Biodegradability Study of Copolyesteramides Based on Diacid Chlorides, Diamines, and Diols

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ABSTRACT: Copolyesteramides were synthesized by polycondensation of diacid chlorides, diamines, and diols of varying methylene group chain length. The composition of the synthesized polymer was determined with nuclear magnetic resonance (NMR) analysis. The biodegradation of the polymers was evaluated both with enzymatic hydrolysis and activated sludge test. The polymers were hydrolyzed with lipases from *Rhizopus arrhizus*, *Rhizopus delemar*, *Candida cylindracea*, and an esterase from hog liver, whereas trypsin and α-chymotrypsin did not have any apparent effect upon them. Enzymatic hydrolysis was found to be greatly affected by the polymer composition and structure. The degradation results obtained from the activated sludge test were in satisfactory agreement with those from enzymatic hydrolysis. The water-soluble parts of hydrolyzed products were more susceptible to degradation of their ester bonds rather than their amide bonds. © 2002 Wiley Periodicals, Inc. J Appl Polym Sci 85: 774–784, 2002

Key words: copolyesteramides; biodegradation; enzymatic hydrolysis; activated sludge

INTRODUCTION

A great part of plastic waste originates from packaging materials such as rubbish bags, agricultural mulch films, food wrappers, and containers. A need for a polymer that breaks down in a controlled manner has been considerably increasing as an approach to solving the acute environmental pollution problem. Biodegradable macromolecules can be tailored specifically for controlled degradation under the inherent environmental stress in biological systems either unaided or by enzyme-assisted mechanisms.[1–3] Aliphatic polyesters are biodegradable and can be potentially used in biomedical, agricultural, and packaging applications, whereas polyamides have not found extensive applications in natural environment because of their lack of biodegradability.[4–24] The introduction of an ester component has been suggested as a promising approach toward increasing the biodegradability of polyamides. Therefore, the synthesis of aliphatic copolyesteramides has been currently one of the main research topics in the biodegradability field. We previously reported the study of the synthesis and properties of novel oligo(esteramide)s based on adipic acid, sebacic acid, octadecanedioic acid, 1,6-hexanediamine, and ϵ-caprolactone.[25–27] Although the study on

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the biodegradability of a polyesteramide derived from α-amino acid has been reported, the effect of the polymer structure on biodegradability, to our knowledge, has not been thoroughly studied yet.[28]

This research aims at investigating the susceptibility to biodegradation of copolyesteramides of varying methylene group chain lengths to compare biodegradability. The effect of several enzymes on the copolymer structure together with its biodegradability in activated sludge was also examined.

EXPERIMENTAL

Materials

1,6-Hexanediamine was dried under vacuum at 60°C for 2 h before use. Other diacid chlorides, diamines, and diols were used as received without any further purification. Solvents were purified by standard methods prior to use.

Measurements

^{1}H nuclear magnetic resonance (NMR) (500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded on a JEOL JNM A-500 spectrometer. The spectra were obtained from chloroform-*d* or D_2O solutions at room temperature. The total organic carbon concentration of aqueous solution (TOC) was measured with a Shimadzu TOC-5000 Total Organic Carbon Analyzer in duplicate. The TOC data stand for average values and were corrected appropriately by taking into account the blank levels.

Polymerization of Polyesters and Copolyesteramides

Polyesters were synthesized by employing bulk polymerization of diacid chlorides and diols. The polymerization of copolyesteramides was carried out by employing interfacial polymerization method. Oligomers having acid chloride terminal groups were first prepared by the polycondensation of two or more equivalents of diacid chlorides with one equivalent of diols. Consequently, diamine was added dropwise to the oligomer solution to produce the corresponding copolymers. The feed ratio of diacids, diols, and diamines varied to control the polymer compositions. The amounts of diacids were the same as the total amounts of diols and diamines. The composition of copolyesteramides was determined with ^{1}H NMR and ^{13}C NMR analysis.

In Scheme 1, the obtained products in Step 1 were the mixture of Cl—[—$CO(CH_2)_aCOO(CH_2)_b$O—]$_m$—$CO(CH_2)_aCO$—Cl and Cl—$CO(CH_2)_aCO$-Cl; in actuality, however, they would be herein represented as follows in the scheme.

$$(m + n)\mathrm{ClCO}\text{—}(\mathrm{CH_2})_a\text{—}\mathrm{COCl} + m\mathrm{HO}\text{—}(\mathrm{CH_2})_b\text{—}\mathrm{OH} \rightarrow \mathrm{Cl}\text{—}[\text{—}\mathrm{CO}(\mathrm{CH_2})_a \times \mathrm{COO}(\mathrm{CH_2})_b\mathrm{O}\text{—}]_m\text{—}\mathrm{CO}(\mathrm{CH_2})_a\mathrm{CO}\text{—}\mathrm{Cl} \quad \text{(Step 1)}$$

$$\mathrm{Cl}\text{—}[\text{—}\mathrm{CO}(\mathrm{CH_2})_a\mathrm{COO}(\mathrm{CH_2})_b\mathrm{O}\text{—}]_m\text{—}\mathrm{CO}(\mathrm{CH_2})_a \times \mathrm{CO}\text{—}\mathrm{Cl} + n\mathrm{H_2N}\text{—}(\mathrm{CH_2})_c\text{—}\mathrm{NH_2} \rightarrow [\text{—}\mathrm{CO}(\mathrm{CH_2})_a\mathrm{COO}(\mathrm{CH_2})_b\mathrm{O}\text{—}]_m[\text{—}\mathrm{CO}(\mathrm{CH_2})_a \times \mathrm{CONH}(\mathrm{CH_2})_c\mathrm{NH}\text{—}]_n \quad \text{(Step 2)}$$

$a = 2, 3, 4; \quad b = 2, 3, 4, 6; \quad c = 2, 3, 4, 6$

Scheme 1 Polymerization of copolyesteramides.

Enzymatic Degradation

Enzymatic hydrolysis tests were conducted as follows: 25 mg of copolymer in 2 mL of phosphate buffer solution (KH_2PO_4/Na_2HPO_4, pH 7.0) was tested in the presence of 200 units (100 units in the case of esterase) of the enzyme at 37°C for 24 h. The TOC value based on the water-soluble degradation products was determined as a first indication for potential biodegradability. The biodegradability data were corrected by subtraction of both blank values: an enzyme blank level and a polymer blank level. The polymer blank level stands for standard nonenzymatic polymer hydrolyzability under the same conditions.

An aqueous portion of enzymatic hydrolysis tests was dried at 60°C overnight in an oven and subjected to NMR analysis using D_2O and solvent to determine the hydrolyzed products component.

Degradation by Activated Sludge

A schematic diagram of the apparatus for an aerobic degradation test has been presented in detail elsewhere.[29] The supernatant (30 mL, mixed liquor suspended solid (MLSS), 30 mg) of standard activated sludge and a polymer sample (0.2 g) were placed in a fermenter containing a carbon-free culture medium (500 mL, pH 7) according to

Table I Characterization of Polyesters[a]

Run	Polymer[b]	T_g (°C)	T_m (°C)	M_n[c] ($\times 10^3$)	M_w[c] ($\times 10^3$)	M_w/M_n	
1	$[—CO(CH_2)_2COO(CH_2)_2O—]_n$	−35	75	1.4	1.6	1.2	Plastic
2	$[—CO(CH_2)_2COO(CH_2)_3O—]_n$	−60	37	2.0	3.4	1.7	Paste
3	$[—CO(CH_2)_2COO(CH_2)_4O—]_n$	—	58	4.2	5.7	1.3	Plastic
4	$[—CO(CH_2)_2COO(CH_2)_6O—]_n$	−38	108	3.4	4.9	1.4	Plastic
5	$[—CO(CH_2)_4COO(CH_2)_3O—]_n$	—	28	4.2	5.6	1.3	Plastic
6	$[—CO(CH_2)_4COO(CH_2)_4O—]_n$	—	58	9.3	18.9	2.0	Plastic
7	$[—CO(CH_2)_4COO(CH_2)_6O—]_n$	—	57	9.2	15.6	1.7	Plastic

[a] Polymerization conditions: diacid chloride: diol = 1 : 1.
[b] $[—CO(CH_2)_aCOO(CH_2)_bO—]_n$; a = 2: succinyl chloride; a = 4: adipoyl chloride; b = 2: ethylene glycol; b = 3: 1,3-propanediol; b = 4: 1,4-butanediol; b = 6: 1,6-hexanediol.
[c] Molecular weight data from DSC analysis.

ASTM D5209-92. The fermenter was incubated at 37°C and aerated with 10–20 mL/min CO_2-free air under 200 rpm magnetic stirring. The evolved CO_2 was collected into a 0.5% sodium hydroxide solution in an absorber and determined by inorganic carbon concentration (IC) measurement for the alkaline solution with a TOC analyzer at every definite time until the evolution rate reached a plateau.

RESULTS AND DISCUSSION

Characterization of Polyesters and Copolyesteramides

The obtained polymers were characterized by NMR, gel permeation chromatography (GPC), and differential scanning calorimetry (DSC) analysis. The NMR spectra of polyesters showed chemical shifts of proton of (C═O)& bond; O—$\underline{CH_2}$ at 4.1 ppm and $\underline{CH_2}$— (C—O)—O at 2.3 ppm. The number average molecular weights of polyesters from GPC measurements ranged from 1.4×10^3 to 9.3×10^3, depending on polymer structure (Table I). From NMR analysis, the number average molecular weights of polymers calculated from peak intensity ratio between inner methylene protons and terminal hydroxyl or carboxyl methylene protons varied within a range from 1.3×10^3 to 7.6×10^3 in agreement with those from the GPC analysis. The difficulty of a 1:1 molar ratio adjustment between diacid chlorides and diols compounds led to low molecular weight of the polyesters synthesized.

The NMR spectra of copolyesteramides showed chemical shifts of proton of (C═O)—NH—$\underline{CH_2}$ at 3.2 ppm and $\underline{CH_2}$—(C═O)—NH at 2.2 ppm. All copolyesteramide copolymers did not dissolve in chloroform, the GPC elution solvent, so no data in terms of their molecular weight distribution could be obtained (Table II). Calculation of peak intensity between inner methylene protons and terminal carboxyl or amino methylene protons from NMR spectra revealed that the number average molecular weight of the copolyesteramides ranged from 4.9×10^3 to 10.1×10^3. However, the copolymers were characterized by high T_m as those of high polymers from polyesters. The ratio of ester/amide composition was determined from the peak intensity at chemical shift 4.1 and 3.2 ppm. Various components of ester/amide in the copolymers can be achieved by control the starting materials molar ratio. Thus, with molar ratio of 2:1:1 of $ClCO(CH_2)_4COCl/HO(CH_2)_6OH/H_2N(CH_2)_6NH_2$, 60/40 of ester/amide copolymer was produced, while the 3:1:2 molar ratio gave 33/67 copolymer.

Enzymatic Hydrolysis of Polyesters

Table III gives a synoptical presentation of the enzymatic biodegradation results of the polyesters with lipase derived from *R. arrhizus* (RA). Biodegradability of the polymers can be calculated as follows:

Polyester synthesized from a reaction of $ClCO(CH_2)_4COCl$ and $HO(CH_2)_6OH$ will be given as an example of theoretical TOC.

$$[—CO(CH_2)_4COO(CH_2)_6O—]_n;$$

$$C_{12}H_{20}O_4 = 228$$

$$C_{12} = 144$$

Table II Characterization of Copolyesteramides

Run	Polymer[a]	OH/NH	T_g (°C)	T_m (°C)	M_n[b] ($\times 10^3$)
1	$[—CO(CH_2)_4COO(CH_2)_6O—]_n$	100/0	—	57	7.6
2	$[—CO(CH_2)_4CONH(CH_2)_6NH—]_n$	0/100	—	84	nd[c]
3	$[—CO(CH_2)_4COO(CH_2)_6O—]_m[—CO(CH_2)_4CONH(CH_2)_2NH—]_n$	76/24	−52	36	6.0
4	$[—CO(CH_2)_4COO(CH_2)_6O—]_m[—CO(CH_2)_4CONH(CH_2)_3NH—]_n$	86/14	—	78	4.9
5	$[—CO(CH_2)_4COO(CH_2)_6O—]_m[—CO(CH_2)_4CONH(CH_2)_4NH—]_n$	59/41	−24	48	8.6
6	$[—CO(CH_2)_4COO(CH_2)_6O—]_m[—CO(CH_2)_4CONH(CH_2)_6NH—]_n$	60/40	—	41	7.8
7	$[—CO(CH_2)_4COO(CH_2)_6O—]_m[—CO(CH_2)_4CONH(CH_2)_6NH—]_n$	50/50	—	52	10.1
8	$[—CO(CH_2)_4COO(CH_2)_6O—]_m[—CO(CH_2)_4CONH(CH_2)_6NH—]_n$	33/67		55	6.4
9	$[—CO(CH_2)_4COO(CH_2)_2O—]_m[—CO(CH_2)_4CONH(CH_2)_6NH—]_n$	55/45	—	92	4.9
10	$[—CO(CH_2)_4COO(CH_2)_2O—]_m[—CO(CH_2)_4CONH(CH_2)_6NH—]_n$	5/95		47	nd

[a] $[—CO(CH_2)_4COO(CH_2)_6O—]_n$: adipoyl chloride/1,6-hexanediol; $[—CO(CH_2)_4CONH(CH_2)_2NH—]_n$: adipoyl chloride/ethylenediamine; $[—CO(CH_2)_aCOO(CH_2)_bO—]_m[—CO(CH_2)_aCONH(CH_2)_cNH—]_n$: $a = 2$: succinyl chloride; $a = 4$: adipoyl chloride; $b = 2$: ethylene glycol; $b = 6$: 1,6-hexanediol; $c = 2$: ethylenediamine; $c = 3$: 1,3-propanediamine; $c = 4$: 1,4-butanediamine; $c = 6$: 1,6-hexanediamine.

[b] M_n from NMR analysis.

[c] nd: Not determined.

The sample used was 25 mg and the solution volume was 2 mL, so the theoretical TOC value is calculated as follows:

$$TOC_{theor} = (25)(1000/2)(144/228) \text{ mg/l}$$

$$= 7895 \text{ ppm}$$

Polyesters containing odd numbers of methylene groups in diol components exhibited high biodegradability (Table III, Run 2 and Run 5). This might be due to their dissimilar structures from those containing even numbers of methylene groups that affected the polymer crystallinity. The biodegradability of other polymers can be summarized as follows:

1. With same numbers of methylene units between carbonyl groups, the polymers exhibited a decrease in biodegradability with an increase in methylene group number in the diols.
2. With same kinds of diols, the synthesized polymers containing longer methylene units in the diacids resulted in a higher biodegradability.
3. Among the polyesters having the same total number of methylene units in its molecules, increase in the numbers of methylene units between the carbonyl groups of the diacid component together with decrease the methylene chain length in the diol component resulted in a considerable increase in polymer biodegradability (Table III, Run 4 and Run 6).

From these results, biodegradability of the synthesized polyesters can be concluded as follows:

Table III Enzymatic Hydrolysis of Polyesters with Lipase from *Rhizopus arrhizus*[a]

Run	Polymer	Control (Polym. + Enzyme) (ppm)	TOC Value (ppm)	Biodegradability (%)
1	$[—CO(CH_2)_2COO(CH_2)_2O—]_n$	160	380	9
2	$[—CO(CH_2)_2COO(CH_2)_3O—]_n$	210	1000	33
3	$[—CO(CH_2)_2COO(CH_2)_4O—]_n$	30	660	26
4	$[—CO(CH_2)_2COO(CH_2)_6O—]_n$	20	50	2
5	$[—CO(CH_2)_4COO(CH_2)_3O—]_n$	6	1536	53
6	$[—CO(CH_2)_4COO(CH_2)_4O—]_n$	24	1762	58
7	$[—CO(CH_2)_4COO(CH_2)_6O—]_n$	20	1280	40

[a] Hydrolysis conditions: Sample 25 mg, buffer solution 2 mL, Lipase 200 units, 37°C, 24 h.

Table IV Enzymatic Hydrolysis of Polyesters with Lipases, Esterase and Trypsin[a]

Run	Polymer	Lipase (RA, %)	Lipase (RD, %)	Lipase (CC, %)	Esterase (HL, %)	Trypsin (PP, %)
1	$[—CO(CH_2)_2COO(CH_2)_2O—]_n$	9	8	5	5	0
2	$[—CO(CH_2)_2COO(CH_2)_3O—]_n$	33	39	0	12	0
3	$[—CO(CH_2)_2COO(CH_2)_4O—]_n$	26	20	2	1	1
4	$[—CO(CH_2)_2COO(CH_2)_6O—]_n$	2	2	1	1	0
5	$[—CO(CH_2)_4COO(CH_2)_3O—]_n$	53	48			
6	$[—CO(CH_2)_4COO(CH_2)_4O—]_n$	58	41			
7	$[—CO(CH_2)_4COO(CH_2)_6O—]_n$	40	7	0	0	0

[a] RA: lipase from *Rhizopus arrhizus* (200 U), RD: lipase from *Rhizopus delemer* (200 U), CC: lipase from *Candida cylindracea* (200 U), HL: esterase from hog liver (100 U), PP: trypsin from porcine pancreas (200 U). Hydrolysis conditions: Sample 25 mg, buffer solution 2 mL, 37°C, 24 h.

1′. In the case of (1), hydrophilicity of the polymer molecules plays an important role to the polymer biodegradability.
2′. The results from (2) revealed that polymer rigidity was a crucial factor for its biodegradability.
3′. From the results of (3), it can be seen that both hydrophilicity and rigidity of the polymer molecules are the main factors controlling the polyester biodegradation. The longer methylene units between carbonyl groups together with the smaller methylene units between two oxygen atoms in the polyesters give rise to the higher the biodegradability.

However, the degradability of polyester produced from $ClCO(CH_2)_2COCl$ /$HO(CH_2)_2OH$ was shown to be rather low, which may due to the rigidity on its molecular structure (Run 1).

To get an insight into the effect of other kinds of enzymes, enzymatic hydrolysis tests were carried out in the presence of lipases from *R. delemar* (RD), *C. cylindracea* (CC), an esterase from hog liver (HD), and trypsin from porcine pancreas (PP). The hydrolysis results are summarized in Table IV. The RD was found to hydrolyze the polymers similarly to the RA except only the case of $[—CO(CH_2)_4COO(CH_2)_6O—]$, upon which RD had a negligible effect (Run 7). Esterase also hydrolyzed polyesters having odd number of methylene groups between diol component similarly to RA and RD, while CC and trypsin had no effect.

An aqueous portion of the enzymatic hydrolysis tests was analyzed with NMR (Table V). It was found that the ester bonds were hydrolyzed to produce acids and alcohol derivatives. In the case of the same acid unit, hydrolyzed products of polyesters having long methylene chain, such as Run 6 and 8 vs Run 4 in Table V, showed high ratio of

Table V Hydrolyzed Products (Water-Soluble Part) of Polyesters[a]

			Peak Intensity		
Run	Polymer	Enzyme	4.2 ppm	3.6 ppm	Ester/Alcohol
1	$[—CO(CH_2)_2COO(CH_2)_3O—]_n$	RA	40	11	78/22
2	$[—CO(CH_2)_2COO(CH_2)_4O—]_n$	RA	40	59	40/60
3	$[—CO(CH_2)_2COO(CH_2)_4O—]_n$	RD	40	20	67/33
4	$[—CO(CH_2)_4COO(CH_2)_3O—]_n$	RA	40	24	62/38
5	$[—CO(CH_2)_4COO(CH_2)_3O—]_n$	RD	40	24	62/38
6	$[—CO(CH_2)_4COO(CH_2)_4O—]_n$	RA	40	30	57/43
7	$[—CO(CH_2)_4COO(CH_2)_4O—]_n$	RD	40	24	62/38
8	$[—CO(CH_2)_4COO(CH_2)_6O—]_n$	RA	40	55	42/58
9	$[—CO(CH_2)_4COO(CH_2)_6O—]_n$	RD	40	22	65/35

[a] Data from ^1H NMR analysis using D_2O as solvent. RA: lipase from *Rhizopus arrhizus.* RD: lipase from *Rhizopus delemer.*

alcohol. These results might be caused by the difference of its solubility to water—that is, oligoesters having long methylene chain have poor solubility compared with short methylene chain oligoesters. Hydrolysis with RD resulted in high ratio of ester/alcohol compared to RA results. One of the considerable reasons is that RD lipase might be effective in homogenous hydrolysis. Thus, the ester bonds were cleaved to give terminal alcohols and carboxylic acids as an increasing of the —CH_2—OH peak intensity at 3.6 ppm.

Another considerable comprehension is in the case of long methylene diol unit: RA has more effective in cleaving the polymers into small oligomer molecules.

Enzymatic Hydrolysis of Copolyesteramides

Table VI gives a synoptical presentation of the enzymatic biodegradation results of the copolymers with several enzymes from *R. arrhizus* (RA), *R. delemar* (RD), *C. cylindracea* (CC), an esterase from hog liver (HD), and trypsin from procine pancreas (PP). The hydrolysis results are summarized in Table VI.

$ClCO(CH_2)_4COCl/HO(CH_2)_6OH/H_2N(CH_2)_6NH_2$ (ester/amide = 53/47) will be given as an example of copolyesteramide theoretical biodegradability TOC calculation.

$$[—CO(CH_2)_4COO(CH_2)_6O—]_m \times [—CO(CH_2)_4CONH(CH_2)_6NH—]_n;$$

$$(C_{12}H_{20}O_4)_m(C_{12}H_{22}O_2N_2)_n = 228m + 226n = 226 + 2m; \quad m + n = 1$$

$$C_{12}(m + n) = 144; \quad m + n = 1$$

The sample used was 25 mg, the solution volume was 2 mL, and ester/amide ratio was 53/47; $m = 0.53$, so the theoretical TOC value is calculated as follows:

$$TOC_{theor} = (25)(1000/2)[144/(226 + 2(0.53))\ mg/l = 7927\ ppm$$

The copolyesteramides were enzymatically hydrolyzed, although their hydrolysis less extensive than the corresponding polyesters. Lipase from RA was most effective with the polyester among them and it was also effective with the copolymers. The CC was found to hydrolyze the copoly-

Table VI Enzymatic Hydrolysis of Copolyesteramides with Lipases, Esterase, and Trypsin[a]

Run	Polymer	*m/n* (%)	Lipase (RA) (%)	Lipase (RD) (%)	Lipase (CC) (%)	Esterase (HL) (%)	Trypsin (PP) (%)
1	$[—CO(CH_2)_4COO(CH_2)_6O—]_n$	100/0	40	7	0	0	0
2	$[—CO(CH_2)_4COO(CH_2)_6O—]_m[—CO(CH_2)_4CONH(CH_2)_2NH—]_n$	76/24	35	1	12	16	0
3	$[—CO(CH_2)_4COO(CH_2)_6O—]_m[—CO(CH_2)_4CONH(CH_2)_3NH—]_n$	86/14	15	1	5	3	0
4	$[—CO(CH_2)_4COO(CH_2)_6O—]_m[—CO(CH_2)_4CONH(CH_2)_4NH—]_n$	59/41	6	~ 0	1	2	0
5	$[—CO(CH_2)_4COO(CH_2)_6O—]_m[—CO(CH_2)_4CONH(CH_2)_6NH—]_n$	33/67	2	7	~ 0	1	0

[a] RA: lipase from *Rhizopus arrhizus* (200 U). RD: lipase from *Rhizopus delemer* (200 U). CC: lipase from *Candida cylindracea* (200 U). HL: esterase from hog liver (100 U). PP: trypsin from porcine pancreas (200 U). Hydrolysis conditions: Sample 25 mg, buffer solution 2 mL, 37°C, 24 h.

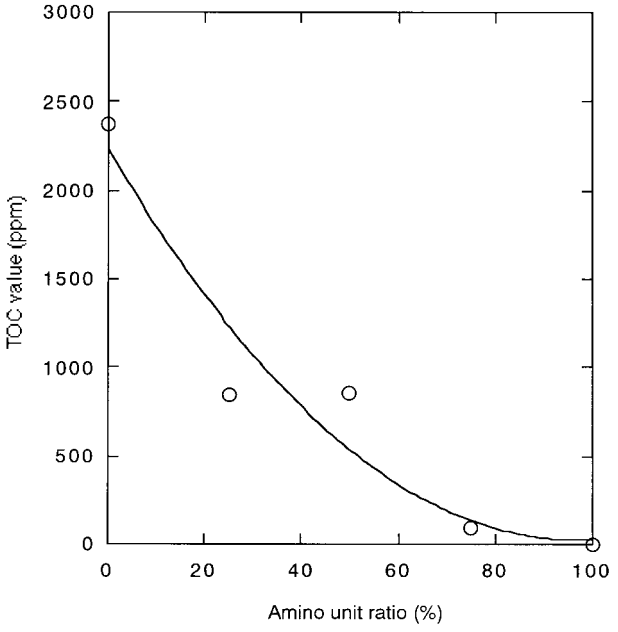

Figure 1 Enzymatic hydrolysis of copolyesteramides, $[—CO(CH_2)_3CO(CH_2)_3O—]_m[—CO(CH_2)_3NH(CH_2)_3NH—]_n$, with lipase from *R. arrhizus*.

mers consisting of a shorter methylene group chain (amide part), whereas RD had a rather negligible effect. Esterase also hydrolyzed the copolymers similarly to CC and RA, while trypsin had no effect whatsoever. This suggests that the substrate specificity of the esterase, which hydrolyzes specifically water-soluble esters, is substantially enhanced in the presence of amide- rich polymers due to their pronounced hydrophilicity.[30]

Enzymatic hydrolysis results of polyesters revealed that hydrophilicity of the polymer molecules and their rigidity, due to the methylene chain length between the carbonyl groups and the oxygen atoms, played an important role in the polymer biodegradability (Table III and IV). Polymers containing a longer methylene chain in the acid component exhibited higher biodegradability. To investigate the relationship between the content of amide unit in the copolyesteramides and its biodegradability, enzymatic hydrolysis of copoly(glutaric acid/1,3-propanediol/1,3-propanediamine)s was carried out with the lipase from RA. The results are summarized in Figure 1. It was found that an increase in the amide component resulted in a decrease in its biodegradability. The copolymer containing more than 50% of amide component showed the reduction on the degradability. The results suggest that polymer rigidity due to the amide component has a greater

Table VII Relationship Between Enzymatic Hydrolysis and Polymer Composition[a]

		TOC Values (ppm)		
Run	Polymer Comp. (Ester/Amide)	Lipase (*R. arrhizus*)	Lipase (*R. delemer*)	α-Chymotrypsin (Bovine Pancrease)
1	Blank level	0	70	50
		$[—CO(CH_2)_4COO(CH_2)_6O—]_m[—CO(CH_2)_4CONH(CH_2)_6NH—]_n$[b]		
2	100/0	1480	257	
3	60/40	650	650	10
4	55/45	610	810	0
5	53/47	80	nd[c]	0
6	50/50	90	40	0
7	33/67	40	20	0
8	0/100	0	0	0
		$[—CO(CH_2)_4COO(CH_2)_2O—]_m[—CO(CH_2)_4CONH(CH_2)_6NH—]_n$[d]		
8	55/45	310	310	0
9	5/95	60	0	0

[a] Hydrolysis conditions: sample 25 mg, buffer solution 2 mL, enzyme 200 units, 37°C, 24 h.
[b] $[—CO(CH_2)_4COO(CH_2)_6O—]_m[—CO(CH_2)_4CONH(CH_2)_6NH—]_n$: adipoyl chloride/1,6-hexanediol/1,6-hexanediamine.
[c] nd = Not determined.
[d] $[—CO(CH_2)_4COO(CH_2)_2O—]_m[—CO(CH_2)_4CONH(CH_2)_6NH—]_n$: adipoyl chloride/ethylene glycol/1,6-hexanediamine.

effect on the enzymatic hydrolyzability than its hydrophilicity.

These results encouraged us to analyze the phenomena based on the correlation between the copolymer composition and the enzymatic hydrolysis of the copolyesteramides. Thus, copolymers $[—CO(CH_2)_4COO(CH_2)_6O—]_m[—CO(CH_2)_4CONH(CH_2)_6NH—]_n$ and $[—CO(CH_2)_4COO(CH_2)_2O—]_m[—CO(CH_2)_4CONH(CH_2)_6NH—]_n$ with varying composition of esters and amides, were tested with RA, RD, and α-chymotrypsin.

From Table VII it was shown that RA effectively hydrolyzed the polyester and the copolyesteramides containing high amount of ester component, but it did not hydrolyze the polyamide. In the case of RD, the enzyme showed smaller efficiency to hydrolyzed polyesters compared with RA, but with the copolyesteramides, RD exhibited high hydrolyzability similar to RA. The results show that in the case of RD, hydrolysis of polymers is enhanced by the hydrophilicity. However, with the copolymer containing more than ca. 50% of amide component, RA and RD had negligible enzymatic hydrolysis effect. Chymotrypsin had no effect on degradability of copolyesteramides.

In the case of different components of copolymers, the ones with shorter methylene chain length in the ester component showed lower biodegradability (Runs 4 and 9). The results revealed that the polymer flexibility is another crucial factor to be taken into account besides its hydrophilicity.

An aqueous portion of the enzymatic hydrolysis tests was analyzed with NMR analysis in order to confirm the water-soluble composition (Table VIII). Those copolyesteramides that gave high TOC values with three kinds of enzymes were chosen as representative samples to determine the difference in the composition of the hydrolyzed products. With RA, an aqueous portion of sample from Run 2 was selected as the highest carbon content among those with different methylene chains between the amine component. Hydrolyzed product from CC of Run 3 was also chosen as the highest TOC among other enzymes except RA. A sample from Run 5 was determined to confirm the hydrolyzed component in aqueous portion of nonbiodegradable copolymers.

From NMR results, it was found that enzymatic hydrolysis occurred exclusively on the ester bonds producing acids and alcohol derivatives compared with the nonenzymatic case. The results confirmed that the enzymes had effectively hydrolyzed the ester bonds more than the amide

Table VIII Hydrolyzed Products (Water-Soluble Part)[a]

Run	Polymer	Enzyme	Peak Intensity			Ester/Alcohol/Amide
			4.2 ppm	3.6 ppm	3.3 ppm	
1	$[—CO(CH_2)_4COO(CH_2)_6O—]_m[—CO(CH_2)_4CONH(CH_2)_2NH—]_n$	None	20	3	4	74/11/15
2	$[—CO(CH_2)_4COO(CH_2)_6O—]_m[—CO(CH_2)_4CONH(CH_2)_2NH—]_n$	RA	20	31	8	34/52/14
3	$[—CO(CH_2)_4COO(CH_2)_6O—]_m[—CO(CH_2)_4CONH(CH_2)_3NH—]_n$	CC	20	23	5	42/48/10
4	$[—CO(CH_2)_4COO(CH_2)_6O—]_m[—CO(CH_2)_4CONH(CH_2)_6NH—]_n$	HL	—	20	—	—/—/—

[a] Data from ^{1}H NMR analysis using D_2O as solvent. RA: lipase from *Rhizopus arrhizus.* CC: lipase from *Candida cylindracea.* HL: esterase from hog liver.

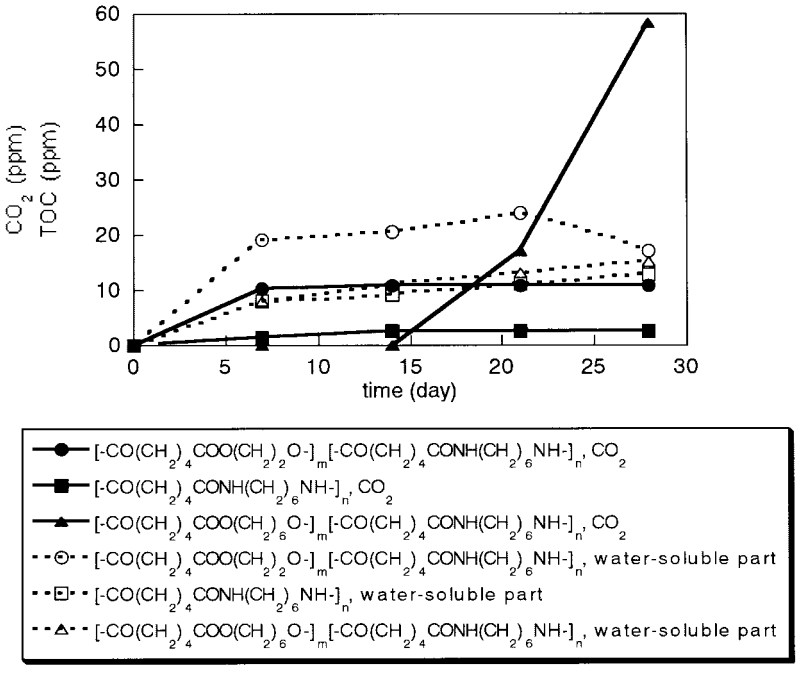

Figure 2 Activated sludge test.

bonds. Thus, 40% of the ester bonds has been transferred to the alcohol, while almost no change was observed in the amide portion (Table VIII, Runs 1 and 2). High concentration of alcohol was also found in the water-soluble part of the hydrolyzed products from CC. No NMR peaks of oligomers were observed in the water-soluble part of the HL enzymatic tests, which is in agreement with the enzymatic hydrolysis results (Table VI, Run 5, and Table VIII, Run 4).

Degradation of Copolyesteramides by Activated Sludge

Studies on biodegradation of copolyesteramides were conducted using a standard activated sludge to compare with those of the enzymatic hydrolysis. Figure 2 (dark lines) shows the results of the biodegradation estimated from the amount of the evolved CO_2. The inorganic carbon concentration values resulted from the experimental values after subtracting the values for the control test, which was conducted in the absence of the copolymer. The polyamide synthesized from adipoyl chloride/1,6-hexanediamine was hardly degraded. Addition of ester component to the polyamide increased the biodegradability. It is noteworthy that, in the case of copolymer synthesized from adipoyl chloride/1,6-hexanediol/1,6-hexanediamine, the biodegradability was drastically increased after 2 weeks. These results indicate that biodegradation of the copolymers by standard activated sludge substantially increased with an increased in the more flexible ester component, which is in agreement with the enzymatic degradation.

Ten milliliters of reaction mixture were taken up from the incubate in the biodegradation testing every week, filtered through 5 Å filter paper, and the TOC based on the water-soluble products in the filtrate was measured. Results are shown in Figure 2 (dot lines). The TOC values from the polymers were ca. 10–20 ppm and remained constant throughout the testing period. This result suggests that the water-soluble products were partially degraded by activated sludge.

The polymer compositions before and after activated sludge tests were analyzed with NMR in order to determine the hydrolysis mechanism. The ratio of ester and amide components from residue polymers after biodegradation by activated sludge test was calculated from the peak ratio of (C═O)& bond;O—$\underline{CH_2}$ at 4.1 ppm and (C═O)& bond;NH—$\underline{CH_2}$ at 3.2 ppm. It was found that the ester/amide ratio was 55/45 before the activated sludge test, while a ratio of 43/57 was found after the test. A decrease in the ester component ratio confirms that hydrolysis of ester

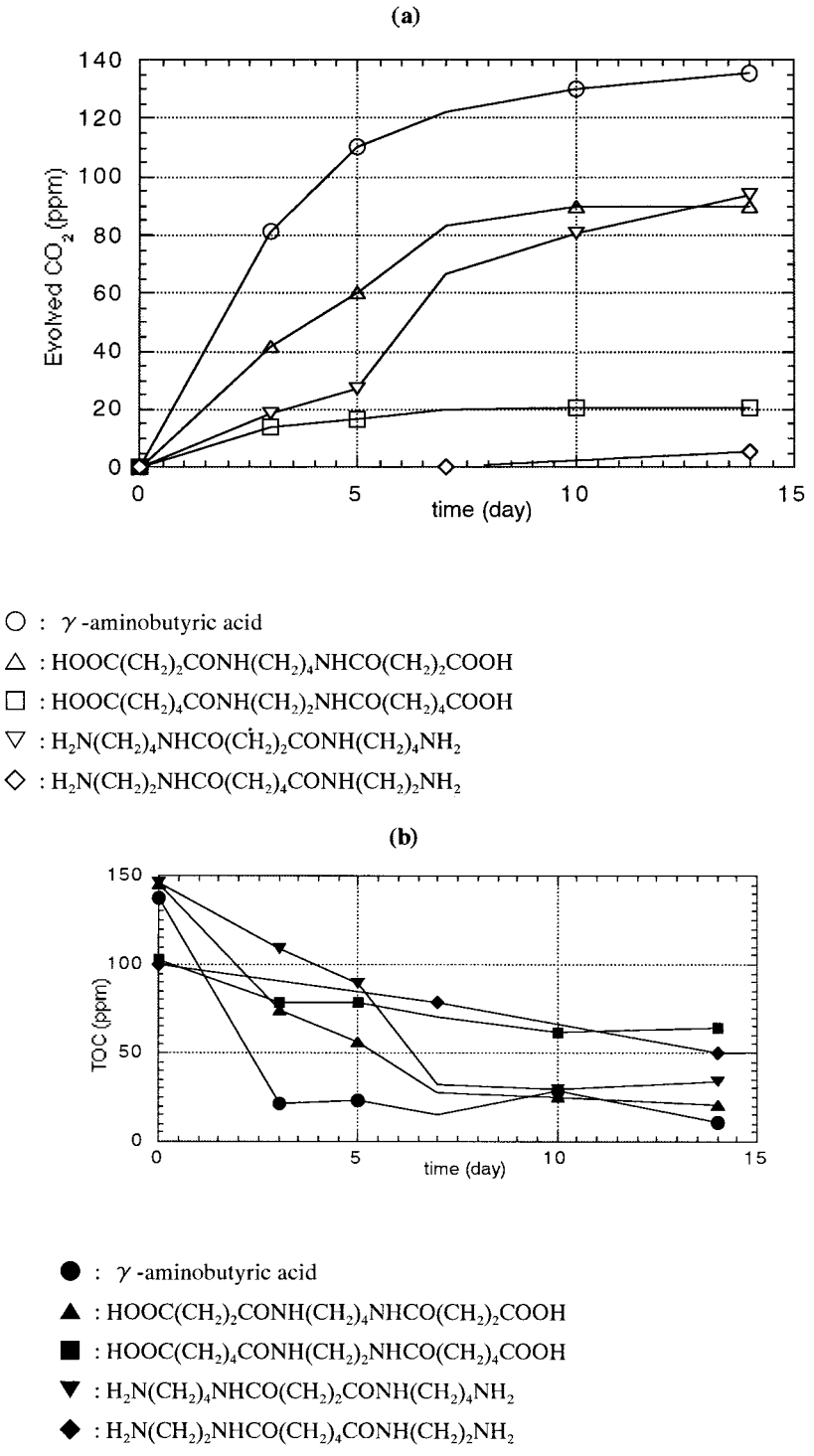

Figure 3 Biodegradation of monomers by activated sludge.

bonds predominantly occurred in agreement with the enzymatic hydrolysis test.

To confirm the degradation of water-soluble components of copolyesteramides, the model compounds containing amide bonds with both terminals carboxylic groups and amino groups with different methylene chain length were synthesized and subjected to an activated sludge test. The results are presented in Figure 3. Figure 3(a) shows that the small molecule γ-aminobutyric

acid degraded faster, as expected. It was of considerable interest that regardless of the length of the whole water-soluble molecules, the oligomers contain a combination of four methylene units between the carbonyl groups, and two methylene units between amino groups exhibited a tedious degradation. The results indicate that at least once the polymers were hydrolyzed into water-soluble components, and their composition was defined for further degradation studies in activated sludge. The total organic carbon results in Figure 3(b) also confirm the decomposition data. A slow decrease in the TOC value of $HOOC-(CH_2)_6CONH(CH_2)_2NHCO(CH_2)_6COOH$ and $H_2N-(CH_2)_2NHCO(CH_2)_6CONH(CH_2)_2NH_2$ verified that these water-soluble molecules did not further degrade in activated sludge, whereas other compounds showed a clear TOC decrease, which confirmed their decomposition into carbon dioxide.

CONCLUSIONS

Biodegradable copolyesteramides were synthesized through polycondensation of diacid chlorides, diamines, and diols of varying methylene chain lengths. The composition of the synthesized copolymers was determined with NMR. Lipases from *Rhizopus arrhizus* and *Rhizopus delemar*, and *Candida cylindracea* and esterase, hydrolyzed the copolymers, whereas trypsin and α-chymotrypsin did not have any effect on them. Enzymatic hydrolysis was found to be greatly affected by the polymer composition and structure, which was in agreement with the activated sludge tests.

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