Biodegradation **11:** 323–329, 2000.
© 2001 *Kluwer Academic Publishers. Printed in the Netherlands.*

Degradation of microbial polyester poly(3-hydroxybutyrate) in environmental samples and in culture

Anupam Manna & A.K. Paul*
Microbiology Laboratory, Department of Botany, University of Calcutta, 35, Ballygunge Circular Road, Calcutta-700019, India (author for correspondence: e-mail: akpaul@cal3.snl.net.in)*

Accepted 11 September 2000

Key words: biodegradation, microbial polyester, poly(3-hydroxybutyrate), poly(3-hydroxybutyrate-co-3-hydroxyvalerate)

Abstract

Poly(3-hydroxybutyrate) [P(3HB)] test-pieces prepared from the polymer produced by *Azotobacter chroococcum* were degraded in natural environments like soil, water, compost and sewage sludge incubated under laboratory conditions. Degradation in terms of % weight loss of the polymer was maximum (45%) in sewage sludge after 200 days of incubation at 30 °C. The P(3HB)-degrading bacterial cultures (36) isolated from degraded test-pieces showed different degrees of degradation in polymer overlayer method. The extent of P(3HB) degradation increases up to 12 days of incubation and was maximum at 30 °C for majority of the cultures. For most efficient cultures the optimum concentration of P(3HB) for degradation was 0.3% (w/v). Supplementation of soluble carbon sources like glucose, fructose and arabinose reduced the degradation while it was almost unaffected with lactose. Though the cultures degraded P(3HB) significantly, they were comparatively less efficient in utilizing copolymer of 3-hydroxybutyrate and 3-hydroxyvalerate [P(3HB-co-3HV)].

Introduction

Poly(3-hydroxybutyrate) [P(3HB)] as well as co-polymers of 3-hydroxybutyrate and 3-hydroxyvalerate [P(3HB-co-3HV)] are polyesters of bacterial origin. These microbial polyesters have properties similar to petrochemical based thermoplastics and are completely degradable in natural environments (Miller & Williams 1987; Mergaert et al. 1992, 1995). Moreover, they are also biocompatible (Brandl et al. 1990; Steinbüchel 1991).

Since the pioneering work of Chowdhury (1963) and Delafield et al. (1965), biodegradation of P(3HB) and P(3HB-co-3HV) has been investigated in different natural environments, such as soils (Mergaert et al. 1992, 1993; Kimura et al. 1994), composts (Gilmore et al. 1992; Mergaert et al. 1994a; Pagga et al. 1995), natural waters (Brandl & Püchner 1992; Doi et al. 1992; Mergaert et al. 1995) and sludges (Briese et al. 1994) as well as under laboratory conditions (Doi et al. 1990, 1992; Mergaert et al. 1992, 1993, 1994; Matavulj et al. 1993). Microorganisms having the ability to depolymerize P(3HB) have also been isolated and characterized from different ecosystems and distinguished into 11 groups based on their substrate specificity (Jendrossek 1996).

The ability to degrade polyhydroxyalkanoates (PHAs) has been recorded from a wide variety of Gram-positive and Gram-negative bacteria (Brandl & Püchner 1992; Mergaert et al. 1993, 1994, 1995). Many P(3HB)-degrading fungi have also been identified (McLellan & Halling 1988; Matavulj & Molitoris 1992). Degradation of P(3HB) is caused by the extracellular depolymerases excreted by a variety of bacteria (Tanio et al. 1982; Nakayama et al. 1985; Shirakura et al. 1986; Jendrossek et al. 1993; Yamada et al. 1993; Klingbeil et al. 1996; Nojima et al. 1996). The products of depolymerase mediated hydrolysis of P(3HB) are monomers or monomer and dimers or a mixture of oligomers (Nakayama et al. 1985). The oligomers are subsequently hydrolyzed to monomers by

oligomer hydrolases (Delafield et al. 1965; Shirakura et al. 1983).

Systematic studies on the biodegradation of poly(3-hydroxybutyrate) in different ecosystems of India have not been worked out so far. Although Manna et al. (1999) have evaluated the ability of some streptomycetes from Indian soil to degrade P(3HB). The purpose of this study was to evaluate the degradation of this unique polymer in different natural environments under controlled laboratory conditions with a view to explore the potentials of P(3HB)-degrading organisms from such environments.

As such, the present communication deals with the degradation of P(3HB) in natural environmental samples like soil, compost, sewage sludge and water under laboratory conditions, isolation of P(3HB)-degrading organisms and evaluation of their efficiency to degrade P(3HB) and P(3HB-co-3HV) in solid-plate assay.

Materials and methods

Source of microbial polyester

The P(3HB) was produced from *Azotobacter chroococcum* MAL-201 isolated and reported from this laboratory (Pal et al. 1998) while P(3HB-co-3HV) was obtained from Monsanto, Billingham, U.K. as generous gift. To obtain P(3HB), cells of *A. chroococcum* were harvested at the late exponential phase of growth, dried at 80 °C and extracted with chloroform for 8–10 hours at room temperature. The P(3HB) extract was filtered, concentrated and precipitated with chilled diethylether. The precipitate was redissolved in chloroform and the process was repeated twice to obtain powdered P(3HB).

Preparation of polyester sheet

About 0.8 g of powdered P(3HB) obtained from *A. chroococcum* MAL-201 was dissolved in 100 ml of chloroform. The P(3HB) solution was then poured into an open, flat glass-tray and allowed to evaporate slowly at 28–30 °C to form a sheet. Sheets of 0.25 mm thickness were then cut into small pieces (20×10 mm) and used for degradation studies.

Degradation of P(3HB) sheet

The pre-weighed test pieces were either buried into freshly collected soil, compost, sludge or dipped into water which were contained in wide-mouth glass jars (height 10 cm, diameter 8 cm, volume 500 ml). The jars were filled either with 450–500 g of soil or sludge, or 200 g of compost or 450 ml of fresh water from a pond. The mouth of the jars were kept open and incubated at three different temperatures under laboratory conditions. Moisture content of samples was maintained by adding sterile distilled water at 24 hours interval. Degradation of P(3HB) was measured by the loss of weight after definite period of incubation.

Isolation of P(3HB)-degrading bacteria

P(3HB)-degrading bacteria were isolated from the degraded P(3HB) sheets following washing and dilution-plating on mineral base medium (Malik & Claus 1978) supplemented with 0.1% (w/v) powdered P(3HB). The P(3HB)-degrading isolates were confirmed by the formation of clear zone around the growth. The organisms were purified by dilution-streaking and maintained on the same medium. The isolates were differentiated based on their colony morphology, micromorphological and physiochemical characteristics.

Evaluation of degradation

The P(3HB)-degrading efficiency of the isolates was assayed on solid plate by the polymer overlayer method as described by Delafield et al. (1965). A thin basal layer of mineral salts agar medium (Malik & Claus 1978) was poured and solidified at low temperature (15 °C). The same agar medium supplemented with 0.2% (w/v) P(3HB) or P(3HB-co-16% 3HV) was then overlayered on the basal layer and allowed to solidify quickly. The overlayered plates were inoculated in form of a streak with a loopful of fresh inoculum of individual isolates and incubated at 37 °C. P(3HB)-degrading ability of the individual isolates was determined by the formation of a clear zone surrounding the growth and the extent of degradation was measured from the width of the clear zone formed.

Results

Degradation of P(3HB) in soils, water, compost and municipal sewage sludge under laboratory conditions were recorded after 100 and 200 days of incubation at 20, 30 and 40 °C (Table 1). All P(3HB) test-pieces lost weight during incubation, but the degree of weight loss varied widely with the temperature and the environment in which the P(3HB) samples were incubated.

Table 1. Degradation of P(3HB) in natural samples under laboratory conditions

Natural sample	Incubation (days)	% weight loss of P(3HB) sheet		
		Incubation temperature (°C)		
		20 ± 2	30 ± 2	40 ± 2
Clay soil	100	10.5 ± 0.7	27.2 ± 1.4	24.3 ± 1.2
	200	19.7 ± 0.8	38.7 ± 2.6	36.5 ± 1.5
Laterite soil	100	15.8 ± 0.7	27.1 ± 1.0	21.8 ± 0.6
	200	21.7 ± 1.0	35.7 ± 1.5	34.0 ± 1.2
Saline soil	100	6.1 ± 0.4	32.0 ± 1.0	23.4 ± 0.8
	200	13.9 ± 0.7	43.5 ± 1.7	39.0 ± 1.4
Sandy soil	100	14.3 ± 0.6	29.4 ± 0.9	24.6 ± 0.8
	200	17.6 ± 1.0	33.5 ± 1.3	26.5 ± 1.3
Tarine soil	100	9.4 ± 0.4	15.9 ± 0.5	11.4 ± 0.5
	200	16.6 ± 0.8	23.9 ± 0.9	20.6 ± 0.9
Fresh water	100	8.8 ± 0.4	14.0 ± 0.6	15.7 ± 0.7
	200	15.8 ± 0.7	29.2 ± 0.8	33.9 ± 0.8
Compost	100	8.5 ± 0.3	15.9 ± 0.5	14.3 ± 0.5
	200	14.8 ± 0.4	24.5 ± 1.0	23.4 ± 0.8
Sewage sludge	100	8.5 ± 0.4	20.6 ± 0.9	19.4 ± 0.5
	200	20.6 ± 0.6	44.7 ± 1.9	41.9 ± 1.4

Values represent the average of triplicate sets ± S.D.

Degradation was maximum (nearly 45% weight loss) in municipal sewage sludge after 200 days of incubation. This was followed by the degradation in soils of saline, clay and laterite types. However, degradation of polymer was comparatively low in water and compost. Irrespective of environments in which samples were buried, incubation temperature influenced the extent of degradation greatly, best degradation being recorded for 30 °C.

Thirty-six P(3HB)-degrading bacterial cultures differing in morphological and physiobiochemical characteristics were isolated from the degraded P(3HB) sheets following dilution-plating method (Table 2). The number of such P(3HB)-degrading organisms were highest in sewage sludge followed by clay and saline soils. Analysis of all 36 bacterial cultures by polymer overlayer method has led to their segregation as 4 potent, 5 moderate and rest 27 as weak P(3HB)-degrading strains depending on their extent of degradation under identical condition. Detailed studies, however, were carried out with isolates belonging to potent and moderate degraders only.

Degradation of P(3HB) as evident from polymer overlayer method has clearly indicated an increase in P(3HB) utilization with increase in incubation period up to 12 days (Figure 1). No significant increase in clear zone formation was recorded beyond this period. The comparative account of the width of clear zone formed after 12 days of incubation have clearly indicated that degradation was maximum with the bacterial isolate TAR-101, followed by isolate SLG-102.

Table 2. Occurrence of P(3HB)-degrading bacteria in different environments

Environment	Moisture content (%)	pH	No. of samples used	No. of P(3HB) degrader
Clay soil	17.5–19.4	7.0–7.3	4	6
Laterite soil	12.4–14.3	5.9–6.2	4	4
Saline soil	13.2–15.8	7.4–8.0	3	5
Sandy soil	16.5–18.7	6.5–6.9	3	4
Tarine soil	19.2–21.2	5.0–6.2	3	4
Fresh water	–	6.6–6.8	3	3
Compost	25.7–27.2	5.8–6.2	3	2
Sewage sludge	42.4–45.1	6.7–7.0	4	8
Total			27	36

Incubation temperature greatly influenced the degradation of P(3HB) in solid medium (Figure 2). With the exception of bacterial isolates SAL-106 and TAR-

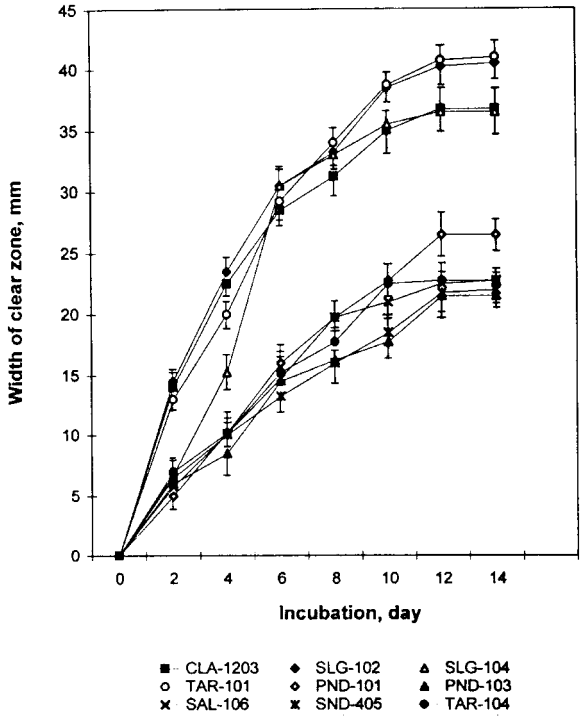

Figure 1. Time profile of poly(3-hydroxybutyrate) degradation by some selected bacterial isolates. The polymer overlayered plates containing 0.2% (w/v) P(3HB) were inoculated with loopful of inoculum and incubated at 37 °C. The width of the clear zone formed around the growth was measured at an interval of 2 days. Each value represents average of triplicate sets.

104, all isolates were much proficient in degrading P(3HB) at 30 °C.

Irrespective of bacterial isolates, P(3HB) degradation increased with increase in concentration of P(3HB) in the medium. The optimum concentration of P(3HB) for degradation was 0.3% (w/v) for all the isolates (Table 3).

Degradation of P(3HB) was affected significantly when the P(3HB) containing medium was supplemented with easily consumable carbon sources. Glucose, fructose and arabinose supplementation lowered the extent of degradation while for most of the isolates it was almost not effected when lactose was supplemented in the medium. Lactose, however, inhibited P(3HB) utilization by isolates SLG-102, SAL-106 and SND-405 (Table 4). It was also evident that supplementation of fructose did not have any effect on P(3HB) degradation by PND-103 and SND-405 as it was for arabinose on PND-10l. All isolates with the exception of PND-103 were comparatively less efficient in degrading P(3HB-co-16% 3HV) (Figure 3).

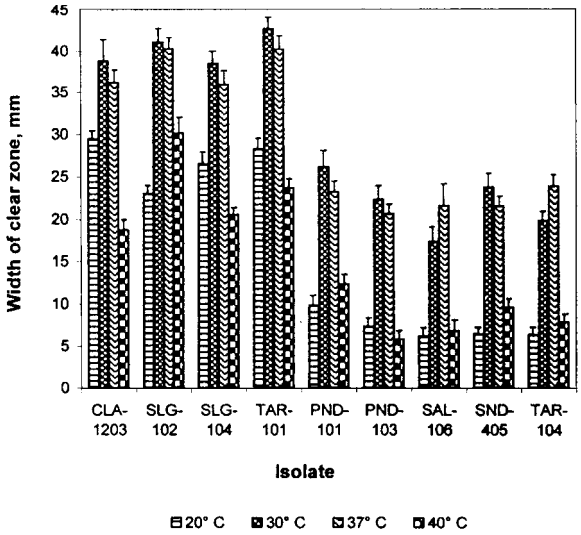

Figure 2. Effect of temperature on poly(3-hydroxybutyrate) degradation by some selected bacterial isolates. The polymer overlayered plates were inoculated with freshly prepared inoculum and incubated at different temperatures for 12 days. The values represent average of triplicate sets.

Table 3. Effect of substrate concentration on P(3HB) degradation

Isolate	Width of clear zone (mm)			
	Concentration of PHB (%, w/v)			
	0.1	0.2	0.3	0.4
CLA-1203	31.2 ± 1.2	36.5 ± 1.3	38.2 ± 1.4	36.0 ± 1.4
SLG-102	34.5 ± 1.1	40.0 ± 1.8	42.2 ± 1.6	39.5 ± 1.4
SLG-104	31.7 ± 1.3	36.5 ± 1.4	39.0 ± 1.5	37.2 ± 1.7
TAR-101	35.5 ± 1.5	40.2 ± 1.7	43.7 ± 1.8	39.2 ± 1.6
PND-101	22.0 ± 0.8	26.2 ± 0.7	27.5 ± 0.9	25.5 ± 1.1
PND-103	18.7 ± 0.6	21.5 ± 0.7	22.5 ± 0.6	21.7 ± 0.5
SAL-106	18.5 ± 0.7	22.5 ± 0.8	24.2 ± 0.9	23.7 ± 0.7
SND-405	17.5 ± 0.4	21.7 ± 0.5	22.5 ± 0.5	20.2 ± 0.4
TAR-104	18.2 ± 0.5	22.5 ± 0.7	23.2 ± 0.6	21.5 ± 0.8

Values are the average of three cultivation experiments ± S.D.

Discussion

Poly(3-hydroxybutyrate)-sheets prepared from the polymer produced by *A. chroococcum* MAL-201 were degraded in all the natural samples incubated under laboratory conditions. Variations in the extent of degradation as evident from percentage weight loss might be due to variation of soil type, qualitative and quantitative differences of the p(3HB)-degrading microbiota and the incubation temperature (Table 1). The highest degradation at 30 °C might have resulted from increased microbial activity and indicated the

Table 4. Effect of different carbon source on degradation of P(3HB)

	Width of clear zone (mm)				
	PHB (control)	Glucose	Fructose	Arabinose	Lactose
CLA-1203	36.7 ± 1.7	22.7 ± 1.1	20.5 ± 0.9	15.2 ± 0.4	36.5 ± 1.4
SLG-102	39.5 ± 1.8	27.5 ± 1.2	37.7 ± 1.4	36.7 ± 1.5	23.2 ± 0.9
SLG-104	36.7 ± 1.6	22.5 ± 1.0	30.2 ± 1.1	16.5 ± 0.8	36.2 ± 1.5
TAR-101	40.5 ± 1.9	32.2 ± 1.4	21.5 ± 0.8	37.5 ± 1.2	40.2 ± 1.8
PND-101	26.2 ± 1.1	18.7 ± 0.6	15.5 ± 0.5	25.5 ± 0.9	26.7 ± 1.2
PND-103	21.5 ± 0.8	16.5 ± 0.5	22.2 ± 0.7	13.7 ± 0.7	20.2 ± 0.8
SAL-106	22.5 ± 1.1	19.2 ± 0.4	15.2 ± 0.4	9.7 ± 0.3	11.5 ± 0.5
SND-405	21.5 ± 0.7	13.0 ± 0.5	20.5 ± 0.9	19.2 ± 0.7	10.7 ± 0.6
TAR-104	22.2 ± 0.7	12.7 ± 0.4	12.0 ± 0.4	10.2 ± 0.4	21.7 ± 1.1

Values are the average of three cultivation experiments ± S.D.

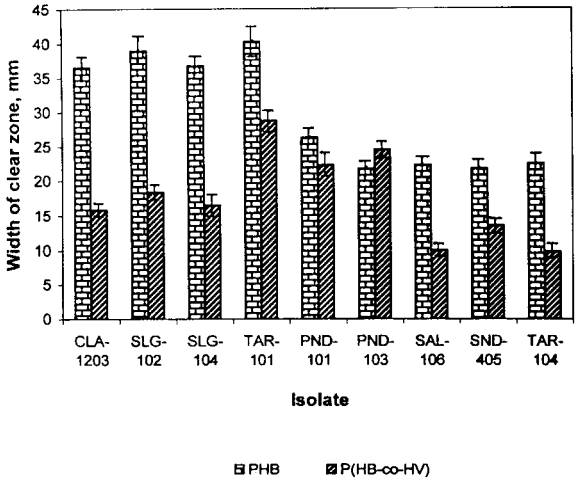

Figure 3. Comparison of degradation of poly(3-hydroxybutyrate) and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) by some selected bacterial isolates. The overlayered plates containing 0.2% P(3HB) or P(3HB-co-16% 3HV) were inoculated in the usual way and incubated at 37 °C for 12 days. The values represent average of triplicate sets.

mesophilic nature of the degrading organisms. Earlier studies of Mergaert et al. (1992, 1993, 1994a) have also demonstrated that P(3HB) are degradable in various soils and composts under laboratory conditions and also in natural waters (1994b).

Thirty-six bacterial cultures that showed clear zones around their colonies on P(3HB)-medium were isolated from the degraded P(3HB) test-pieces and segregated as potent, moderate and weak P(3HB)-degrading strains. There was no remarkable quantitative difference on the occurrence of PHB-degrading organisms per sample (Table 2). The isolates, however, differed qualitatively although their detail taxonomic consideration was not undertaken during the present study.

Time profile of P(3HB) degradation in solid medium (Fig. I) indicated a gradual increase in the formation of clear zone surrounding the bacterial growth. Such an increase of clear zone formation could be explained by release of extracellular P(3HB) depolymerases by all the isolates up to a period of 12 days. Furthermore, the stop of clear zone increase beyond 12 days may probably be due to inhibition of diffusion of extracellular enzymes. The optimum temperature for such degradation was 30 °C for most of the isolates. In contrast to our findings, Mergaert et al. (1993) have claimed that microbial degradation is enhanced at higher temperature.

The effect of substrate concentration on P(3HB) degradation revealed an optimum concentration of 0.3% P(3HB) for all the isolates (Table 3). The decrease in width of clear zone at higher concentration of P(3HB) in the growth medium seems to be due to saturation of extracellular depolymerase by the substrate in the immediate vicinity of the microbial growth. Substrate concentration above the optimum level, as a rule suppresses the activity of the enzyme.

Results of supplementation studies (Table 4) have indicated that the synthesis of P(3HB) depolymerases by the isolates is regulated by soluble carbon sources. The reduction of clear-zone formation in comparison to the control (with the polymer alone) indicates repression of P(3HB) depolymerase, the extent of such repression appeared to be influenced by the type of carbon source supplemented in the medium. Jendrossek et al. (1993) have also mentioned that most P(3HB)-degrading bacteria repress P(3HB) depolymerase gene expression in the presence of a soluble

carbon source that permits high growth rates. After exhaustion of the readily available nutrients, the synthesis of PHA depolymerases is derepressed in many strains and halo formation begins.

It was evident (Figure 3) that the rate of degradation of P(3HB)-co-16% 3HV) by most of the isolates was much lower than that of homopolymer of (3-hydroxybutyric acid). In contrast to these findings, Mergaert et al. (1992, 1993, 1994) found that P(3HB-co-3HV) was degraded at higher rate than homopolymer in soil and composts. On the other hand, Doi et al. (1990) using the purified enzyme from *Alcaligenes faecalis* (Tanio et al. 1982) found that the degradation rate of P(3HB-co-3HV) with 4–21% 3HV content was lower than that of the homopolymer. Several studies have confirmed the secretion of different depolymerases with different substrate specificities (Müller & Jendrossek 1993; Jendrossek et al. 1993; Schirmer et al. 1993). Moreover, it has also been argued that the difference in the degradation rates are due to differences in crystallinity and chemical composition (Holland 1987; Doi et al. 1992).

Acknowledgment

The authors are grateful to the Council of Scientific and Industrial Research, New Delhi for financial assistance.

References

Brandl H, Gross RA, Lenz RW & Fuller RC (1990) Plastic from bacteria and for bacteria: poly(β-hydroxyalkanoates) as natural, biocompatible and biodegradable polyesters. Adv. Biochem. Eng. Biotechnol. 41: 77–93

Brandl H & Püchner P (1992) Biodegradation of plastic bottles made from "Biopol" in an aquatic ecosystem under in situ conditions. Biodegradation 2: 237–243

Briese BH, Jendrossek D & Schlegel HG (1994) Degradation of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) by aerobic sewage sludge. FEMS Microbial. Lett. 117: 101–111

Chowdhury AA (1963) Poly-β-hydroxybuttersäure abbauende Bakterien und Exoenzym. Arch. Microbiol. 47: 167–200

Delafield FP, Doudoroff N, Palleroni NJ, Lusty J & Contopoulos R (1965) Decomposition of polyβ-hydroxybutyrate by *Pseudomonas*. J. Bacteriol. 90: 1455–1466

Doi Y (1990) Microbial polyesters. VCH Publishers, New York

Doi Y, Kanesawa Y & Tanahashi N (1992) Biodegradation of microbial polyesters in the marine environments. Polym. Degrad. Stab. 36: 173–177

Gilmore DF, Antoun S, Lenz RW, Goodwin S, Austin R & Fuller RC (1992) The fate of "biodegradable" plastics in municipal leaf compost. J. Ind. Microbiol. 10: 199–206

Holland SJ, Jolly AM, Yasin N & Tighe BJ (1987) Polymers for biodegradable medical devices. Biomaterials 8: 289–295

Jendrossek D, Knoke I, Habibian RH, Steinbuchel A & Schlegel HG (1993) Degradation of poly(3-hydroxybutyrate), PHB, by bacteria and purification of a novel PHB depolymerase of *Comamonas* sp. J. Environ. Polym. Degrad. 1: 53–63

Jendrossek D, Schirmer A & Schlegel HG (1996) Biodegradation of polyhydroxyalkanoic acids. Appl. Microbiol. Biotechnol. 46: 451–463

Kimura M, Toyota K, Iwatsuki N & Sawada H (1994) Effects of soil conditions on biodegradation of plastics and responsible microorganisms. In: Doi Y & Fukada K (Eds) Biodegradable plastics and polymers, (pp 92–106). Elsevier, Amsterdam, London, New York, Tokyo

Klingbeil B, Kroppenstedt R & Jendrossek D (1996) Taxonomical identification of *Streptomyces exfoliatus* K10 and characterization of its poly(3-hydroxybutyrate) depolymerase gene. FEMS Microbial. Lett. 142: 215–221

Malik KA & Claus D (1978) A method for the demonstration of extracellular hydrolysis of poly-β-hydroxybutyrate. J. Appl. Bacteriol. 45: 143–146

Manna A, Giri P & Paul AK (1999) Degradation of poly(3-hydroxybutyrate) by soil streptomycetes. W. J. Microbiol. Biotechnol. 15: 705–709

Matavulj N & Molitoris HP (1992) Fungal degradation of polyhydroxy-alkanoates and a semiquantitative assay for screening their degradation by terrestrial fungi. FEMS Microbiol. Rev. 103: 323–332

Matavulj M, Moss ST & Molitoris HP (1993) Degradation of poly-β-hydroxyalkanoate based plastics in natural environments. In: Schlegel HG & Steinbuchel A (Eds) Proceedings of the International Symposium on Bacterial Polyhydroxyalkanoates (pp 465–466)

McLellan DW & Halling PJ (1988) Acid-tolerant poly(3-hydroxybutyrate) hydrolases from moulds. FEMS Microbiol. Lett. 52: 215–218

Mergaert J, Anderson C, Wouters A, Swings J & Kersters K (1992) Biodegradation of polyhydroxyalkanoates. FEMS Microbiol. Rev. 103: 317–322

Mergaert J, Webb A, Anderson C, Wouters A & Swings J (1993) Microbial degradation of poly(3-hydroxybutyrate) and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) in soils. Appl. Environ. Microbiol. 59: 3233–3238

Mergaert J, Anderson C, Wouters A & Swings J (1994) Microbial degradation of poly(3-hydroxybutyrate) and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) in compost. J. Environ. Polym. Degrad. 2: 177–183

Mergaert J, Wouters A & Swings J (1994) Estimation of the intrinsic biodiversity among poly(3-hydroxyalkanoates) degrading streptomycetes using gas chromatographic analysis of fatty acids. Syst. Appl. Microbiol. 17: 601–612

Mergaert J, Wouters A, Anderson C & Swings J (1995) In situ biodegradation of poly(3-hydroxybutyrate) and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) in natural waters. Can. J. Microbiol. 41(Suppl 1): 154–159

Miller ND & Williams DF (1987) On the biodegradation of poly-β-hydroxybutyrate (PHB) homopolymer and poly-β-hydroxybutyrate-hydroxyvalerate copolymers. Biomaterials 8: 129–137

Müller B & Jendrossek D (1993) Purification and properties of poly(3-hydroxyvaleric acid) depolymerase from *Pseudomonas lemoignei*. Appl. Microbiol. Biotechnol. 38: 487–492

Nakayama K, Saito K, Fukui T, Shirakura Y & Tomita K (1985) Purification and properties of extracellular poly(3-

hydroxybutyrate) depolymerases from *Pseudomonas lemoignei*. Biochem. Biophys. Acta 827: 63–72

Nojima S, Mineki S & Iida M (1996) Purification and characterization of extracellular poly(3-hydroxybutyrate)-depolymerases produced by *Agrobacterium* sp K-03. J. Ferment. Bioeng. 81: 72–75

Pagga U, Beimborn DB, Boelens J& De Wilde B (1995) Determination of the aerobic biodegradability of polymeric material in a laboratory controlled composting test. Chemosphere 31: 4475–4487

Pal S, Manna A & Paul AK (1998) Nutritional and cultural conditions for production of poly-3-hydroxybutyric acid by *Azotobacter chroococcum*. Folia Microbiol. 43: 177–181

Schirmer A, Jendrossek D & Schlegel HG (1993) Degradation of poly(3-hydroxyoctanoic acid) [P(3HO)] by bacteria: purification and properties of a P(3HO) depolymerase from *Pseudomonas fluorescens* GK13. Appl. Environ. Microbiol. 59: 1220–1227

Shirakura Y, Fukui T, Tanio T, Nakayema K, Matsuno R & Tomita K (1983) An extracellular D(-)3-hydroxybutyrate oligomer hydrolase from *Alcaligenes faecalis*. Biochem. Biophys. Acta 748: 331–339

Shirakura Y, Fukui T, Saito T, Okamoto Y, Narikawa T, Koide K, Tomita K, Takemasa T & Massamune S (1986) Degradation of poly(3-hydroxybutyrate) by depolymerase from *Alcaligenes faecalis* T_1. Biochem. Biophys. Acta 880: 46–53

Steinbüchel A (1991) Polyhydroxyalkanoic acids. In: Byrom D (Ed) Biomaterials (pp 123–213). Macmillan, Basingstoke, London

Tanio T, Fukui T, Shirakura Y, Saito T, Tomita K & Kaiho T (1982) An extracellular poly(3-hydroxybutyrate) depolymerase from *Alcaligenes faecalis*. Eur. J. Biochem. 124: 71–77

Yamada K, Mukai K & Doi Y (1993) Enzymatic degradation of poly(hydroxyalkanoates) by *Pseudomonas pickettii*. Int. J. Biol. Macromol. 15: 215–220