JOURNAL OF APPLIED POLYMER SCIENCE VOL. 18, PP. 3571–3579 (1974)

Microbial Degradation of Polyesters: Polycaprolactone Degraded by *P. pullulans**

R. D. FIELDS,† F. RODRIGUEZ, and R. K. FINN, *School of Chemical Engineering, Olin Hall, Cornell University, Ithaca, New York 14850*

Synopsis

Polycaprolactone is degraded by the mold *P. pullulans* in the presence of other nutrients. The weight loss from solid polymer films covered by a nutrient agar gel on which colonies are growing is used to establish comparative rates of degradation. There is substantial loss (16 mg/cm² surface area) from a whole polymer of low (2,000) molecular weight in three weeks at 30°C. A high (30,000) molecular weight whole polymer degrades about 0.15 as much in the same time period. A fraction in the same range (38,000) but with a narrower molecular weight distribution shows no significant loss. This indicates that whole polymers of high molecular weight may lose only a portion of their distribution by microbial degradation in short-term tests. This hypothesis is tested by making mixtures of high (61,000) molecular weight with low (2,000) molecular weight polymer. Degradation is directly proportional to the low molecular weight content in these short-term tests with a single species of mold. Other workers have shown previously that in long-term, soil-burial tests, even a high (40,000) molecular weight polycaprolactone is essentially completely degraded after one year.

INTRODUCTION

Some alternatives for the disposal of solid municipal wastes are recycling, incineration, and burial. Although polymers in the form of fibers, packaging materials, and discarded objects form a small fraction of such waste (about 2% to 5%), they present unique problems in disposal. As far as polymers are concerned, the technology for the first two alternatives is known. Plastics, fiber, or rubber, once segregated, can be recycled for the original use or a less demanding use. Incineration is a problem only insofar as corrosion problems might arise in some cases. However, for the third alternative, a major block is the fact that synthetic polymers do not easily biodegrade on being buried in the soil in the manner that paper and wood do.[1–3]

It is the purpose of the present work to explore those polymers and microorganisms which have shown some promise as biodegradation systems, and to try to establish some principles by which more susceptible polymers and more aggressive agents might be designed.

As a class, polyesters are mentioned more frequently in the literature as being attacked by microorganisms than any other polymer.[4–8] However,

* Presented before the Division of Polymer Chemistry, A.C.S., Chicago, 1973.

† Present address: Eastman Kodak Co., Rochester, New York.

TABLE I
Typical Physical Properties of Polycaprolactone (Union Carbide Corp., PCL-700)[a]

Molecular weight (nominal)	40,000
1% Secant modulus	50,000
Elongation at break, %	750
Yield stress, psi	1,600
Tensile strength, psi	3,500
Glass transition temperature °C	−60
Melting temperature, °C	63

[a] From Brode and Koleske.[10]

most of the studies use low molecular weight materials, often without characterizing the molecular weight or its distribution. The work reported here is confined to polycaprolactone and to the fungus *Pullularia pullulans* (ATCC 9348 Culture of *P. Pullulans*, supplied by Dr. Arthur Kaplan, Army Natick Laboratories). The properties of the highest molecular weight polymer available are given in Table I.

Soil burial, which would be the ultimate fate of solid polymer wastes, suffers as a laboratory technique in terms of reproducibility, requirements for samples of large size and uniform dimensions, and time. Soil burial tests usually are carried out for months or years and do not lend themselves to sequential programming of tests. A second method is the "Clear Zone" technique. In this, a water-immiscible polymer is suspended in a nutrient-agar medium poured into Petri dishes. The dilute suspension is hazy. As a colony of cells grows on the surface of the gel, a clear zone may occur in the medium surrounding the colony. From this, one can infer that the polymer in the zone has been broken down into fragments that are soluble or that have been metabolized. Usually this would occur by the excretion of an extracellular enzyme by the organisms. The enzyme is presumed to hydrolyze the polyester. More than one enzyme may well be involved.

The clear zone method was used to screen organisms and to obtain some from naturally occurring communities. However, most of the work here involves a more quantitative method which, while simple, is described in some detail because it has not been used often in polymer biodegradation studies.

Since a single mold was used here, it is of some interest to compare the severity of the test with soil burial. Potts et al.[8] measured weight losses of 8% to 12% on polycaprolactone buried in soil for two months. Depending on the configuration of the test, the work reported here achieved weight losses of up to 12% in three weeks with *P. pullulans*. Although no long-term tests were run with *P. pullulans*, the relative ratings measured in these short-term tests (less than six seeks) thus would seem to be applicable in large degree to the more realistic environment of soil burial.

QUANTITATIVE METHOD FOR DEGRADING POLYMERS

Polycaprolactone is melted in Petri dishes, forming a layer on the bottom of the dish (Fig. 1). Residual water in the polymer is removed by drying in

TABLE II
Mineral Salts Used for Culturing Molds[a]

NH_4Cl	1.0 gm/l
K_2HPO_4	1.0 gm/l
$MgSO_4 \cdot 7H_2O$	0.2 gm/l
$FeSO_4 \cdot 7H_2O$	50 mg/l
$CaCl_2$	20 mg/l
$MnCl_2 \cdot 4H_2O$	2 mg/l
Na_2SO_4	0.1 gm/l
$ZnSO_4 \cdot 7H_2O$	2.0 mg/l
pH = 7.0	

[a] From Stanier et al.[11]

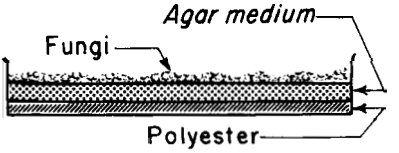

Fig. 1. Method for degrading polyesters involving polymer recovery.

a vacuum oven, and then the films are weighed. A nutrient agar medium at 50°C (low enough not to melt the films) is poured over the polymers. The plates are then inoculated with a suspension of *P. pullulans* and incubated at 30°C. Sterile plates are wetted with 1% mercuric chloride solution to ensure that they remain sterile. Since low molecular weight polyesters are slowly hydrolyzed by water, these sterile plates distinguish between enzymatic hydrolysis and hydrolysis due solely to the moisture present.

For the first experiments, a culture medium of 0.5% yeast extract, 0.5% glucose, 1.5% agar, and mineral salts (Table II) was used. Sodium succinate was substituted for glucose in the rest of the work. Glucose is a carbon source that is very easily utilized by many organisms, and its presence may repress enzymes that would break down the polyesters. The pH of all culture media was adjusted to 7.0.

After the polymers have been degraded, the agar and microorganisms are washed off and the plates dried in a vacuum oven. The plates are then reweighed to see how much of the polyester was lost. Other properties such as the tensile strength and intrinsic viscosity of the polymer can also be measured.

This experimental method has several advantages over the clear zone method. High molecular weight polymers which do not easily form suspensions can be used, and quantitative results are obtained. The clear zone method is good for isolation of polymer-utilizing organisms. The advantages that the weight loss method has over soil burial are: (1) only microbial degradation and simple hydrolysis are involved; (2) the organisms used are defined; (3) conditions for microbial growth are controlled with respect to nutrients, minerals, moisture, and temperature; (4)

hydrolytic degradation of sterile samples can be distinguished from microbial attack.

DEPENDENCE OF PCL DEGRADATION RATE ON TIME AND MOLECULAR WEIGHT

Films of 1,250, 2,000, 15,000, and 30,000 molecular weight polycaprolactone (Union Carbide's PCP-0230, PCP-0240, PCL-300, and PCL-700) were degraded by the method just described. The particular sample of PCL-700 used in initial studies had a molecular weight of 30,000 rather than the 40,000 usually supplied. The weight loss for three inoculated plates and one sterile plate was measured after 3, 7, 14, 22, and 42 days of incubation. *P. pullulans* was the fungus used. Figure 2 shows the weight loss with time for various molecular weights. The weight loss is expressed as mg/cm^2 of surface area. Since the Petri dishes have an area of about 60 cm^2, a weight loss of 10 mg/cm^2 amounts to a little over 0.5 g from the entire plate. These are corrected for hydrolysis. It is quite obvious that the degradation rate falls rapidly with increasing molecular weight as we would expect from previous work. An increase in molecular weight undoubtedly means a less hydrophylic surface, if only from the decreased concentration of hydroxyl (and possibly carboxyl) groups as the molecular weight increases. The fact that the weight loss is almost linear with time is encouraging. It means that a comparison at a shorter time is likely to be valid also at longer times of growth. This result was not entirely expected since there is no information available about the schedule on which enzymes are released under these circumstances. At least the result with the lowest molecular weight polymer indicates that either the enzyme concentration remains nearly constant or, more likely, that some other step in the reaction is rate controlling.

Another way of examining the same results is to cross-plot weight loss versus molecular weight at various times of growth (Fig. 3). There is the expected drop in degradation with increased molecular weight. Within any one run, duplicate plates agree within 10% or so. Each point is based on the average from three plates. However, when the culture has aged some time, a replicate run does not necessarily give identical results. The

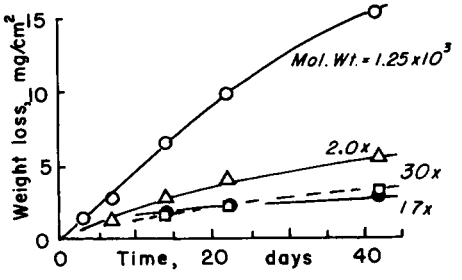

Fig. 2. Degradation of polycaprolactone by *P. pullulans* at 30°C.

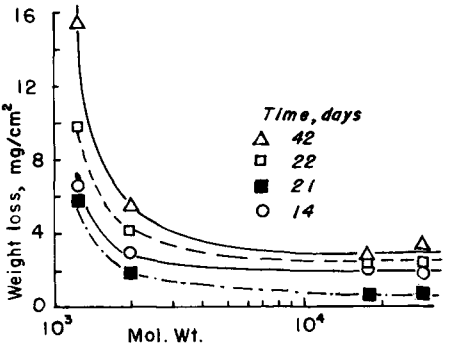

Fig. 3. Degradation of polycaprolactone. Crossplot of Fig. 3.

second three-week run shown in Figure 3 shows the same qualitative trends as the earlier one, but it is not the same on a quantitative basis.

HYDROLYTIC STABILITY OF PCL

Polycaprolactone with molecular weight less than 2000 is hydrolyzed slowly by water. To find the weight loss of such polymers attributable to enzymatic depolymerization, the weight loss of sterile samples has to be subtracted from the weight loss of inoculated ones. This was done for the data in Figures 1 and 2. The sterile samples of high molecular weight showed negligible weight losses.

The pH of all culture media was adjusted to 7.0 before the plates were inoculated. When microorganisms grow, they may change the pH of their environment. The waste products from fungi causes their cultures to become more acidic, but the sterile samples remain at a constant pH. An experiment was run to see if the weight loss that had been attributed to enzymatic hydrolysis was not just acid-catalyzed hydrolysis.

Films of a polycaprolactone with a molecular weight of 2000 were incubated in a series of media with initial pH values of 3.0, 4.0, 5.5, or 7.0. A citrate–phosphate buffer was used to control the pH. The weight loss remained constant (2.5 mg/cm²) down to pH 4.0 and then rose slightly (to 3.0 mg/cm²) at pH 3.0. A pH of 3.0 is lower than would be expected normally in a buffered fungial culture. Hence, the decrease of a culture's pH does not affect the rate of simple hydrolysis and can be ignored.

DEPENDENCE OF DEGRADATION RATE ON MOLECULAR WEIGHT DISTRIBUTION

Degradation of Whole and Fractionated Polycaprolactone

The highest molecular weight polymer (PCL-700) was fractionated by successive precipitations of the polymer from acetone with water. Table III shows the volume of water used to precipitate each fraction and the molecular weight of the fraction. Virtually all (99.5%) of the polymer was recovered.

TABLE III
Fractionation of PCL-700 at 24°C

No.	Wt., g[a]	$[\eta]$, dl/g	$M_v \times 10^{-3}$	Volume water/ Volume acetone
1	0.23	gel	—	0.100
2	3.42	0.84	61	0.108
3	2.72	0.74	53	0.113
4	2.90	0.64	44	0.117
5	2.43	0.57	38	0.121
6	4.32	0.49	32	0.126
7	3.36	0.43	27	0.130
8	6.90	0.29	17	0.135
9	3.54	0.12	5.7	recovered by evaporation

[a] Total 29.82 g recovered $[\eta] = \Sigma \mathrm{Wt}_i[\eta]_i/\Sigma \mathrm{Wt}_i = 0.48$ dl/g; $[\eta]$ (original whole polymer) = 0.47 dl/g. In benzene, 30°C: $[\eta] = 0.994 \times 10^{-4} (M_v)^{0.82}$ (ref. 10).

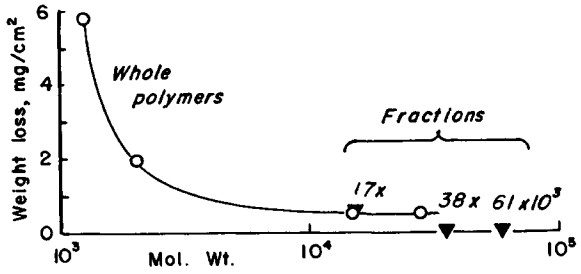

Fig. 4. Comparison of whole polymers with fractions 2, 5, and 8 (Table III).

Films of three fractions and the whole sample were then degraded by *P. pullulans*. Figure 4 shows the weight loss for the fractions and whole polymers. At an equivalent molecular weight, the two higher fractions lost significantly less weight than the whole polymer. In Germany about ten years ago, Jen-Hao and Schwartz[9] published a brief paper in which the degradation of polyethylene was studied with similar results. When whole polymers were used, even high molecular weights showed initial support for growth, expressed as bacterial counts. However, the rate dropped off rapidly for molecular weights above 5000. When polymer samples which had been biodegraded somewhat previously were rerun, the initial growth for molecular weights greater than 4800 was much diminished and differed insignificantly from a carbon-free control. It is impossible to make any quantitative comparisons between polycaprolactone and polyethylene since very few details of organisms and growth parameters were given by the German workers.

Degradation of Polymer with a Bimodal Distribution

The inference drawn from the comparison of fractions with whole polymer is that the lower molecular weight components may be degraded without

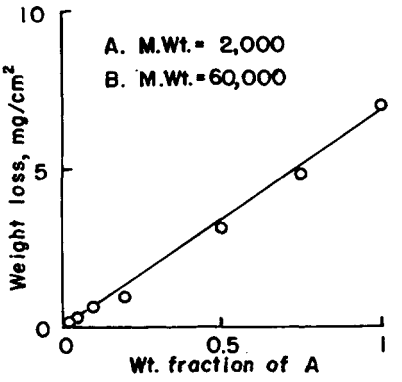

Fig. 5. Degradation of polycaprolactone mixtures for 21 days.

affecting the higher components. One way of testing this idea is to make up mixtures of a high molecular weight fraction which shows essentially no degradation by itself with a low molecular weight whole polymer.

A high molecular weight sample of polymer, relatively free of low molecular weights, was obtained from PCL-700 by twice precipitating a fraction from acetone with water. The fraction amounted to 28% of the whole polymer. The molecular weight of the fraction (from viscosity measurement) was 60,000.

This fraction together with PCP-0240, a 2000 molecular weight polymer, was then dissolved in benzene to give a series of mixtures with high molecular weight percentages of 0, 25, 50, 80, 90, 95, and 98. Evaporating these solutions in Petri dishes left behind films of polymer with a bimodal distribution.

The films were weighed and then degraded by *P. pullulans* for 21 days. Figure 5 shows the weight loss versus fraction of low molecular weight polymer. It appears probable that the whole polymer degrades more than the fractions of equal average molecular weight because the lower molecular weight portions are selectively degraded. One might expect that the presence of low molecular weights could build up a supply of enzymes which would, in turn, attack the high molecular weights. The results with mixtures indicate no such effect. On the other hand, the presence of high molecular weights seems to offer no protective barrier to prevent the degradation of low molecular weights at the same rate at which undiluted polymer degrades.

It should be pointed out that this test result does not mean that high molecular weight polycaprolactone never degrades. Potts et al.[8] have reported that a thin-walled container of the polymer with a molecular weight of 40,000 almost completely disappeared after being buried a year in soil. The weight loss was almost linear with time over that period. The combination of soil conditions and longer times may alter the conclusions reached in the present work. The containers were placed in flower pots which were watered daily. A continuation of ordinary hydrolysis may have

TABLE IV
Physical Properties of Degraded PCL-700

	Original	Sterile	Inoculated
Ultimate tensile strength, psi	2035 ± 15	1280 ± 12.5	920 ± 160
Decrease in thickness, 0.001 in.		1.5 ± 1.7	11.7 ± 8.3
Elongation at break, %	8.2	5.8	4.0
Modulus, psi	2.5×10^4	2.2×10^4	2.3×10^4

aggravated conditions. A sterile sample was not included in the report and pH measurements were not mentioned. However, even if these were not involved, the mixed culture medium may be important. With a mixture of microorganisms rather than a single mold, a second species may be able to flourish after the first has started to wane. This process, continued by several species, could lead to the progressive degradation of high molecular weight polymer. Although tests with single molds and sterile controls are important for understanding the mechanism of degradation, it must be acknowledged that soil burial tests are far more impressive in assessing the commercial application of prospective container materials.

CHANGES IN PHYSICAL PROPERTIES

The ultimate tensile strength and Young's modulus of degraded polymer were also measured. The polymer (PCL-700, M_v = 30,000) was molded into a sheet and then cut into dumbbells. Ten dumbbells were degraded in a medium inoculated with *P. pullulans*, and another ten were incubated in a sterile medium. After three weeks of degradation, their strength and modulus were measured with an Instron testing machine. Table IV shows the decrease in the strength of degraded polycaprolactone.

The surface of the sterile samples was smooth, but the inoculated ones were quite eroded and irregular. Even though as much as 20% of the sample was hydrolyzed away, the strength of the remaining material did not differ much from that of the sterile sample. The strength is a property of the sample and failure occurs at cracks or stress concentration points in the sample. The modulus, however, is not as dependent on the sample used, but is more a property of the material. The modulus for the original, sterile, and inoculated samples were essentially the same.

CONCLUSIONS

The behavior of the system qualitatively resembles that of polyethylene.[9] The molecular weight at which degradation became negligible was rather low (about 4000) in the case of polyethylene. The molecular weight at which degradation becomes negligible is above 15,000 for a fractionated polycaprolactone (Fig. 2). Since the techniques for judging degradation were not the same, a comparison of the two polymers cannot be carried much further. In a practical sense, it is important to remember also that

polar polymers such as the polyesters and polyamides generally have useful structural properties at molecular weights far lower than those necessary in nonpolar polymers such as polyethylene and polypropylene.

References

1. F. Rodriguez, *Chem. Technol.*, **1**, 409 (1971).
2. *Degradability of Polymers and Plastics*, Conference Preprint Booklet, The Plastics Institute, London, 1973.
3. P. Engler and S. H. Carr, *J. Polym. Sci., Polym. Phys.*, **11**, 313 (1973).
4. S. Berk, H. Ebert, and L. Teitell, *Ind. Eng. Chem.*, **49**, 1115 (1957).
5. R. T. Darby and A. M. Kaplan, *Appl. Microbiol.*, **16**, 900 (1968).
6. A. M. Kaplan, R. T. Darby, M. Greenberger, and M. R. Rogers, *Devel. Ind. Microbiol.*, **9**, 201 (1968).
7. R. E. Klausmeier, *Soc. Chem. Ind. (London) Monogr.*, **23**, 232 (1966).
8. J. E. Potts, R. A. Clendinning, W. B. Ackart, and W. D. Niegisch, in *Polymers and Ecological Problems*, James Guillet, Ed., Plenum Press, New York, 1973, p. 61.
9. L. Jen-Hao and A. Schwartz, *Kunstoffe*, **51**, 317 (1961).
10. G. L. Brode and J. V. Koleske, *J. Macromol. Sci. Chem.*, **A-6** 1109 (1972).
11. R. Y. Stanier, M. Doudoroff, and E. A. Adelberg, *The Microbial World*, Prentice-Hall, Engelwood, N.J., 1957.

Received March 4, 1974
Revised June 3, 1974