Environmental Degradation of Biodegradable Polyesters. IV. The Effects of Pores and Surface Hydrophilicity on the Biodegradation of Poly(ϵ-caprolactone) and Poly[(*R*)-3-hydroxybutyrate] Films in Controlled Seawater

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ABSTRACT: Poly(ϵ-caprolactone) (PCL) and poly[(*R*)-3-hydroxybutyrate] (*R*-PHB) films with pores and hydrophilic surfaces were prepared by the water extraction of poly(ethylene oxide) from as-cast blend films (1:1) and by the alkali treatment of as-cast nonporous films, respectively. These films, as well as as-cast nonporous PCL and *R*-PHB films, were biodegraded in static seawater kept at 25°C, and their biodegradation was monitored with gravimetry, gel permeation chromatography (GPC), and scanning electron microscopy. The pores or highly hydrophilic surfaces of the PCL and *R*-PHB films enhanced their biodegradation in seawater. Moreover, GPC measurements could be used to trace the biodegradation in seawater when the biodegradation proceeded to a great extent. © 2003 Wiley Periodicals, Inc. J Appl Polym Sci 90: 587–593, 2003

Key words: biodegradable; biomaterials; degradation; processing; polyesters

INTRODUCTION

The environmental degradation of biodegradable aliphatic polyesters has intensively been investigated to obtain information useful for their environmental applications.[1–14] The natural and controlled environmental degradation of representative biodegradable polyesters such as poly(ϵ-caprolactone) (PCL),[4,6,8,12,13] poly[(*R*)-3-hydroxybutyrate] (*R*-PHB),[2,4,6,12,13] and poly(L-lactide), that is, poly(L-lactic acid) (PLLA),[12,13] has been studied in seawater,[2,6,8,12,13] river water,[4,6] and lake water.[6]

In our previous studies, we investigated the biodegradation of PCL, *R*-PHB, and PLLA films in controlled static[12] and natural dynamic seawater.[13] The following conclusions were reached:

1. The biodegradabilities of the aliphatic polyesters in controlled static seawater decreased in the following order when estimated by changes in the weight loss, tensile strength, and Young's modulus: PCL > *R*-PHB > PLLA.
2. The biodegradation of PCL and *R*-PHB in controlled static seawater proceeded via surface erosion mechanisms inhomogeneously on the film surface by the attached marine microbes.
3. The biodegradation of PLLA in controlled static seawater was insignificant even after immersion in controlled seawater for 10 weeks, and the initial crystallinity (X_c) had a negligible effect on the biodegradability of PLLA, with the exception of changes to its tensile properties.
4. Natural dynamic seawater caused mechanical destruction or degradation of the aliphatic polyester films, and this resulted in their seemingly accelerated (bio)degradation, compared with that in controlled static seawater, when the (bio) degradation was estimated with gravimetry and tensile testing.

Doi and coworkers[4,6] revealed that the biodegradabilities of *R*-PHB copolymers in sea and river water could be controlled through variations in the comonomer structure and fraction. Despite these intensive studies, however, the effects of the pores and surface hydrophilicity of these biodegradable polyesters on their biodegradation in seawater have not been reported so far, although their enzymatic hydrolyzabilities have been studied.[15–17] Pore formation and increased surface hydrophilicity are expected to enhance the biodegradabilities of aliphatic polyesters in seawater. The former factor increases the biodegradable surface area per unit of weight, whereas the latter

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enhances the attachment of the environmental microbes without changing the bulk properties and characteristics, such as the mechanical and thermal properties and molecular weight.

The purpose of this study was to elucidate the effects of the pores and surface hydrophilicity of biodegradable aliphatic polyesters in seawater. For this purpose, environmentally highly biodegradable polyesters, PCL and *R*-PHB, were selected as model biodegradable polyesters. We did not use PLLA in this study because its biodegradation rate in seawater was reported to be very low.[12,13] The porous PCL and *R*-PHB films were prepared by the water extraction of poly(ethylene oxide) (PEO) from their blends,[15,18] whereas the surface hydrophilicities of PCL and *R*-PHB films were enhanced by alkali treatment (surface hydrolysis).[16,19] These films were biodegraded in static seawater (kept at 25°C), and their biodegradation was monitored with gravimetry, gel permeation chromatography (GPC), and scanning electron microscopy (SEM).

EXPERIMENTAL

Materials

PCL was synthesized by the ring-opening polymerization of ϵ-caprolactone in bulk at 140°C for 72 h with stannous octoate (0.03 wt %) as a polymerization catalyst.[7,20,21] ϵ-Caprolactone (guaranteed-grade) was purchased from Nacalai Tesque, Inc. (Kyoto, Japan) and purified by distillation under reduced pressure before polymerization. The polymerized PCL was purified by precipitation with methylene chloride and methanol as solvent and nonsolvent, respectively. *R*-PHB (natural origin) was purchased from Sigma–Aldrich Co. (Milwaukee, WI) and was purified by precipitation with chloroform and *n*-hexane as solvent and nonsolvent, respectively. The purified polymers were dried *in vacuo* for 1 week before film preparation.

Nonporous films about 50 and 200 μm thick were prepared by solution casting with methylene chloride and chloroform as solvents for PCL and *R*-PHB, respectively. The polymer solutions, with a polymer concentration of 1.0 g/dL, were cast onto petri dishes, and this was followed by solvent evaporation at room temperature for approximately 1 week and subsequent drying *in vacuo* for another week. The films with high surface hydrophilicities were prepared by the immersion of the as-cast nonporous films in a 4.0*N* NaOH solution (Nacalai Tesque) at 37°C for 3 h. The alkali-treated films were washed thoroughly with distilled water (HPLC-grade; Nacalai Tesque) and were dried *in vacuo* for 1 week.

Porous films about 50 and 200 μm thick were prepared by the casting of a 1 g/dL mixed solution of purified PCL or PHB with PEO [Aldrich; weight-average molecular weight (M_w) = 7.0×10^4 and weight-average molecular weight/number-average molecular weight (M_w/M_n) = 1.6; 50/50 (w/w)] with methylene chloride as a solvent; this was followed by slow solvent evaporation at room temperature for about 1 week and subsequent drying *in vacuo* for another week. The period of time for the solvent evaporation was set to be 1 week for complete phase separation between the two polymers during solvent evaporation.[7,20,21] The extraction of PEO from the films was performed in distilled water (HPLC-grade; Nacalai Tesque) at room temperature for 1 week, with the distilled water changed once a day.[15,18] After the extraction, the films were dried *in vacuo* for 1 week. The average water-extracted ratios (i.e., approximate porosities) of the porous PCL and *R*-PHB films were 49.7 and 48.2 wt %, respectively; this means that most of the PEO chains were eluted from the blend films during water extraction, and in this way, porous structures were formed in the films.

The as-cast nonporous and porous PCL and R-PHB films are denoted PCL-AC and PCL-P films and *R*-PHB-AC and *R*-PHB-P films, respectively, whereas the as-cast nonporous PCL and *R*-PHB films treated in 4.0*N* NaOH solutions for 3 h are denoted PCL-H and *R*-PHB-H films, respectively. All the prepared films were stored in a desiccator at room temperature for at least 1 month so that they could reach the equilibrium state before the physical measurements and biodegradation.

Biodegradation in controlled static seawater

The biodegradation of the films (18 mm × 30 mm × 50 μm) in seawater was performed according to the procedure reported in part I of this series.[12] That is, the films covered with meshes were immersed in aerated seawater kept at 25°C for predetermined periods of up to 35 days. Seawater from the Pacific Ocean (Terasawa-cho, Toyohashi, Aichi, Japan) was used for the biodegradation after the sedimentation of solid particles for 1 h. The seawater was replaced with fresh seawater from the ocean every 2 days. The biodegradation test started on October 6, 2000.

Measurements and observation

After the biodegradation test, the films were washed thoroughly with distilled water (HPLC-grade; Nacalai Tesque) at room temperature, and this was followed by drying under reduced pressure for at least 2 weeks. M_w's, M_n's, and the molecular weight distributions of the films (50 μm thick) were evaluated in chloroform at 40°C with a Tosoh GPC system (RI-8020 refractive-index monitor, Tokyo, Japan) with two TSKgel columns (GMH_{XL}) with polystyrene as a standard.

TABLE I
Characteristics and Properties of PCL and *R*-RPHB Films Before and After Biodegradation in Seawater at 25°C

Sample	Biodegradation time (days)	$M_w/10^5$ (g/mol)	M_w/M_n	T_m (°C)	X_c (%)	θ_a (°C)	θ_r (°C)	TS (kg/mm^2)	YM (kg/mm^2)	EB (%)
PCL-AC	0	1.81	1.63	63	56	86.0	53.8	0.88	35	60.3
	35	1.66	1.83	—	—	—	—	—	—	—
PCL-P	0	1.87	1.62	63	65	—[a]	—[a]	0.71	11	447.5
	7[b]	1.82	2.97	—	—	—	—	—	—	—
PCL-H	0	1.84	1.68	63	54	63.4	23.4	0.55	33	21.3
	28[b]	1.44	3.63	—	—	—	—	—	—	—
R-PHB-AC	0	5.19	2.24	178	67	84.6	53.0	2.07	168	3.6
	35	5.39	1.71	—	—	—	—	—	—	—
R-PHB-P	0	5.36	2.18	178	64	—[a]	—[a]	0.82	78	2.3
	35	1.73	2.22	—	—	—	—	—	—	—
R-PHB-H	0	5.80	1.88	178	64	76.7	24.1	1.98	178	1.4
	35	5.28	2.00	—	—	—	—	—	—	—

TS = tensile strength; YM = Young's modulus; EB = elongation at break.
[a] Unmeasurable because the pores were known to cause large deviations in the contact angles from the real values.
[b] Unmeasurable for the specimens biodegraded for the periods of time exceeding the shown periods because of the disturbace by the adsorbed marine microbes and substances on the film surface or on insufficient amount of the specimen after biodegradation.

The melting temperature (T_m) and enthalpy of melting (ΔH_m) of the films (50 μm thick) were determined with a Shimadzu DT-50 differential scanning calorimeter (Kyoto, Japan). The films were heated at a rate of 10°C/min under a nitrogen gas flow at a rate of 50 mL/min for the DSC measurements. T_m and ΔH_m were calibrated with benzophenone, indium, and tin as standards. The X_c values of the films were evaluated according to the following equation:

$$X_c(\%) = 100 \cdot \Delta H_{\mathrm{m}}/\Delta H_{\mathrm{m}}(100\%) \quad (1)$$

where $\Delta H_m(100\%)$ is the ΔH_m value of a polymer crystal of infinite thickness. The $\Delta H_m(100\%)$ values of PCL and *R*-PHB have been reported to be 142 (J/g of polymer)[22] and 146 (J/g of polymer),[23] respectively.

The advancing and receding contact angles (θ_a and θ_r, respectively) of the as-cast and NaOH-treated films (15 mm × 30 mm × 200 μm) before the biodegradation were measured with the Wilhelmy plate method[24] with a DCA-100 (A&D Co., Ltd., Tokyo, Japan). The measurements were performed in distilled water (HPLC-grade; Nakarai Tesque, Inc.) at 25°C and a film speed of 20 mm/min. The contact angles are indices for the net densities of hydrophilic carboxyl and hydroxyl groups of the film surface. However, θ_r may be an inappropriate index because of the absorption of water during the first advancing measurement. Accordingly, θ_a is regarded as the main index for the hydrophilicity, whereas θ_r is also shown in Table I for reference.

The tensile properties of the films (3 mm × 30 mm × 200 μm) were measured at 25°C and 50% relative humidity with a tensile tester (EZ Test, Shimadzu) at a crosshead speed of 100%/min (20 mm/min). The initial length between the two gauges was always kept at 20 mm. The morphology of the films was studied with a Hitachi X-650 SEM instrument (Tokyo, Japan). The films for SEM observation (50 μm thick) were coated with Pt to a thickness of about 20 nm.

RESULTS AND DISCUSSION

Pore formation and alkali treatment

Mechanical properties

The alkali treatment in this study was performed in a 4*N* NaOH solution for a period as short as 4 h because the previous study revealed that the treatment for relatively long periods exceeding 16 h caused the formation of cracks and pores on PCL films,[16] which induced a dramatic decrease in its mechanical properties. Figure 1 shows the relative mechanical properties of the as-cast nonporous, porous, and alkali-treated films before biodegradation in controlled seawater. The absolute values of the mechanical properties are shown in Table I (biodegradation time = 0 day). In Figure 1(a), the relative mechanical properties are given on a logarithmic scale. For the PCL films, pore formation insignificantly changed the tensile strength, but it dramatically increased the elongation at break and reduced Young's modulus. In contrast, for *R*-PHB films, pore formation reduced all the mechanical properties. The difference in the effect of pore formation on the mechanical properties could be ascribed to the brittleness of the *R*-PHB films compared with that of the PCL films, as shown by their glass-transition temperatures (T_g's): *R*-PHB has a high T_g (4°C)[25] compared with that of PCL (−60°C).[19,20] It is

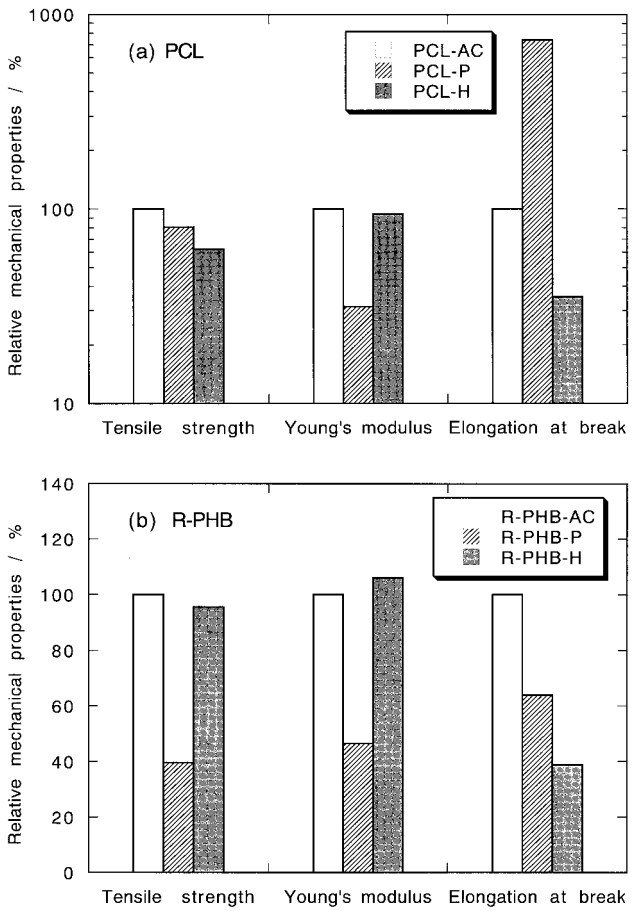

Figure 1 Relative mechanical properties of the as-cast nonporous, porous, and alkali-treated (a) PCL and (b) *R*-PHB films before biodegradation in controlled seawater.

probable that the pores in the brittle *R*-PHB films acted as defects and, consequently, reduced the mechanical properties.

However, in the case of the PCL films, the surface treatment negligibly altered Young's modulus but significantly decreased the tensile strength and the elongation at break. This is comparable to reported results.[16] In the case of the *R*-PHB films, the surface treatment insignificantly changed the tensile strength and Young's modulus but dramatically decreased the elongation at break. Although the weight-loss values by the alkali treatment were small [0.2 and 1.0 wt % for PCL-H and *R*-PHB-H films (200 μm thick)], the decrease in the elongation at break of both the PCL-H and *R*-PHB-H films could be attributed to the formation of pores and/or cracks by the alkali treatment, as shown later in this article and suggested earlier.[16]

Hydrophilicity

As shown in Table I (biodegradation time = 0 day), θ_a of the PCL-AC film decreased from 86.0 to 63.4° by the alkali treatment for 3 h, whereas that of the *R*-PHB-AC film decreased from 84.6 to 76.7° by the same treatment. This indicates that the hydrophilicity of the *R*-PHB film and that of the PCL film[16] could be enhanced by the alkali treatment. The θ_a value was lower for the PCL-H film than for the *R*-PHB-H film. This difference, despite the similar initial θ_a values before the treatment and the larger weight loss during the treatment of the *R*-PHB-AC film compared with that of the PCL-AC film, may be explained by a lower hydrolyzability of the chains on the lateral surfaces of the crystalline regions present on the *R*-PHB film surface compared with that on the PCL film surface.

The pore formation and the surface treatment produced no significant changes in the molecular weights and X_c values of the PCL and *R*-PHB films (Table I), with the exception of the slightly higher X_c value of the PCL-P film compared with that of the PCL-AC film. This meant that the pure effects of pore formation and increased surface hydrophilicities on the biodegradation of films in seawater could be studied with these specimens.

Biodegradation in seawater

Weight losses

Figure 2 gives the weight losses (%) of the as-cast nonporous, porous, and alkali-treated PCL and *R*-PHB films biodegraded in controlled seawater as a function of the degradation time. Significant weight losses were observed for all the PCL and *R*-PHB films for biodegradation periods exceeding 7 and 14 days, respectively. The weight losses of the PCL-AC and PCL-P films increased monotonically and finally reached 14 and 88%, respectively, whereas that of the PCL-H film gave a maximum value of 73% at 21 days and then decreased to 53% at 35 days. In contrast, the weight loss of the *R*-PHB-AC film increased slowly and finally reached 9% at 35 days, whereas those of the *R*-PHB-AC and *R*-PHB-H films increased after an induction period of 14 days and then saturated around 25 and 55%, respectively, for biodegradation periods above 30 days. These findings indicate that the pore formation and increased hydrophilicities enhanced the biodegradabilities of the PCL and *R*-PHB films in seawater. The weight-loss values of the porous PCL-P film were 8.9, 17.9, 6.6, and 6.2 times those of the nonporous PCL-AC film for biodegradation periods of 7, 14, 21, and 35 days, respectively. This enhancement of biodegradability by the pore formation is comparable to the result reported for the *Rhizopus arrhizus* lipase-catalyzed hydrolysis of nonporous and porous PCL films prepared by the same procedure, for which the weight-loss values of the porous PCL film (the porosity was about 50%) were 34.3, 9.6, and 5.7 times those of the nonporous PCL film at degradation periods of 24, 48, and 72 h, respectively.[15]

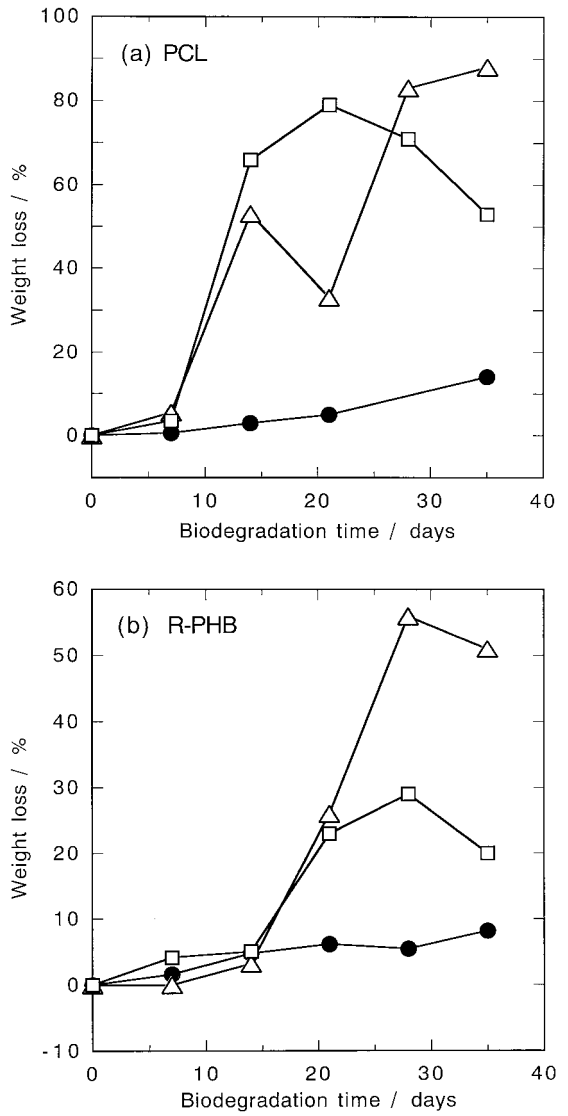

Figure 2 Weight losses of the as-cast nonporous, porous, and alkali-treated (a) PCL and (b) *R*-PHB films biodegraded in controlled seawater as a function of the degradation time: (●) PCL-AC and *R*-PHB-AC films, (△) PCL-P and *R*-PHB-P films, and (□) PCL-H and *R*-PHB-H films.

As the X_c values of the PCL-AC and *R*-PHB-AC films before biodegradation were similar, as shown in Table I, a comparison of their weight-loss values has significance. The final weight loss at 35 days was higher for the PCL-AC films than for the *R*-PHB-AC films. This means that the biodegradability of the PCL-AC film in controlled seawater was higher than that of the *R*-PHB-AC film, in good agreement with reported results.[12] The decrease in the weight-loss values of the PCL-H film and the *R*-PHB-H and *R*-PHB-P films for the biodegradation periods exceeding 21 and 28 days, respectively, was attributable to the imperfect removal of the attached marine microbes and substances due to their strong adhesion. This can be seen in the SEM photograph of the PCL-H film after biodegradation (shown later).

Molecular weight changes

The M_w and M_w/M_n values of the PCL and *R*-PHB films before and after biodegradation for 35 days are listed in Table I. The M_w and M_w/M_n values of the PCL-P and PCL-H films became unmeasurable for biodegradation periods exceeding 7 and 28 days because of the disturbance by the adsorbed marine microbes and substances on the film surface or the insufficient amount of the specimen after biodegradation. The slight decrease in M_w of the PCL-AC film at 35 days and the significant decrease in M_w and the increase in M_w/M_n of the PCL-H films even at 28 days support the result determined with gravimetry, that the biodegradability was higher for the PCL-H film than for the PCL-AC film. The increase in M_w/M_n of the PCL-P film from 1.62 to 2.97 even at 7 days strongly suggests that the porous PCL-P film had the highest biodegradability among the PCL films. Such an increase in M_w/M_n was reported for the PCL films hydrolyzed enzymatically in the presence of *R. arrhizus* lipase.[26]

After biodegradation for 35 days, the changes in M_w and M_w/M_n of the *R*-PHB-AC and *R*-PHB-H films were negligibly small, whereas a significant decrease was observed for M_w of the *R*-PHB-P film. These findings confirm that the biodegradability of the *R*-PHB-P film was the highest among the *R*-PHB films. The result obtained here indicates that the biodegradation of biodegradable polyester films in seawater is traceable by GPC measurements when the biodegradation proceeds to a great extent, as for the porous PCL-P and *R*-PHB-P films. This is in marked contrast to the results reported for PCL and *R*-PHB films biodegraded in seawater to a small extent.[12,13]

Morphological changes

Figures 3 and 4 provide SEM photographs of the as-cast nonporous, porous, and alkali-treated PCL and *R*-PHB films before and after biodegradation in controlled seawater for 35 days. Before biodegradation, the PCL-AC and PCL-H films had smooth and slightly rough surfaces, respectively, with the boundaries of spherulites, whereas numerous pores with a maximum size of about 30 μm were observed on the surface of the PCL-P film (Fig. 3). The slightly rough surface of the PCL-H film was attributable to selective alkaline hydrolysis and the subsequent removal of the chains in the amorphous region. After the biodegradation, the predominant degradation and subsequent removal of the chains occurred in the amorphous region of the PCL-AC film, and so a spherulitic structure of the film could be seen on the film surface in addition to the boundaries of spherulites. Similar biodegradation took place in the PCL-H film, but the surface roughness was higher for the PCL-H film than for the

PCL-AC film, probably because of the larger weight loss of the PCL-H film by biodegradation. Consequently, the spherulitic structure was clearer for the PCL-H film than for the PCL-AC film. Moreover, microbes and/or adhesives were seen on the surface of the PCL-H film after biodegradation, and these must have been the cause of the decreased weight-loss values at biodegradation periods exceeding 21 days. In contrast, after biodegradation, the structure of the PCL-P film collapsed to a great extent, and the film surface was completely biodegraded and removed.

Before the biodegradation, the *R*-PHB-AC and *R*-PHB-H films had similar vague surface morphologies, in contrast to the nonbiodegraded PCL-AC and PCL-H films, whereas pores with a maximum size of 80 μm were observed on the surface of the *R*-PHB-P film (Fig. 4). After the biodegradation, some microbes and/or adhesives were seen on the surfaces of *R*-PHB-AC and *R*-PHB-H films, and so the surface structures of these films became vaguer. This was in marked contrast to the biodegraded PCL-AC and PCL-H films, for which the spherulitic structure became evident in comparison with the nonbiodegraded films. It seems that after the biodegradation, the surface of the *R*-PHB-P film remained unchanged, with the pore sizes not varying, but some cracks and microbes and/or adhesives were observed on the film surfaces, in contrast to the biodegraded PCL-P film, the surface of which was biodegraded and removed.

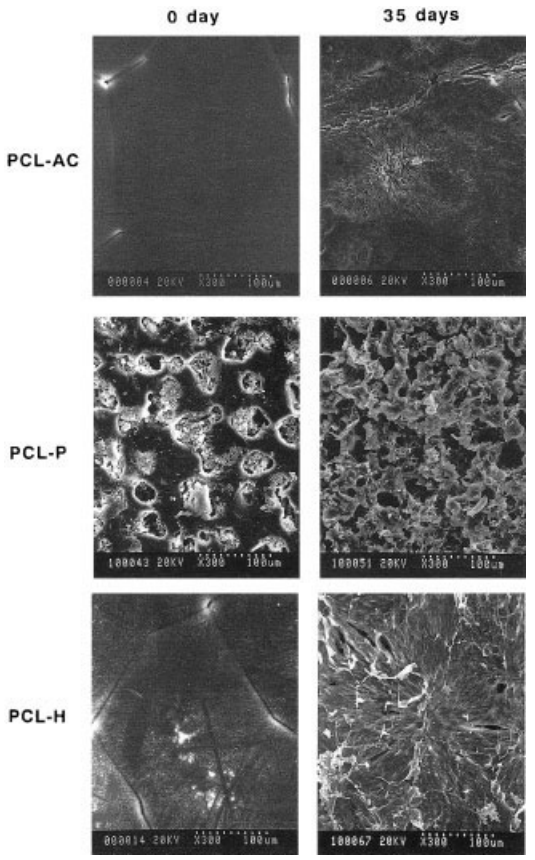

Figure 3 SEM photographs of the as-cast nonporous, porous, and alkali-treated PCL films before and after biodegradation in controlled seawater for 35 days. The length between the left and right dots in the photographs is 100 μm.

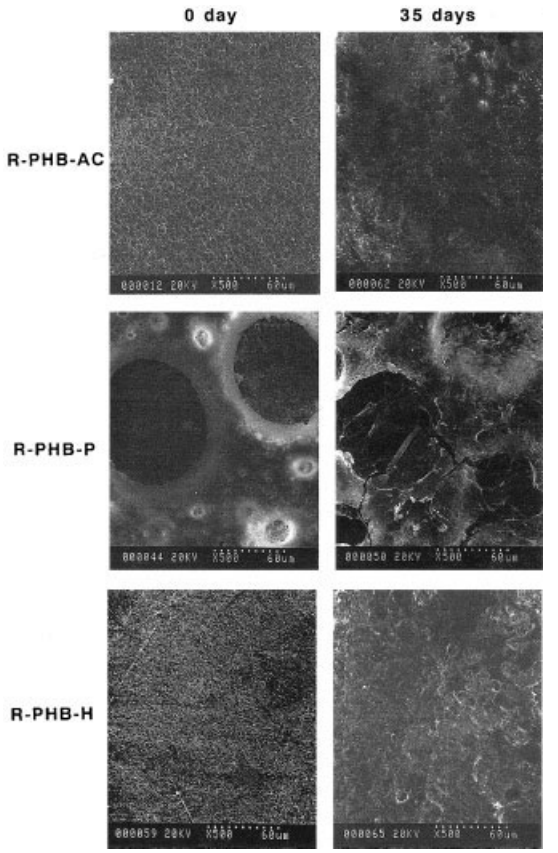

Figure 4 SEM photographs of the as-cast nonporous, porous, and alkali-treated *R*-PHB films before and after biodegradation in controlled seawater for 35 days. The length between the left and right dots in the photographs is 60 μm.

CONCLUSIONS

Pore formation and increased surface hydrophilicity powerfully enhanced the biodegradabilities of PCL and *R*-PHB films in seawater. Moreover, GPC measurements could trace the biodegradation in seawater when the biodegradation proceeded to a great extent.

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