# Biodegradable Polymers Based on Renewable Resources VIII. Environmental and Enzymatic Degradability of Copolycarbonates Containing 1,4 : 3,6-Dianhydrohexitols

**Makito Yokoe,[1] Keigo Aoi,[1] Masahiko Okada[2]**

[1]*Graduate School of Bioagricultural Sciences, Nagoya University, Chikusa-ku, Nagoya 464-8601, Japan*
[2]*College of Bioscience and Biotechnology, Chubu University, 1200 Matsumoto-cho, Kasugai, Aichi 487-8501, Japan*

Received 2 November 2004; accepted 3 April 2005
DOI 10.1002/app.22339
Published online in Wiley InterScience (www.interscience.wiley.com).

**ABSTRACT:** Environmental and enzymatic degradations were investigated on a series of copolycarbonates consisting of equimolar amounts of 1,4 : 3,6-dianhydrohexitols (1,4 : 3,6-dianhydro-D-glucitol (**1a**) and 1,4 : 3,6-dianhydro-D-mannitol (**1b**)) and alkylene diols (1,4-butanediol, 1,6-hexanediol, 1,8-octanediol, and 1,10-decanediol) or oligo(ethylene glycol)s (di-, tri-, and tetraethylene glycols). Fourteen different copolycarbonates with number average molecular weights in the range of 1.1–4.2 $\times$ $10^4$ were prepared by solution polycondensation as described in our previous article. Biodegradability of the copolycarbonates was assessed by soil burial degradation tests in composted soil at 27 °C and by enzymatic degradation tests in a phosphate buffer solution at 37 °C. In general, biodegradability of the copolycarbonates increased with increasing chain lengths of the methylene groups of alkylene diols or of the oxyethylene groups of the oligo(ethylene glycol)s. SEM observations of the film surfaces of polymers recovered from soil burial indicated that the copolycarbonates were degraded by microorganisms in soil. In enzymatic degradation, the copolycarbonates containing alkylene diol components showed high degradability with *Pseudomonas* sp. lipase, whereas the copolycarbonates containing oligo(ethylene glycol) components were not degraded at all. The enzymatic degradability of the copolycarbonates is discussed with reference to the geometrical structure around the carbonate linkages and the microstructure and hydrophobicity of the polymer chains.
 J Appl Polym Sci 98: 1679–1687, 2005

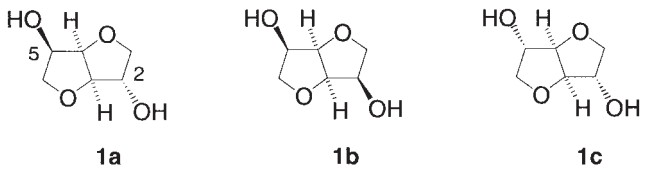
**Key words:** biodegradability; polycarbonate; renewable resources; dianhydrohexitol; soil burial degradation

## INTRODUCTION

Biodegradable polymers have gained considerable attention in recent years due to increasing interest in their environmental, biomedical, and agricultural applications.[1–5] In particular, biodegradable polymers have been investigated from the standpoint of environmental sustainability with a target of effectively utilizing renewable biomass resources as raw materials.[6–8] For example, cornstarch-based poly(L-lactic acid) has received much attention as a carbon-neutral material and is widely used in packaging film, containers, fibers for clothing, and other applications.

Among a wide variety of candidate compounds based on plant-based biomass resources, 1,4 : 3,6-dianhydrohexitols, i.e., 1,4 : 3,6-dianhydro-D-glucitol (**1a**), 1,4 : 3,6-dianhydro-D-mannitol (**1b**), and 1,4 : 3,6-dianhydro-L-iditol (**1c**) as shown in Structure 1, are full of promise in applications such as drug intermediates, nonionic surfactants, plasticizers, and polymer components.[9,10] In particular, compounds **1a** and **1b** are readily available from D-glucose and D-mannose, respectively, by hydrogenation followed by acid-catalyzed intramolecular dehydration.[11] Compound **1c** can be derived from L-idose or L-fructose, but due to the rarity of these sugars, **1c** is actually obtained from **1b** via Mitsunobu reaction. These carbohydrate-based stereoisomeric diols **1a**–**1c** have been used for synthesizing polymers and their biodegradation behaviors have been investigated in detail. Since the first synthesis of polyesters containing dianhydrohexitols by Thiem and Lüders,[12] a broad variety of polymers containing dianhydrohexitols has been synthesized by several research groups.[13–25] We have reported the syntheses of polyesters,[18–21] poly(ester amide)s,[23] and poly(ester carbonate)s[25] based on dianhydrohexitols and have found that the polymers are easily degraded under environmental conditions and in the presence of enzymes in phosphate buffer solution. Recently, we synthesized copolycarbonates by polycondensations of several combinations of 2,5-bis((*p*-nitro)phenoxycarbonyl) derivatives of dianhydrohexitols **1a** and **1b**

*Correspondence to:* M. Okada (okada-m@isc.chubu.ac.jp).
Contract grant sponsor: Ministry of Education, Culture, Sports, Science, and Technology; contract grant number: 11217208
Contract grant sponsor: Japan Society for the Promotion of Science; contract grant number: 16550113.



**Structure 1**

with alkylene diols (1,4-butanediol, 1,6-hexanediol, 1,8-octanediol, and 1,10-decanediol). On the basis of the NMR analysis of the resulting polymers, we demonstrated that the copolycarbonates of two different microstructures, in which equimolar amounts of dianhydrohexitol and alkylene diol moieties were distributed randomly or alternately, respectively, along the polymer chain, were successfully prepared under proper selection of the polymerization conditions.[26]

Previous studies concerning degradation of polycarbonates were mostly performed with aliphatic polycarbonates and their copolymers. For example, Nakano and coworkers[27,28] reported that poly(ethylene carbonate) (PEC) underwent rapid enzyme-mediated bioabsorption *in vivo*, and introduction of a methyl substituent, i.e., poly(1,2-propylene carbonate), completely suppressed the enzymatic attack. Pitt and colleagues[29] studied the *in vitro* and *in vivo* degradation of poly(trimethylene carbonate) (PTMC) and demonstrated that the enzymatic degradability of PTMC was lower than that of PEC. Biodegradability of PTMC was also investigated by Albertsson and Eklund[30] and Kricheldorf and Weegen-Schulz[31] However, biodegradation of polycarbonates based on carbohydrate derivatives has scarcely been reported so far.

The object of the present study is to examine the degradation behavior of the copolycarbonates containing dianhydrohexitols as one of the diol components and to discuss the effects of their structure and thermal property on their biodegradability in soil and under enzymatic hydrolysis conditions.

## EXPERIMENTAL

### Materials

Commercially available 1,4 : 3,6-dianhydro-D-glucitol (**1a**) (Tokyo Kasei Kogyo) and 1,4 : 3,6-dianhydro-D-mannitol (**1b**) (Aldrich) were purified by repeated recrystallization from chloroform and chloroform/hexane (2 : 1, v/v), respectively. 4-(Dimethylamino)pyridine (DMAP) was purified by recrystallization from toluene. Diethylene glycol, triethylene glycol, and tetraethylene glycol were purified by distillation under reduced pressure. All other liquid reagents and solvents were dried and purified by distillation. 1,4 : 3,6-Dianhydro-2,5-bis-*O*-(*p*-nitorophenoxycarbonyl)-D-glucitol (**2a**) and 1,4 : 3,6-dianhydro-2,5-bis-*O*-(*p*-nitrophenoxycarbonyl)-D-mannitol (**2b**) were prepared according to the method described previously.[26]

### Measurements

The number and weight average molecular weights of the copolycarbonates were estimated by size-exclusion chromatography (SEC) using chloroform as eluent at 40 °C. The SEC system (Tosoh) was equipped with TSK-GEL G2000$H_{XL}$, G3000$H_{XL}$, and G5000$H_{XL}$ columns and standard polystyrene was used for calibration. $^1$H- and $^{13}$C-NMR spectra were taken at 300 and 75.4 MHz, respectively, using a Varian 3–2 Gemini 2000 with deuteriochloroform as solvent and tetramethylsilane as an internal reference. Differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) were performed with Seiko Instruments DSC 6200 and TGA 6000 systems, respectively. DSC analyses were carried out at a heating rate of 5 °C/min under a nitrogen atmosphere. Scanning electron microscopy (SEM) observations of the film surfaces of copolycarbonates recovered from soil burial were carried out using a Hitachi *S*-2150 scanning electron microscope after Pt coating of the films. Total organic carbon (TOC) concentrations in aqueous solutions after enzymatic degradation of copolycarbonates were determined with a Shimadzu TOC-5000A.

### Polymer synthesis

Preparation of copolycarbonates was carried out by the method reported previously.[26] Briefly, bis(*p*-nitrophenyl) carbonate **2a** or **2b**, an equimolar amount of diol, and 10 mol % of DMAP were weighed in a glass vessel. The mixture was dissolved in sulfolane, and *N*,*N*-diisopropylethylamine (DIPEA) was added to the solution. The solution was heated to 60 °C for 24 h under a nitrogen atmosphere. The reaction mixture was diluted with chloroform and poured into methanol containing a small amount of 1*N* hydrochloric acid to precipitate polymer. The isolated polymer was purified by repeated reprecipitation using chloroform and methanol as a solvent–precipitant pair and then dried *in vacuo*.

### Preparation of model compounds

To a solution of **1a** (0.67 g, 4.55 mmol) and pyridine (7.35 g, 9.29 mmol) dissolved in dry chloroform (10 mL) was added dropwise *n*-octyl chloroformate (1.77 g, 9.17 mmol) at 0 °C. After 1 h, the reaction mixture was allowed to warm to room temperature and stirred for 5 h. The mixture was then repeatedly washed with 1*N* hydrochloric acid and distilled water and dried over anhydrous $Na_2SO_4$. After removal of the salt by filtration, the filtrate was evaporated to afford color-

less oil. The product was purified by flash column chromatography (hexane/ethyl acetate = 10/1, v/v) to yield model compound **7a** (yield: 72%). Model compound **7b** was prepared from **1b** by the same procedure (yield: 98%).

*7a.* $^1$H-NMR (300 MHz, $CDCl_3$): δ (ppm) = 5.16–5.15 (m, 1H, H-2), 5.14–5.11 (t, 1H, H-5, $J_{5,4} = J_{5,6b} = 5.4$ Hz), 4.97–4.94 (t, 1H, H-4, $J_{4,5} = J_{4,3} = 5.4$ Hz), 4.62–4.61 (d, 1H, H-3, $J_{3,4} = 4.8$ Hz), 4.25–4.19 (m, 4H, -OCOOC$H_2$-), 4.17–4.07 (m, 2H, H-1), 4.03–3.92 (m, 2H, H-6), 1.81–1.67 (m, 4H, -$OCH_2CH_2$-), 1.50–1.25 (br, 20H, -$OCH_2CH_2(CH_2)_5CH_3$), 1.05–0.92 (m, 6H, -$OCH_2CH_2(CH_2)_5CH_3$).

*7b.* $^1$H-NMR (300 MHz, $CDCl_3$): δ (ppm) = 5.04–4.95 (m, 2H, H-2 and H-5), 4.74–4.71 (m, 2H, H-3 and H-4), 4.18–4.12 (t, 4H, -OCOOC$H_2$-, $J$ = 5.1 Hz), 4.10–3.87 (m, 4H, H-1 and H-6), 1.72–1.63 (m, 4H, -$OCH_2CH_2$-), 1.36–1.28 (br, 20H, -$OCH_2CH_2(CH_2)_5CH_3$), 0.90–0.86 (m, 6H, -$OCH_2CH_2(CH_2)_5CH_3$).

### Soil burial test

Soil burial degradation tests were undertaken on polycarbonate films (10 × 10 mm; thickness, 100–150 μm). The films were prepared by casting a solution onto a glass plate. They were buried 1 cm beneath the surface in soil stored in a desiccator in which the relative humidity was adjusted to 70–80% with a saturated aqueous ammonium nitrate solution. The soil, which had been composted for more than 10 years, was taken from the Nagoya University farm. The desiccator was placed in a room thermostated at 27 °C. At specified intervals during the degradation process, the films were taken out, washed with water, and dried. When soil adhered to the sample surface could not be removed by washing, the samples were extracted with chloroform and the soil was separated by filtration. After the evaporation of the chloroform, the residue was dried to a constant weight under reduced pressure. The recovered polymer was characterized by weight and SEC measurements.

### Enzymatic degradation

The enzymes used in this investigation were *Pseudomonas* sp. lipoprotein lipase, *Streptmyces rochei* carboxylesterase (Wako Chemical), *Pseudomonas* sp. lipase, porcine pancreas lipase, porcine liver esterase, *Candida rugosa* lipase, *Tritirachium album* proteinase K, *Bacillus subitilis* proteinase (Sigma Chemical), and *Rhizopus delemer* lipase (Seikagaku Kogyo). All enzymes were used as received. Each polymer sample (25 mg) was added to a test tube with a screw cap, and a small amount of chloroform was added to dissolve the polymer. By rotation of the test tube and slow evaporation of the solvent, the bottom part (height from the bottom, about 1.5 cm) of the inner wall of the test tube was coated with a thin film. After being dried, 2 mL of 1/15*M* phosphate buffer solution (pH 7.0) containing enzyme (25 or 250 units) was added to the test tube. The test tube was incubated for 24 h at 37 °C. As blank tests, tubes containing either the polymer only or the enzyme only were incubated under the same conditions. The reaction mixture was filtered through a Millipore filter (pore size, 0.45 μm). A 100-μL aliquot of the filtrate was taken and diluted with distilled water to 10 mL. The TOC concentrations in the aqueous solutions were then determined.

## RESULTS AND DISCUSSION

### Synthesis of copolycarbonates

Copolycarbonates **3a**-**3d** or **4a**-**4d** were prepared by solution polycondensations of **2a** or **2b** with four different alkylene diols (1,4-butanediol, 1,6-hexanediol, 1,8-octanediol, and 1,10-decanediol) according to the procedure published previously, as shown in Scheme 1.[26] The synthetic method provides copolycarbonates with alternating structure of dianhydrohexitol and alkylene diol units. Table I summarizes some of the characterization data of the alternating copolycarbonates used for the biodegradation tests. The number average molecular weights ($M_n$'s) of copolycarbonates **3a**–**3d** and **4a**–**4d** were in the range of 1.1–3.3 × $10^4$ as estimated by SEC.

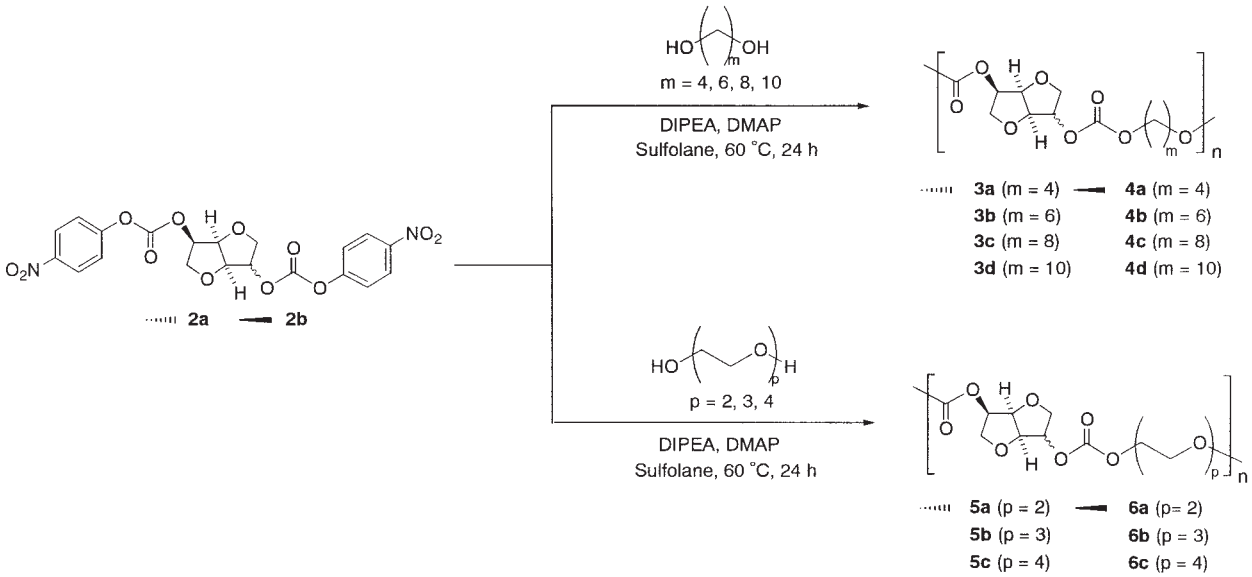
Copolycarbonates **5a**–**5c** and **6a**–**6c** were synthesized by the polycondensations of **1a** and **1b**, respectively, with oligo(ethylene glycol)s (diethylene glycol, triethylene glycol, and tetraethylene glycol) (Scheme 1). The results of the polycondensations and the thermal properties of the product copolycarbonates **5a**–**5c** and **6a**–**6c** are presented in Table II. In both series, the copolycarbonates containing oligo(ethylene glycol) were obtained in high yields with $M_n$'s up to 4.2 × $10^4$.

According to DSC measurement, all polycarbonates described above were amorphous and the glass transition temperatures ($T_g$'s) decreased with increasing methylene or oxyethylene chain length of alkylene diols or oligo(ethylene glycol)s, respectively.

### Biodegradability of copolycarbonates

#### Soil burial degradation

Figure 1(a) shows the results of soil burial degradation at 27 °C for the series of copolycarbonates **3a**–**3d** based on **1a** and alkylene diols. The films of copolycarbonates **3c** and **3d** completely disappeared after soil burial for 35 days. The copolycarbonates became less degradable with decreasing methylene chain length of the alkylene diol components. In fact, copolycarbonate **3a** derived from **1a** and 1,4-butanediol was only slightly degraded even after soil burial for 50 days. The lower degradability of **3a** seems to arise from the higher $T_g$




**Scheme 1**

compared with other copolycarbonates. The $T_g$ of **3a** (59 °C) is higher than the temperature at which the degradation experiment (27 °C) was carried out. As is evident from the $T_g$ data in Table I, only copolycarbonate **3a** is in a glassy state at the experimental temperature. Hence, it seems likely that **3a** is less susceptible to attack by enzymes secreted from microorganisms in soil, due to the lesser mobility of the polymer chain, compared with other copolycarbonates **3b**–**3d**.

The change in the recovered polymer weight during the soil burial degradation of the series of copolycarbonates **4a**–**4d** based on **1b** and alkylene diols is depicted in Figure 1(b). A trend similar to that found for **3a**–**3d** was observed in the soil burial degradation of **4a**–**4d**, i.e., the copolycarbonates containing **1b** were less degradable with decreasing methylene chain length of the alkylene diol components. Copolycarbonates **4c** and **4d** containing 1,8-octanediol and 1,10-decanediol, respectively, were completely degraded in soil within 25 days.

Figure 2(a) shows a SEM micrograph of the film surface of copolycarbonate **3b** after soil burial test for 35 days. Actinomycetes eroding the surface of the film were observed. Figure 2(b) is a SEM micrograph of the film surface of copolycarbonate **4b** after soil burial for 25 days, indicating that the film surface was eroded by filamentous fungi. These SEM observations strongly support that these polycarbonates are biodegradable in soil.

The soil burial degradation of the series of the copolycarbonates based on dianhydrohexitols and oligo(ethylene glycol)s was also studied in a similar manner. Figure 3(a,b) depicts the change in residual poly-

**TABLE I**
**Synthesis and Characterization of Copolycarbonates from Dianhydrohexitols and Alkylene Diols[a]**

| Polymer | $m$[b] | Yield (%) | $M_n$[c], ($\times 10^4$) | $M_w/M_n$[c] | $T_g$[d] (°C) | $T_m$[d] (°C) | $T_{d5}$[e] (°C) |
|---|---|---|---|---|---|---|---|
| **3a** | 4 | 98 | 2.4 | 1.8 | 59 | n.d.[f] | 311 |
| **3b** | 6 | 97 | 2.3 | 1.7 | 20 | n.d. | 323 |
| **3c** | 8 | 93 | 2.3 | 1.8 | 23 | n.d. | 329 |
| **3d** | 10 | 95 | 3.4 | 2.1 | 7 | n.d. | 331 |
| **4a** | 4 | 87 | 1.1 | 1.8 | 39 | n.d. | 293 |
| **4b** | 6 | 91 | 2.1 | 1.7 | 27 | n.d. | 314 |
| **4c** | 8 | 84 | 1.7 | 1.8 | 16 | n.d. | 322 |
| **4d** | 10 | 86 | 1.8 | 2.1 | 6 | n.d. | 329 |

[a] All reactions were carried out in sulfolane with 2 equiv of DIPEA and 10 mol% of DMAP at 60 °C for 24 h.
[b] Number of methylene groups in alkylene diols.
[c] Estimated by SEC in chloroform (polystyrene standard).
[d] Determined by DSC at the heating rate of 5 °C/min.
[e] $T_{d5}$ denotes 5% weight loss temperature determined by TGA.
[f] Not detected.

**TABLE II**
**Synthesis and Characterization of Copolycarbonates from Dianhydrohexitols and Oligo(ethylene glycol)s**

| Polymer | $p$[b] | Yield (%) | $M_n$[c] ($\times 10^4$) | $M_w/M_n$[c] | $T_g$[d] (°C) | $T_m$[d] (°C) | $T_{d5}$[e] (°C) |
|---|---|---|---|---|---|---|---|
| **5a** | 2 | 96 | 3.0 | 2.0 | 48 | n.d.[f] | 315 |
| **5b** | 3 | 97 | 4.2 | 2.1 | 18 | n.d. | 324 |
| **5c** | 4 | 86 | 3.8 | 1.9 | 10 | n.d. | 324 |
| **6a** | 2 | 95 | 2.1 | 2.0 | 45 | n.d. | 319 |
| **6b** | 3 | 91 | 3.4 | 2.0 | 16 | n.d. | 312 |
| **6c** | 4 | 81 | 2.8 | 1.9 | 9 | n.d. | 292 |

[a] All reactions were carried out in sulfolane with 2 equiv of DIPEA and 10 mol% of DMAP at 60 °C for 24 h.
[b] Number of oxyethylene groups of oligo(ethylene glycol)s.
[c] Estimated by SEC in chloroform (polystyrene standard).
[d] Determined by DSC at the heating rate of 5 °C/min.
[e] $T_{d5}$ denotes 5% weight loss temperature determined by TGA.
[f] Not detected.

mer weight of **5a**–**5c** and **6a**–**6c**, respectively, during the soil burial tests. Both series of the copolycarbonates containing the oligo(ethylene glycol)s showed similar degradation behavior in soil. Copolycarbonates **5c** and **6c** containing tetraethylene glycol vanished completely in the soil within 10 days, in sharp contrast to the other copolycarbonates that degraded rather slowly. The rapid weight losses of **5c** and **6c** are not due to their dissolution in water contained in the soil, because any residual polymer was not found in the chloroform extracts even after thorough extraction of the soil with chloroform. However, it is not clear at present why the rate of degradation was so markedly different between the copolycarbonates containing tetraethylene glycol and those containing di- or triethylene glycol moieties.

Figure 4(a,b) shows SEM micrographs of the film surfaces of **5a** and **6b** recovered after soil burial for 40 and 10 days, respectively. Many bacteria and actinomycetes eroding the films can be clearly seen in Figure 4(a,b), respectively, although the weight losses of these sample films were less than 20%. These findings clearly demonstrate that the copolycarbonates containing diethylene glycol or triethylene glycol were degraded by microorganisms in soil, although rather slowly.

## Enzymatic degradation of copolycarbonates

Enzymatic degradation of the copolycarbonates was monitored by TOC measurements of phosphate buffer solutions containing water-soluble degradation products. The TOC values were corrected for concurrent nonenzymatic hydrolysis by subtracting the TOC values of the blank test without the enzyme from the observed values with enzyme.

Figure 5 graphically represents the results of the enzymatic degradation of copolycarbonates **3a**–**3d** and **4a**–**4d** using *Pseudomonas* sp. lipase. For all these polymers, the TOC values due to nonenzymatic hydrolysis were negligibly small. Among the four different copolycarbonates based on **1a**, copolycarbonates **3b** and **3c** containing 1,6-hexanediol and 1,8-octanediol, respectively, showed somewhat higher enzymatic degradability than the other two polycarbonates **3a** and **3d** [Fig. 5(a)]. Presumably, the difference in the de-

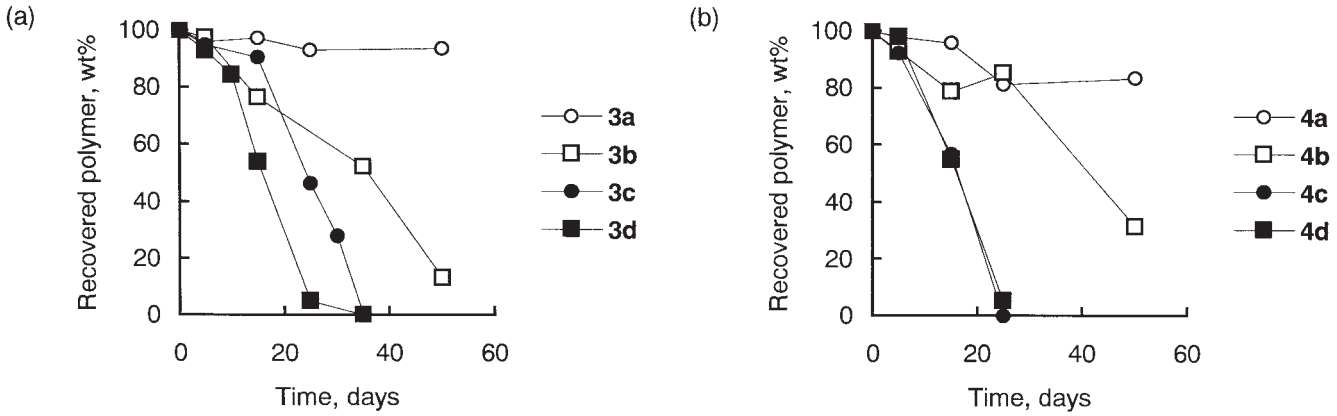



**Figure 1** Recovery (wt %) of copolycarbonates in soil burial tests. (a) **3a**–**3d**, (b) **4a**–**4d.** Film thickness, 100–150 $\mu$m; temperature, 27 °C; humidity, 70–80%.

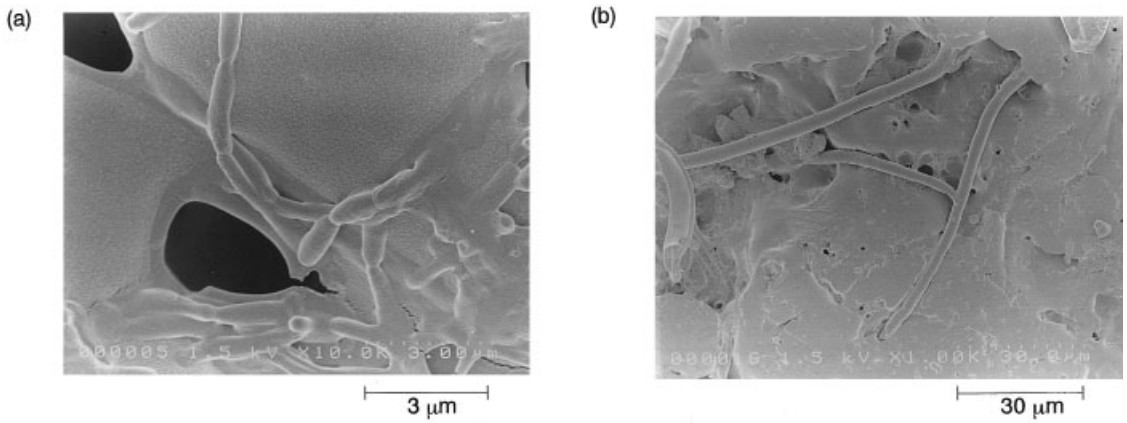



**Figure 2** Scanning electron micrographs of the copolycarbonate films. (a) **3b** recovered after soil burial for 35 days, (b) **4b** recovered after soil burial for 25 days.

gradability between these polycarbonates is ascribable to the substrate specificity of the enzyme. The enzymatic degradation of aliphatic polyesters by lipases is well known to be affected by the methylene chain length between the ester linkages. We reported previously that the polyesters prepared from **1a** and aliphatic dicarboxylic acids of the methylene chain lengths of 6 and 8 exhibited higher enzymatic degradability than the polyesters of the methylene chain lengths of 4 and 10 when *Pseudomonas* sp. lipase was used as the enzyme.[21]

The enzymatic degradability of copolycarbonates **4a–4d** was lower than that of **3a–3d** [Fig. 5(b)]. The lower enzymatic degradability of **4a–4d** can be only partly explained by the substrate specificity of the enzyme as described above, because the enzymatic degradability of **4a** was too low compared with that of **3a**. In addition to the effects of substrate specificity of the enzyme, the geometrical structure around the carbonate linkages of the copolycarbonates should affect the enzymatic degradability. That is, the copolycarbonates derived from **1b** with the two hydroxyl groups both in the *endo*-position, are sterically hindered, so that the attack by enzyme would be less likely to occur, compared with the polycarbonates derived from **1a** having one of the two hydroxyl groups fixed in the *endo*-position and the other in the *exo*-position. It appears that the shorter the methylene chain length of the alkylene diol component, the greater the effect of such steric hindrance. This point will be discussed later in a section dealing with enzymatic degradation of model compounds.

Enzymatic degradability of the copolycarbonates is also affected by the microstructures of the polymer chains. Copolycarbonates with "scrambled" structures, in which equimolar amounts of dianhydrohexitol and alkylene diol moieties were nearly randomly distributed along the polymer chains, were prepared by bulk polycondensation with 2,5-bis(phenoxycarbonyl) substituted 1,4 : 3,6-dianhydrohexitols and alkylene diols according to the method described in our previous paper.[26] We found that the scrambled co-

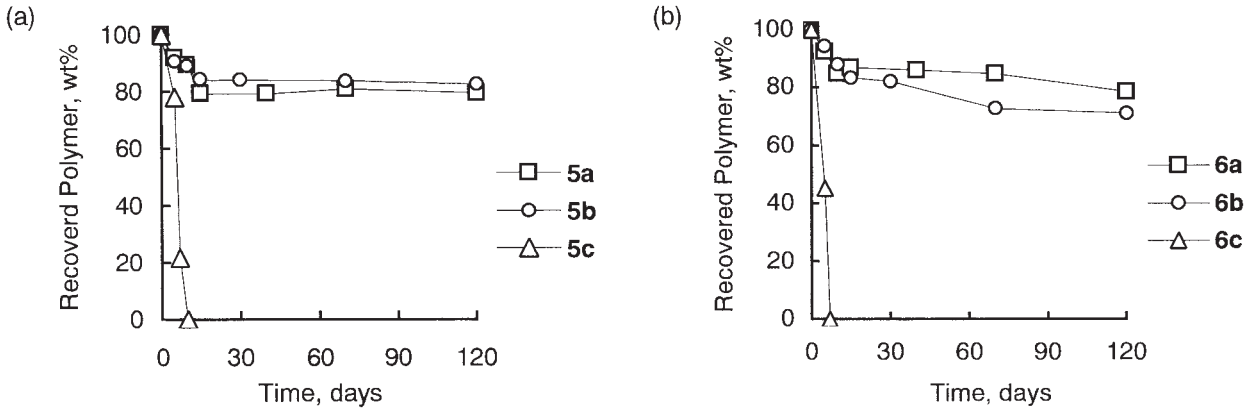



**Figure 3** Recovery (wt %) of copolycarbonates in soil burial tests. (a) **5a–5c**, (b) **6a–6c.** Film thickness, 100–150 μm; temperature, 27 °C; humidity, 70–80%.

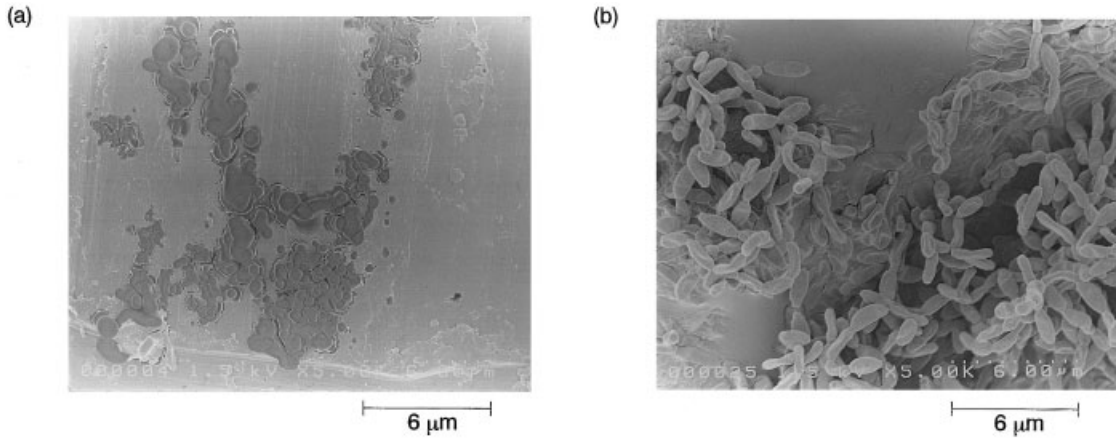



**Figure 4** Scanning electron micrographs of the copolycarbonate films. (a) **5a** recovered after soil burial for 40 days, (b) **6b** recovered after soil burial for 10 days.

polycarbonates were much less degradable by enzyme than the corresponding "well-defined" copolycarbonates consisting of alternating dianhydrohexitol and alkylene diol moieties. For example, when *Pseudomonas* sp. lipase was used as an enzyme, the TOC value of **3c** with scrambled structure after enzymatic degradation was only 340 ppm, whereas that of the corresponding well-defined **3c** was 1380 ppm under the same conditions. A similar tendency was also observed not only for the other **1a**-based copolycarbonates with different methylene chain lengths, but also for the copolycarbonates containing **1b**. Furthermore, also when porcine pancreas lipase was used, the scrambled copolycarbonates generally showed lower degradability than the corresponding alternating copolycarbonates. Considering that there is, if any, little difference in the thermal properties between the scrambled and alternating polycarbonates, it appears that the variation in the enzymatic degradability of the copolycarbonates depends on their microstructure, i.e., the sequence distribution of the dianhydrohexitol and alkylene diol units. However, it still remains to be clarified why the enzymatic degradation of the well-defined copolycarbonates is faster than that of the corresponding scrambled copolycarbonates.

Enzymatic degradability of alternating copolycarbonates **5a–5c** and **6a–6c** containing oligo(ethylene glycol)s was investigated using various enzymes. Interestingly, these copolycarbonates were hardly degraded when they were treated with *Pseudomonas* sp. lipase, *Pseudomonas* sp. lipoprotein lipase, *Candida rugosa* lipase, *Rhizopus delemer* lipase, porcine liver esterase, *Streptmyces rochei* carboxylesterase, *Tritirachium album* proteinase K, and *Bacillus subitilis* proteinase. In general, degradation of polymers by lipases proceeds via two steps, namely, adsorption of an enzyme on a hydrophobic scaffold of polymer, followed by cleavage of the polymer chain by the active site of the adsorbed enzyme. Thus, the findings that the copolycarbonates containing oligo(ethylene glycol) moi-

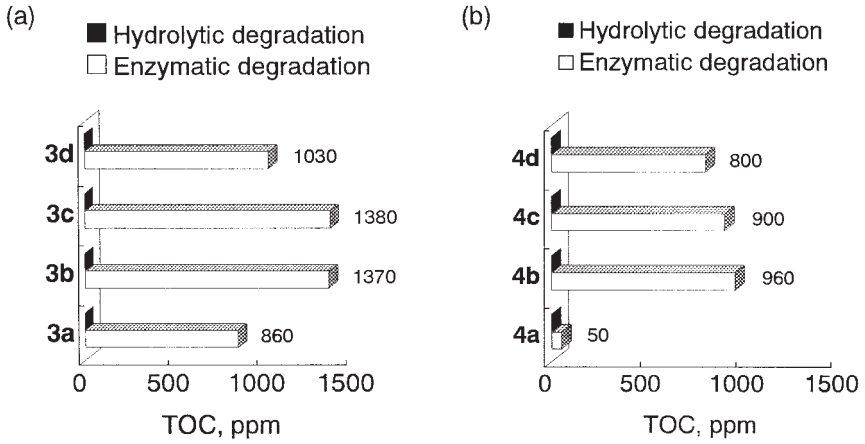
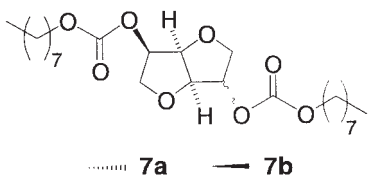



**Figure 5** Enzymatic degradation of copolycarbonates by *Pseudomonas* sp. lipase. (a) **3a–3d** and (b) **4a–4d.** Conditions: enzyme conc., 12.5 unit/mL; sample, 25 mg; phosphate buffer, pH 7.0, 2 mL; temp., 37 °C; incubated for 24 h.




**Structure 2**

eties hardly underwent enzymatic degradation by lipases are explained by the lack of an appropriate hydrophobic scaffold on the oxyethylene chains, in contrast to the methylene chains.

### Enzymatic degradation of model compounds

To find a clue to clarify the enzymatic degradation mechanism of the copolycarbonates, model compounds **7a** and **7b** in Structure 2 were prepared by the reactions of **1a** and **1b**, respectively, with *n*-octyl chloroformate. The model compounds were enzymatically hydrolyzed by porcine pancreas lipase in phosphate buffer at 37 °C for 72 h, and the resulting water-soluble products were examined by $^1$H-NMR and MS. Figure 6 shows the $^1$H-NMR spectrum of the isolated degradation product of model compound **7a**. It is noteworthy that the carbonate linkage remained at the *O*(2)-position, i.e., the *exo*-position, of the 1,4 : 3,6-dianhydro-D-glucitol units, whereas no carbonate linkages remained at the *O*(5)-position, i.e., the *endo*-position. In other words, the enzyme hydrolyzed the carbonate linkage of **7a** preferentially at the *endo*-position. It seems reasonable to assume that this regiospecific cleavage arises from the steric requirements of the binding site of the enzyme.

In contrast, model compound **7b** was only slightly degraded by the lipase under the same conditions, and most of the model compound molecules were recovered. The findings support the above-mentioned assumption that the copolycarbonates with the carbonate linkages fixed in the *endo*-position of 1,4 : 3,6-dianhydro-D-mannitol moieties are sterically hindered, so that the carbonate linkages are less likely to be attacked by enzymes. Needless to say, the results on the enzymatic degradation of the model compounds do not necessarily exclude the enzymatic cleavage of the carbonate linkage fixed in the *exo*-position of a 1,4 : 3,6-dianhydro-D-glucitol moiety nor the enzymatic cleavage of the *endo*-carbonate linkages of a 1,4 : 3,6-dianhydro-D-mannitol moiety. Rather, considering the results on the enzymatic degradation of the copolycarbonates described in the foregoing section, it would be more appropriate to assume that these carbonate linkages also undergo enzymatic hydrolysis, although very sluggishly.

## CONCLUSIONS

According to the degradation tests in composted soil described above, all copolycarbonates containing 1,4 : 3,6-dianhydrohexitol and aliphatic diol moieties are potentially biodegradable, and their biodegradability increases with increasing chain lengths of the methylene groups of alkylene diols or of the oxyethylene groups of oligo(ethylene glycol)s. The enzymatic degradation tests of the copolycarbonates and model compounds have revealed that not only the geometrical structures around the carbonate linkages to the 1,4 : 3,6-dianhydrohexitol moieties (i.e., *exo*, *endo*- or *endo*, *endo*-) but also the microstrucure of the copolycarbonate chains (i.e., random or alternating arrangement of the two different components) are important factors in determining their biodegradability. The present work leads us to conclude that the environmental and en-

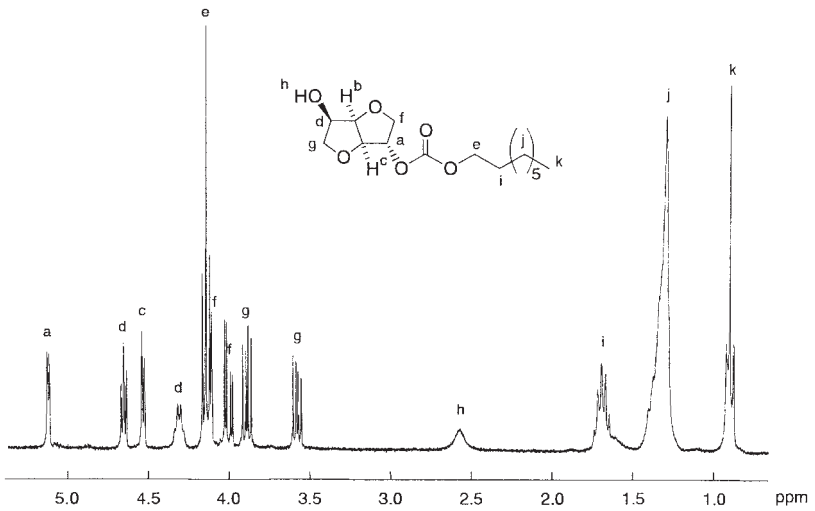



**Figure 6** $^1$H-NMR spectrum of the degradation product of model compound **7a** ($CDCl_3$, 300 MHz).

zymatic biodegradability of the copolycarbonates based on 1,4 : 3,6-dianhydrohexitols can be widely varied and controlled by properly selecting diol comonomers and polycondensation methods. The mechanism of the biodegradation of the copolycarbonates remains a subject of future investigation.

## References


1. Okada, M. Prog Polym Sci 2002, 27, 87.
2. Gross, R. A.; Kalra, B. Sience 2002, 297, 803.
3. Amass, W.; Amass, A.; Tighe, B. Polym Int 1998, 47, 89.
4. Chandra, R.; Rustgi, R. Prog Polm Sci 1998, 23, 1273.
5. Chiellini, E.; Solaro, R. Adv Mater 1996, 8, 305.
6. Narayan, R. In Emerging Technologies for Materials and Chemicals from Biomass; Rowell, R. W.; Schultz, T. P.; Narayan, R., Eds.; ACS Symposium Series 476; American Chem Society: Washington, DC, 1992; p 1.
7. Dirlikov, S. K. In Emerging Technologies for Materials and Chemicals from Biomass; Rowell, R. W.; Schultz, T. P.; Narayan, R., Eds.; ACS Symposium Series 476; American Chem Society: Washington, DC, 1992; p 231.
8. Lindblad, M. S.; Liu, Y.; Albertsson, A.-C.; Ranucci, E.; Karlsson, S. Adv Polym Sci 2002, 157, 139.
9. Stoss, P.; Hemmer, R. Adv Carbohydr Chem Biochem 1991, 49, 93.
10. Kricheldorf, H. R. J Macromol Sci Rev Macromol Chem Phys C 1997, 37, 599.
11. Wiggins, L. F. Adv Carbohydr Chem 1950, 5, 191.
12. Thiem, J.; Lüders, H. Polym Bull 1984, 11, 365.
13. Thiem, J.; Lüders, H. Makromol Chem 1986, 187, 2775.
14. Storbeck, R.; Rehan, M.; Ballauff, M. Makromol Chem 1993, 194, 53.
15. Braun, M.; Bergman, M. J Prakt Chem 1992, 334, 298.
16. Majdodub, M.; Loupy, A.; Fléche, G. Eur Polym Mater 1994, 30, 1431.
17. Cognet-Georjon, E.; Mechin, F.; Pascault, J.-P. Macromol Chem Phys 1995, 196, 3753.
18. Okada, M.; Okada, Y.; Aoi, K. J Polym Sci Part A Polym Chem 1995, 33, 2813.
19. Okada, M.; Okada, Y.; Tao, A.; Aoi, K. J Appl Polym Sci 1996, 62, 2257.
20. Okada, M.; Tachikawa, K.; Aoi, K. J Appl Polym Sci 1999, 74, 3342.
21. Okada, M.; Tsunoda, K.; Tachikawa, K.; Aoi, K. J Appl Polym Sci 2000, 77, 338.
22. Gomurashvili, Z.; Kricheldorf, H. R.; Katsarava, R. J Macromol Sci Pure Appl Chem A 2000, 37, 215.
23. Okada, M.; Yamada, M.; Yokoe, M.; Aoi, K. J Appl Polym Sci 2001, 81, 2721.
24. Bachmann, F.; Reimer, J.; Ruppenstein, M.; Thiem, J. Macromol Chem Phys 2001, 202, 3410.
25. Okada, M.; Yokoe, M.; Aoi, K. J Appl Polym Sci 2002, 86, 872.
26. Yokoe, M.; Okada, M.; Aoi, K. J Polym Sci Part A Polym Chem 2003, 41, 2312.
27. Kawaguchi, T.; Nakano, M.; Juni, K.; Yoshida, Y. Chem Pharm Bull 1983, 31, 1440.
28. Nakano, M. J Synth Org Chem Japan (Yuki Gosei Kagaku Kyoukaishi) 1984, 42, 665.
29. Zhu, K. Z.; Hendren, R. W.; Jensen, K.; Pitt, C. G. Macromolecules 1991, 24, 1736.
30. Albertsson, A. C.; Eklund, M. J Appl Polym Sci 1995, 57, 87.
31. Kricheldorf, H. R.; Weegen-Schulz, B. J Polym Sci A Polym Chem 1995, 33, 2193.