Biodegradability of Polyesters Having Tetrahydropyran Rings in Their Backbones

MASAHIKO OKADA,* SHINJI ITO, KEIGO AOI, and MIHARU ATSUMI†

Faculty of Agriculture, Nagoya University, Chikusa, Nagoya 464-01, Japan

SYNOPSIS

Biodegradability of several homo- and copolyesters, containing tetrahydropyran rings in their backbones with or without pendant groups, was investigated by degradation tests, both in soil and in an activated sludge. These polyesters were hydrolytically degraded to lower molecular weight compounds, and eventually to hydroxytetrahydropyran carboxylic acids, at different rates, depending on their molecular structure. Quantitative determination of carbon dioxide, generated during the treatment with the activated sludge, showed that the hydrolysates from polyesters **2** and **10**, without pendant alkoxycarbonyl groups, were catabolized by microorganisms. It was concluded from these results that at least the polyesters without pendant groups were biodegradable, and that polyester **10**, consisting of 2,6-linked tetrahydropyran rings, underwent biodegradation more readily than polyester **2**, consisting of 2,5-linked tetrahydropyran rings. © 1994 John Wiley & Sons, Inc.

INTRODUCTION

Biodegradable polymers are classified into the following three categories[1-3]: (1) polymers produced by microorganisms, such as poly(hydroxyalkanoate)s, poly(lysine), etc., (2) naturally occurring polymers constituting plants and animals, such as cellulose, chitin, etc., and (3) chemically synthesized polymers, such as poly(ϵ-caprolactone), poly(ethylene glycol), poly(α-amino acid), etc. Among biodegradable synthetic polymers, aliphatic polyesters are perhaps the most promising. Poly(glycolic acid),[4] poly(β-propiolactone),[5] poly(β-butyrolactone),[6,7] poly(ϵ-caprolactone),[8] poly(tetramethylene adipate),[9] their copolyesters, and poly(esterurethane)s[10] as well, belong to the category of biodegradable synthetic polyesters. One of the advantages of chemically synthesized biodegradable polymers is that their degradation rates can be controlled at will, so as to meet specific requirements by chemical modification of the structure.

In our laboratory, efforts have been directed toward the synthesis of a series of potentially biodegradable polyesters with oxacyclic moieties in their main chains, mainly by ring-opening polymerization of bicyclic oxalactones.[11-14] In the preceding article in this series,[15] we reported spontaneous hydrolysis of various homo- and copolyesters, having tetrahydropyran rings in their backbones in a neutral phosphate buffer solution. These polyesters were hydrolytically degraded, rapidly or gradually, depending on the molecular structure, to yield eventually the corresponding hydroxytetrahydropyran carboxylic acids. The present study is concerned with the biodegradability of these polyesters in relation to their molecular structure.

There are many factors affecting the biodegradability of plastic materials. They are related to primary structure (chemical composition, molecular weight, molecular weight distribution, etc.), higher-order structure (melting point, glass transition point, crystallinity, crystal structure, etc.), and surface conditions (surface area, hydrophilicity, hydrohobicity, etc.).[16] Since these factors are interrelated in a complicated way, it is not easy to clarify structure–biodegradability relationships for a wide range of polymers.

Generally applicable, standard methods for evaluating the biodegradability of polymeric materials

* To whom correspondence should be addressed.
† *Present address:* Tokyo Woman's Christian University, Zempukuji, Suginami, Tokyo 167, Japan.

Journal of Applied Polymer Science, Vol. 51, 1045–1051 (1994)
 CCC 0021-8995/94/061045-07

has not yet been established. Soil burial and activated sludge tests are commonly employed as qualitative methods. Needless to say, these tests are obscure, because microorganisms present in soil or activated sludge differ significantly, depending on the soil or activated sludge in which the biodegradation test is carried out. Furthermore, there still remains uncertainty as to whether these tests are closely correlated with what happens in the real world. However, since there is no other convenient method to evaluate biodegradability, we adopted the soil burial and activated sludge tests for the present investigation.

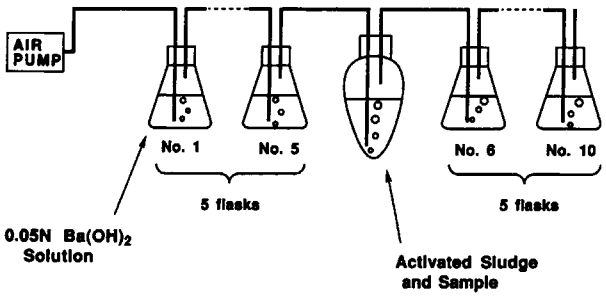


Figure 1 An apparatus for carbon dioxide evolution test.

EXPERIMENTAL

Polyesters

All the polyesters used in the present investigation were prepared by cationic ring-opening polymerization of the corresponding bicyclic oxalactones, with boron trifluoride etherate as an initiator, and were purified as described in the preceding article in this series.[15] The molecular weights of polyesters were estimated by gel permeation chromatography (column, TSK-gel G2000HXL and G3000HXL; eluent, chloroform; polystyrene standard).

Soil Burial Test

Films (thickness, 50–70 μm) were prepared by casting a chloroform solution of polyester. Samples (0.8 $\times$ 2.5 cm) were buried in soil in a box. The box was placed in a desiccator, in which the relative humidity was adjusted to 60–70% by a saturated solution of ammonium nitrate. The desiccator was kept in a room thermostated at 27°C. At intervals, the samples were taken out, washed with water, and dried to a constant weight. The weights and molecular weights of the recovered samples were determined.

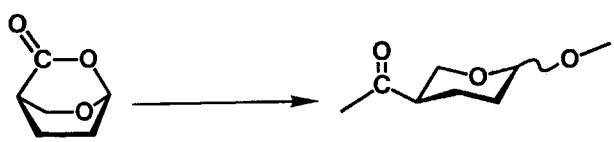
Determination of Carbon Dioxide during Biodegradation in an Activated Sludge

An apparatus, used for biodegradation in an activated sludge, is illustrated in Figure 1. A sample of 6-hydroxytetrahydropyran-2-carboxylic acid (1.0 g) was charged in a flask containing an activated sludge (200 mL). The activated sludge (pH = 6.7–6.8) was obtained from a sewage treatment plant in Meitoku, Nagoya. Air from a pump was passed through 0.05 N aqueous barium hydroxide solution in five flasks connected in series (No. 1 to No. 5), and the resulting carbon dioxide-free air was bubbled into the activated sludge at a constant flow rate of 25 mL/min at 27°C. Carbon dioxide, generated during catabolism, was quantitatively captured by passing the gas from the activated sludge through 0.05 N aqueous barium hydroxide solution in five flasks connected in series (No. 6 to No. 10). At intervals, barium carbonate, precipitated in the flasks, was collected on a filter, was dried, and was weighed. There was no precipitation of barium carbonate in flasks No. 9 and No. 10. This result means that carbon dioxide was completely captured by the aqueous barium hydroxide solution in the three flasks No. 6 to No. 8. The amount of carbon dioxide, evolved by catabolism, was calculated from the weight of barium carbonate corrected by subtracting the weight of barium carbonate in a control run without sample from the total weight of barium carbonate.

RESULTS AND DISCUSSION

Degradation in Soil

A soil burial test on polyester films was carried out in a room controlled at 27°C. The degradation was followed by monitoring the molecular weights and molecular weight distributions of the remaining polymers by GPC. Figure 2 demonstrates the change of GPC profiles with soil burial time for a film of polyester **2** (M_n, 2.3 $\times$ 10^4 and film thickness, 50 μm), derived from a bicyclic oxalactone **1**.



1 2

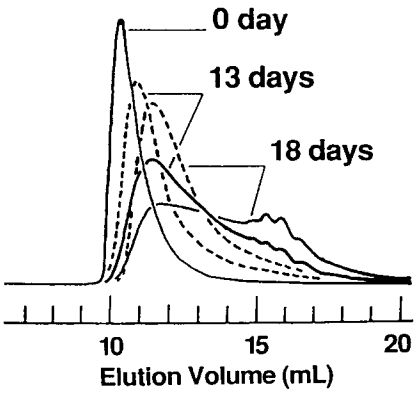


Figure 2 GPC profiles of polyester **2** before and after soil burial and hydrolysis tests. M_n of polyester **2**, 2.3 $\times 10^4$; film thickness, 50 μm. (——) soil burial test (temp., 27°C). (-----) hydrolysis test (phosphate buffer, pH 7.5; temp., 27°C).

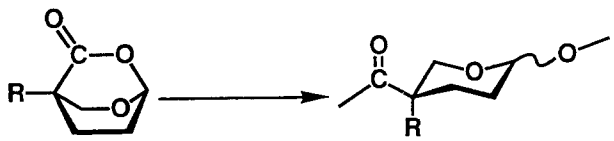
Several cracks already appeared on the film after the soil burial for 9 days, and the film was disintegrated into small pieces after 18 days. As the GPC curves in Figure 2 clearly indicate, the molecular weight of the recovered polymer decreased and the molecular weight distribution was broadened with increasing burial time. Several peaks, appearing in the low molecular weight region of the GPC curves, are ascribed to oligomeric compounds formed by degradation.

From the comparison of the GPC curves in the soil burial test with those in the spontaneous hydrolysis in a phosphate buffer solution of the same film (dotted curves in Fig. 2), the degradation in soil seems to proceed faster than the hydrolytic degradation. However, such a conclusion, drawn from this comparison, might be questionable, because the GPC curves in the hydrolysis test are for the water-insoluble polymers, which are free of water-soluble low molecular weight compounds, whereas the GPC curves in the soil burial test are for the whole polymer, including water-soluble low molecular weight compounds. In order to confirm the validity of the comparison, the sample, which had been buried in soil for 13 days, was immersed in a phosphate buffer solution (pH 7.5) at 27°C for 6 h to remove water-soluble compounds. The GPC curve of the sample thus treated was essentially the same as that shown in Figure 2, except that the peaks, appearing in the low molecular weight region, became only slightly weaker. This finding, therefore, allows us to compare directly the GPC curves in the two different series of experiments.

There is a possibility that the enhanced degradation rate in the soil burial test arises from the higher acidity in soil. In fact, the acidity of the soil used for the experiment was estimated to be pH = 5.8 by the pH measurement on a mixture of the soil and distilled water (1 : 2.5, by weight). However, as will be described below, the molecular weight decrease of some polyesters with pendant groups in soil was found to be practically the same as, or slower than, that in a phosphate buffer solution. Therefore, we cannot exclude the possibility that biodegradation contributes, at least in part, to the faster decrease in the molecular weight of the film of polyester **2** in soil.

The degradation rate in soil depends on the film thickness and molecular weight of the sample. For instance, a film of polyester **2** (M_n, 3.5 $\times 10^4$ and film thickness, 70 μm), retained its shape after soil burial for 24 days. The film degraded more slowly than that used for the experiment demonstrated in Figure 2, and the recovery of polymer was about 20% after 55 days.

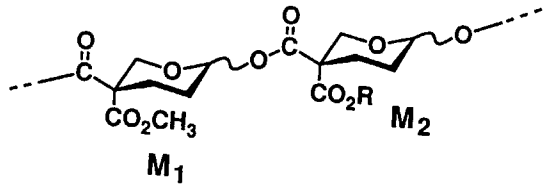
Polyesters **5** and **6**, having a pendant methoxycarbonyl or benzyloxycarbonyl group in its repeating unit, respectively, were prepared from the corresponding bicyclic oxalactones **3** and **4**. These polyesters, particularly **6**, were degraded in soil more slowly than polyester **2**. Figure 3 compares the molecular weight decrease in soil with that in a phosphate buffer solution for polyester **5** (M_n, 4.1 $\times 10^4$ and film thickness, 70 μm). The degradation curves were similar to each other, indicating that polyester **5** was hydrolytically degraded in soil. The shape of the film was retained after soil burial for 24 days. The recovery of polymer was still more than 75% after 55 days, which was much higher than the recovery for polyester **2** under similar conditions.



3, R=CO_2CH_3
4, R=$CO_2CH_2C_6H_5$

5, R=CO_2CH_3
6, R=$CO_2CH_2C_6H_5$

Copolyesters **7** and **8**, in which 4% of the pendant methoxycarbonyl groups were replaced by benzyloxycarbonyl groups or carboxyl groups, respectively, were prepared by copolymerization of **3** and **4**, followed by catalytic hydrogenolysis. Degradation of films of these copolyesters in soil was compared with that in a phosphate buffer solution. As Figure 4 demonstrates, the decrease of molecular weight in soil was somewhat slower than that in the buffer solution for both copolyesters **7** and **8**.



7, R=$CH_2C_6H_5$
8, R=H

Although a strict comparison cannot be made because of the considerable difference in the initial molecular weights of the two samples, the degradation of copolyester **8** with pendant hydrophilic carboxyl groups in soil was faster than copolyester **7**, with the same amount of pendant hydrophobic benzyloxycarbonyl groups. As in the case of homopolyester **5**, the data given in Figure 4 imply that both copolyesters **7** and **8** were hydrolytically degraded in soil, biodegradation being less likely to take place.

All the polyesters, so far described, consist of 2,5-linked tetrahydropyran rings. As described in the previous article,[15] 2,6-linked polyester **10**, prepared from 6,8-dioxabicyclo[3.2.1]octan-7-one (**9**) and copolyester **11** of **1** and **9**, underwent spontaneous hydrolysis more readily than did polyester **2**. Figure 5 shows the marked effect of the incorporation of 2,6-linked tetrahydropyran units on the degradation of polyester **2**. Copolyester **11**, having only a small fraction (3 mol %) of 2,6-linked tetrahydropyran units, was degraded in soil more rapidly than was homopolyester **2**. Presumably, the accelerating effect of the 2,6-linked tetrahydropyran unit mainly originates from its higher hydrophilicity, as discussed in the previous article.[15] Furthermore, it is noteworthy that the degradation of copolyester **11** in soil proceeded faster than the hydrolytic degradation in the phosphate buffer solution.

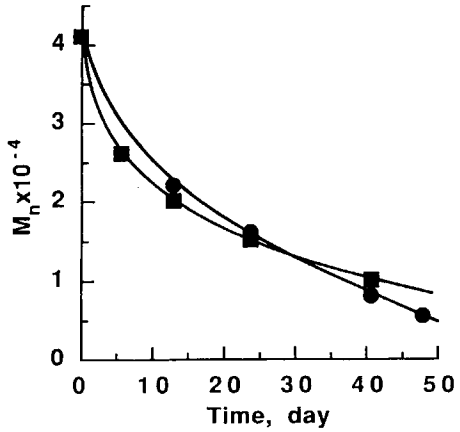


Figure 3 Changes of the molecular weight of polyester **5** with time in soil burial and hydrolysis tests. M_n of polyester **5**, 4.1×10^4; film thickness, 70 μm. (●) soil burial test (temp., 27°C). (■) hydrolysis test (phosphate buffer, pH 7.5; temp., 27°C).

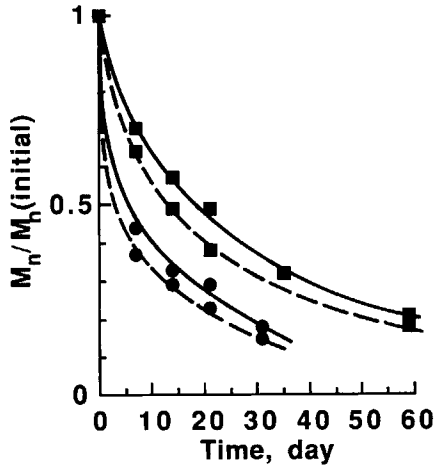
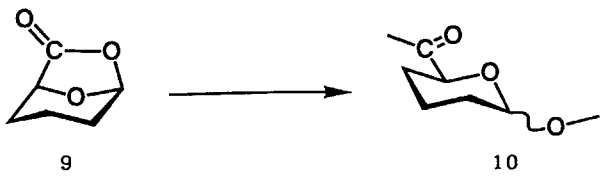
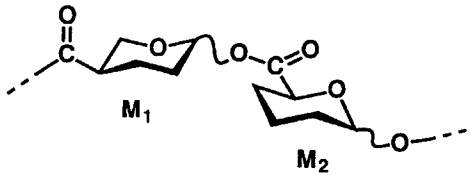


Figure 4 Changes of the molecular weights of copolyesters **7** and **8** with time in soil burial and hydrolysis tests. (■) **7**, R=$CH_2C_6H_5$; $M_1 : M_2 = 96 : 4$; M_n, 4.7×10^4; film thickness, 50 μm. (●) **8**, R=H; M_n, 2.7×10^4; film thickness, 50 μm. (——) soil burial test (temp., 27°C). (-----) hydrolysis test (phosphate buffer, pH 7.5; temp., 27°C).



9 10



11

The soil burial degradation of the polyesters, containing tetrahydropyran rings in their backbones, are summarized as follows.

1. Unsubstituted polyester **2** was degraded in soil faster than in the phosphate buffer solution, whereas homo- and copolyesters, having pendant alkoxycarbonyl groups, were degraded in soil as fast as, or more slowly than,

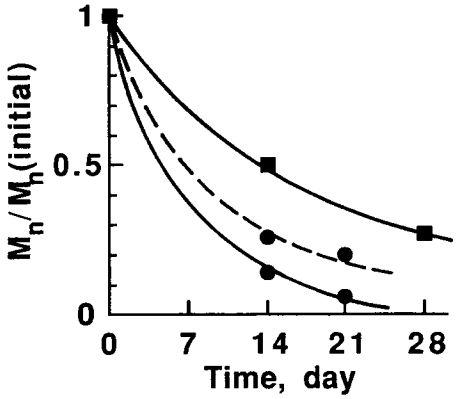


Figure 5 Changes of the molecular weights of polyester **2** and copolyester **11** with time in soil burial and hydrolysis tests. (■) polyester **2**; M_n, 4.4×10^4; film thickness, 70 μm. (●) copolyester **11**; $M_1 : M_2 = 97 : 3$; M, 4.7×10^4; film thickness, 70 μm. (——) soil burial test (temp., 27°C). (-----) hydrolysis test (phosphate buffer, pH 7.5; temp., 27°C).

they were hydrolyzed in the phosphate buffer solution.

2. Incorporation of a small fraction of 2,6-linked tetrahydropyran units into polyester **2**, consisting of the 2,5-linked tetrahydropyran backbone, significantly enhanced the degradation in soil.
3. Hydrolysis plays a major role in the soil burial degradation, although biodegradation may contribute to some extent to the degradation of the polyesters without pendant groups.

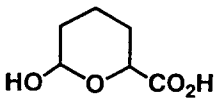
Degradation in Activated Sludge

Biodegradation of polymers consists of two processes, that is, (1) a degradation process, in which polymers are degraded to lower molecular weight compounds and (2) a catabolism process, in which the lower molecular weight compounds are converted to water, carbon dioxide, etc.

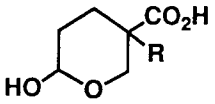
Table I summarizes the results on the degradation of four homopolyesters, **2, 5, 6,** and **10,** in an activated sludge. The water-soluble part in Table I refers to hydrolysates that are chiefly composed of the respective hydroxytetrahydropyran carboxylic acids, and the chloroform-soluble part denotes water-insoluble polymers. In the case of polyester **10,** neither the water-soluble part nor the chloroform-soluble part remained after the treatment in the activated sludge for 30 days. In other words, not only polyester **10,** but also the final hydrolysis product, 6-hydroxytetrahydropyran-2-carboxylic acid, are degraded relatively easily. In contrast, although most of polyesters **2** and **5** were degraded, there were still considerable amounts of water-soluble hydrolysates. This means that these polyesters are hydrolyzed to water-soluble low molecular weight compounds, but the catabolism of the hydrolysates takes place reluctantly, particularly for polyester **5.** Polyester **6,** with pendant hydrophobic benzyloxycarbonyl groups, was hardly degraded under the conditions examined.

The data presented in Table I clearly demonstrate that the degradation of these polyesters can be separated into two stages, that is: the cleavage of the backbones giving rise to low molecular weight compounds, and the subsequent catabolism of the cleavage products. The main reaction involved in the first stage is the hydrolytic cleavage of acetal-ester linkages in the backbones and, hence, it is more favorable for more hydrophilic polyesters. The catabolism process in the second stage is highly dependent on the hydrolysates.

Figure 6 compares degradation of four hydroxytetrahydropyran carboxylic acids **12–15** in an activated sludge. These carboxylic acids are the final hydrolysis products of polyesters **10, 2, 5,** and **6,** respectively. The carboxylic acid **12** was rapidly degraded and completely disappeared in two weeks, whereas the carboxylic acids **14** and **15,** bearing an alkoxycarbonyl group, degraded reluctantly. The carboxylic acid **13** showed an intermediate degradability. Obviously, a hydrophobic substituent on the tetrahydropyran ring significantly reduces the biodegradability in the activated sludge.



12



13, R=H
14, R=CO_2CH_3
15, R=$CO_2CH_2C_6H_5$

Table I Degradation of Polyester (2, 5, 6, and 10) in an Activated Sludge[a]

Polyester	Water-Soluble Part (mg)	Chloroform-Soluble Part (mg)
10	0	0
2	26	1
5	34	4
6	0	44

[a] For 30 days at 20°C; initial weight of polyester, 50 mg; activated sludge, 20 mL.

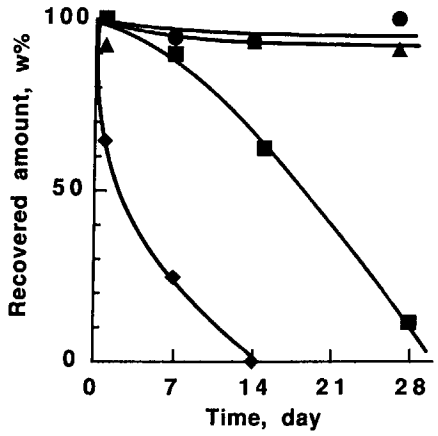


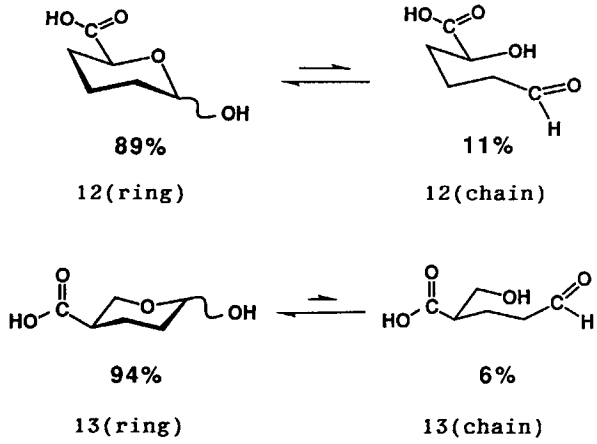
Figure 6 Biodegradation of 6-hydroxytetrahydropyran carboxylic acids (**12**, **13**, **14**, and **15**) in an activated sludge at 27°C. Sample, 100 mg; activated sludge, 20 mL. (♦) **12**, (■) **13**, (●) **14**, (▲) **15**.

Direct and confirmative evidence for biodegradation can be obtained by quantitative analysis of carbon dioxide evolved during catabolism. In the present investigation, **12** and **13** were treated in an activated sludge under aerobic conditions, and the carbon dioxide that evolved was quantitatively determined as barium carbonate (see Fig. 1). The results are graphically represented in Figure 7.

The ordinate of these figures represents the percent of the cumulative amount of carbon dioxide to the theoretical amount of carbon dioxide, calculated on the assumption that all the carbons in the sample are converted to carbon dioxide. In the case of **12**, the total amount of carbon dioxide evolved in the catabolism increased rapidly after a short induction period and reached 73% after 28 days. In the case of **13**, the total amount of carbon dioxide increased gradually after a longer induction period and reached 52% after 28 days. Since the evolution of carbon dioxide was still continuing, although slowly, after 28 days, it appeared that most of the samples were converted to carbon dioxide and water during prolonged catabolism in the activated sludge.

As shown in Figure 7, the amount of carbon dioxide in the initial stage became negative, that is, the amount of carbon dioxide evolved in the run with **12** or **13** was smaller than that in a control experiment without sample. This phenomenon can be explained as follows. The hydroxytetrahydropyran carboxylic acids **12** and **13** are probably transformed to lower molecular weight carboxylic acids and/or alcohols before they are finally converted to carbon dioxide and water. In this process, oxygen absorbed by microorganisms in the activated sludge is temporarily stored as carboxyl and/or hydroxyl groups and, hence, the amount of carbon dioxide generated in the run with **12** or **13** becomes less than the amount of carbon dioxide for the control run in the initial stage of the catabolism.

The evolution of carbon dioxide in the catabolism of **13** in the activated sludge was slower, and the induction period was longer, than in the case of **12**. The difference could be interpreted in terms of the following three factors: (1) ring-chain equilibrium, (2) molecular structure of the open chain form, and (3) hydrophilicity.



The proportions of the ring and open-chain forms in the ring-chain equilibria were estimated by ^{13}C-NMR spectroscopy to be 89 : 11 for **12** and 94 : 6 for **13**. In the glycolysis pathway, glucose is converted to lactate through the process in which fructose 1,6-diphosphate (open chain form) is decom-

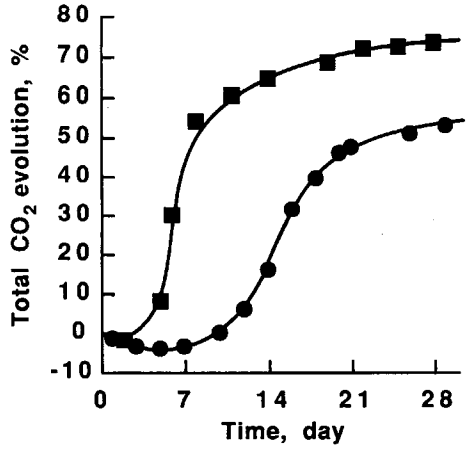


Figure 7 Evolution of carbon dioxide from 6-hydroxytetrahydropyran carboxylic acids (**12** and **13**) in an activated sludge at 27°C. Sample, 1.0 g; activated sludge, 200 mL. (■) **12**, (●) **13**.

posed to dihydroxyacetone phosphate and glyceraldehyde 3-phosphate.[17] On the analogy of the glycolysis pathway, it would appear that the open chain forms of **12** and **13** play an important role in their catabolism. If this is the case, **12** is more favorable for catabolism, because of its higher proportion of the open chain form.

The open-chain form of **12** has an α-hydroxy carboxylic acid moiety at one of the terminals of the molecule. Lactic acid, malic acid, and the enol-forms of pyruvic acid and oxalacetic acid, all of which are common intermediates in metabolism, also have a terminal α-hydroxy carboxylic acid moiety. Therefore, it would be reasonable to assume that **12** undergoes catabolism more readily than **13,** of which open-chain form is an inner β-hydroxy carboxylic acid.

With regard to the third factor, microorganisms are more likely to interact with hydrophilic polymers. As described in the previous article,[15] polyester **10,** consisting of 2,6-linked tetrahydropyran rings, is more hydrophilic than polyester **2,** consisting of 2,5-linked tetrahydropyran rings. Probably, the hydrolysis product **12** of the former polyester is more hydrophilic than the hydrolysis product **13** of the latter polyester. In other words, **12** has a higher affinity to microorganisms and is more susceptible to biodegradation.

In summary, the biodegradability of homo- and copolyesters, containing tetrahydropyran rings in their backbones, have been evaluated by the degradation experiments in soil and in the activated sludge. The degradation can be separated into two processes, that is, the cleavage of polyester chains to hydroxytetrahydropyran carboxylic acids, and their catabolism to carbon dioxide and water. The former process occurs mostly by hydrolysis because of the acetal-ester backbone structure that is sensitive to hydrolysis, although biodegradation may contribute, to some extent, in the degradation of polyesters **2** and **10** without pendant groups. The latter process is strongly dependent on the structure of the hydroxytetrahydropyran carboxylic acids: The catabolism rates were in the order of **12** > **13** ≫ **14** and **15.** These findings have led us to the conclusion that at least polyesters **2** and **10,** without pendant alkoxycarbonyl groups, are biodegradable, and that the biodegradability of 2,5-linked polyester **2** can be enhanced by the incorporation of an appropriate amount of highly biodegradable 2,6-linked structural units by copolymerization of **1** with **9.**

Financial support from the Ministry of Education, Science, and Culture, Japan (Grant-in-Aid for General Scientific Research, No. 02650666) is greatly appreciated.

REFERENCES

1. A.-C. Albertsson and S. Karlsson, *Comprehensive Polymer Science, First Supplement,* S. L. Aggarwall and S. Russo, Eds., Pergamon, Oxford, 1992, p. 285.
2. S. J. Huang, *Comprehensive Polymer Science,* Vol. 6, G. C. Eastmond, A. Ledwith, S. Russo, and P. Sigwalt, Eds., Pergamon, Oxford, 1989, p. 597.
3. S. J. Huang, *Encyclopedia of Polymer Science and Engineering,* Vol. 2, 2nd Ed., Wiley, New York, 1985, p. 220.
4. C. C. Chu, *Ann. Surg.,* **195,** 55 (1982).
5. T. Mathisen and A.-C. Albertsson, *J. Appl. Polym. Sci.,* **42,** 2365 (1991).
6. Y. Doi and N. Teranishi, *Macromolecules,* **24,** 5732 (1991).
7. J. E. Kemnitzer, S. P. McCarthy, and R. A. Cross, *Macromolecules,* **25,** 5927 (1992).
8. Y. Tokiwa, T. Suzuki, and T. Ando, *J. Appl. Polym. Sci.,* **24,** 1701 (1979).
9. A.-C. Albertsson and O. Ljungquist, *Acta Polym.,* **39,** 95 (1988).
10. P. Bruim, G. J. Veenstra, A. J. Nijenhuis, and A. J. Pennings, *Makromol. Chem. Rapid Commun.,* **9,** 589 (1988).
11. M. Atsumi and M. Okada, *Polym. J.,* **24,** 1109 (1992).
12. M. Okada, K. Aoi, W. Mizuno, and S. Ito, *J. Polym. Sci. Part A Polym. Chem.,* **31,** 1135 (1993).
13. M. Okada, H. Sumitomo, M. Atsumi, H. K. Hall, Jr., and R. B. Ortega, *Macromolecules,* **19,** 503 (1986).
14. M. Okada, H. Sumitomo, M. Atsumi, and H. K. Hall, Jr., *Macromolecules,* **20,** 1199 (1987).
15. M. Okada, S. Ito, K. Aoi, and M. Atsumi, *J. Appl. Polym. Sci.,* the preceding article in this series.
16. H. Nishida and Y. Tokiwa, *J. Appl. Polym. Sci.,* **46,** 1467 (1992).
17. A. L. Lehninger, *Principle of Biochemistry,* Worth, New York, 1982, p. 397.

Received March 20, 1993
Accepted July 20, 1993