RESEARCH ARTICLE

Biodegradability of chemically synthesized syndiotactic poly(β-[R]-hydroxybutyrate) in soil of Northeast China

Guo-Xia Zhang [1], **Ji-Dong Gu** [2*]

[1] Guangdong Provincial Key Laboratory of Tropical Disease Research, School of Public Health and Tropical Medicine, Southern Medical University, Guangzhou 510515, China
[2] Laboratory of Environmental Microbiology and Toxicology, Department of Ecology & Biodiversity, The University of Hong Kong, Pokfulam Road, Hong Kong, China

Abstract: Degradability of syndiotactic poly ([R]-β-hydroxybutyrate) (*syn*-PHB), a chemically synthesized PHB, was investigated in this study by incubation of the polymer films in a soil of northeastern China. During incubation, progressive weight loss of the *syn*-PHB films and a corresponding decrease of molecular weight were observed over the 90 days of incubation indicating the biodegradation of *syn*-PHB and a random cleavage of the ester bonds. Microorganisms isolated and identified from the partially degraded films included *Pseudomonas* spp., *Alcaligenese* sp., and *Comamonas* sp.. Our results suggest that chemically synthesized *syn*-PHB is biodegradable under aerobic conditions in soil.
Keywords: biodegradable plastics, polyhydroxyalkanoates, soil test, synthetic, biodegradability

*Correspondence to:
Ji-Dong Gu, Laboratory of Environmental Microbiology and Toxicology, Department of Ecology & Biodiversity, The University of Hong Kong, Pokfulam Road, Hong Kong, China; E-mail: jdgu@hku.hk

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1. Introduction

Many bacteria are capable of synthesizing poly (alkanoates) as an energy storage (Brandl et al., 1990; Doi, 1990; Stenbüchel, 1991). One example is the crystalline poly ([R]-β-hydroxybutyrates) (PHBs) which can be degraded under both aerobic (Doi, 1990; Gilmore et al., 1992; Wirsen and Jannasch, 1993) and anaerobic conditions (Budwill et al., 1992; Mas-Castellà et al., 1995). Bacterial PHBs have only the [R] stereochemical configuration and the thermoplastics are isotactic, in contrast chemical synthesis may result in an array of stereochemical configurations depending on the catalysts and reaction condition.

The difference in stereochemistry [R] and [S] forms or their proportion affects the resulting polymer degradability (Kenmitzer et al., 1992; 1993). [R]-PHBs are the preferably forms by bacteria, but [S]-PHBs are hardly degraded *in vivo* experiment. Among syndiotactic, atactic and isotactic PHBs, *syn*-PHB has a predominantly alteration stereochemical sequence along the polymer chain, it provides a unique opportunity to explore the relationship between stereochemistry and biodegradability.

Polymer degradation can be tested in a range of selective environmental conditions and some of the typical ones include composting, activated sludge and soil incubation (Gross et al., 1993; Gu et al., 1993a, b; Gu and Mitchell, 2013). More sophisticated and sensitive testing can also be made using electrochemical impedance spectroscopy to detect the physical property change in the test specimens (Gu et al., 2000). However, information on the degradability of the chemically synthesized PHB is currently not available in the literature. The objectives of this study were to determine the degradability of *syn*-PHB under soil exposure in laboratory incubation and measure the molecular changes during the processes.

2. Materials and Methods

2.1. Synthesis of *syn*-PHB

The polymer *syn*-PHB was synthesized in bulk using a modified procedure as described by Kemnitzer et al. (1992). The synthesis processes involved tri-*n*-butyltin methoxide ($Sn(n\text{-}Bu)_3OCH_3$) to catalyze the ring-opening polymerization of racemic β-butyrolactone (BL) to form poly (β-hydroxybutyraye) (PHB) with syndiotactic (syn) placement. The polymer was characterized and had a M_w 1.5×10^5, M_w/M_n 1.73, racemic diad 0.62. Films were made by compression modeling at 76 °C. Each specimen had a dimension of 0.56 mm×3 mm×13 mm and the average sample weight was 22±2 mg. Each sample was identified with a colored nylon threat as label.

2.2. Test Conditions

The soil, classified as a black soil, was taken from the surface 15 cm of an agricultural soil at Heilongjiang in northeastern China. Physical and chemical analyses of the soil were: soil bulk density 2.56 g/cm^3, porosity 59.8%, cation

exchange capacity 30.2 m.e.q. per 100 g, soil total organic carbon 2.07%, total nitrogen 0.31%, total phosphorus 0.18%, available NO_3-N 18.1 μg/g soil, available P (P_2O_5) 30 μg/g soil, and pH 7.53. The soil was sieved through a 2 mm size sieve and visible plant debris and gravels were removed. Each 250-mL Erlenmeyer flask was added with 50 g soil, 1.0mL of a mineral salt medium. The mineral salt medium consisted of (g/L): K_2HPO_4, 0.8 g; KH_2PO_4, 0.2 g; $CaSO_4{\cdot}2H_2O$, 0.05 g; $MgSO_4{\cdot}7H_2O$, 0.5 g; $FeSO_4{\cdot}7H_2O$, 0.01 g; and $(NH_4)_2SO_4$, 1.0 g. The soil moisture content was adjusted to 60% of water holding capacity of the soil to allow a maximal aeration and also biological activity.

Polymer films were mixed into the soil matrix for incubation, at the same time tetracycline (5 μg/g air-dried soil) and nystatin (5 μg/g air-dried soil) were incorporated into the abiological controls post sterilization successively for 3 times with a 24-hr interval to inhibit potential growth of microorganisms. The flasks were incubated at 35°C in the dark throughout the duration of the experiment. At 5-day intervals, the total weight of the flask was measured and the difference from the previous weighing was taken as evaporation of moisture, and sterile de-ionized water was added to bring back to the previously recorded weight. At monthly intervals, triplicate polymer samples were taken from each treatment for measuring the weight and molecular weight.

2.3. Polymer Analysis

Polymer films were first immersed in de-ionized water and adsorbed soil particles were removed. After washing the external surfaces, the polymer films were dried at 35°C in a vacuum oven till constant weight. The weight loss was calculated as the difference in weight changes between initial and the sampling and expressed as mg mm^{-2}. Then, the samples were used for polymer molecular weight analysis using gel permeation chromatography as described early (Gu et al., 1994). The specimens were dissolved in chloroform (0.3%, w/v) and then 125 μL were used in analysis. Polystyrene standards with low polydispersity (Polysciences, Inc., Warrington, PA) wee used to generate calibration curve. Additional samples from each treatment were taken at the end of this study for preparation of scanning electron microscopy. The detailed method of sample preparation involving fixation in glutaraldehyde, OsO_4, critical-point drying were described elsewhere (Gu et al., 1996). Specimens were observed using a Leica Cambridge S440 SEM.

Additional samples were also prepared for isolation of bacteria utilizing the *syn*-PHB as the sole source of carbon and energy. When isolates were purified through streaking, each of them was tested for biochemical profile using API 20 NE (bio Merieux, France) following the manufacturer's manual. Bacterial isolates were preserved in DMSO at -70°C.

3. Results and Discussion

The *syn*-PHB polymer was successfully synthesized using the procedure as detailed by Kemnitzer et al. (1992) with minor modifications. The finished product is in powder form and has M_w 1.5×10^5, M_w/M_n 1.74 (also see Table 1) and racemic diad 0.62. This polymer was further processed through a compression molding to form thick film for subsequent biodegradability test.

Table 1. Molecular weight characterization of *syn*-PHB films before and after exposure to soil and those exposed to abiotic control

	Time (d)	M_w ($\times10^5$)	M_n ($\times10^5$)	M_w/M_n
Without Exposure	0	1.47	0.84	1.75
Bioactive	30	0.78	0.51	1.54
	60	0.15	0.11	1.36
Abiotic Control	30	1.34	0.88	1.53
	60	1.09	0.73	1.49

During incubation in the soil from the northeastern China, polymer films of *syn*-PHB showed weight loss progressively in the biologically active flasks for the 90-day period of the study whereas only minor weight loss (7.3%) was observed associated with the control (Figure 1). By the end of the study, more than 80% weight loss were observed in the bioactive flasks, suggesting that chemically synthesized *syn*-PHB is degradable in soils. Degradation rate of the *syn*-PHB was 0.80 μg/mm^2/d and 0.88%/d in the biologically active flasks for the duration of the 90-day incubation. Previous investigation concluded that PHB degradation is soil dependent and degradation rate is generally higher in acidic soils than calcareous ones (Barak et al., 1991; Mergaert et al., 1993). *syn*-PHB was previously shown to be hydrolyzable by extracellular enzymes of *Penicillium funiculosum* (Kemnitzer et al., 1992) and by aqueous water (Doi,1990). Bacterial PHBs were previously reported to be degraded in thermophilic composting (Gilmore et al., 1992), soil (Barak et al., 1991; Mergaert et al., 1993), marine (Wirsen and Jannasch, 1993) and sewage sludge (Budwill et al., 1992). In the current study, the controls showed approximately 7.3% weight loss by the end of 90 days of incubation presumably due to surface erosion and hydrolysis (Figure 1).

When the partially degraded *syn*-PHB films were recovered and prepared for SEM examination, dense bacterial population were observed colonizing the polymer surfaces and causing dissolution of the polymer matrice over time of incubation (Figure 2). At the same time, films from the control only showed soil particles adhered on surfaces but no apparent bacterial population (Figure 2). The data further confirmed the degradation of *syn*-PHB in this study was carried out by native microbial population in the soil. Further analysis of the molecular weights of recovered residual polymer films demonstrated that a decrease in molecular weights, M_w and M_n, was evident during the progression of experiment, providing strong evidence on the breakage of polymer chain structures. In addition, the narrow range of polydispersity, M_w/W_n, values indicated that the polymer chain scission sites are a random and non-specific.

Bacteria isolated from the surfaces of partially degraded *syn*-PHB were dominated by *Pseudomonas* spp., *Alcaligenese* sp., and *Comamonas* sp. in this study. Similar results

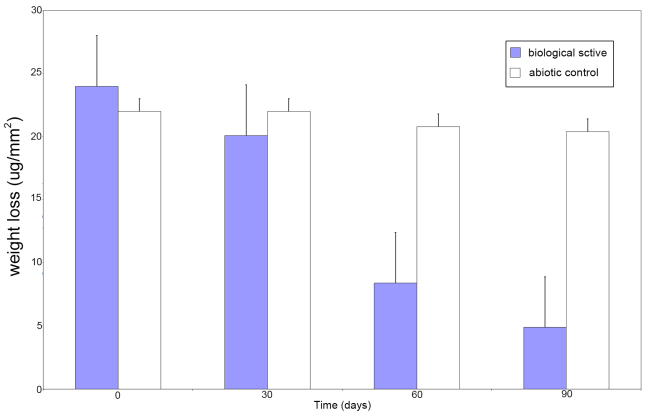

Figure 1. Weight loss of chemically synthesized *syn*-PHB films after exposure to a biologically active soil incubated at 35°C and the controls. The soil was kept at 60% of water holding capacity and loss of moisture was adjusted by adding sterile water every 5 days. Treatment was carried out in triplicate.

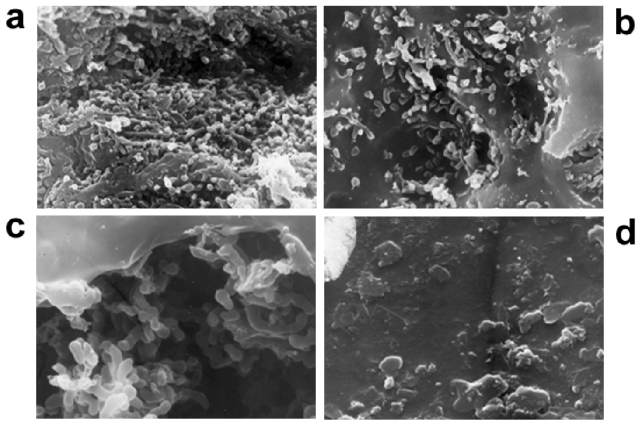

Figure 2. Scanning electron micrographs (SEMs) of *syn*-PHB films during incubation in a soil from biologically active flask (a,30 days; b, 60 days; c, 90 days) and from abiotic controls (d). (scale bar: a and b, 10 μm; c and d, 5 μm)

on microflora associated with PHB degradation were also reported (Mergaert et al., 1993). *Pseudomonas lemoignei* (Chowdhury, 1963) and *Alcaligenes faecalis* (Saito et al., 1989) have been known for their role in degradation of microbial synthesized polymers. Degradation of PHB is expected to occur naturally due to enzymatic processes (Anderson and Dawes, 1990). Extracellular PHB depolymerases are capable of hydrolyzing highly crystalline PHB while intracellular PHB depolymerases are only active on amorphous elastomer (Anderson and Dawes, 1990; Doi, 1990). Since chemically synthesized *syn*-PHB is structurally slight different from bacterial PHB, their environmental fate is of great concern when large quantities of the materials are potentially disposed of into the environment including MSW composting facilities and landfills. From this study, aerobic soil bacteria are capable of degrading chemically synthesized *syn*-PHB in a reasonable period of time providing evidence on degradability of the chemically synthesized *syn*-PHB.

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References

Anderson, A.J. and Dawes, E.A., 1990. Occurrence, metabolism, metabolic role, and industrial uses of bacterial polyhydroxyalkanoates. Microbiological Reviews, 54(4), 450-472.

Barak, P., Coquet, Y., Halbach, T.R. and Molina, J.A.E., 1991. Biodegradability of polyhydroxybutyrate (co-hydroxyvalerate) and starch-incorporated polyethylene plastic films in s oils. Journal of Environmental Quality, 20(1), 173-179. https://doi.org/10.2134/jeq1991.00472425002000010028x.

Brandl, H., Gross, R.A., Lenz, R.W. and Fuller, R.C., 1990. Plastics from bacteria and for bacteria: poly (β-hydroxyalkanoates) as natural, biocompatible, and biodegradable polyesters. Advances in Biochemical Engineering/Biotechnology, 41, 77-93. https://doi.org/10.1007/BFb0010232.

Budwill, K., Fedorak, P.M. and Page, W.J., 1992. Methanogenic degradation of poly (3-hydroxyalkanoates). Applied and Environmental Microbiology, 58(4), 1398-1401.

Chowdhury, A.A., 1963. Poly-β-hydroxybuttersaure abbauende Bakterien und exo-enzyme. Archives of Microbiology, 47(2), 167-200.

Doi, Y., 1990. Microbial Polyesters. New York: VCH Publishers.

Gilmore, D.F., Antoun, S., Lenz, R.W., Goodwin, S., Austin, R. and Fuller, R.C., 1992. The fate of biodegradable'plastics in municipal leaf compost. Journal of Industrial Microbiology & Biotechnology, 10(3), 199-206. https://doi.org/10.1007/BF01569767.

Gross, R.A., Gu, J.-D., Eberiel, D.T., Nelson, M. and McCarthy, S.P., 1993. Cellulose acetate biodegradability in simulated composting and anaerobic bioreactors as well as by a bacterial isolate derived from compost. pp. 257-279. *In*: Kaplan D, Thomas E and Ching C (eds), Fundamentals of Biodegradable Materials and Packaging. Technomic Publishing Co., Inc., Lancaster, PA, USA.

Gu, J.-D., Eberiel, D.T., McCarthy, S.P. and Gross, R.A., 1993a. Cellulose acetate biodegradability upon exposure to simulated aerobic composting and anaerobic bioreactor environments. Journal of Polymers and the Environment, 1(2), 143-153.

Gu, J.-D., Eberiel, D.T., McCarthy, S.P. and Gross, R.A., 1993b. Degradation and mineralization of cellulose acetate in simulated thermophilic compost environments. Journal of Polymers and the Environment, 1(4),281-291. https://doi.org/10.1007/BF01458295.

Gu, J.-D., Ford, T.E. and Mitchell, R., 1996. Susceptibility of electronic insulating polyimides to microbial degradation. Journal of Applied Polymer Science, 62(7), 1029-1034.

Gu, J.-D., Ford, T.E., Mitton, D.B., and Mitchell, R. (2000) Microbiological degradation of polymeric materials. pp. 439-460. *In*: Winston R (ed), Uhlig's Corrosion Handbook (2nd ed) , Wiley, New York.

Gu, J.-D., Ford, T.E., Mitton, D.B., and Mitchell, R. (2000) Microbiological degradation of materials: general processes. pp. 349-365. *In*: Winston R (ed), Uhlig's Corrosion Handbook (2nd ed) , Wiley, New York.

Gu, J.-D. and Mitchell, R. (2013) Biodeterioration. *In*: Rosenberg E, DeLong EF, Lory S, Stackebrandt E and Thompson F (eds), pp. 309-341. The Prokaryotes: Applied Bacteriology and Biotechnology. Springer-Verlag, New York.

Gu, J.-D., Yang, S.J., Welton, R., Eberiel, D., McCarthy, S.P. and Gross, R.A., 1994. Effect of environmental parameters on the degradability of polymer films in laboratory-scale composting reactors. Journal of Polymers and the Environment, 2(2), 129-135.

Kemnitzer, J.E., McCarthy, S.P. and Gross, R.A., 1992. Poly (β-hydroxybutyrate) stereoisomers: a model study of the effects of stereochemical and morphological variables on polymer biological degradability. Macromolecules, 25(22), 5927-5934. https://doi.org/10.1021/ma00048a011.

Kemnitzer, J.E., McCarthy, S.P. and Gross, R.A., 1993. Syndiospecific ring-opening polymerization of β-butyrolactone to form predominantly syndiotactic poly (β-hydroxybutyrate) using tin (IV) catalysts. Macromolecules, 26(23), 6143-6150. https://doi.org/10.1021/ma00075a001.

Lave, L.B., Hendrickson, C. and McMichael, F., 1994. Recycling decisions and green design. Environmental Science and Technology, 28(1), 18A-24A. https://doi.org/10.1021/es00050a001.

Mas-Castellà, J., Urmeneta, J., Lafuente, R., Navarrete, A. and Guerrero, R., 1995. Biodegradation of poly-β-hydroxyalkanoates in anaerobic sediments. International Biodeterioration & Biodegradation, 35(1-3), 155-174. https://doi.org/10.1016/0964-8305(95)00066-E.

Mergaert, J., Webb, A., Anderson, C., Wouters, A. and Swings, J., 1993. Microbial degradation of poly (3-hydroxybutyrate) and poly (3-hydroxybutyrate-co-3-hydroxyvalerate) in soils. Applied and Environmental Microbiology, 59(10), 3233-3238.

Saito, T., Suzuki, K., Yamamoto, J., Fukui, T., Miwa, K., Tomita, K., Nakanishi, S., Odani, S., Suzuki, J. and Ishikawa, K., 1989. Cloning, nucleotide sequence, and expression in *Escherichia coli* of the gene for poly (3-hydroxybutyrate) depolymerase from *Alcaligenes faecalis*. Journal of Bacteriology, 171(1), 184-189. https://doi.org/10.1128/jb.171.1.184-189.1989.

Stenbüchel, A. 1991. Polyhydroxyalkanoic acids. pp. 123-213. *In*: Byrom D (ed), Biomaterials: Novel Materails from Biological Sources, Stockton Press, New York.

Suflita, J.M., Gerba, C.P., Ham, R.K., Palmisano, A.C., Rathje, W.L. and Robinson, J.A., 1992. The world's largest landfill: a multidisplinary investigation. Environmental Science & Technology, 26(9), 1486-1494.

Sullivan, B.K., Oviatt, C.A., Klein-MacPhee, G. (1993) Fate and effects of a starch-based biodegradable plastic substitute in the marine environment. pp. 281-296. *In*: Kaplan D, Thomas E and Ching C (eds), Fundamentals of Biodegradable Materials and Packaging. Technomic Publishing Co., Inc., Lancaster, PA, USA.

Wirsen, C.O. and Jannasch,H.W. (1993) Microbial degradation of a starch-based biopolymer in the marine environment. pp. 297-310. *In*: Kaplan D, Thomas E and Ching C (eds), Fundamentals of Biodegradable Materials and Packaging. Technomic Publishing Co., Inc., Lancaster, PA, USA.