OH group interacting with the oxygen atom through hydrogen bonding. The 2874, 2934, 2978, and 2994 cm^{-1} absorption bands are typical of the bonded OH group and may be a component of the COOH dimer group. In the 3000-2500 cm^{-1} range, there is a group of weak bands typical of the dimers of carboxylic acids.

In contrast to P(3HB) IR spectra, the IR spectra of peat (Fig. 3 b) contain absorption bands of the –S-S- bond groups (521 cm^{-1}); –C-O- groups characteristic of carbohydrates (1029 cm^{-1}); deformation vibrations of -CH$_2$- groups (718 cm^{-1}); skeletal vibrations of the benzene ring C=C (lignin), C=N, N – H (1513 and 1597 cm^{-1}), and stretching of the –OH groups at 3300 cm^{-1}.

The IR spectra of clay (Fig. 3 c) contain absorption bands in the 776 and 795 cm^{-1} range, corresponding to stretching and symmetrical vibrations of Si-O-Si(Al) and Fe-O in α-Fe$_2$O$_3$; stretching vibrations of (AI-0), (Si-O-Al), and -A1$_2$0$_3$ in the 692 см$^{-1}$ range. In the 978 cm^{-1} range, there are absorption bands corresponding to (O-Si) deformation vibrations. The absorption frequency observed at 1635 cm^{-1} is indicative of deformation vibrations of H-O-H, which suggest water sorption on the surface of the mineral. The absorption band at 3615 and 3392 cm^{-1} corresponds to stretching and symmetric vibrations of AlO-H for clay bound water. The 3619 cm^{-1} band corresponds to stretching of intramolecular hydroxyls located on the surface of octahedral and tetrahedral sheets [34].

IR spectra of birch wood flour (Fig. 3 d) show plane bending of the guaiacyl ring of lignin of leaves (1031 cm^{-1}), stretching of -C-O-;-C-O-C- (1235 cm^{-1}), skeletal vibrations of the syringyl ring of lignin of leaves (1324 cm^{-1}), stretching and skeletal vibrations of benzene ring (1422 cm^{-1}, 1504 cm^{-1}, 1593 cm^{-1}), stretching of -C=O of conjugated and unconjugated groups (1652 cm^{-1}, 1734 cm^{-1}), and intramolecular and intermolecular stretching of –OH groups and water (3337-3342 cm^{-1}).

3.2. Characterization of P(3HB)/filler blends

Powdered P(3HB) and fillers were processed by cold pressing to produce pellets with a diameter of 6 mm and height 3 mm and granules with a diameter of 2 mm and length of between 2 and 4 mm.

Elemental analysis of the samples was done using a scanning electron microscope equipped with a system for X-ray spectrometry (Table 2). The initial P(3HB) contained only carbon (66.96 at.%) and oxygen (33.04 at.%). The P(3HB)/wood flour blend also contained silicon, aluminum, iron, calcium, and sulfur; P(3HB)/peat blends additionally contained Mg and K. The elemental composition of P(3HB)/clay blends was the most diverse. They contained 11 elements: in addition to the elements mentioned above, analysis detected sodium, titanium and manganese.

Table 2.- Elemental composition of P(3HB) and blend (P(3HB)/filler = 70/30) samples (atomic %)

Element	P(3HB)	P(3HB)/wood flour	P(3HB)/peat	P(3HB)/clay
C	66.96 ± 6.5	67.03 ± 6.4	63.32 ± 6.2	44.80 ± 3.8
O	33.04 ± 4.5	32.03 ± 4.2	36.52 ± 4.9	43.39 ± 4.7
Si	–	0.26	0.03	6.71 ± 0.5
Al	–	0.13	0.02	2.98 ± 0.2
Fe	–	0.22 ± 0.1	–	0.84 ± 0.1
Mg	–	–	0.03	0.41 ± 0.1
K	–	–	0.07	0.34
Ca	–	0.26	–	0.29
Na	–	–	–	0.12
Ti	–	–	–	0.09
Mn	–	–	–	0.05
S	–	0.08	–	–

(-) not detected

Morphology of blends.

Pellets and granules prepared from blends with fillers that had different properties exhibited diverse surface structures (Fig. 4). SEM images showed porous and rough surface microstructures. The surface of the P(3HB)/wood flour pellets showed rectangular sawdust particles embedded in the surface layer; on the surface of the P(3HB)/peat pellets, there were asymmetrical heteraxial inclusions. On P(3HB)/clay pellets, clay inclusions were uniformly distributed over the surface.
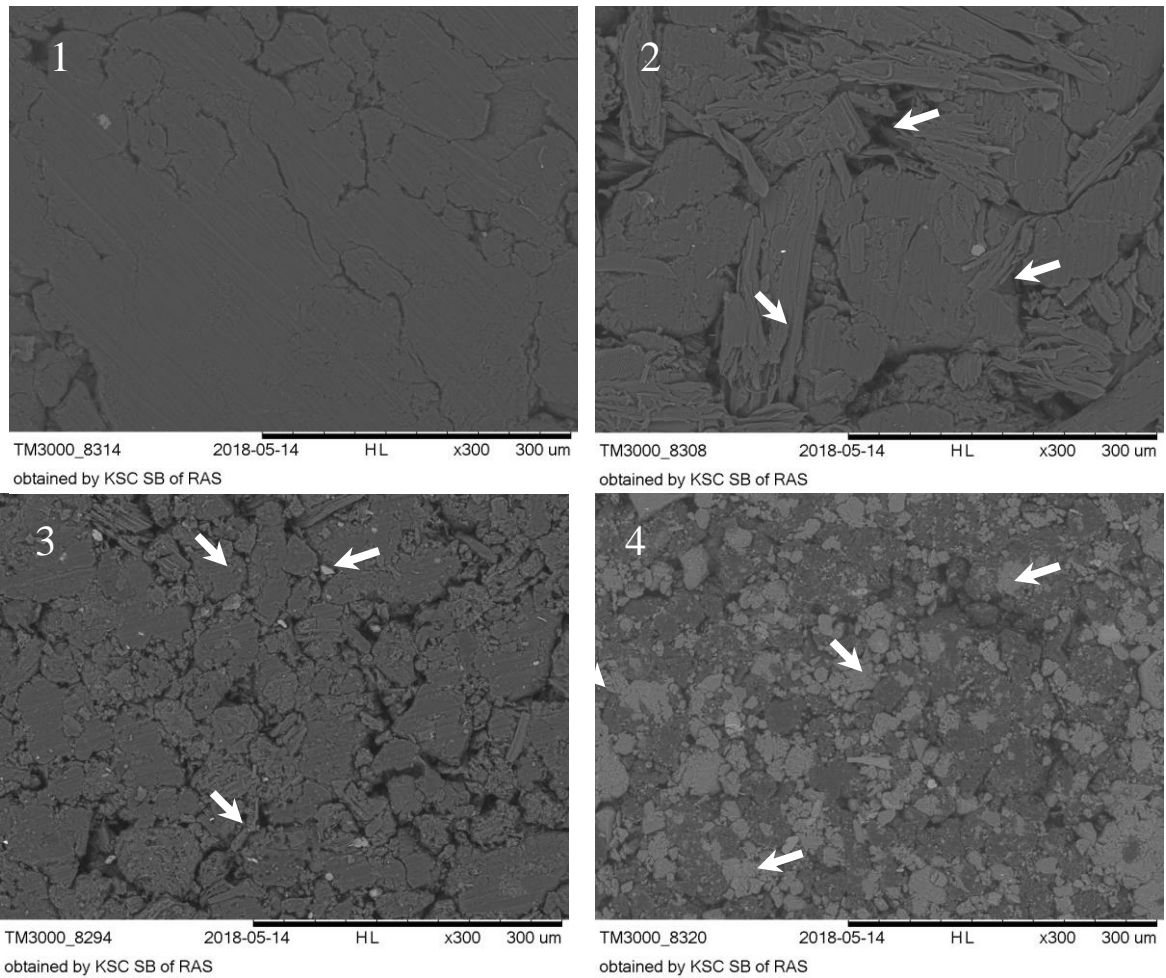
Fig. 4. SEM images of pressed pellets (P(3HB)/filler = 70/30): 1-P(3HB); 2-P(3HB)/wood flour; 3- P(3HB)/peat; 4-P(3HB)/clay

The filling of the hydrophobic P(3HB) with the hydrophilic natural materials considerably enhanced the water absorption capacity of the blends (Fig. 6).
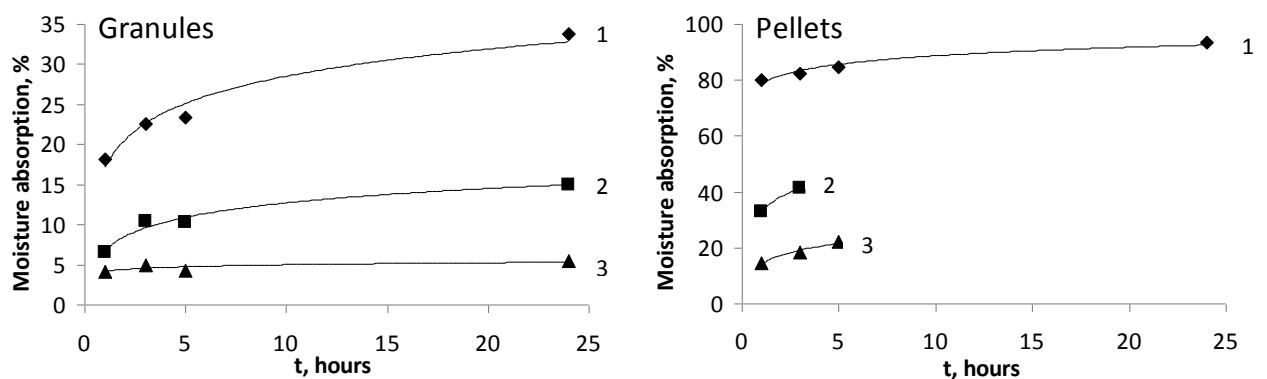

Fig. 6. Moisture absorption: 1 – P(3HB)/wood flour; 2 – P(3HB)/peat; 3 –P(3HB)/clay.

Pellets showed higher moisture absorption. The moisture absorption by the P(3HB)/wood flour pellets reached 93.5% in 24 h. The moisture absorption by P(3HB)/peat and P(3HB)/clay pellets measured after the first 3 and 5 h was 41.3 and 22.9%, respectively; then, water-filled pellets became deformed. Granules absorbed less moisture than pellets. P(3HB)/wood flour granules absorbed the largest amount of water – 33.8% for 24 h, while moisture absorption by P(3HB)/clay granules was the lowest – 5.4% for 24 h. In contrast to pellets, granules retained

their shape. The reason for destruction of pellets was that they had been prepared from the mechanically mixed blend, in which polymer and filler particles were located next to each other. Upon contact with water, the more hydrophilic filler absorbed moisture and swelled. That swelling damaged the polymer matrix and, subsequently, the pellet was destroyed. During preparation of granules, polymer particles encapsulated filler particles, thus reducing the hydrophilicity of the blend and moisture absorption of the granules.

IR spectrophotometry

The blends were characterized using DSC, X-ray examination, and IR spectroscopy. IR spectra of the blends are shown in Figure 5.
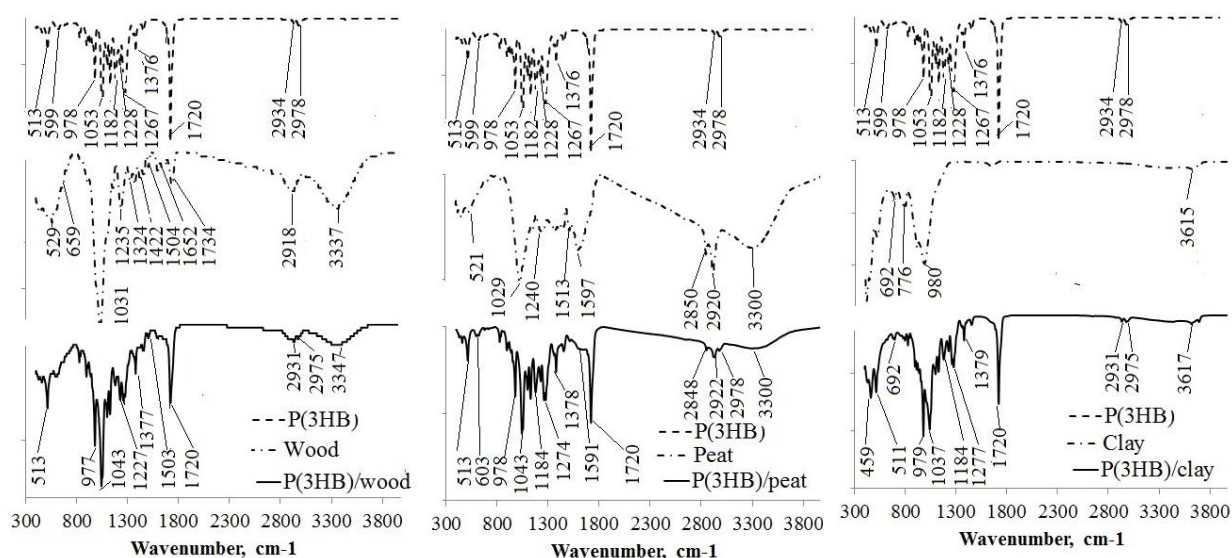

Fig. 5 FT-IR spectra of the blends (P(3HB)/filler = 70/30): 1 – P(3HB)/wood flour; 2 – P(3HB)/peat; 3 – P(3HB)/clay.

IR spectrometry showed that characteristic absorption bands of initial components were separately retained in all blends: for P(3HB) those were CH, CH_2, CH_3, C-OH, and COOH groups; for peat – the additional and characteristic S-S-, C=C, C=N, N – H groups; for clay – Si-O-Si(AL) and Fe-O in α-Fe_2O_3; for birch wood flour – -C-O-;-C-O-C-. In the spectra of the blends, absorption bands were either separate bands or coinciding peaks, some of them transformed. An insignificant difference in numbers was within the limits of spectral error – 4 cm^{-1} (accumulation of 256 scans) and could result from increased moisture content of some of the samples. No new significant absorption bands were revealed in the blends, suggesting that no new chemical bonds were formed and that the blends prepared by the techniques employed in this study were physical mixtures.

X-Ray analysis

Blending of P(3HB) with the fillers changed the properties of the polymer. The blends became progressively amorphous as the percentage of the fillers was increased (10, 30, and 50% fillings of the polymer with natural materials were tested). The degree of crystallinity of P(3HB) was decreased more noticeably in the blends with peat and wood flour (materials with low C_x). The C_x of the P(3HB)/peat and P(3HB)/wood flour blends with 30% filler decreased to 47 and 48%, respectively; the C_x of the blends with 50% of the same fillers decreased to 42 and 44%, respectively. That is, amorphous phase prevailed in these blends (Fig. 1 b, Table 1). In the P(3HB)/clay blends, the crystalline phase prevailed at all levels of filling of the polymer with clay; the C_x was no less than 56%. Changes in the crystallization kinetics of the polymer in the blends were associated with the rather high percentages of fillers (30 and 50%). This must have decreased the free volume necessary for nucleation of spherulites to occur, preventing the development of the crystalline phase and, thus, reducing it.

DSC analysis

Thermograms of the blends were taken in a wide range of temperatures including boundaries of melting and thermal decomposition (Fig. 2). DSC was used as one of the most informative methods for determining thermal properties of polymers and polymer-based blends, as melting behavior of components of the blend provides evidence of the degree of miscibility and interactions of the components. Our results showed that the fillers did not significantly affect the temperatures of melting and thermal decomposition of P(3HB) but considerably decreased enthalpy of melting (Table 1). Compared to enthalpy of melting of P(3HB) (89.3 J/g), the enthalpy of melting of the blends containing 30% clay, peat, and wood flour decreased to 38.5, 59.7, and 51.3 J/g and to 29.8, 48.7, and 45.4°C for the blends with 50% of the fillers. Melting temperature was not affected by fillers, as it is largely the property of the polymer matrix. The components of the blend (polymer and filler) are mixed mechanically, the chemical structure of the polymer remains unchanged, and, thus, the filler does not produce any effect on the melting temperature. The peak of degradation for the P(3HB)/clay and P(3HB)/peat blends with 30% of the filler was at 283 and 292°C, i.e. the temperatures insignificantly different from the degradation temperature of pure polymer (287°C); for the blends containing 50% of the fillers, the peaks of degradation were at 276 and 290°C, respectively. The lower degradation temperature of the blend containing 50% clay can be explained using dispersion concept. The increase in the amount of clay in the blend impaired the dispersion of the filler in the polymer matrix and clay particles could agglomerate. This secondary structure in the polymer matrix could accumulate heat from external sources and, eventually, trigger a more rapid onset of decomposition.

For the P(3HB)/peat blend, thermograms showed the onset of thermal decomposition at 225°C, in contrast to pure polymer (270°C) and the P(3HB)/clay blend (272°C) (Fig. 2 b). For the P(3HB)/wood flour blend, the degradation peak was observed at 273°C (at a temperature 14°C lower than the temperature for pure polymer), and the onset of decomposition occurred at 259°C. A possible reason for the lower temperatures for decomposition of the P(3HB)/wood flour blend could be accumulation of wood flour decomposition products in the melt, which could catalyze destruction of the polymer matrix. For the P(3HB)/clay and P(3HB)/peat blends, no appreciable change in the temperature of crystallization was noted. By contrast, for the P(3HB)/wood flour blend, the temperature of crystallization was insignificantly lower (106°C). Wood flour particles could serve as defect centers and prevent polymer chains from folding, producing smaller crystals and causing the temperature of crystallization to decrease. In conditions of the thermal analysis, glass transition temperature was not registered. This fact was noted for many times previously for P(3HB) samples we synthesized [30,31]. Because glass transition process is defined by the amorphous phase, high P(3HB) crystallinity may complicate detection of this parameter.

3.3. A physical/mechanical study

Analysis of physical/mechanical properties of the pellets prepared from initial materials and blends is shown in Figure 6 (granules had very low values and, therefore, they were not studied). Young's moduli of the pellets prepared from initial materials varied considerably, and the highest value was obtained for P(3HB) pellets (350 MPa). Young's moduli of all fillers were lower: 2890 MPa for wood flour pellets, 138 MPa for peat pellets, and 50 MPa for clay pellets. Under downward force applied to pellets prepared from blends, compressive strength of the pellets consistently decreased as the percentage of the filler increased. P(3HB)/wood flour pellets showed the highest resistance: Young's moduli of the pellets with 30% and 50% of the filler reached 310 and 276 MPa, respectively. The P(3HB)/peat and P(3HB)/clay pellets with 50% of the fillers showed the lowest values of Young's modulus: 150 and 76 MPa, respectively. The reason for that could be amorphization of the blends: the amount of the polymer, in which crystalline phase prevailed (75%), decreased and the amount of the filler, with the prevailing amorphous phase, increased.
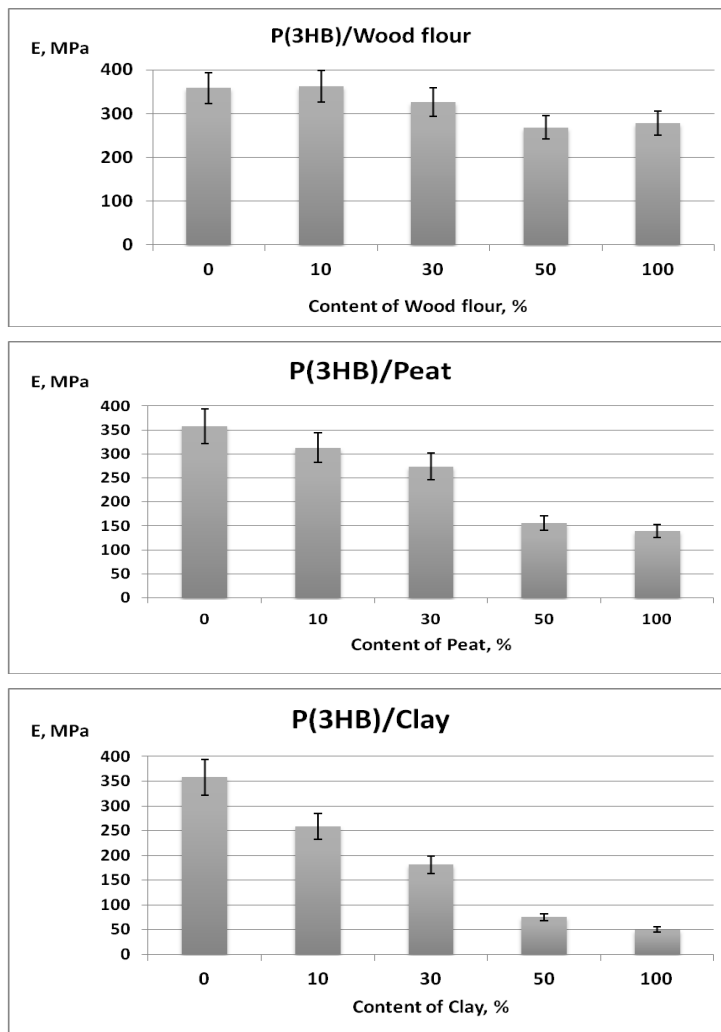
P(3HB)/Wood flour

E, MPa

Content of Wood flour, %

P(3HB)/Peat

E, MPa

Content of Peat, %

P(3HB)/Clay

E, MPa

Content of Clay, %

Fig. 6. Young's modulus of the pressed pellets prepared from blends of P(3HB) and fillers mixed in different proportions

3.4. Degradation of pellets and granules prepared from blends in soil

As the blends prepared and tested in this study can be considered as potential carriers for pesticides and fertilizers for soil application and as materials for fabricating degradable packaging and other goods, it is important to study their degradation in soil. Figure 7 is a photograph of pellets and granules degraded for 35 d in agro-transformed (field) soil; Figure 8 is their SEM photographs; Figure 9 shows their degradation kinetics.
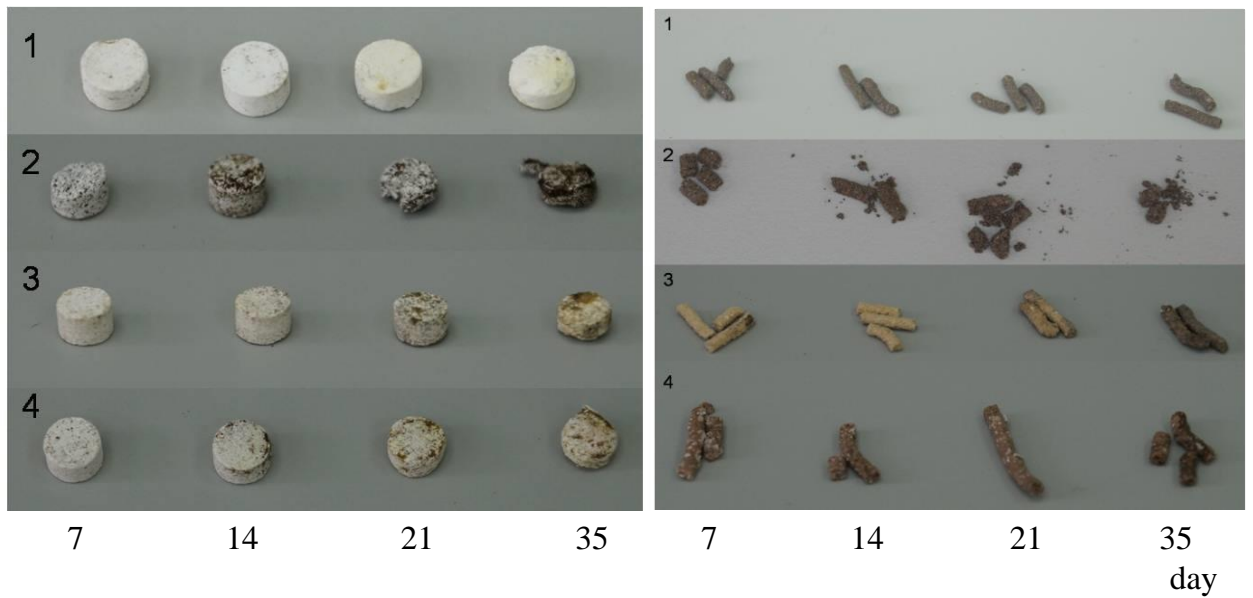
Fig. 7. Photographs of pellets and granules at different time points of their degradation in soil: 1 – P(3HB), 2 – P(3HB)/peat, 3 – P(3HB)/wood flour, 4 – P(3HB)/clay
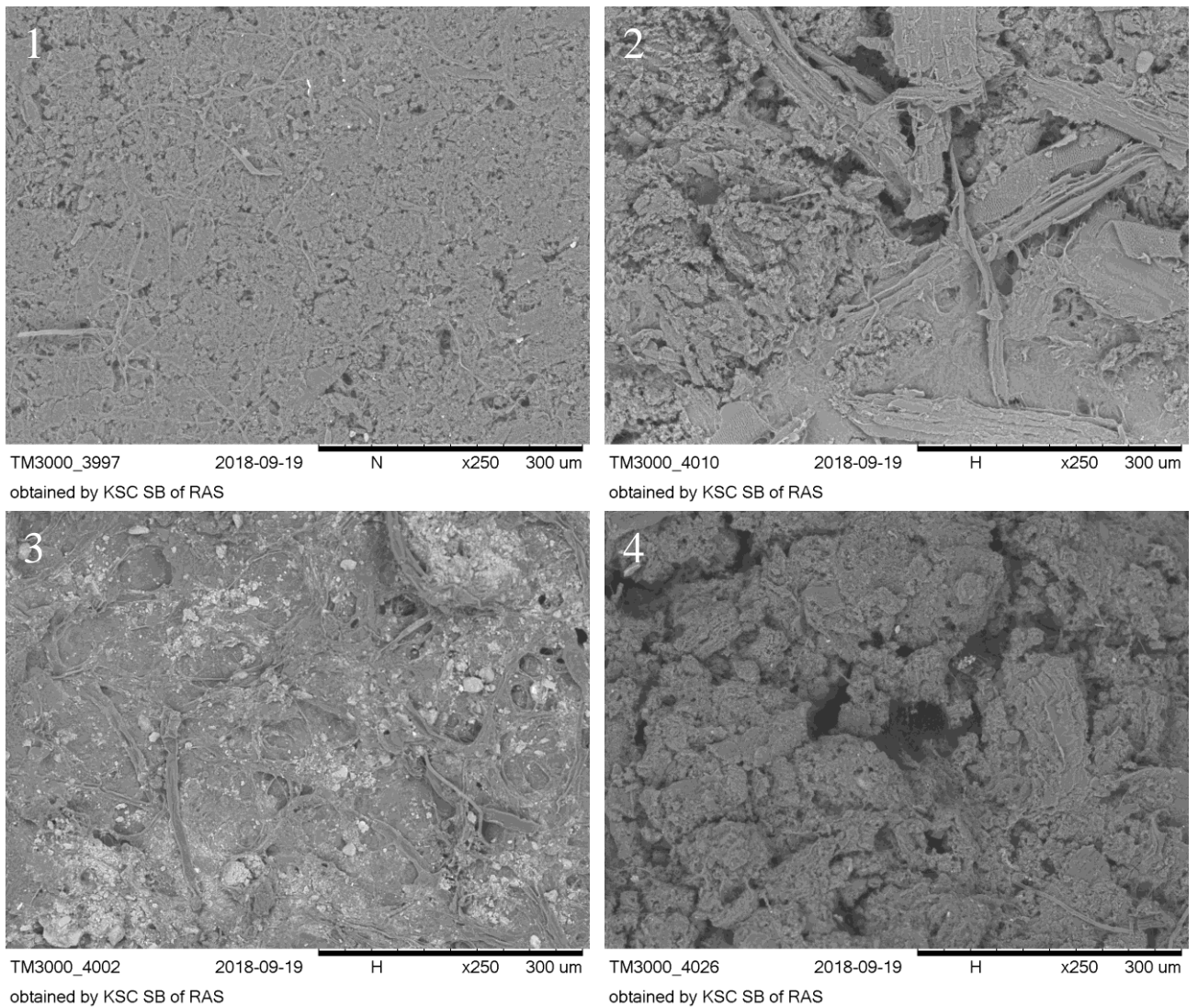

Fig. 8. SEM images of pressed pellets (P(3HB)/filler = 70/30) at different time points of their degradation in soil: 1-P(3HB); 2- P(3HB)/wood flour; 3- P(3HB)/peat; 4-P(3HB)/clay
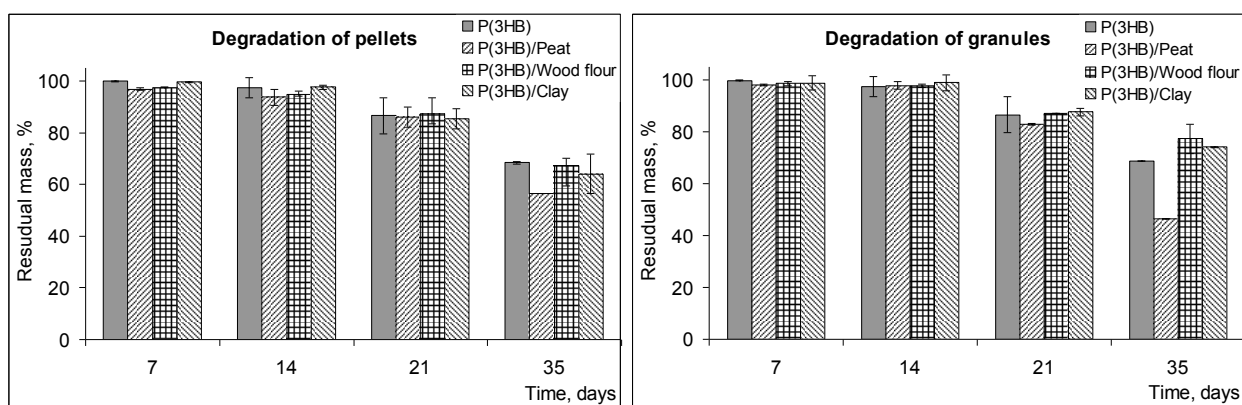
Fig. 9. Kinetics of degradation of experimental pellets and granules in soil

Pellets and granules of all compositions were degraded at high rates after 3 weeks of incubation in soil (Fig. 9 a). By the end of the experiment, residual mass of the pellets had decreased to 68% for P(3HB), 56.4% for P(3HB)/peat, 67% for P(3HB)/wood flour, and 64% for P(3HB)/clay. Granules exhibited a similar mass loss (Fig. 9 b); high biodegradation rates were also observed after 3 weeks of incubation in soil. At the end of the experiment, residual mass of the granules of P(3HB) was 68.4%, P(3HB)/peat 46.4%, P(3HB)/wood flour 77%, and P(3HB)/clay 74%. Both pellets and granules made from the P(3HB)/peat blend showed a somewhat more considerable mass loss. However, all pellets and granules during the experiment showed comparable mass loss, which was mainly caused by polymer degradation. The mass loss of the specimens except P(3HB)/peat blends was about 30-40% for 35 d, suggesting slow destruction of all products in soil.

3.4. Discussion

A relatively new line of research is filling of polyhydroxyalkanoates with natural materials to improve properties of the polymers and reduce their cost, in order to widen the range and increase the scale of their applications. This is particularly important for the large-scale use of PHAs in technical areas and agriculture.

The fillers of P(3HB) used in the present study were clay, birch wood flour, and peat. These materials were chosen for the following reasons. Clay is a common and relatively cheap natural material; it has attracted a good deal of attention among researchers as material for nanocomposites. Wood flour, as well as other wood waste, is produced in great quantities everywhere, and, thus, this is another potentially useful material for manufacturing hybrids. Peat is relatively safe for the environment and it is abundant; burial of peat in soil as a component of biodegradable packaging or carrier of fertilizers or other chemicals is safe for soil biota and even improves its structure.

The literature search revealed few studies that describe results of using natural materials, including waste, as fillers. Angelini et al. [26] reported using a lignocellulosic biomass (LC), biowaste from the second generation bioethanol production process as a filler of P(3HB). LC was processed in order to isolate its main components: holocellulose (HC) and acid-insoluble lignin (IL). LC and its derivatives were introduced as fillers at 30 wt.% in P(3HB). The blends were prepared by a solvent-assisted method. The addition of IL to P(3HB) affected its rheological properties, significantly enhancing melt stability due to the formation of a percolated filler network within the matrix. The DSC analysis showed that LC and HC promoted the polymer melt crystallization, potentially enabling control of physical aging of P(3HB). To sum up, that study provided an insight into the effect of a lignocellulosic biowaste and its derivatives on the properties of a naturally occurring, biodegradable polyester.

Other studies [27-29] discuss properties and potential uses of wood waste and PHA to fabricate wood particle boards. The studies proved that PHAs could serve as nontoxic binding

material instead of toxic formaldehyde resins. Addition of 15 to 30% PHA to wood flour and chips enhanced their mechanical strength: modulus of rupture rose from 5.27 to 6.58 MPa and internal bond strength from 0.18 to 0.22 MPa. Water resistance of the material increased, too: water absorption decreased from 115.48 to 85.62%. The authors concluded that such mixes were promising materials for construction industry. A similar conclusion was reached in another study [35], in which PHAs were tested as adhesive materials, and the particleboards made from oil palm trunks with addition of PHA were stronger than the boards fabricated without adding PHA.

Studies by Bakri et al. [36,37] describe using acacia wood and discuss the positive eco-economic aspect of using wood waste in combination with degradable polymers, PHAs. The authors proposed producing stronger and naturally degradable hybrids based on wood waste and thermoplastic PHAs and polylactides.

Studies by Maiti et al. [20,21] reported preparation of P(3HB)/clay composites by melt extrusion. Clay was pre-modified with octadecyl ammonium. Well-ordered intercalated clay nanocomposites with decreased d-spacing were produced. Mechanical tests showed that the fluoromicas led to higher enhancement than montmorillonite. Bittmann et al. [38] studied the effects of clay fillers on surface modification, dispersive state, heat and dynamic mechanical properties of P(3HB-co-3HV) copolymers. Montmorillonite and bentonite were used as clay component. In a study by Lim et al. [22], P(3HB) composites with clay fillers were prepared by suspending organomodified montmorillonite (OMMT, a commercial preparation) in a polymer solution; the authors noted intercalation of polymer chains into layered structures of clay and enhanced thermal stability of the composite compared to the initial P(3HB). The study by Torres-Giner et al. [23] deals with grafting of sepiolite nanoclay onto poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate) biopolymer by melt compounding. The newly formed sepiolite-grafted P(3HB-*co*-4HB) (sepiolite-*g*-P(3HB-*co*-4HB)) nanocomposite showed higher thermal, thermomechanical, and mechanical performance than the equivalent ungrafted nanocomposite. Melt grafting of sepiolite at different weight contents (1, 3, and 5 wt.%) increased the thermal stability and stiffness of P(3HB-*co*-4HB) without impairing its ductility and toughness.

In the present study, P(3HB) was blended with three natural materials, the blends were used to prepare pellets and granules, and their properties were studied.

IR spectroscopy was used to reveal possible structural differences between the blends. The IR spectra of the initial P(3HB), each of the three natural materials, and their blends were taken in the 400-4000 cm^{-1} range. The spectra of all blends retained characteristic absorption bands of the initial components: for P(3HB) those were CH, CH_2, CH_3, C-OH, and COOH groups; for peat – the additional and characteristic S-S-, C=C, C=N, N – H groups; for clay – Si-O-Si(Al) and Fe-O in α-Fe_2O_3; for birch wood flour – -C-O-;-C-O-C-. No new absorption bands were visible in the spectra of the blends, suggesting that no new chemical bonds were formed and the blends prepared by the methods employed in this study, without using chemical reagents and heating, were physical mixtures. However, in the study by Torres-Giner et al. [23], in which composites of sepiolite nanoclay and P(3HB-co-4HB) copolymer were produced by melt compounding, during thermal extrusion, the reactive coupling agent successfully established strong chemical ''bridges'' between the biopolymer and sepiolite by Si – O - C bonds. As a result, it generated a new hybrid nanostructure, i.e., a sepiolite-grafted P(3HB-co-4HB)(sepiolite-g-P(3HB-co-4HB)) nanocomposite. Thus, the technique of processing of the PHA/filler blend is a more important factor determining the type of the blend than the chemical composition of the material.

A scanning electron microscope equipped with a system for X-ray spectrometry was used to perform elemental analysis of the initial materials and their blends, which showed considerable differences between the samples. The P(3HB)/wood flour blend contained not only carbon and oxygen but also Si, Al, Fe, Ca, and S; P(3HB)/peat blends additionally contained Mg and K. The elemental composition of P(3HB)/clay blends was the most diverse. They contained 11 elements: in addition to the elements mentioned above, analysis detected Na, Ti, and Mn.

Materials used as fillers for P(3HB) had different structure and properties and, thus, produced different effects on the morphology and properties of the blends. First of all, the initially hydrophobic polymer blended with hydrophilic fillers acquired the ability to sorb water. Pellets prepared from P(3HB)/wood flour blends had higher water absorption capacity than the products of P(3HB)/peat and P(3HB)/clay blends. As the amounts of fillers in the blends were increased, the degree of crystallinity of the polymer decreased, especially in the P(3HB) blends with wood flour and peat, whose C_x values were initially the lowest. These blends mainly consisted of the amorphous phase. These results are in good agreement with the data reported in a study by Thiré et al. [39], suggesting that attapulgite clay decreased the degree of crystallinity of P(3HB-co-3HV); the authors also noted an insignificant decrease in the temperature of crystallization and the melting point of the blend with an increase in clay concentration from 1 to 5%.

The study of thermal properties of the blends did not show any significant influence of the fillers on the temperatures of melting and thermal decomposition and the position of the T_{melt} and T_{degr} peaks in thermograms. However, the onset of thermal decomposition of the blends was shifted to the left relative to the initial P(3HB). The enthalpy of melting of the blends was significantly decreased (by a factor of 1.5-2.2). It is difficult to compare results obtained in this study with literature data because of data scarcity and different techniques used to process blends into products. Results of our study, however, are consistent with the data reported in a study by Angelini et al. [26], in which unprocessed wood flour was used as a filler, and thermograms showed an earlier onset of degradation of the blend compared to pure P(3HB). The temperature properties of the P(3HB)/clay blends obtained in this study are in good agreement with the data reported in a study by Lim et al. (2003), in which P(3HB)/clay blends were prepared by dissolving the components together and the subsequent removal of the solvent. The authors of that study described formation of intercalated structures, but did not reveal any effect of the filler on P(3HB) properties, which was confirmed by IR spectra that showed the presence of two different phases.

We did not find any available studies of P(3HB) blends with peat. Peat was used as a filler in composites with polylactide and polypropylene [40] and was found to affect crystallization of polylactide. Tensile strength of peat composites was lower than that of cellulose composites, but peat composites had higher impact strength. The authors concluded that polymer/peat composites were promising materials for agriculture.

The study of physicochemical properties of P(3HB) blends with the three natural materials did not reveal any dramatic impairment of the properties of the polymer. However, Young's moduli of all blends decreased as the amount of the filler in the blend increased. The most significant decrease in Young's modulus was observed in the P(3HB)/peat and P(3HB)/clay blends containing 50% of the filler: the parameter dropped to 150 and 76 MPa, respectively, compared to Young's modulus of the initial polymer of approximately 360 MPa. These results differ from the data reported in a study by Bensalah et al. [34], which addressed the effect of clay on thermal and mechanical properties of polypropylene (PP) – a polymer whose basic properties are similar to those of P(3HB). The authors of that study showed that clay used as a filler enhanced commercial properties of PP, increasing Young's modulus. Just like in our study, the addition of different amounts of clay (between 5 and 20%) to PP did not affect T_{melt}, insignificantly decreased crystallinity, and considerably decreased enthalpy.

As P(3HB) blends can be considered as potential carriers for pesticides and fertilizers for soil application and as materials for fabricating degradable packaging and other goods, it is important to study their degradation behavior in soil. Degradation of P(3HB) blends with natural materials was first investigated in this study, showing that the mass loss of the blends over 35 days of incubation in soil varied between 30 and 50% of the initial mass of the products, depending on the type of the filler.

4. Conclusions

Natural materials – clay, birch wood flour, and peat – were used as fillers for degradable poly-3-hydroxybutyrate. All materials were ground into powder with particle size of 200 μm. The P(3HB)/filler dry powder and wet paste (40% moisture content in ethanol) were cold-pressed to produce pellets and granules. All initial materials and blends were investigated using IR spectroscopy, DSC, X-ray analysis, electron microscopy with microanalysis and X-ray spectrometry. Analysis of IR spectra showed that no chemical bonds were established between the polymer and fillers and that the blends were physical mixtures. Their temperature characteristics and degrees of crystallinity were lower than those of P(3HB), suggesting different crystallization kinetics of the blends. The hydrophobic P(3HB) filled with the natural materials acquired the ability to sorb moisture; Young's modulus of the blends was lower than that of P(3HB). Degradation behavior of pellets and granules prepared from P(3HB)/filler blends in soil was similar to degradation behavior of pure polymer. The mass loss of all pellets and granules over 35 days of incubation in soil varied between 30 and 50% of the initial mass of the products, depending on the type of the filler. Thus, the blends prepared and tested in this study can be used to fabricate degradable packaging, greenhouse accessories, and delivery systems for fertilizers and pesticides.

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