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Biodegradation of Polyhydroxyalkanoates in Natural Soils

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The review reports studies of polyhydroxyalkanoate (PHA) biodegradation behavior in natural soils of different climatic zones. Degradation of different types of PHA is strongly influenced by the temperature, humidity, type of soil, amount of precipitation and the density of microbial populations. Micromycetes are considered to be the most efficient soil PHA degraders, and Penicillium is the most typical genus. But many bacterial species also participate in biodegradation. In all environments most PHA degrading soil microorganisms degrade short-chain PHAs only. Increasing the degrees of crystallinity of degrading PHAs suggests preferential disintegration of their amorphous phase in the soil as compared with crystalline phase.

Keywords: polyhydroxyalkanoates (PHAs), PHA properties, PHA biodegradation, PHA degrading microorganisms, soil.

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Биодеградация полигидроксиалканоатов в природных почвах

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Обзор обобщает результаты исследований по биодеградации полигидроксиалканоатов (ПГА) в природных почвах различных климатических зон. Деградация различных типов ПГА находится под влиянием температуры, влажности и типа почвы, количества осадков и плотности микробной популяции. Наиболее эффективными почвенными деструкторами ПГА являются микромицеты, среди которых широко распространены представители рода Penicillium. Однако значительное количество видов бактерий также принимает участие в биодеградации. Во всех экосистемах микроорганизмы-деструкторы короткоцепочечных ПГА являются значительно более распространенными по сравнению с деструкторами среднецепочечных полимеров. Увеличение степени кристалличности ПГА по мере разрушения свидетельствует о преимущественном разрушении в почве аморфной фазы полимеров по сравнению с кристаллической.

Ключевые слова: полигидроксиалканоаты (ПГА), свойства ПГА, биодеградация ПГА, микроорганизмы-деструкторы ПГА, почва.

Introduction

Polyhydroxyalkanoates (PHAs), representing the developing industry of degradable bioplastics, are good candidates to gradually replace synthetic polymers. As the outputs of PHAs increase, studies examining degradation of these polymers in natural environments acquire increasing significance. Results obtained in laboratory experiments cannot be used to construct prognostic models and predict PHA behavior and degradation in diverse and changeable natural ecosystems. This can only be achieved in integrated studies, which should answer the following key questions:

How does the microbial community composition in a given environment influence the process of PHA degradation and what microorganisms are the most effective PHA degraders under given conditions?

How do the chemical composition of a PHA, the process used to prepare PHA-based devices, and the shape and size of the devices influence the PHA degradation rate?

How do the macro- and microstructure of PHAs and their properties (crystallinity, molecular weight, polydispersity) change during degradation?

Do the physicochemical conditions of the environment (temperature, pH, oxygen availability, salinity, etc.) considerably affect this process?

How will the process of PHA degradation be affected by weather and climate of different regions?

Analysis of the available literature shows that rather few authors reported integrated studies of various aspects of PHA degradation, which is a very complex process.

Most of the studies were performed in laboratory, and they mainly addressed the mechanism of interaction between the PHA supramolecular structure and PHA-depolymerizing enzymes, the structure and molecular organization of various depolymerases (Kim et al., 2007) and microorganisms secreting extracellular PHA depolymerases.

Aerobic and anaerobic PHA degrading bacteria have been isolated from various ecosystems such as soil, compost, aerobic and anaerobic sewage sludge, fresh and sea water, estuarine sediments, and air (Kumagava et al., 1992; Imam et al., 1999; Kusaka et al., 1999; Quinteros et al., 1999; Volova et al., 2006; Shah et al., 2007; Volova et al., 2010). Thus, PHA degrading microorganisms, including P(3HB) degrading bacteria, are present in all terrestrial and aquatic ecosystems.

Laboratory studies of PHA degradation in soil

Soil is the natural environment with the greatest capacity for PHA degradation. However, most of the studies addressing PHA degradation in soil were carried out in laboratory (Bonartseva et al., 2003; Mergaert et al., 1993; Erkske et al., 2006; Suyama et al., 1998; Woolnough et al., 2008) and some of them used isolated cultures of PHA degrading microorganisms (Mokeeva et al., 2002; Bhatt et al., 2008; Colak, Güner, 2004; Nishida and Tokiwa, 1993). Samples of soil suspension were used as laboratory microcosms to study degradation of polymer films based on two PHA types (poly(3-hydroxybutyrate) and copolymers of 3-hydroxybutyrate and 3-hydroxyvalerate) under stable temperature and moisture conditions (Volova et al., 1992; 1996). Degradation of both types of PHA was strongly influences by the temperature, but no pH effect was observed. Copolymer samples were degraded with a higher rate than homogenous P(3HB), and as the molar fraction of 3HV increased, the difference in degradation rates became more significant.

The disintegration under composting conditions of films based on poly(lactic acid)-poly(hydroxybutyrate) (PLA-PHB) blends and intended for food packaging was studied by Arrieta et al. (2014). Two different plasticizers, PEG and ATBC, were used to limit the inherent brittleness of both biopolymers. Formulations based on plasticized PLA/PHB blends were successfully disintegrated under composting conditions in less than one month, stating their biodegradable character.

Field studies of PHA degradation in soil

Influence of soil type on the degradation rate

There are very few published data on PHA biodegradation in soil under field conditions. One of the first studies that addressed PHA degradation under natural conditions showed (Mukai, Doi, 1993) that a golf tee made of the polymer was almost completely degraded in soil within four weeks; unfortunately, the authors of this study did not describe either the exact composition of the PHA or the soil characteristics. There are data, however, suggesting that the type of the soil is an essential factor affecting PHA degradation. Mergaert and coauthors (1994) studied biodegradation of P(3HB) and P(3HB-co-3HV)s with different molar fractions of 3-hydroxyvalerate (10 and 20 %) in household compost heaps and showed that significant mass loss was only recorded in the P(3HB-co-3HV) specimens with a high 3HB percent (20 mol. %). Lim et al. (2005) studied degradation of the polymer consisting of 3-hydroxyhexanoate, 3-hydroxyoctanoate, 3-hydroxydecanoate, 3-hydroxydodecanoate, 3-hydroxytetradecano-

ate, and 3-hydroxyhexadecanoate in forest and mangrove soils. After 112 d of burial, there was 16.7 % reduction in gross weight of the films buried in acidic forest soil, 3.0 % in the ones buried in alkaline forest soil, and 4.5 % in those buried in mangrove soil. Sridewi and coauthors reported (2006) that the weight of P(3HB) and P(3HB-co-3HHx) films was reduced in 7 days after burial in the mangrove soil. All PHA types were degrading with similar rates. The half-life was 42 days for all PHA samples on soil surface and 28 days for the samples buried 20 cm deep in the soil. Yew and co-authors showed (2006) that PHA degradation rate in the garden soil was influenced by the burial depth and the density of microbial populations. The mass of P(3HB) films decreased by 55 % and 25 % in the soils with microorganism concentrations $1.0{\times}10^8$ and $3.2{\times}10^6$ CFU/g soil, respectively, within 43 days. The degradation rate of the films placed on the soil surface was 50 % slower.

Environmental degradation of the bioplastics samples was conducted in "mature soil" under controlled conditions (Woolnough et al., 2013). Combining PHB and P(HB-co-HV) films with the anti-fouling agent 4,5-dichloro-2-n-octyl-4-isothiazolin-3-one (DCOI, 10 % w/w) reduced biofouling and postponed the onset of weight loss by up to 100 days, a 10-fold increase compared to unmodified films where the microbial coverage was significant. It have been shown the "switch" that initiates film weight loss, and its subsequent reduced rate, depended on the DCOI loading to control biofouling.

Wang et al. (2005) studied degradation of (3HB-co-3HV) films in natural media and reported the highest degradation rate in activated sludge (residual mass 10.87 % of the initial mass at day 480 of the experiment) and lower rates in farm soil (84.32 %) and in the infertile garden soil (98.88 %). Rapid degradation of (3HB-co-3HV) and its blends with atactic P(3HB) was observed in compost containing activated sludge (almost 100 % for 6 weeks) and in soil environment of activated sludge (Rutkowska et al., 2008; Arcos-Hernandez et al., 2012).

Influence of polymer composition and molecular weight on the degradation rate

Copolymer degradation is determined by the chemical structure of its monomer units (Sudesh et al., 2000). The data on the effect of polymer composition on its degradation rate are, however, rather contradictory. Some authors reported quicker degradation of P(3HB-co-3HV) compared with P(3HB) (Madden et al., 1998; Rizzarelli et al., 2004). Weng at al. (2013) investigated the degradation of P(3HB,4HB) and P(3HB,4HB)/PLA blends under real soil conditions. The order of biodegradability was as follows: PHA-100 [P(3HB,4HB)] > PHA-75 [P(3HB,4HB)/PLA, 75/25] > PHA-50 [P(3HB,4HB)/PLA, 50/50] > PHA-25 [P(3HB,4HB)/PLA, 25/75] > PLA, which correlates well with the PLA content.

On the other hand, comparison of the degradation rates of poly(3-hydroxybutyrate) and its copolymers by the depolymerase of *Alcaligenes faecalis* showed that the erosion rates decreased in the order P(3HB-co-4HB) > P(3HB) > P(3HB-co-3HV) (Doi et al., 1992). An opposite order was obtained in experiments with PHA depolymerases from *P. lemoignei* and in the *in situ* soil studies (Kanesawa et al., 1994; Mergaert et al., 1994). It can be assumed that in natural environments, differences in PHA degradation rates may be caused by differences between depolymerizing enzymes secreted by the diverse microbial community, as degradation of structurally dissimilar PHAs is largely determined by the specificity of the active site in the depolymerase catalytic domain (Shinomiya, 1998; Kasuya et al., 1999; Gumel at al., 2013).

The available data on the effect of PHA properties such as molar mass on its degradation are inconsistent. PHA properties, however, are related to the molar mass of the polymer and, specifically, to its M_w (weight average molecular weight). Several authors reported a direct relationship between the M_w of a polymer and its degradation (Quinteros et al., 1999; Mokeeva et al., 2002; Bonartseva et al., 2003; Bonartsev et al., 2009). The only way to correctly determine Mw is to use high-performance gel permeation chromatography.

PHA-degrading microorganisms

Degradation of polymer samples (biomass loss, changes in the surface and structure) by both mixed populations of microorganisms (Woolnough et al., 2008) and single-species isolates (Reddy et al., 2008; Bhatt et al., 2008) has been described. Fungi degrade PHAs more actively than bacteria, due to higher mobility of fungal PHA depolymerases (Reddy et al., 2008). PHA depolymerases of some soil microorganisms have been isolated and their properties have been studied (Colak, Güner, 2004; Reddy et al., 2003).

In order to gain insight into PHA biodegradation patterns and mechanisms of this process, it is important to isolate and identify PHA degrading microorganisms. Among PHA degraders that have been described in the literature are representatives of various genera: *Bacillus*, *Pseudomonas*, *Alcaligenes*, *Comamonas*, *Rhodococcus*, *Rhodocyclus*, *Syntrophomonas*, *Ilyobacter* (Jendrossek et al., 1996), *Terrabacter*, *Terracoccus*, *Brevibacillus*, *Agrobacterium*, *Duganella*, *Ralstonia*, *Matsuebacter*, *Rhodoferax*, *Variovorax*, *Acinetobacter*, *Pseudomonas*, *Bacillus*, *Azospirillum*, *Mycobacterium*, *Streptomyces* etc. (Suyama et al., 1998; Jendrossek, Handrick, 2002; Mergaert, Swings, 1996; Bonartsev et al., 2009; Volova et al., 2006). Fungi are considered to be the most efficient PHA degraders: Ascomycetes, Basidiomycetes, Deuteromycetes, Zygomycetes (Matavulj, Molitoris, 1992) and Mixomycetes, Mastigiomycetes, *Penicillium, Fusarium* (Brucato, Wong, 1991; Mokeeva et al., 2002; Kim, Rhee, 2003). The higher degradation capacity of fungi is accounted for by the fact that fungal PHA-depolymerases are more mobile than PHA-depolymerases secreted by bacteria (Reddy et al., 2008). Most soil PHA degraders are believed to be capable of degrading short-chain PHAs, i.e. ones that consist of monomers containing not more than 5 carbon atoms. Only a few of them can degrade medium-chain PHAs, and this is accounted for by the substrate specificity of PHA extracellular depolymerases (Kim et al., 2003, 2007). Authors of earlier studies showed that under their study conditions, most PHA degrading soil microorganisms degraded short-chain PHAs, but the portion of degraders of medium-chain PHAs was rather small: 0.8 % to 18 % of all PHA degraders (Nishida and Tokiwa, 1993; Suyama et al., 1998).

It is noteworthy that isolation of PHA degraders is often performed by analyzing the media (soil or compost) in which polymer specimens have been maintained and microorganisms isolated from biofilms on the surface of polymers, by inoculating them onto standard microbiological media. Among the microorganisms isolated, there may be commensal organisms, which utilize monomers and other degradation products of high-molecular-mass PHAs and which exist in the medium due to the vital activity of primary and true PHA degraders. A reliable way to isolate true PHA degraders is to use the clear-zone technique (Mergaert et al., 1993), which involves inoculation of the isolates onto mineral agar that contains PHA as sole carbon source. Clear zones are formed around colonies of microorganisms with PHA-depolymerase activity on the surface of the agar medium, as a result of polymer degradation.

An integrated approach to the investigation of PHA degradation: the studies in Siberian soils

The discrepancies between the data reported by different authors at different times must be due to dissimilarities in PHA specimens used: they were synthesized by different producers on different media, the amounts of residual impurities (such as lipids) in the samples were not equal, the polymers were processed by various techniques, exposure conditions were not the same, and, finally, the effects of degradation were determined using different methods. The degree of crystallinity and PHA molecular weight can be determined using X-ray structure analysis and high-performance liquid chromatography, but the data of some authors are based on indirect evidence, obtained by differential thermal analysis and viscometry.

An integrated approach to the investigation of PHA degradation by soil microorganisms under natural conditions was employed in publications by Boyandin et al. (2012, 2013). The authors studied this process in different climates and soils, taking into account the diversity of soil microbial communities, shapes of polymer samples and methods of their preparation, and the chemical composition of the PHAs tested.

The studies in Siberian soils (Boyandin et al., 2012) addressed degradation of PHAs with different chemical structures in the form of film discs and pressed pellets by soil microorganisms inhabiting the rhizosphere of coniferous and broadleaved trees under varying soil temperature conditions. Experiments were performed under natural conditions, in the arboretum at the V.N. Sukachev Institute of Forest SB RAS (Krasnoyarsk) during two field seasons, which differed in temperature conditions.

The initial microbial communities in the soils under the two tree species differed in both their total counts and their compositions. The total count of aerobic microflora was higher under the larch than under the birch, amounting to $(1.47\pm0.08)\times10^9$ of colony-forming units per gram (CFU/g) of soil. After 3 months, the total counts of bacteria in the soil were $(5.11 \pm0.42)\times10^9$ CFU/g soil under the larch and $(2.21\pm0.24)\times10^9$ CFU/g soil under the birch. The counts of prototrophs and oligotrophs were also higher in soil samples collected under the larch, in which oligotrophic index (PA/FPA) reached 0.75, indicating high rates of nutrient uptake and assimilation.

The bacterial component of the soil microbial community in the larch rhizosphere was represented by the following dominant bacteria: *Alcaligenes* (25.0 %), *Aureobacterium* (15.9 %), *Pseudomonas* (4.5 %), *Cellulomonas* (52.3 %), and *Acinetobacter* (2.3 %). The microbial community in the soil under the birch was more diverse. The dominant species were *Pimelobacter* (48.4 %), *Actinomyces* (16.1 %), and *Micrococcus* (9.7 %); *Flavimonas, Mycobacterium, Corynebacterium* and *Arthrobacter* were present in small numbers. In the soil under the larch fungi were represented by *Acremonium* (1.8 % of the total count), *Mucor* (5.5 %), *Verticillium* (18.2 %), and the dominant species, *Penicillium* (74.5 %). In the soil under the birch, fungal flora was more diverse: in addition to the species inhabiting the soil under the larch (*Penicillium* and *Verticillium*), we identified *Cladosporium* (1.7 %), *Hyphoderma* (1.7 %), *Pytium* (3.3 %), *Cephalosporium* (8.3 %), and *Beltrania* (31.7 %); *Beltrania* and *Penicillium* were the dominant species. The total counts of the fungi inhabiting soils under the larch and under the birch were similar.

The total counts of microorganisms on the surface of polymer specimens were two orders of magnitude higher than the counts in the control soil samples in 2007 and one order of magnitude higher in 2010. In the control soil samples collected under the larch, the dominant species belonged to the genus *Micrococcus.* Other isolated

species were *Bacillus* spore-forming rods and arthrobacteria. Gram-negative microflora was represented by *Acinetobacter, Flavobacterium* and *Pseudomonas*. In soil samples removed from the surface of polymer specimens, proportions of microorganisms were different. The bacterial community of the soil in the larch rhizosphere was dominated by *Agrobacterium* species followed by *Cellulomonas. Alcaligenes, Aureobacterium, Acinetobacter, Pseudomonas,* and *Arthrobacter* were present in small numbers. Analysis of the control soil samples collected in the birch rhizosphere and those removed from the surface of the polymer specimens buried under the birch yielded similar results. The bacterial community of the soil in the birch rhizosphere was dominated by *Micrococcus* species. The soil from the polymer film surface was mainly inhabited by *Bacillus* species, with some representatives of *Arthrobacter, Micrococcus, Nocardia, Actinomyces, Pimelobacter,* and *Alcaligenes.*

PHA degradation behavior was influenced by properties of the soils under the trees and characteristics of the microbial communities. In 2007, in the soil under the larch, which was moister and housed more microorganisms, PHA degradation rates were higher than those recorded under the birch. By the end of the experiment, the residual mass of P(3HB) specimens had decreased to 45 % of their initial mass, and the residual mass of P(3HB-co-3HV) specimens – to 22 %; the half-lives of these polymers were 83 d and 68.5 d and their average mass losses for the field season 0.325 and 0.44 mg/d, respectively (Fig. 1).

In the soil of the birch rhizosphere, degradation rates of both PHA types were lower, in spite of the great variety of the fungi present in this soil. At day 109 of the exposure, the residual masses of P(3HB) and P(3HB-co-3HV) specimens amounted to 84 % and 74 % of their initial masses, respectively, with the mass losses of the homopolymer and the copolymer 0.097 and 0.15 mg/d.

In 2010, PHA degradation rates were lower than during the 2007 field season. At the end of the field experiment, the residual mass of the specimens amounted to 89.9 % and 74 % for P(3PHB) and P(3HB-co-3HV) specimens buried under the larch and to 91.4 % and 89 % for the specimens buried under the birch. As in 2010 the mass loss was so small, we failed to find any reliable differences in the degradation of the two PHAs used in this study.

Thus, at temperate latitudes (Siberia, Krasnoyarsk) with markedly continental climate, in the soddy-carbonate soil of the arboretum, during

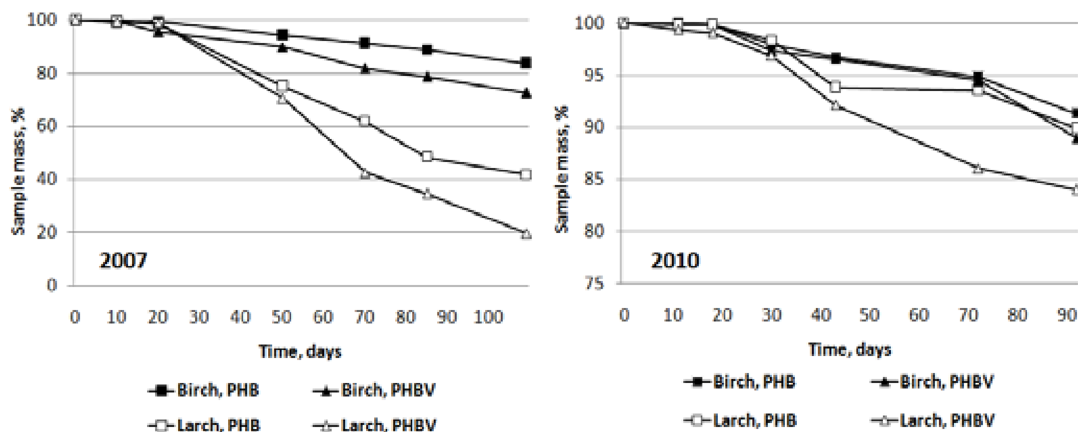

Fig. 1. Dynamics of the mass of polymer specimens in Siberian soil

the warmer summer season, P(3HB-co-3HV) copolymer films were degraded faster than the higher-crystallinity P(3HB) specimens. These results are in good agreement with the data reported by other authors and those obtained in our previous studies, which show that PHA copolymer specimens are degraded in biological media faster than the homopolymer of 3-hydroxybutyrate (Volova et al., 1992; 1996; Mergaert et al., 1993; 1994; Woolnough et al., 2008), but contradict the data reported by Rosa and coauthors (2003). Degradation rates of P(3HB) recorded by these authors were higher than P(3HB-co-3HV) degradation rates, and they explained their results as being due to specific surface structure and properties of their specimens.

PHA biodegradability is influenced not only by the chemical composition of the polymer and the temperature of the environment, but also by the polymer stereoconfiguration, crystallinity, and molecular weight (Nishida, Tokiwa, 1993; Jendrossek, Handrick, 2002). PHA specimens used in this study had different degrees of crystallinity.

X-ray structure analysis performed at the end of the field experiment showed increases in the degrees of crystallinity of both PHAs, suggesting preferential disintegration of the amorphous phase of both PHAs in the soil under the study conditions, which resulted in a higher degree of crystallinity of the specimens. This result is consistent with the data reported by a number of authors (Abe et al., 1998; Sridewi et al., 2006).

In contrast to some other degradable polymers (polysaccharides, polylactides), PHAs undergo true biological degradation, which occurs via the cellular and the humoral pathways and is effected by phagocytes and PHA-depolymerizing enzymes secreted by microflora (Kim et al., 2007). The total counts and composition of control soil microbial communities examined at the end of the field experiments were considerably different from those of microbial communities of soil samples removed from the surface of polymer specimens.

The counts of prototrophs increased by about 3 times compared to the initial counts in the soils under both tree species. The fact that the counts of oligotrophs in the soil under the birch increased to $(3.81 \pm 0.71) \times 10^8$ CFO/g soil suggested greater activity of this group of microorganisms. By contrast, the counts of oligotrophs and oligotrophic index in the soil under the larch decreased. The reason may be that high-density populations of hydrolytic bacteria (primary degraders of organic matter) in the soil under the larch could suppress the development of oligotrophs.

True PHA degraders were isolated by inoculating samples onto diagnostic agar that contained 0.25 % PHA powder as sole carbon source. Growth of microorganisms with PHA-depolymerase activity was accompanied by the formation of clear zones around colonies of microorganisms. In addition to conventional morphological and biochemical examination, PHA degrading bacteria were identified by 16S rRNA gene sequence analysis. This approach enabled identifying primary PHA degraders: 16 bacterial and 5 fungal isolates (Boyandin et al., 2012). Inoculation of the samples onto the diagnostic medium confirmed PHA-depolymerase activity of species belonging to the genera *Penicillium*, *Paecilomyces*, *Acremonium*, *Verticillium* and *Zygosporium*. Mokeeva and coauthors in their recent study (2002) described a wider range of fungi degrading PHAs, which included representatives of *Penicillium*, *Aspergillus*, *Paecilomyces*, *Acremonium*, *Verticillium*, *Cephalosporium*, *Trichoderma*, *Chaetomium*, and *Aureobasidium*, but the authors of that study did not inoculate samples onto the diagnostic medium, i.e. their data were not based on the use of the clear-zone technique.

PHA degraders in the soil of the larch rhizosphere were mainly represented by *Paecilomyces*

lilacinus, amounting to 81.5 %. This species was also described as a polymer degrader by Sang and coauthors (2002). The fungi localized on polymer surface in the birch rhizosphere were dominated by *Penicillium* sp. BP-1 and *Penicillium* sp. BP-2, totally amounting to 81 %. Lopez-Llorca and coauthors (1993) also emphasized that *Penicillium* species were the major PHA degraders (up to 88 % of the isolates). This is consistent with the literature data on *Penicillium* predominance among micromycetes of northern soils (Egorova, 1986). Soil samples removed from the surface of polymer films contained 36.5 and 4 times higher total counts of micromycetes in the rhizosphere of the birch and that of the larch, respectively, than in the control samples. Thus, fungi are actively involved in PHA degradation under natural conditions, and there are literature data confirming this conclusion (Mergaert et al., 1993; Sang et al., 2002; Lee et al., 2005).

Not all isolated fungi exhibited P(3HB) depolymerase activity. The occurrence of clear zones around colonies on the diagnostic medium was recorded for *Penicillium, Paecilomyces, Acremonium, Verticillium,* and *Zygosporium* species. At the end of the growth season, control samples contained representatives of the genera *Penicillium, Paecilomyces, Aureobasidium,* and *Verticillium*, with *Penicillium* fungi remaining the dominant genus.

Based on similarities of morphological types, 16 strains of bacteria capable of PHA biodegradation were selected, which were subsequently identified based on the combination of morphological, cultural, biochemical, and molecular-genetic properties. Strains IBP-SB5, IBP-SL5, IBP-SL9, and IBP-SL10 were identified as *Variovorax* species; strains IBP-SB4, IBP-SB7, IBP-SB9, and IBP-SL14 as *Stenotrophomonas* species; strains IBP-SB14, IBP-SL6, IBP-SL7, and IBP-SL13 as *Acinetobacter* species; strains IBP-SB6 and IBP-SB8 as *Pseudomonas* species; and strains IBP-SL8 and IBP-SL11 as *Bacillus* species (Boyandin et al., 2012).

The integrated study of biodegradation of two PHA types by soil microbial communities of different structures performed in Siberia conditions showed that PHA degradation is influenced by both polymer chemical composition and soil parameters: temperature, moisture content, and composition of microbial community.

An integrated approach to the investigation of PHA degradation: the studies in tropical soils

Another study of PHA degradation in the soil was performed in the tropics (Boyandin et al., 2013).

Biodegradation of PHAs of two types – poly(3-hydroxybutyrate) (P(3HB)) and poly(3-hydroxybutyrate-co-3-hydroxyvalerate)(P(3HB-co-3HV)) – was analyzed in soils at field laboratories in the environs of Hanoi (Vietnam) and Nha Trang (Vietnam). The air and soil temperatures and humidity in both study sites were similar throughout the study season. Precipitation at Hanoi was, however, almost an order of magnitude higher than in Nha Trang.

PHAs of all types were degraded at higher rates in the soil of the study site at Hanoi. In Nha Trang PHAs were degraded at lower rates because of lower precipitation amounts in this area in summer.

PHA films were more prone to degradation than pressed pellets. At the end of the experiment (after 184 days of soil exposure), degradation of P(3HB) films reached more than 97 %, and P(3HB-co-3HV) films were 33 % degraded, while the pressed pellets were 42 and 23 % degraded, respectively. In the more arid area (Nha Trang), the mass loss of P(3HB) and P(3HB-co-3HV) films was 16 and 7 % and that of the pressed pellets – 18 and 3 %. (Fig. 2)

P(3HB) specimens were degraded faster than P(3HB-co-3HV) films and pellets. This is

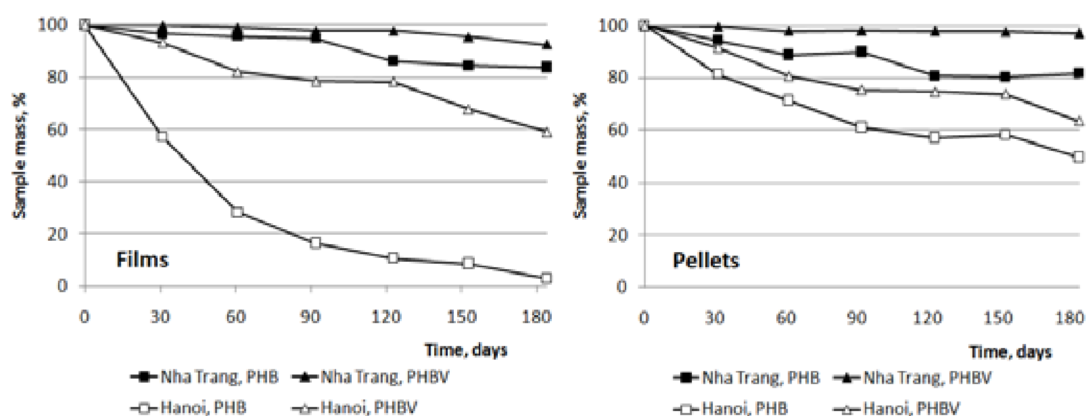

Fig. 2. Dynamics of the mass of polymer specimens in Vietnamese soil

consistent with the data reported by other authors, showing that P(3HB) was degraded with higher rates than P(3HB-co-3HV) by most isolates of PHA degrading bacteria (Manna, Paul, 2000), actinomycetes (Manna et al., 1999), and fungi (Sanyal et al., 2006; McLellan, Halling, 1988). Rosa et al. (2003) also reported higher biodegradation rates of P(3HB) granules compared with P(3HB-co-3HV) and polycaprolactone, and they explained their results as being due to specific surface structure and properties of their specimens. Feng et al. (2004) showed that as 3HV fraction of P(3HB-co-3HV) increased from 8 to 98 mol %, enzymatic hydrolysis of the copolymer by the depolymerase from *Ralstonia pickettii* occurred with higher rates. The copolymer containing more than 80 % 3HV was not degraded by the depolymerase from *Acidovorax* sp.

Several authors reported faster degradation of P(3HB-co-3HHx) compared with P(3HB) and P(3HB-co-3HV), suggesting that this difference was caused by dissimilar structures of the polymers (Wang et al., 2004; Sridewi et al., 2006; Morse et al., 2011). Other authors reported faster degradation of copolymers compared with P(3HB) (Volova et al., 1992; 1996; Mergaert et al., 1992; 1993; 1994; Ya-Wu Wang et al., 2004). This can be accounted for by the diversity of depolymerases with different substrate specificity and by dissimilarities in polymer crystallinities (Manna, Paul, 2000).

Microbial communities of the soils in the two study areas in Vietnam were significantly different. Microbial populations of the Hanoi soil were dominated by *Acinetobacter calcoaceticus, Arthrobacter artocyaneus, Bacillus aerophilus, Bacillus megaterium, Bacillus* sp., *Brevibacillus agri, Brevibacillus invocatus, Chromobacterium violaceum, Cupriavidus gilardii, Mycobacterium fortuitum, Ochrobactrum anthropi, Staphylococcus arlettae, Staphylococcus haemoliticus, Staphylococcus pasteuri, Pseudomonas acephalitica,* and *Rodococcus equi*; while the major species in the Nha Trang soil were *Bacillus cereus, Bacillus megaterium, Bacillus mycoides, Brevibacillus agri, Gordonia terrari,* and *Microbacterium paraoxydans.*

The total counts of bacteria from the biofilm on the surface of polymer specimens showed that their concentration was one or two orders of magnitude higher than in the control soil. Analysis of fungi on Saburo medium showed that the counts of fungi on the surface of all PHA specimens were higher than in the control soil; the difference was more pronounced in the experiment in Nha Trang, reaching 2 or 3 orders of magnitude.

Thus, fungi actively degrade polymers. Examination of control soil samples and PHA surface biofilms proved that the soils of the study areas contained PHA degrading microorganisms and that they were more numerous on polymer surface.

The compositions of microbial communities in the two study sites differed significantly. PHA degrading bacteria dominated in the soils in Nha Trang, while PHA degrading fungi were major PHA degraders in the soil at Hanoi. This may be accounted for by differences in soil parameters such as pH: at Hanoi, the soil was weakly acidic (pH = 5.48), which is a favorable condition for the development of fungi, while in Nha Trang, soil pH was close to neutral (6.63).

The clear-zone technique was used to identify and examine PHA degrading soil microorganisms; 62 isolates of heterotrophic bacteria, 23 isolates of actinomycetes, and 74 isolates of microscopic fungi were selected. Colonies of different morphological types were quantitatively differentiated. Eight to ten isolates for each type of the colony were cultured and their morphological and cultural parameters were compared. Bacteria were additionally analyzed for their physiological and biochemical properties, using conventional tests (catalyse, oxidase, ptotease, and amylase activities, fermentation of carbohydrates: glucose, sucrose, lactose, maltose, and mannitol). Bacteria and fungi were identified by DNA extraction, amplification, and determination of nucleotide sequences of the sites encoding the 16S and 28S rRNA genes. The nucleotide sequences obtained were compared with the sequences in the GenBank, EMBL and DDBJ databases, using the BLAST tool for the search for sequences with high homology, of the NCBI Web site (http://www.ncbi.nlm.nih.gov/BLAST/). PHA degrading microorganisms were identified based on their cultural, morphological, biochemical, and molecular-genetic parameters.

Determination of the species composition of microbial communities showed that PHA degrading bacteria were dominated by Gram-negative rods of *Burkholderia* sp.; they were isolated from the samples of both study areas. Actinobacteria of the genus *Streptomyces* were also present in the samples of both areas.

Other PHA degraders isolated from the samples at Nha Trang were *Bacillus, Cupriavidus* and *Mycobacterium* spp.; *Nocardiopsis* actinobacteria were isolated from the soil at Hanoi. *Gongronella butleri* and *Penicillium* sp. were the fungi found in both study areas. *Acremonium recifei, Paecilomyces lilacinus,* and *Trichoderma pseudokoningii* were only isolated from the soil at Hanoi.

The major PHA degrading soil microorganisms were additionally identified using sequencing of the 16S rRNA gene. Having compared nucleotide sequences of the 16S rRNA gene segment of the isolated bacterial strains – true PHA degraders – with the sequences in the GenBank, we revealed high homology with the sequences of some previously identified strains of prokaryotes and fungi.

Thus, polymer biodegradation in soils – both Siberian and tropical – is performed by bacteria and fungi. Bacteria inhabiting these soils belonged to different genera (except *Bacillus*), while most of the fungi in both Siberian and Vietnamese soils were represented by *Penicillium, Paecilomyces,* and *Acremonium.* (Table 1).

Conclusion

Studies of PHA degradation in different soils showed that PHA biodegradation is influenced by the chemical structure of the polymer, its geometry and the technique used to process it; climate and weather, the type of the natural ecosystem and its microbial component in particular, as the factor determining the mechanism of PHA biodegradation: preferential attack of the amorphous

Table 1. The occurrence of PHA degrading microorganisms in samples of Siberian and tropical soils

Soil samples		PHA degrading microorganisms	
		Bacteria	Fungi
Siberian soil	Larch	*Acientobacter* sp. *Acientobacter schindleri* *Bacillus* sp. *Pseudomonas* sp. *Stenotrophomonas maltophilia* *Variovorax paradoxus*	*Acremonium butyri* *Penicillium* sp. *Purpureocillium lilacinum* *Zygosporium masonii*
	Birch	*Acientobacter* sp. *Bacillus* sp. *Pseudomonas* sp. *Stenotrophomonas rhizophilia* *Variovorax paradoxus*	*Penicillium* sp. *Purpureocillium lilacinum* *Verticillium lateritium*
Vietnamese soil	Hanoi	*Burkholderia* sp. *Nocardiopsis* sp. *Streptomyces* sp.	*Acremonium recifei* *Gongronella butleri* *Penicillium* sp. *Purpureocillium lilacinum* *Trichoderma pseudokoningii*
	Nha Trang	*Bacillus cereus* *Burkholderia* sp. *Cupriavidus* sp. *Mycobacterium* sp. *Streptomyces* sp.	*Gongronella butleri* *Penicillium oxalicum* *Penicillium* sp.

regions of the polymer or equal degradation of both crystalline and amorphous phases. PHA degrading microorganisms that dominate microbial populations in some soil ecosystems have been isolated and identified.

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