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Biodegradation of Polystyrene, Poly(methyl methacrylate), and Phenol Formaldehyde

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The biodegradation of three synthetic ^{14}C-labeled polymers, poly(methyl methacrylate), phenol formaldehyde, and polystyrene, was studied with 17 species of fungi in axenic cultures, five groups of soil invertebrates, and a variety of mixed microbial communities including sludges, soils, manures, garbages, and decaying plastics. Extremely low decomposition rates were found. The addition of cellulose and minerals failed to increase decomposition rates significantly.

Until recently, biodegradation of synthetic polymers had been assessed either by plate tests, with visual evaluation, or by observations on changes in physical characteristics of plastics placed into soil or aquatic systems (9, 13). The physical characteristics, such as hardness or tensile strength, may relate to shifts in conformation or bond breakage at vulnerable regions of macromolecules but do not necessarily provide evidence for biodegradation which would lead to assimilation and respiration. Similarly, the plating assays do not provide direct evidence for metabolic involvement. Neither type of assay is sufficiently sensitive to detect chemical decomposition processes which may be occurring at a very slow rate.

More recently, ^{14}C-labeled plastics have been synthesized to study their degradation (1, 6) and provide sensitivity better than 0.001%. Guillet et al. (6) studied the biodegradation of ^{14}C-labeled polystyrene in soil before and after photodegradation. Tsuchii et al. (16) followed the microbial decomposition of styrene oligomers. In this study we examined a wide range of microbiological systems to determine whether a potential existed for degrading ^{14}C-labeled polystyrene, poly(methyl methacrylate), and phenol formaldehyde.

Fungi were grown in 2.5% malt extract on orbital shakers at 28 to 30°C. They were harvested aseptically with cheesecloth and homogenized in 0.85% potassium chloride with a Waring blender. Suspensions, 1.0 ml, were added to 125-ml Erlenmeyer flasks containing 0.045 μCi of one of the polymers and either 2.0% malt extract or 100 mg of cellulose with 10 ml of fungal medium. The fungal medium consisted of 2.0 g of $NH_4H_2PO_4$, 0.6 g of KH_2PO_4, 0.4 g of K_2HPO_4, 0.3 g of Na_2HPO_4, 0.2 g of $MgSO_4$, 0.7 g of $CaCl_2$, 12.0 mg of $FeSO_4 \cdot 7H_2O$, 6.6 mg $ZnSO_4 \cdot 7H_2O$, 5.0 mg of $MnSO_4 \cdot H_2O$, 1.0 mg of $CoCl_2 \cdot 6H_2O$, 1.0 mg of $CuSO_4 \cdot 5H_2O$, 0.1 mg of thiamine · HCl, and 1.0 g of yeast extract in 1.0 liter of distilled water adjusted to pH 5.0 (modified from 2). Flasks were incubated at 28 to 30°C for 5 weeks without shaking. Vials (diameter, 5.5 by 1.5 cm) were suspended with wire from the lip of the flasks, which were sealed tightly with a rubber stopper. A strip of filter paper (3 by 3 cm) with 0.05 ml of 20 N NaOH was placed in each vial to trap $^{14}CO_2$. The strips were changed twice weekly during the 5-week period.

The fungi used were as follows. *Coriolus hirsutus* (Wolf. ex Fr.) Quel, *Gloeophyllum trabeum* (Pers. ex Fr.) Murr., *Coriolus versicolor* (L. ex Fr.), *Poria placenta* (Fr.) Cke., *Bjerkandera adusta* Willd. ex Fr., *Daedalea quercina* L. ex Fr., and *Phellinus pini* (Thore ex Fr.) A. Ames were obtained from Frances Lombard, Forest Products Laboratory, Madison, Wis.; *Aureobasidium pullulans* (DeBary) Arnaud, *Fomes annosus* (Fr.) Cke., *Peniophora gigantea* (Fr.) Massee, *Fomes everhartii* (Ell. and Gell.) var. Schr. and Spauld., and *Poria xantha* (Fr.) Uce were obtained from R. Zabel of this College and are named as in Hepting (8); *Aspergillus fumigatus* Fres., *Paecilomyces varioti* Bainer, *Trichoderma koningii* Dud., *Penicillium variable* Sopp, and *Aspergillus niger* van Tiegh were obtained from R. Ziobro and C. J. Wang of this college.

Five groups of soil invertebrates were tested to determine whether they harbored procaryotic organisms capable of degrading one or more of the three plastics: an isopod (*Isopoda*), *Oniscus asellus* L.; a millipede (*Diplopoda*), *Diploiulus* sp.; a snail (*Gastropoda*), *Oxychilus draparnaldi* Beck; the slugs (*Gastropoda*) *Limax maximus* Linne and *Deroceras reticulatum* Muller;

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and the earthworms (*Oligochaeta*) *Eisenia foetida* (Savingny), *Eudrilus eugeniae* Kingberg, and a *Pheretima* spp. The animals were collected near the college and used on the day of their capture. Experiments were run for 14 days in 125-ml Erlenmeyer flasks and traps were changed on days 1, 2, 4, 7, 10, and 14. The invertebrates were fed 0.045 μCi of each polymer. The polymers were offered to the earthworms on 1.5 g of horse manue, to the isopod and millipede on a section (2 by 2 cm) of decayed box elder leaf (*Acer negundo*) and to the slugs and snail on a piece (2 by 2 cm) of lettuce (*Lactuca sativa*). These quantities of food and polymer were consumed within week 1 of testing.

Mixed microbial systems in the form of soils, manures, decaying plastics, and garbage were obtained locally and used fresh. Activated and anaerobic sludges were obtained from the Limestone-Meadowbrook and Ley Creek Wastewater Treatment Plants, Onondaga County, N.Y., and from the pulp sludge from a paper mill, respectively. Samples weighing 10 g were tested in 125-ml flasks containing 0.045 μCi of one of the polymers, with and without 100 g of cellulose and 10 ml of salts solution adjusted to pH 7.0. The salts solution consisted of 1.0 g of K_2HPO_4, 1.0 g of KH_2PO_4, 0.1 g of $MgSO_4$, 0.1 g of NaCl, and 3.0 g of NH_4H_2PO in 1 liter of tap water. A new supply of cellulose and salts was added every 4 weeks during the 11-week test period. Flasks were flooded with 5 N H_2SO_4 to release residual $^{14}CO_2$ into the traps at the end of the experiment.

Phenol formaldehyde (0.0027 μCi/mg) was synthesized from ^{14}C-ring-labeled phenol (purchased from Amersham, Arlington Heights, Ill.) with formaldehyde in a stepwise polymerization reaction (15). Poly(methyl methacrylate) (0.0095 μCi/mg) was synthesized by bulk radical polymerization with azobisisobutyronitrile from [^{14}C]methyl methacrylate monomer (purchased from Amersham, Arlington Heights, Ill.) (15). Polystyrene (0.0017 μCi/mg) was synthesized by emulsion polymerization of [8-^{14}C]styrene (purchased from Research Products Intern. Corp., Elk Grove Village, Ill.) with persulfate (15). All polymers were in granular form and less than 0.5 mm in diameter.

Radioactivity was measured in a Beckman LS 100C-liquid scintillation counter in a solution containing 5.0 g of PPO (2,5-diphenyloxazole), 0.4 g of POPOP [1-4-bis-(5-phenyloxazolyl)-benzene], 6.5 ml of monoethanolamine, 500 ml of toluene, and methanol to 1 liter. Each vial was corrected for quench with an external standard and for base line rates of $^{14}CO_2$ release from sterile controls consisting of the polymers in 10 ml of distilled water. All fungal and mixed microbial systems were run with two or three repetitions, whereas invertebrates were run with four or five repetitions.

Fungi. Fungi in axenic cultures demonstrated very limited ability to degrade the polymers during 35 days. As a group, the 17 different fungi degraded from 0 to 0.29%, 0 to 0.17%, and 0 to 0.24% of poly(methyl methacrylate), phenol formaldehyde and polystyrene, respectively. *A. pullulans,* commonly associated with decaying acrylic paints (14, 19), converted only about 0.1% of the poly(methyl methacrylate) polymer to $^{14}CO_2$ in 35 days. Despite the ability of many of these fungi to degrade the complex aromatic plant polymer lignin (3; D. L. Kaplan and R. Hartenstein, Siol Biol. Biochem., in press), they were unable to decompose the synthetic plastic polymers.

Invertebrates. Neither the eight soil invertebrates nor the microbes egested by them in their fecal pellets were able to degrade any of the polymers. Soil invertebrates are also unable to degrade lignin (12).

Mixed microbial communities. During 11 weeks in silt loam, cow manure, activated sludge, or decaying plastics (a mixture of plastics and adhering debris in various stages of decomposition collected from fields) or 5 weeks in anaerobic sludge, pulp mill sludge, horse manure, garbage, garden soil, or farm soil, all three polymers were highly recalcitrant to biological decay. Figure 1 presents data on the decomposition of polystyrene in the silt loam and in activated sludge from the aeration tanks. In the different microbial systems, total decomposition of the polymers during 5 or 11 weeks ranged from 0.04 to 0.57%, 0 to 0.15%, and 0 to 0.15% for polystyrene, phenol formaldehyde, and poly(methyl methacrylate), respectively.

It is clear that all three polymers are highly recalcitrant to biological decay. Polystyrene appeared to be destroyed most rapidly, but even here (Fig. 1) the trivial percentage decomposed in any system, together with the decomposition kinetics of a rapid early output of $^{14}CO_2$ followed by virtual cessation of activity despite periodic renewal of nutrients, suggests that only oligomeric residues or trace impurities were destroyed. Guillet et al. (6) also found virtually no biodegradation of polystyrene and less than 0.01% $^{14}CO_2$ during 8 weeks in garden soil. Rates and total quantities of $^{14}CO_2$ production from poly(methyl methacrylate) and phenol formaldehyde in this study were even lower than for polystyrene. Albertsson and Ranby (1) found 0.005 to 0.10% degradation per month for polyethylenes. In comparison to these synthetic polymers, rates of decompositions of lignins in soils range from about 2 to 15% (4, 7, 11) during 1

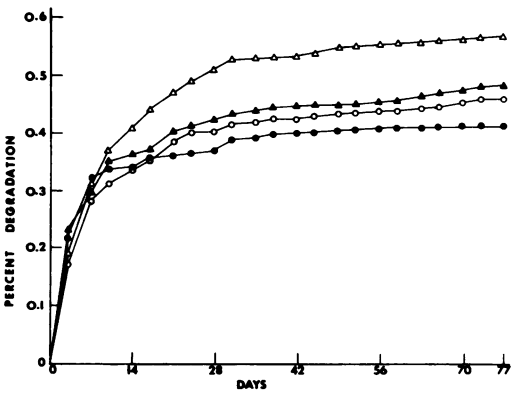

FIG. 1. *Decomposition of polystyrene in soil and activated sludge (aeration tank). Symbols: ○, soil; ●, soil with cellulose and salts; △, sludge; ▲, sludge with cellulose and salts.*

month and in axenic cultures of white-rot basidiomycetes up to 7% (Kaplan and Hartenstein, in press). The rates at which cellulose and chitin decompose vary with environmental circumstances (5), but cellulose in horse manure may decompose as rapidly as 2% per day (17), and the enzymatic activity of cellulose (18), like that of chitinase (10), is measurable by chemical rather than more sensitive radiochemical procedures in hours instead of weeks or months.

The results of this study, in which numerous heterogeneous microbial communities failed to effect biodegradation of the plastics tested, suggest that the evolution of the required biochemical catalysts within these microbial communities has not yet developed.

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