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Biodegradation of microbial polyesters in the marine environment

Yoshiharu Doi, Youko Kanesawa, Naoki Tanahashi

Research Laboratory of Resources Utilization, Tokyo Institute of Technology, Nagatsuta, Midori-ku, Yokohama 227, Japan

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Yoshiharu Kumagai

Sumitomo Metal Industries Ltd, 16 Sunayama, Hasaki-cho, Kashima-gun, Ibaraki 314-02, Japan

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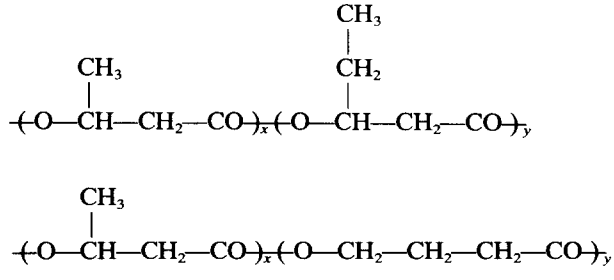
The biodegradation of microbial copolyesters, poly(3-hydroxybutyrate-co-3-hydroxyvalerate)(P(3HB-co-3HV)) and poly(3-hydroxybutyrate-co-4-hydroxybutyrate) (P(3HB-co-4HB)), were studied under marine exposure conditions in sea water during the period from January to December. The processes of biodegradation were analyzed by monitoring the time-dependent changes in weight loss (erosion), molecular weight and mechanical strength of films, plates and fibers. All the samples exposed in sea water were degraded via surface dissolution. The rate of surface erosion was almost independent of the copolymer compositions of P(3HB-co-3HV) and P(3HB-co-4HB) samples, but markedly dependent upon the temperature of the sea water. Two strains of actinomycetes (*Streptomyces*) capable of using P(3HB) as the sole carbon source have been isolated from sea sediment.

INTRODUCTION

Several hundred thousand tonnes of plastics have been reported to be discarded into marine environments every year.[1] It has been estimated that one million marine animals are killed every year either by choking on floating plastic items or by becoming entangled in plastic debris.[1,2] The development of biodegradable plastics is the key to solving the problems caused by marine plastic debris.

The microbial poly(hydroxyalkanoate) family of polyesters are biodegradable thermoplastics produced from various carbon substrates by microorganisms.[3] These microbial polyesters have recently attracted industrial attention as large-scale biotechnological products.[4,5] A copolyester of 3-hydroxybutyrate (3HB) and 3-hydroxyvalerate (3HV) has been produced by *Alcaligenes eutrophus* from propionic acid[4,6] and pentanoic acid.[7] Imperial Chemical Industries (ICI Biological Products, Billingham, UK) has produced the P(3HB-co-3HV) copolymer commercially by a fermentation process.[4,5] Recently, the copolyester of 3HB and 4-hydroxybutyrate (4HB) has been produced by *A. eutrophus* from 4-hydroxybutyric acid[8,9] and γ-butyrolactone.[10,11]



The physical and thermal properties of these two families of copolyesters have been shown to be regulated by the copolymer compositions.[12–15] The microbial copolyesters can be formed into

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films, plates and fibers by traditional processing techniques.[16]

Products made from microbial polyesters are known to be degradable in soil or sludge.[3,4] Under optimum conditions the degradation rate is fast. Some microorganisms, such as bacteria and actinomycetes, excrete extracellular P(3HB) depolymerases that hydrolyze environmental polyesters, and the resulting products are absorbed into the cells and utilized as nutrients.[17,18] An extracellular P(3HB) depolymerase has been isolated from *A. faecalis*,[19] and the rate of enzymatic surface erosion has been found to decrease in the order: P(3HB-co-4HB) > P(3HB) > P(3HB-co-3HV).[20]

The objective of the present study is to determine quantitatively the biodegradability of films, plates and fibers of microbial polyesters under marine exposure conditions in sea water. The microbial polyesters have been evaluated for physical and chemical degradation including both surface and internal changes.

EXPERIMENTAL

Microbial polyester samples

Ten polyester samples were produced by a controlled fermentation.[7,11] Sample 1 of P(3HB) homopolymer was produced from butyric acid by *A. eutrophus*.[7] Samples 2–8 of P(3HB-co-3HV) copolyesters were produced by *A. eutrophus* from butyric and pentanoic acids.[7] Samples 9 and 10 of P(3HB-co-4HB) copolyesters were produced by *A. eutrophus* from fructose and γ-butyrolactone.[11]

The compositions of copolyesters were determined by analysis of the ^{1}H NMR spectra.[8] Table 1 shows the compositions, molecular weights and densities of the microbial polyesters used in this study.

The films (50–150 μm thick) of samples 1, 2, 7, 8, 9 and 10 were prepared by conventional solvent-casting techniques from chloroform solutions. The plates (2050–2100 μm thick) of samples 3, 4 and 6 were melt-extruded. The monofilament fiber (260 μm diameter) of sample 5 was melt-spun.

Exposure conditions

Biodegradation experiments were carried out at the exposure facility at Kanagawa Prefectural Fishery Experiment Station at Jogashima, Japan. All experimental samples (5 cm × 10 cm in size), placed nylon nets within a stainless steel cage, were positioned at a water depth of 1·5 m in an outdoor tank (10 × 10 m × 3 m in depth), with fresh sea water continuously flowing through the tank from the sea. Figure 1 shows the monthly mean temperature (high, mean and low) of the sea water during the period of exposure, January–December 1990. The lowest mean

Table 1. Compositions and molecular weights of microbial poly(hydroxyalkanoate) samples

Sample no.	Composition (mol%)[a]			Molecular weight[b]		Density[c] (g/ml)
	3HB	3HV	4HB	$\bar{M}_n \times 10^{-3}$	$\bar{M}_w/\bar{M}_n$	
1	100	0	0	768	1·9	1·250
2	96	4	0	186	2·6	
3	91	9	0	144	2·0	
4	87	13	0	121	2·0	
5	86	14	0	138	2·2	
6	85	15	0	102	2·1	
7	79	21	0	238	3·0	1·231
8	39	61	0	244	3·5	
9	94	0	6	494	2·1	
10	90	0	10	197	2·6	1·232

[a] 3HB, 3-hydroxybutyrate; 3HV, 3-hydroxyvalerate; 4HB, 4-hydroxybutyrate.
[b] Determined by GPC.
[c] Determined at 25°C.

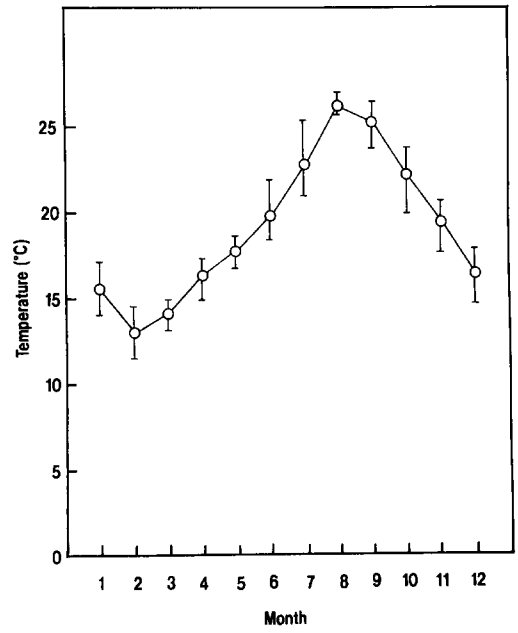

Fig. 1. Average temperature of the sea water (January–December 1990) in the exposure facility at Kanagawa Prefectural Fishery Experiment Station in Jogashima, Japan.

temperature was 13·0°C in February, and the highest was 26·1°C in August.

The exposed samples were washed with distilled water and dried to constant weight *in vacuo* at room temperature. The extent of surface erosion was determined from weight loss and thickness loss.

Analytical procedures

All molecular weight data were obtained at 40°C using a Shimadzu 6A GPC system and a 6A refractive index detector with a Shodex 80M column. Chloroform was used as eluant at a flow rate of 0·5 ml/min, and a sample concentration of 1·0 mg/ml was used. Polystyrene standards with low polydispersity were used to construct a calibration curve.

Mechanical properties were determined using an Imada SV-50 tensile machine. Single loading experiments were carried out in air at 23°C, and the strain rate was 20 mm/min.

The densities of the polyester films were measured at 25°C using a density gradient column containing mixtures of toluene and carbon tetrachloride as the column liquid.

The ^{1}H NMR analyses of the polyesters were carried out on a JEOL FX-100 spectrometer. The 100 MHz ^{1}H NMR spectra were recorded at 27°C using a $CDCl_3$ solution of polyester (5 mg/ml) with 45° pulse (15 μs), 5 s pulse repetition, 1000 Hz spectral width, 8 K data points and 200 accumulations.

RESULTS AND DISCUSSION

Surface erosion

All samples of microbial polyesters exposed in sea water were degraded via surface dissolution. Figure 2 shows the surface erosions of solvent-cast films of five polyester samples 1, 2, 7, 8 and 10 for the three-week period 20 June–12 July (22 ± 3°C) in sea water. The weight and thickness of the films decreased and the surface of the films was apparently blemished. A scanning electron micrograph of the films showed that no appreciable change took place in the inside of the film. After three weeks exposure, films of samples 1, 2, 8 and 10 had lost 13–16 μm of film thickness. On the other hand, sample 7 lost 22 μm of film thickness. The rate of surface

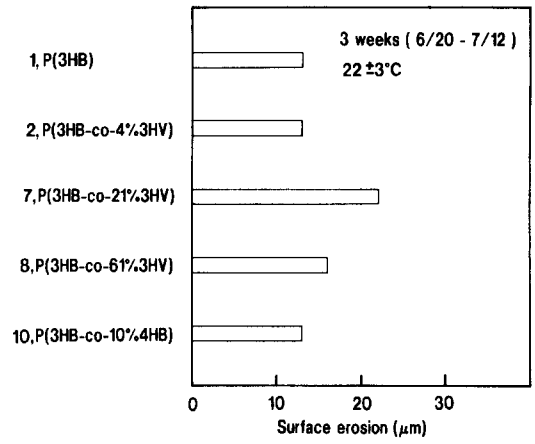

Fig. 2. Surface erosion of solvent-cast films (initial thickness: 50–100 μm) of five polyester samples 1, 2, 7, 8 and 10 for three weeks (20 June–12 July) in sea water.

erosion was almost independent of the copolymer composition, except for sample 7, P(3HB-co-21% 3HV).

The films of P(3HB-co-4HB) samples 9 and 10 were exposed for eight weeks in sea water during two seasons (18 January–15 March and 21 August–19 October). Figure 3 shows the surface erosions of the films after eight weeks in sea water. The film erosions of samples 9 and 10 after eight weeks from 18 January to 15 March (14 ± 2°C) were 31 and 33 μm, respectively. In contrast, the film thicknesses of samples 9 and 10 lost respectively 55 and 60 μm during eight weeks from 21 August to 19 October (24 ± 3°C). The rate of surface erosion was almost independent of

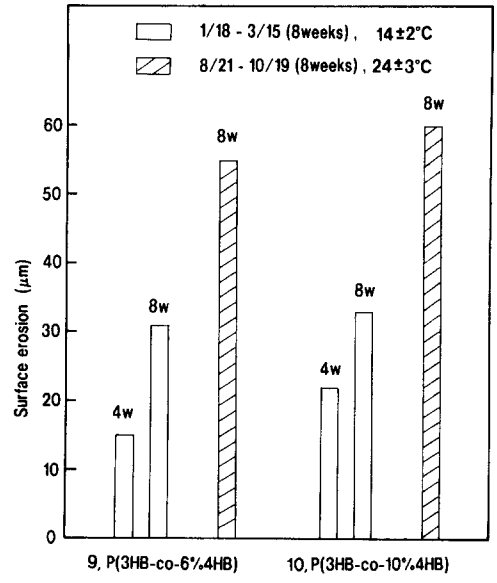

Fig. 3. Surface erosion of solvent-cast films (initial thickness: 100–150 μm) of P(3HB-co-4HB) samples 9 and 10 in sea water for eight weeks during two seasons (18 January–15 March and 21 August–19 October).

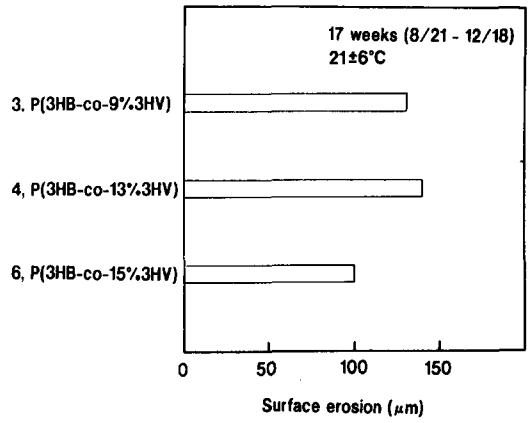

Fig. 4. Surface erosions of melt-extruded plates (initial thickness: 2050–2100 μm) of P(3HB-co-3HV) samples 3, 4 and 6 for 17 weeks (21 August–18 December) in sea water.

the copolymer composition of P(3HB-co-4HB) films, but strongly dependent on the temperature of the sea water.

Figure 4 shows the surface erosions of melt-extruded plates of P(3HB-co-3HV) samples 3, 4 and 6 in sea water for 17 weeks from 21 August to 18 December (21 ± 6°C). The P(3HB-co-3HV) plates lost 100–140 μm of plate thickness in 17 weeks.

Molecular weight and mechanical properties

Table 2 shows the changes in molecular weights and mechanical properties of P(3HB-co-4HB) films 9 and 10 exposed in sea water during the period 18 January–15 March. The number-average molecular weights ($\bar{M}_n$) of the residual films decreased slightly as the surface erosion proceeded, while the polydispersities ($\bar{M}_w/\bar{M}_n$) remained almost unchanged. The strain at break of the films decreased during the exposure in sea water, and the material gradually turned brittle. It was difficult to determine accurate values of stress at break of the films exposed in sea water.

Table 3. Changes in weight, molecular weight and mechanical properties of the fiber of P(3HB-co-14% 3HV) sample 5 in sea water during the period 18 January–15 March[a]

Time (weeks)	Retention of weight (%)	Molecular weight		Strain at break (%)	Stress at break (MPa)	Youngs modulus (MPa)
		$\bar{M}_n \times 10^{-3}$	$\bar{M}_w/\bar{M}_n$			
0	100	138	2·2	67	110	1 100
4	75	136	1·9	13	75	1 400
8	35	134	1·9	0	0	—

[a] The initial diameter of monofilament fiber was 260 μm.

The degradation process of melt-spun fiber (260 μm diameter) of P(3HB-co-14% 3HV), sample 5, was studied in sea water for eight weeks from 15 January to 15 March (14 ± 2°C). Table 3 summarizes the weight loss, molecular weight and mechanical properties of the fiber. The weight of fiber decreased to only 35% of the initial weight after eight weeks in sea water. The strain and stress at break of the fiber rapidly decreased in sea water, and both values reached zero after eight weeks.

Isolation of P(3HB)-degrading microorganisms

The degradation of microbial polyesters in sea water may involve a simple hydrolytic degradation process in addition to a microbial (enzymatic) degradation. The degradation of

Table 2. Changes in thickness, molecular weight and mechanical properties of P(3HB-co-4HB) films in sea water during the period 18 January–15 March

Sample[a] no.	Time (weeks)	Thickness (μm)	Molecular weight		Strain at yield (%)	Stress at yield (MPa)	Strain at break (%)	Stress at break (MPa)
			$\bar{M}_n \times 10^{-3}$	$\bar{M}_w/\bar{M}_n$				
9	0	100	494	2·1	8	25	51	26
9	4	85	398	1·8	5	20	16	n.d.[b]
9	8	69	360	1·9	3	16	25	n.d.[b]
10	0	150	197	2·6	13	19	523	24
10	4	128	186	2·6	6	17	48	n.d.[b]
10	8	117	186	2·6	5	19	50	n.d.[b]

[a] Initial thicknesses of films were 100 and 150 μm for samples 9 and 10, respectively.
[b] It was difficult to determine accurate values of stress at break.

P(3HB) and P(3HB-co-10% 4HB) films was studied at 37°C for four weeks in sea water that had been treated for 15 min at 120°C. No weight loss of films was observed in the pretreated sea water, indicating that a simple hydrolytic degradation process does not contribute to the degradation of microbial polyesters in the marine environment.

An attempt has been made to isolate P(3HB)-degrading microorganisms from the sediment on the surface of P(3HB) film exposed in sea water. The sediment was incubated in a medium containing P(3HB) granules as the sole carbon source at 30°C for seven days until the turbid medium became clear. Then, two stains were isolated on the turbid P(3HB)-mineral-agar plates by the formation of clear zones surrounding the colonies due to the excretion of extracellular P(3HB) depolymerases, and characterized as actinomycetes (*Streptomyces*).

The rate of enzymatic degradation in sea water was little dependent upon the copolymer compositions of P(3HB-co-3HV) and P(3HB-co-4HB) samples, as shown in Figs 2–4. This result may indicate that the extracellular P(3HB) depolymerases from *Streptomyces* have a broad specificity on the degradation of microbial polyesters.

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