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AEROBIC BIODEGRADATION OF SYNTHETIC AND NATURAL POLYMERIC MATERIALS: A COMPONENT OF INTEGRATED SOLID-WASTE MANAGEMENT

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ABSTRACT

Solid waste collection, handling, and processing systems are changing worldwide as concerns increase regarding the environmental impact of solid waste. Recycling, waste to energy, and composting options are all expected to increase significantly as available landfill capacity decreases. As composting becomes an important method of managing solid waste, information about the biodegradability of polymeric materials and products is required in order to understand the fate of polymers in the environment. This paper describes the development of a tiered testing strategy for assessing the aerobic biodegradability and compostability of synthetic polymers. This testing strategy is demonstrated with polycaprolactone (PCL), a polymeric material that is generally regarded as biodegradable and compostable. PCL biodegradation was assessed in screening-level tests using the OECD 301B and ASTM D5338 test methods. In addition, ^{14}C-PCL was synthesized and used in confirmatory-level biodegradation tests that were conducted under realistic composting conditions. These studies show that screening-level biodegradation tests can provide knowledge of the inherent biodegradability of polymers, but the rate of biodegradation and the ultimate fate of polymeric materials in environmental matrices must be determined under realistic test conditions.

INTRODUCTION

Concerns about the management of municipal solid waste (MSW) continue to grow around the world. As waste management authorities are forced to deal with the problems associated with solid waste accumulating in landfills and with operation of mass-burn incineration systems, alternative solid waste collection, handling, and processing systems are being considered. An emerging principle that unifies these diverse options, called integrated waste management, requires that we process the various fractions of the solid waste streams according to the unique characteristics of the fractions. The application of this principle means that we will recycle those items that can be recovered for value, compost the organic biodegradable fraction that includes soiled paper, food, and yard waste, and incinerate the remaining organic fraction of the waste stream to recover energy. The remaining MSW, which will be small and largely inorganic (<30–40% of the waste stream), can then be landfilled safely. In addition to recovering useful materials, the advantages of this approach include the use of smaller facilities for the same total waste flow, a better match between facility design and waste characteristics, less variability in the waste fed to the facilities, and the resultant residue is likely to be more stable than the original waste streams.

Composting will become increasingly important as a method of solid-waste management since this technique can handle up to 60–70% of the waste stream, converting the organic fraction into a useful product called compost [2]. Composting can be described as a method of solid-waste management whereby the organic component of the waste stream is biodegraded under controlled conditions. As composting becomes more prevalent, manufacturers will need to determine the compostability of their materials and products that will be disposed via solid waste. After consultation with technical experts and consideration of US Federal Trade Commission guidelines, we have defined a compostable material as:

> An organic material which undergoes physical, chemical, thermal, and/or biological degradation in a municipal solid waste composting facility such that the material will break down into, or otherwise become part of, usable finished compost, and which ultimately will completely biodegrade in the environment in a manner similar to known compostable materials in municipal solid waste such as paper and yard waste.

One of the key elements of the compostable material definition requires that we assess the biodegradability of a test material. Biodegradation results when an organic material is used by microorganisms as a nutrient or when microorganisms carry out cometabolism. When microorganisms use the chemical as a carbon and energy source, they metabolize the chemical through biochemical reactions. Some of these reactions, which are catabolic, result in complete mineralization of the chemical to products such as CO_2 and CH_4 (under anaerobic conditions). Other reactions, which are anabolic, result in incorporation of the carbon, nitrogen, and other elements into new biomass.

During cometabolism, the chemical is not used as a nutrient and the microbial population responsible for biodegradation does not increase. Cometabolism typically occurs when a material is nonspecifically metabolized by enzymes meant for other compounds [4]. The result is usually only a partial breakdown of the material. On the basis of our review of the polymer biodegradation literature, we are not aware of any studies that clearly document the cometabolism of synthetic polymers.

In addition to being mineralized, another fate exists for carbon derived from chemicals that are biodegraded, particularly with polymers in compost and soil. This fate involves humification, in which metabolites as well as new and old biomass become incorporated into humic materials. This process is biologically and chemically catalyzed slowly over time and is a major sink for a significant portion of many organic compounds that enter compost and soil. Therefore, mineralization in soil, measured as the yield of CO_2 or CH_4 can be quite low [10].

Among the important criteria for compostability of new materials, biodegradation and disintegration need to be determined early in the evaluation process. We have developed a tiered testing approach toward the evaluation of new materials (Table 1). This approach allows us to focus the correct level of resources on the materials of greatest interest and potential. The objective of this paper is to describe screening-level and confirmatory-level test methods that can be used in the initial stages of the tiered testing approach and to highlight advantages and shortcomings with each method. These test methods are demonstrated with polycaprolactone (PCL), an aliphatic polyester that is generally regarded as biodegradable [3].

TABLE 1. Tiered Testing Approach for Compostability Assessment

1. Screening
    A. Inherent Biodegradability
        Are the repeating subunits of a polymeric material inherently biodegradable?
        Is the polymer inherently biodegradable?
    B. Physical and Chemical Disintegration
        Does the material disintegrate under composting conditions?
        Do the chemical characteristics of the material change after exposure to a compost environment?
2. Confirmation
    A. Practical Biodegradability
        Does the material actually biodegrade in compost and soil under realistic conditions?
    B. Is the composted polymer/material safe?
3. Field Testing
    A. Fate and Effects Testing in MSW Compost Facilities
        Does the material/product show expected physical and chemical disintegration in situ over time?
    B. Fate and Effects Testing in Terrestrial Ecosystems
        Do persistent intermediates or residues accumulate over time?

MATERIALS AND METHODS

Sample Preparation

PCL resins were obtained from Union Carbide (Tone 787), and PCL films were 1-3 mil melt-blown films. PCL resins and films were pulverized with a Spex 6700 freezer mill (Spex Industries, Metuchen, NJ) using a stainless steel impactor, two steel end plugs, and a steel center cylinder. Film samples larger than the diameter of the vial were precut to approximately 0.5 cm squares. The grinding vial was filled with approximately 0.5 g of sample. The vial was placed in the cylindrical compartment within the mill and immersed in liquid nitrogen for approximately 1 minute, and the impactor was started. After 5 minutes of grinding the vial was removed from the liquid nitrogen and the sample particle size was determined. An additional 5 minutes of grinding was usually required. Sample surface area was determined using a Quantachrome Quantasorb Sorption System (Syosset, New York) that is based on gas absorption. The system was equipped with a Spectra-Physics SP4100 recorder (San Jose, California). Ultrahigh purity nitrogen was used for the adsorbate gas and ultrahigh purity krypton (0.078%) in helium as the carrier gas. Three contiguous replicates were tested for each sample. A Tekmar A-10 analytical mill (Cincinnati, Ohio) was also used to grind PCL film. The film was precut to approximately 0.5 cm squares. The mill lid and sample container, including sample, were cooled with a small volume of liquid nitrogen. After the liquid nitrogen evaporated, the mill was quickly assembled, pulsed for 10 seconds, unassembled, and the sample was brushed into the sample container. This procedure was repeated as necessary.

Radiolabeled-PCL was synthesized using [u-^{14}C]-ϵ-caprolactone prepared from ^{14}C-cyclohexanone (New England Nuclear) by a Baeyer–Villiger oxidation. The polymer was then prepared by bulk polymerization of a mixture of the labeled lactone and unlabeled caprolactone with a dialkylaluminum isopropoxide initiator in a manner similar to those described by Teyssié and coworkers [5]. The [u-^{14}C]-PCL was precipitated from methylene chloride in methanol and stored and used as a fluffy powder. Combustion analysis of the [u-^{14}C]-PCL was performed using a Packard 307 Sample Oxidizer (Meridin, Connecticut). Chromatographic analysis of the [u-^{14}C]-PCL was performed using a Water's Millennium Chromatography System (Marlborough, Massachusetts) equipped with a Model 410 differential refractometer detector, Shodex KF 804L columns, and a tetrahydrofuran mobile phase. The effluent from the columns was collected at 0.5 minute intervals and counted on a Beckman LS7800 (Fullerton, California) liquid scintillation counter to determine the distribution of radioactivity in the [u-^{14}C]-PCL sample.

Screening-Level Biodegradation Studies

The Sturm test, which measures CO_2 production as the biodegradation endpoint, is an aqueous aerated test that uses the supernatant from settled activated sludge as the source of microbial inoculum [9]. The modified Sturm test, designated 301B by the Organization for Economic Cooperation and Development [6], was used in this study. PCL samples were tested at a concentration of 110 mg/L in this test.

The compost-CO_2 test is an aerobic biodegradation test that uses stabilized mature compost derived from the organic fraction of municipal solid waste (MSW) as the microbial inoculum and the test matrix. This test simulates an intensive aerobic composting process and was done according to the procedure designated D5338 by the American Standards and Test Methods Society [1]. PCL was tested in triplicate at a concentration of 10% (w/w).

Confirmatory Biodegradation Studies

The extent of degradation of [u-^{14}C]-PCL was followed in a mature compost matrix that was equivalent to the compost used in the ASTM D5338 test method and was produced in a pilot-scale compost facility [8]. After adjustment of the moisture level to 50% (w/w), 100 g compost was added to 500 mL, three-neck, round bottom flasks. Each flask was fitted with a stirring shaft, a condenser, and an air sparger. The flasks were submerged in either a 45 or 55°C water bath, and experiments were conducted isothermally. Radiolabeled-PCL was added to test flasks at concentrations $<1\%$ (w/w) and tested in duplicate. A CO_2-free air supply was connected to each flask, and mineralized $^{14}CO_2$ was collected in a base trapping system. The flasks were connected to a CO_2 scrubbing train that consisted of two absorber bottles in series that were filled with 100 mL ethylene glycol monomethyl ether:ethanolamine (7:1). The CO_2 produced in each test flask reacted with the base in the absorber bottles, and the amount of CO_2 produced was determined by scintillation counting of the base traps. Periodically the absorber nearest the test flasks is removed for analysis. The remaining absorber bottle is moved one place closer to the test flasks and a new absorber bottle with fresh solution is added to the end of the trapping system. Scintillation counting was done using a Beckman LS7800 (Fullerton, California) operated in an external reference mode.

RESULTS AND DISCUSSION

Numerous methods have been developed to determine the biodegradability of natural and synthetic polymers. For example, Cain [3] reviewed the PCL biodegradation literature and noted that microbial growth, enrichment culture, and enzymatic depolymerization assays have been used to assess the biodegradability of this material. While all of these methods facilitate the elucidation of the mechanisms by which PCL is biodegraded, these methods generally produce results that are of limited value in assessing the environmental fate of a material and they do not provide direct evidence of complete biodegradation. In addition, the lack of consistent standard methods to assess polymer biodegradability has often resulted in confusion and misinterpretation among scientists and the lay public [7]. The tiered testing approach described in Table 1 was developed in order to assess the compostability and environmental safety of a large number of new synthetic polymers in an economically feasible manner. This tiered approach includes initial screening-level tests that determine the inherent biodegradability of a material followed by confirmatory laboratory and field testing where the practical biodegradability of a material is determined.

Screening-Level Studies

Knowledge of the inherent biodegradability of a polymer and its repeating subunits and degradation products is the first step of the tiered testing strategy described in Table 1. In some cases, such as the case for PCL, this information can be obtained by a literature review. However, the inherent biodegradability of new materials that have no prior testing history can be assessed by several screening-level tests. The modified Sturm test was originally developed in our laboratories in the early 1970s and is an example of a screening-level test [9]. Test compounds are placed in an inorganic medium and inoculated with the supernatant of homogenized activated sludge-mixed liquor. This test measures the mineralization of the test material, and the results are expressed as the percentage of theoretical CO_2 that could be produced if all of the carbon in the test material was mineralized to CO_2. The Sturm test was initially developed to assess the biodegradability of relatively low molecular weight materials that are discarded down the drain. Therefore, the Sturm test is ideal for testing the biodegradability of low molecular weight polymer repeat units or degradation products. However, due to the paucity of alternative methods, the Sturm test has often been used by default for testing high molecular weight polymer biodegradability. Because of the limitations associated with testing polymeric materials in the Sturm test, we investigated ways to improve polymeric material preparation and test reproducibility.

To study the effect of surface area on the biodegradability of PCL, we prepared a series of PCL samples with different surface areas and tested their biodegradability using the modified-Sturm test (OECD 301B). Increasing the surface area of PCL resulted in an increase in the rate and extent of CO_2 production for surface areas up to 0.122 m^2 per gram of sample (Table 2). Grinding PCL samples at liquid N_2 temperatures resulted in smaller particle sizes and larger surface areas without significant loss in molecular weight (Table 2). Despite optimizing the surface area of PCL, the maximum CO_2 yield obtained in this study was 63% TCO_2 after 180 days in the modified-Sturm test (Table 2). During this extended study the test chambers were reinoculated at periodic intervals in order to maintain an active microbial culture. While increasing the biomass level could facilitate the rate of biodegradation in the modified-Sturm test, the low biomass levels used in this test are required in order to obtain a good signal-to-noise ratio.

Since the conditions of the Sturm test were not originally optimized for poly-

TABLE 2. Effect of Surface Area on PCL Biodegradation in the Modified Sturm Test

Surface area (m^2/g)	MW (daltons)	Preparation technique	CO_2 production, % theoretical at 180 d
0.0035	109,500	Resin pellets	8.4
0.0112	99,400	Extruded film	36.6
0.0303	100,100	Film/analytical mill	37.4
0.0863	105,300	Pellets/cryogenic grinder	40.1
0.1216	104,300	Film/cryogenic grinder	62.6

meric materials, there are several limitations that must be considered. The length of polymer studies are often problematic since the microorganisms may die-off over time. The Sturm test was originally designed to run for 1 month, and tests requiring longer duration should be reinoculated. In addition, while an activated sludge inoculum is very diverse, it is primarily composed of bacterial populations, and the conditions of the Sturm test are not conducive to growth of filamentous microorganisms (e.g., actinomycetes and fungi). These groups of microorganisms are known to play key roles in the biodegradation of natural polymeric materials such as plant litter. Because of these limitations, alternative screening-level tests such as the ASTM 5338 Compost-CO_2 test were tried.

An alternative screening-level approach involves the use of a relatively high biomass level where the optimum CO_2 signal-to-noise ratio is maintained by increasing the test substance concentration. The biodegradability of a PCL sample ground with an analytical mill was tested in a compost matrix, containing a relatively broad diversity of microorganisms, using the ASTM D5338 test method. PCL degraded to an overall extent of 45% when considering all replicates (Fig. 1). However, one reactor acidified early in the test run and PCL biodegradation was halted. Biodegradation resumed after excess acid produced by the rapid hydrolysis of the PCL was degraded by the compost microorganisms. Neglecting the acidified reactor, the biodegradation after 45 days was 54% TCO_2. The ASTM D5338 method facilitates the exposure of the test material to a broad diversity of microorganisms in a high biomass system, but the high test substance concentration (10%, w/w) required to achieve good signal-to-noise ratios was problematic in the acidified reactor. Likewise, the artificially imposed temperature regime was noted to cause drastic shifts in microbial respiration (Fig. 1). While these results suggest that PCL is inherently biodegradable, they also point out the need for replicate analyses and demonstrate how different test conditions can yield different results in screening-level tests.

Confirmatory Studies

The second step of the tiered testing strategy outlined in Table 1 is the assessment of the practical biodegradability of a polymeric material. Biodegradation tests conducted at this level are designed to confirm the results that were obtained using

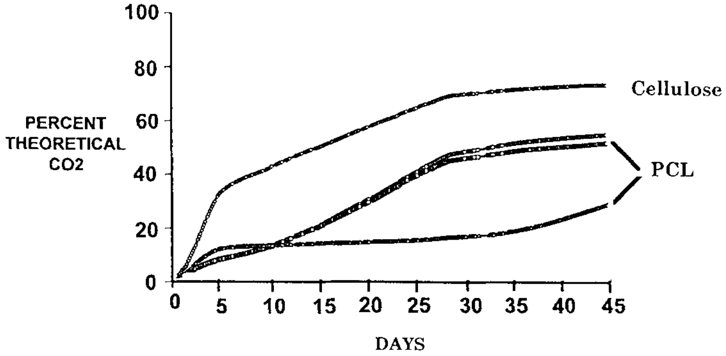

FIG. 1. Biodegradation of PCL in the compost CO_2 test (ASTM D5338). The incubation temperature was controlled in order to simulate an intensive aerobic composting process (Days 0-1, 35°C; Days 1-5, 58°C; Days 5-28, 50°C; Days 28-45, 35°C).

screening-level test methods, and they attempt to simulate actual environmental systems. In the context of integrated waste management we chose to study the biodegradation of polymeric materials in an aerobic compost environment.

Biodegradation test conditions that mimic a compost environment involve high biomass levels in a solid matrix, low test substance concentrations, and require the use of sensitive analytical techniques. One common technique involves the use of isotopically labeled test materials. A uniformly ^{14}C-labeled PCL was prepared by "ring-opening" polymerization and was determined to have a specific activity 0.975 mCi/g. Chromatographic analysis of the [u-^{14}C]-PCL indicated that the M_n and M_w were 79,138 and 98,084, respectively, relative to polystyrene standards. The distribution of radioactivity in the various molecular weight fractions of the [u-^{14}C]-PCL was associated with a single peak, indicating radiochemical purity (Fig. 2). In addition, chromatographic analysis of the [u-^{14}C]-PCL indicated that only background levels of radioactivity were detected before and after the main peak.

The results of confirmatory-level biodegradation experiments using [u-^{14}C]-PCL in a mature compost matrix are shown in Figs. 3 and 4. These experiments were conducted under test conditions that mimic municipal solid waste compost conditions, and the results confirm the screening-level results that suggested PCL is inherently biodegradable. The combination of high biomass, relatively low concentration of test material, and elevated incubation temperatures facilitated the complete and relatively rapid biodegradation of PCL, indicating that PCL is practically biodegradable in a compost environment. Maintenance of isothermal conditions in these experiments facilitated kinetic analysis of the biodegradation data. The results of PCL biodegradation at 45 and 55°C are consistent with the general rule that higher temperatures that do not kill microorganisms or denature enzymes will result in higher metabolic activities. If the biodegradation of materials like PCL can be modeled by the Arrhenius equation, we may be able to predict the biodegradation of PCL and other similar polymers at ambient temperatures from studies run at elevated temperatures. However, in the case of PCL, the relative roles of biotic and

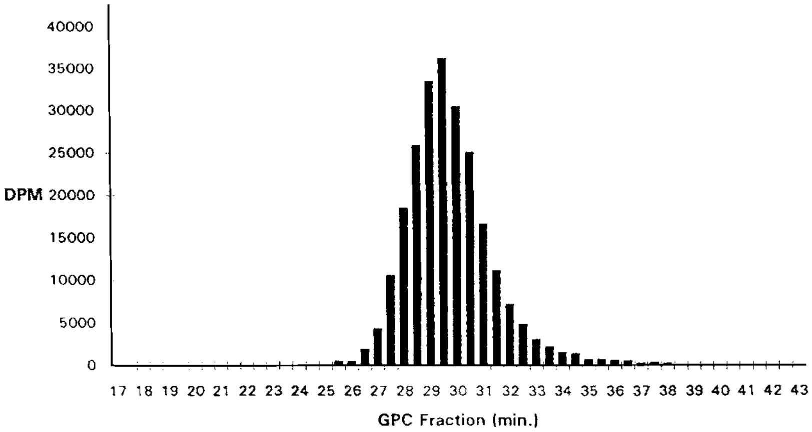

FIG. 2. Distribution of radioactivity in fractions collected from a [u-^{14}C]-PCL sample separated by gel permeation chromatography.

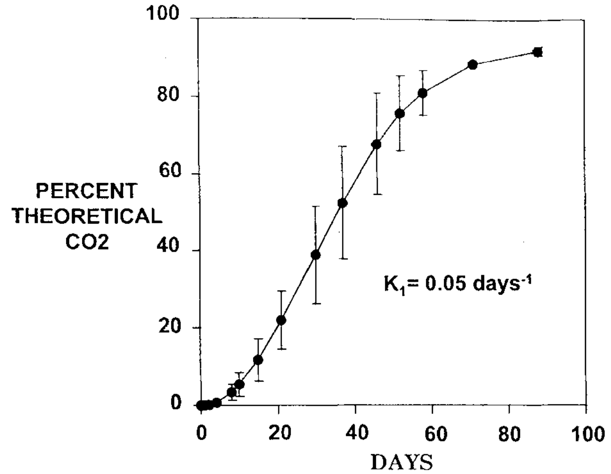

FIG. 3. Biodegradation of PCL in 45°C compost $^{14}CO_2$ test. These data are the means and standard errors of the values obtained for two replicates. K_1 is the first-order rate constant (days $^{-1}$).

abiotic ester hydrolysis of PCL and the interactive effects that temperature may have with other factors such as T_m requires further testing.

Both the screening-level and confirmatory tests used in this study measured the mineralization of the test material to CO_2 as the primary biodegradation endpoint. While PCL was completely mineralized in the [u-^{14}C]-PCL compost test, many other slowly degrading synthetic polymers will require alternative biodegradation endpoints, such as disappearance of parent compound, appearance of interme-

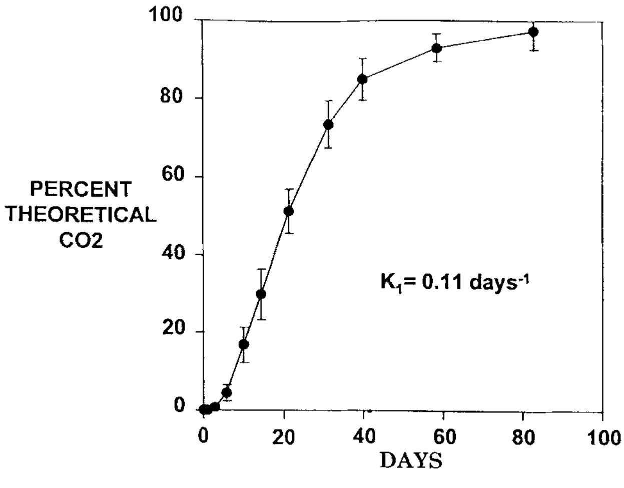

FIG. 4. Biodegradation of PCL in 55°C compost $^{14}CO_2$ test. These data are the means and standard errors of the values obtained for two replicates. K_1 is the first-order rate constant (days $^{-1}$).

diates/metabolites, and incorporation into biomass or humic materials. Both the screening-level and confirmatory tests used in this study have the common limitation that they were in-vitro batch systems which ultimately encounter nutrient limitations and lack mechanisms for product removal. While closed batch systems facilitate mass balance determinations, the artificial conditions used in these tests do not mimic the open systems found in the environment and may ultimately limit the biodegradability of a test material. Alternative confirmatory-level biodegradation tests using mesocosm designs or field tests using in-situ conditions are required to achieve an open system.

CONCLUSIONS

As composting becomes an important method of managing solid waste, information about the biodegradability of polymeric materials and products is required in order to understand the fate of polymers in the environment. The implementation of a logical testing approach that includes consistent biodegradation test methods is critical to the development of compostable materials and testing claims of biodegradability. On the basis of initial experiments conducted with PCL, a synthetic polymer that is generally regarded as biodegradable, we conclude that screening-level tests provide useful information but that biodegradation test results can be highly dependent on the conditions of the test. Care must be taken to design screening-level tests to provide information on the inherent biodegradability of a polymer and not practical biodegradability. As noted in the compost-CO_2 test, attempts to generate both types of information tend to complicate the execution of the screening-level test and confuse the interpretation of results. The rate of biodegradation of complex high MW polymers can be very slow, and screening-level tests may provide misleading or false negative results. Confirmatory-level testing should be performed under realistic and relevant conditions in order to facilitate the extrapolation of results to the environment.

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