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Biodegradation of polyhydroxyalkanoate-based plastic (BIOPOL) under different environmental conditions

I. weight loss of substrate

by

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1 Introduction

The current focus of interest on environmental pollution caused by discarded non-biodegradable petrochemical-based plastics lead to investigations on the replacement of such synthetic polymers by biodegradable ones (HARTLEY 1987, LAFFERTY et al. 1988, STEINBÜCHEL & SCHLEGEL 1988, ANDERSON & DAWES 1990, BRANDL et al. 1990, NEUMEIER 1994, RATHBERGER 1995, STRAUBINGER 1995, WALK 1996).

Besides glycogen, polyhydroxyalkanoates (PHA) with poly-β-hydroxybutyrate (PHB) as their most abundant form are the major bacterial carbon and energy storage materials. It was found that PHB and its copolymers with poly-β-hydroxyvalerate (PHV) and other PHAs have thermoplastic properties. In addition, this material can be produced using renewable resources, thereby saving fossil resources. This resulted in the investigation of these bioplastics (DOI et al. 1986, DOI et al. 1987, BRANDL et al. 1988, MARCHESSAULT et al. 1988, HAYWOOD et al. 1989, BYROM 1990, DOI et al. 1990a, ICI 1990).

One of the most important properties of PHAs is their complete microbial biodegradability to carbon dioxide, water and energy without any toxic byproducts (ANDERSON & DAWES 1990, BRANDL et al. 1990, ICI 1990). Recent environmental

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concerns lead to intensified investigations of plastic materials that are non-polluting and are designed for biological degradability.

BIOPOL® (Zeneca BioProducts), a copolymer of PHB and PHV, has been commercially produced and is used as packaging material. *In vitro* bacterial degradation of BIOPOL and several other PHAs has been proven (MERRICK & DOUDOROFF 1964, HIPPE & SCHLEGEL 1967, MACRAE & WILKINSON 1968, FEDULOVA et al. 1980, TANIO et al. 1982, JENDROSSEK et al. 1992, SCHIRMER et al. 1993) and was reviewed recently by JENDROSSEK et al. (1996). PHA degradation has been recorded in nature and attributed mainly to bacteria (LAFFERTY et al. 1988, ANDERSON & DAWES 1990, BRANDL et al. 1990, MOLITORIS et al. 1996). Although fungi, the other potent group of the degrading microbiota, may play an important role in PHA breakdown (DELAFIELD et al. 1965, LEPIDI et al. 1972), only recently, fungal breakdown of PHA has attracted considerable attention (MCLELLAN & HALLING 1988, DAVE et al. 1990, MATAVULJ & MOLITORIS 1991, 1992, NEUMEIER et al. 1994).

One of the first indications of microbial, including fungal, degradation of PHAs in nature was published by LEPIDI et al. (1972), who found that microfungi accumulated radioactive compounds originating from bacterially synthesized ^{14}C-labelled PHB.

Fungi were probably involved in the degradation of BIOPOL shampoo bottles in a lake at the sediment-water interface investigated by BRANDL & PÜCHNER (1990). That terrestrial and marine fungi are able to degrade BIOPOL, even under marine conditions, was shown first by MOLITORIS et al. (1995). More recently, fungal breakdown of thermoplasts, including PHA has been shown under marine, including simulated deep sea conditions (GONDA et al. 2000).

The degradation of BIOPOL and its blends with other plastic materials such as polycaprolactone, polystyrene, poly-L-lactide and polystyrene-co-acrylonitrile by PHA depolymerase from the culture supernatant of the fungus *Penicillium funiculosum* THOM was investigated by DAVE et al. (1990). They found higher weight losses of the blends tested with increasing content of the BIOPOL component. Similar results were reported by GASSNER & OWEN (1992), who found that only the BIOPOL component of blends with ethylene-vinylacetate was degraded by soil microorganisms.

In a few cases the enzymatic breakdown process of PHB by fungi has been investigated in detail (BRUCATO & WONG 1991, NEUMEIER 1997).

In addition to the type of microbiota involved, the rate of biodegradation depends also on many other factors, notably those related to the ease of microbial colonization. Environmental factors, such as humidity, temperature, electrochemical reaction, biological oxygen demand and supply of other nutrients (nitrogen,

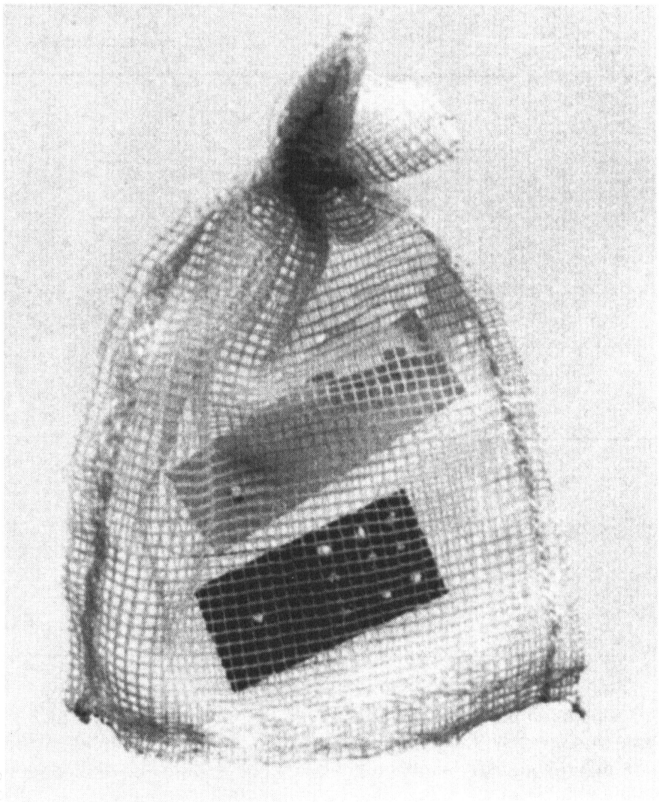
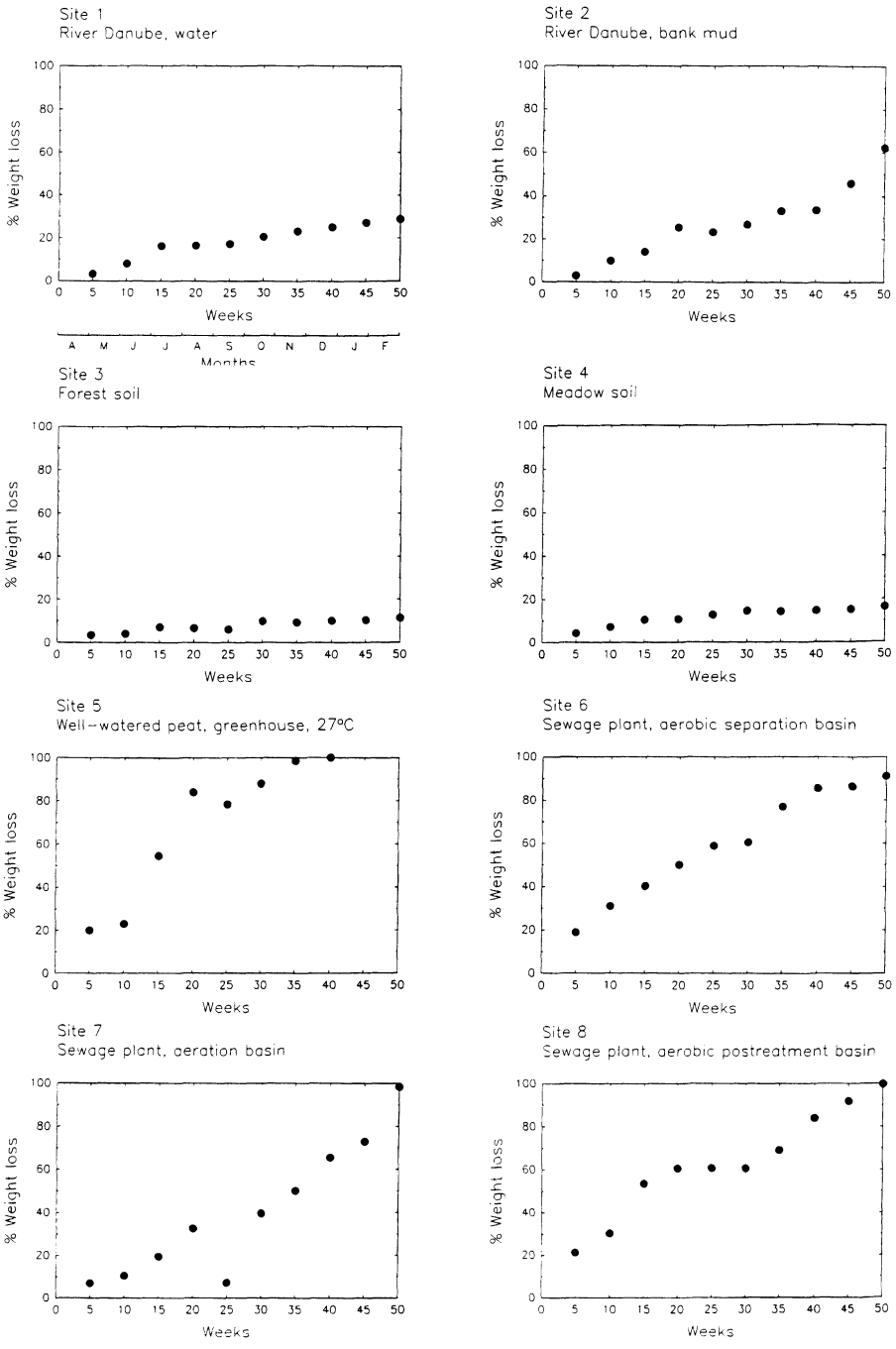


Fig. 1: Sample bag consisting of undegradable polyvinylchloride netting containing the BIOPOL strip and an undegradable identification tag.

phosphorus), are also important (HOLMES 1988, ANDERSON & DAWES 1990, BRANDL et al. 1990).

Preliminary results of PHA degradation by fungi under natural conditions have been reported by MOLITORIS et al. (1992) and MATAVULJ et al. (1993). The lack of specific evidence from experiments in nature on the microbiota involved, on the environmental conditions necessary and on the breakdown process of PHA itself, stimulated this field study of the degradation of BIOPOL at several sites with different environmental conditions.

Over a period of a year, BIOPOL strips were exposed in different natural environments. The samples were investigated for their appearance and weight loss (a), the adhering microbiota was analyzed, fungal cultures isolated (b) and the degradation process was followed by light and scanning electron microscopy (c). This paper deals with the first part of our investigations.


Site 1
River Danube, water
Site 2
River Danube, bank mud
Site 3
Forest soil
Site 4
Meadow soil
Site 5
Well-watered peat, greenhouse, 27°C
Site 6
Sewage plant, aerobic separation basin
Site 7
Sewage plant, aeration basin
Site 8
Sewage plant, aerobic postreatment basin
% Weight loss
Weeks
A M J J A S O N D J F
Months

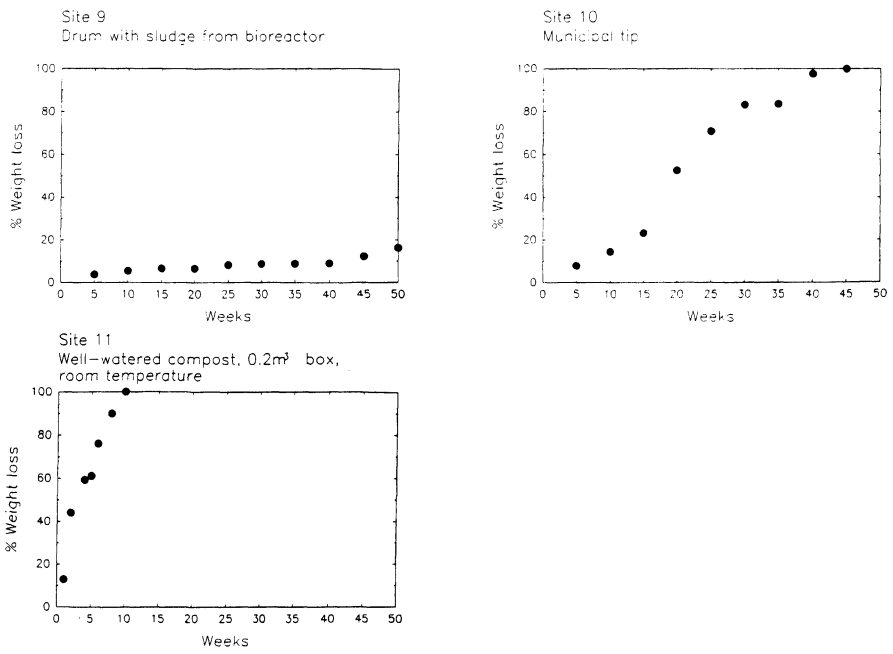


Plate 1: sites 1 to 11: Weight loss (%) of BIOPOL strips after exposure (weeks) in different environments (sites 1 to 11, see legend to Fig. 3). For site 1 the months are added to the abscissa to enable correlation of retrieval times with the seasons of the year.

2 Material and Methods

Preweighed strips (30 × 10 × 1 mm) of BIOPOL bottles, used for packaging of WELLA shampoo, were placed in sacks made from non-degradable polyvinylchloride netting (Fig. 1) and exposed for one year under 11 different environmental conditions at a depth of approximately 20 to 40 cm. The 11 exposure sites were chosen to represent conditions where biodegradation of such discarded plastic material would take place: 1) River Danube water, 2) river Danube bank mud, 3) forest soil, 4) meadow soil, 5) greenhouse, well-watered peat (constant temperature 27 °C), 6) sewage plant, aerobic separation basin (Fig. 2), 7) sewage plant, aeration basin, 8) sewage plant, aerobic post-treatment basin, 9) sewage plant, drum with sludge from anaerobic bioreactor, 10) municipal tip. In a separate experiment (site 11) the strips were incubated in a drum filled with well-watered compost (temperature 20 to 24 °C, semianaerobic).

The BIOPOL strips were exposed starting the first week of April (see time scale in Plate 1) and recovered at five-weekly intervals for up to 50 weeks. After

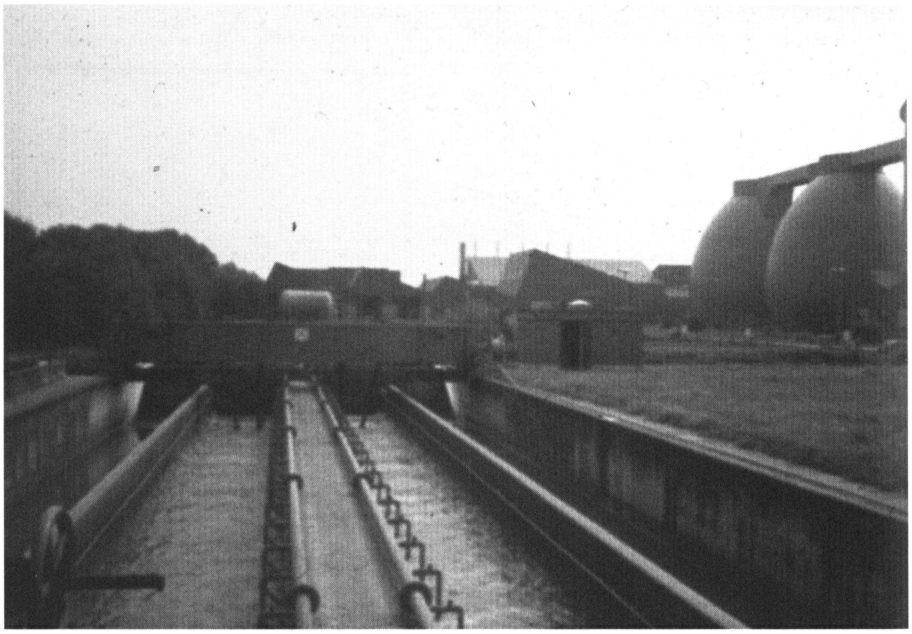


Fig. 2: Sewage plant, aerobic separation basin (site 6).

retrieval, the samples were gently rinsed with water to remove adhering material and dried to constant weight at 40 °C. The weight loss was followed gravimetrically and changes of the material were observed visually and by microscopy and photography.

3 Results

Results of the microbial degradation for pieces of BIOPOL shampoo bottles during 50 weeks exposure to different environmental conditions is shown in Fig. 3 (sites 1 - 10), Fig. 4 (greenhouse compost, site 11) and in Plate 1 (sites 1 - 11). The fastest degradation, indicating the highest degrading efficiency of the microorganisms involved, was observed in well-watered compost (site 11), where a more or less constant and elevated temperature was maintained throughout the year. Under such conditions, complete degradation of BIOPOL bottles samples occurred within only 10 weeks (Fig. 4, Plate 1). Peat from a greenhouse (site 5) where again elevated humidity and temperature were maintained, resulted in total degradation of the plastic strips after 35 weeks (Fig. 3, Plate 1).

The municipal tip (site 10) showed 100 % breakdown of PHA-based plastic after 40 weeks of exposure (Fig. 3, site 10, Plate 1).

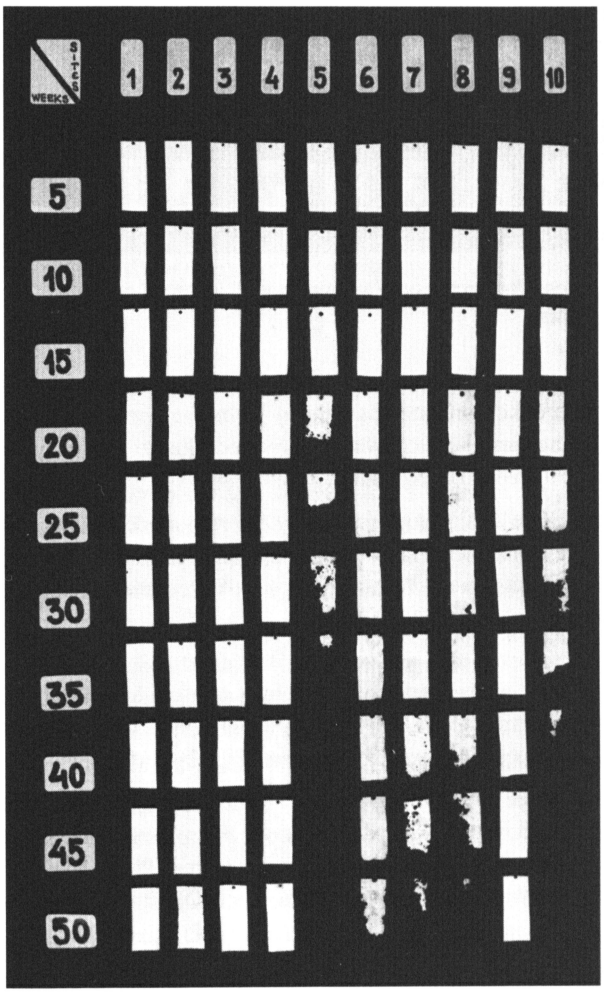


Fig. 3: Incubation sites (# 1 to 10), retrieval times (weeks) and morphology of BIOPOL strips after exposure. Sites: 1) Danube water; 2) Danube bank mud; 3) Forest soil; 4) Meadow soil; 5) Greenhouse, well-watered peat; 6) Sewage plant, aerobic separation basin; 7) Sewage plant, aeration basin; 8) Sewage plant, aerobic post-treatment basin; 9) Sewage plant, sludge from anaerobic bioreactor; 10) Municipal tip.

A relatively high rate of degradation was found in all of the three basins of the sewage plant system (sites 6 - 8, Fig. 3, Plate 1). Under aerobic conditions the total degradation in the aerobic separation basin (site 6 Fig. 2) and in the aerobic posttreatment basin (site 8) was achieved after 50 weeks. Under the more turbulent and oxidative conditions of the aeration basin (site 7) the samples were degraded after 45 weeks. The BIOPOL strips exposed to microbial enzymatic breakdown in

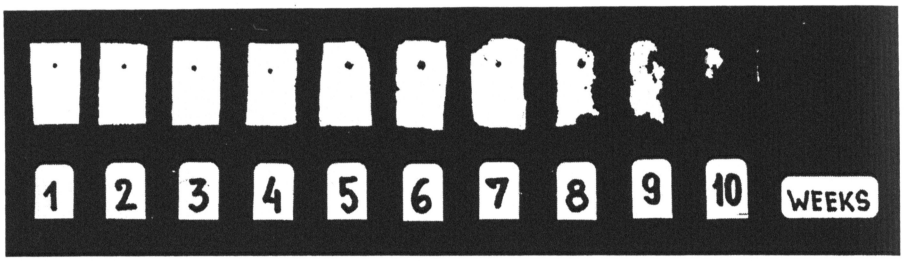


Fig. 4: BIOPOL strips after exposure (weeks) in well-watered compost (site 11, see text for further information).

a drum with activated sludge from the anaerobic sewage plant bioreactor (site 9), showed a slower breakdown rate than those from the aerobic sewage plant sites (Fig. 3, Plate 1). The samples exposed to activated sludge lost only about 16 % of their weight after 50 weeks of exposure (Fig. 3, Plate 1).

The graphs in Plate 1, show more clearly the rate of degradation of the plastic under natural field conditions. These indicate that less humid environments such as forest soil (site 3) and meadow soil (site 4), offered the poorest conditions for BIOPOL degradation.

The lowest breakdown rate was recorded in forest soil (site 3, Fig. 3, Plate 1), where only about 11 % weight loss of samples was recorded after 50 weeks of exposure. Meadow soil (site 4) was slightly more effective with about 16 % weight loss of BIOPOL bottles strips in the same time (Fig. 3, Plate 1).

River Danube water (Fig. 3, site 1) and the constantly wet Danube bank mud (Fig. 3, site 2) offered better natural conditions for plastic degradation with one third of material being broken down in Danube water (Plate 1, site 1) and about two thirds in the bank mud of this river (Plate 1, site 2) after 50 weeks exposure.

Comparison of degradation activity and environmental conditions of those sites exposed to seasonal changes (all sites except of site 5 = peat and site 11 = compost) showed that strongest degradation occurred between June and October, with peaks around July and October (Plate 1).

Visual observation (Figs 3, 4) and light microscopy (data not shown) showed differences in the breakdown pattern. While samples exposed to microbial attack in aqueous conditions (site 1, 2, 6, 7, 8, 9) were eroded more evenly and more homogeneously over the whole surface, the bottle strips recovered from dryer and more aerobic conditions (sites 3, 4), but also those from well-watered compost (site 11) and peat (site 5) were degraded more in randomly distributed and deeper erosion troughs.

Macroscopic and microscopic observations revealed also protective effects of the surface colours, originating from a paint cover or lettering of the bottle. The thin layer (film) of apparently nonbiodegradable colour prevented in those specific areas the microbial action and breakdown of the plastic.

Differences in the erosion of the two different surfaces of the bottles (inside/outside) were noticed. The smoother inside surface turned out to be more resistant to the microbial/enzymatic attack than the rougher outside surface. Similarly, the thickness of the plastic-strips, depending on the part of the bottle cut for the strip, also seemed to be a factor affecting the biodegradation rate of the investigated material (see discussion).

4 Discussion

It is difficult to compare our results of the environmental biodegradation of BIOPOL samples with the results of other authors because they used as substrate either pure polyesters (HOLMES 1985, DOI et al. 1990a, MATAVULJ & MOLITORIS 1991) or used this and similar substrates in the form of thin films (DOI et al. 1990b, MAYER 1990) both of which are more susceptible to the enzymatic depolymerization owing to the chemical purity or their greater surface area.

To improve comparability in testing the biodegradability of new biosynthetic plastic material, MAYER (1990) suggested the use of a standardized testing system. Recently, a semiquantitative test for the determination of PHA biodegradation was described for screening of terrestrial fungi (MATAVULJ & MOLITORIS 1992).

Based on the literature (HOLMES 1985, 1988, HARTLEY 1987, ANDERSON & DAWES 1990, BRANDL & PÜCHNER 1990, BRANDL et al. 1990, DAWES 1990, DOI et al. 1990a, MAYER 1990) and our own investigations (MATAVULJ & MOLITORIS 1991, 1992), the environmental factors influencing the rate of degradation of biosynthetic plastic polyesters can be divided into four groups:

1) **Material parameters** including chemical composition of the polymer (homopolymer or copolymer) used as substrate (HOLMES 1988, DOI et al. 1989, KUNIOKA et al. 1989, ANDERSON & DAWES 1990, BRANDL et al. 1990, DOI et al. 1990b, MOLITORIS et al. 1996), its molecular weight (HOLMES 1988, BRANDL et al. 1990, MOLITORIS et al. 1996), presence of long-pendant groups in the substrate molecule (BRANDL et al. 1990, MOLITORIS et al. 1996), degree of cristallinity (HOLMES 1988, ANDERSON & DAWES 1990), level of orientation (HOLMES 1988, BRANDL et al. 1990) and additional components in the plastic material (BRANDL et al. 1990) such as conditioners, fillers, pigments. Additional physicochemical properties of the substrate that may influence degradation include surface tension (HOLMES 1988), texture and porosity (HOLMES 1985, 1988, BRANDL et al. 1990), volume and specific surface (HOLMES 1988, BRANDL & PÜCHNER 1990, BRANDL et al.

1990) and blending with other biodegradable or nondegradable materials (DAVE et al. 1990, GASSNER & OWEN 1992).

2) **Physicochemical conditions of the environment** constitute factors that also influence microbial growth and activity. They include humidity (BRANDL et al. 1990, MATAVULJ & MOLITORIS 1991), temperature (HOLMES 1985, 1988, BRANDL & PÜCHNER 1990, BRANDL et al. 1990), presence/absence of oxygen (HOLMES 1985, 1988, BRANDL & PÜCHNER 1990), hydrostatic pressure (BRANDL & PÜCHNER 1990, GONDA et al. 2000), light (BRANDL et al. 1990), ionic strength (HOLMES 1988) and the availability of other nutrients essential for growth (HOLMES 1985, 1988). Other factors such as UV radiation (BRANDL et al. 1990), turbulence (HOLMES 1985, 1988), pH (HOLMES 1988), are also discussed in relation to the chemical or mechanical breakdown of plastics.

3) **Composition and abundance of microbial populations** (bacteria, actinomycetes, fungi) (HOLMES 1985, 1988, BRANDL et al. 1990) and of other organisms (mites, nematodes, insects) (HOLMES 1988) are potentially responsible for polymer breakdown and/or consumption.

4) **Combination of the factors** mentioned above, that affect the mechanism of biodegradation. These begin with the contact of the surrounding material with the substrate to be biodegraded (HOLMES 1985, 1988), followed by attachment of the microbiota (HOLMES 1988, MOLITORIS et al. 1996), colonization of the surface of the plastic material (HOLMES 1985, 1988, MOLITORIS et al. 1996), induction and synthesis of the degrading enzymes, formation of the enzyme-substrate complex, penetration of microorganisms into the substrate, water availability (HOLMES 1988, MC LELLAN & HALLING 1988, BRANDL et al. 1990) and finally activators/inhibitors of enzyme activity.

The results presented in this paper showed that water is a prerequisite for enhanced microbial growth and the enzyme-substrate contact. The highest degradation rate of BIOPOL was found in well-watered systems such as compost (site 11) and well-watered peat (site 5). Water is available in surplus in the sewage plant sites (sites 6 to 9) where (with the exception of sludge from the bioreactor, site 9) good degradation of samples was observed.

Water availability, however, resulted in a high degradation of the plastics only when combined with elevated temperature (compost: site 11, peat: site 5), and/or the availability of additional nutrients (sewage plant sites, Danube bank mud = site 2). Therefore it is not surprising that the relatively oligotrophic Danube water (site 1), combined with relatively constant and low temperature, resulted in one of the lowest breakdown rates found in these investigations.

Interestingly, highest degradation under field conditions occurred between May and October when the temperature and humidity were highest and favoured microbial degradation activities (Plate 1).

Conversely, limited availability of water was apparently one of the main reasons for a relatively slow degradation rate of BIOPOL in both, forest and meadow soils (sites 3 and 4, respectively), despite a rich microbial population (data not shown). This result is possibly correlated with other factors causing low microbial enzymatic activity (e.g. composition of the microbial community, specimen contact with the surrounding soil). Slow degradation rates of 1 mm thick sheets of pure PHB homopolymers in soil (75 weeks) in an aerobic sewage site (60 weeks) have been reported by HOLMES (1988) and BRANDL et al. (1990). In contrast, the abundance of microbial populations, adapted to the degradation and use of organics of widely differing biodegradability, such as that inhabiting a municipal tip, was apparently one of the main reasons for the high degradation rate of BIOPOL specimens buried in the landfill site (site 10).

The slower degradation of BIOPOL reported in this paper as compared to pure PHB, could be explained by an apparently slower microbial degradation of BIOPOL or PHB-co-PHV compared to that of the pure PHB homopolymer as reported earlier for bacteria (JENDROSSEK et al. 1992) and for fungi (MATAVULJ & MOLITORIS 1991, 1992).

In some cases (HOLMES 1985) anaerobic conditions were found to be more effective for PHA degradation than aerobic ones. HOLMES (1988) reported the total degradation of 1 mm mouldings of PHA in anaerobic sewage systems within a 6-week-period. In contrast, one of the lowest BIOPOL degradation rates in our experiments was found in activated sludge. This can possibly be explained by the static and semiaerobic and therefore suboptimal degradation conditions for the specific aerophilic microbial population, but possibly also by the total absence of fungi which prefer predominantly aerobic conditions. To clarify this possibility, the composition, number and degradative activity of the microbial population around the incubated samples in the different sites was analyzed and will be reported in a following paper.

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6 Abstract

The current problems with decreasing fossil resources and increasing environmental pollution by petrochemical-based plastics have stimulated investigations to

find biosynthetic materials which are also biodegradable. Polyhydroxyalkanoates (PHA) produced by bacteria meet these requirements. Thermoplastic materials already have been produced commercially and are used as base for a wide range of applications. BIOPOL® (Zeneca BioProducts) is such a new material. In order to investigate their potential for degradation in nature, BIOPOL strips were subjected to eleven different environmental conditions. Their degradation, based on weight loss, was followed at 5-weekly intervals for 50 weeks (if not indicated otherwise). The results are given as % weight loss of the substrate in order of decreasing activity: 1) Well-watered compost about 22 °C (100 %, 10 weeks); 2) Well-watered peat, greenhouse, 27 °C (100 %, 40 weeks); 3) Municipal tip (100 %, 45 weeks); 4) Sewage plant, aerobic posttreatment basin (100 %, 50 weeks); 5) Sewage plant, aeration basin (98 %); 6) Sewage plant, aerobic separation basin (91 %); 7) River Danube, sludge mud (82 %); 8) River Danube, water (29 %); 9) Sewage plant, sludge from anaerobic bioreactor (16 %); 10) Meadow soil (16 %); 11) Forest soil (11 %). Constantly higher temperatures and humidity seem to enhance the degradation process.

Keywords

Polyhydroxyalkanoates (PHA), poly-β-hydroxybutyrate (PHB), poly-β-hydroxyvalerate (PHV), BIOPOL, fungi, degradation.

7 Zusammenfassung

Die Abnahme fossiler Brennstoffe und die Zunahme der Umweltverschmutzung durch petrochemische Kunststoffe führten zu einer verstärkten Suche nach entsprechenden biosynthetischen Materialien. Polyhydroxyalkanoate (PHA) bakterieller Herkunft erfüllen diese Forderungen. Diese thermoplastischen Kunststoffe werden bereits kommerziell hergestellt und werden vielseitig eingesetzt. BIOPOL® (Zeneca Bioproducts) ist ein derartiges Material. Um seine biologische Abbaubarkeit zu untersuchen, wurden vorgewogene BIOPOL Streifen an elf Standorten unterschiedlichen Umweltbedingungen unterworfen. Ihr Abbau wurde in 5-Wochenabständen als Gewichtsverlust über eine Gesamtdauer von etwa einem Jahr verfolgt. Die Ergebnisse als % Gewichtsverlust nach einer Inkubationszeit von 50 Wochen (soweit nicht anders angegeben) sind in der Reihenfolge abnehmender Abbauaktivität folgende: 1) Gut durchfeuchteter Kompost bei 22 °C (100 %, 10 Wochen), 2) Gut durchfeuchteter Torf, Gewächshaus bei 27 °C (100 %, 40 Wochen), 3) Mülldeponie (100 %, 45 Wochen), 4) Kläranlage, aerobes Nachklärbecken (100 %, 50 Wochen), 5) Kläranlage, Durchlüftungsbecken (98 %), 6) Kläranlage, durchlüftetes Absetzbecken (91 %), 7) Donau, Uferschlamm (82 %), 8) Donauwasser (29 %), 9) Kläranlage, Klärschlamm vom Bioreaktor (16 %), 10)

Wiesenboden (16 %), 11) Waldboden (11 %). Der Abbau scheint durch höhere Temperaturen und Feuchtigkeit gefördert zu werden.

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