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BIODEGRADATION OF POLYCAPROLACTONE BY MICROORGANISMS FROM AN INDUSTRIAL COMPOST OF HOUSEHOLD REFUSE. PART II

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ABSTRACT

The rate of biodegradation of polycaprolactone samples, hydroxy (M_n = 4000 and 37000) and methoxy (M_n = 4000) terminated, by mixed cultures of microorganisms from a suspension of compost, and by a pure culture of an actinomycete isolated from it, has been monitored by the measurement of oxygen consumption. The dependence on polymer molecular weight suggests that initiation of degradation occurs in the vicinity of chain ends. Further analysis of the residues allows primary considerations about the bacterial metabolism of the substrate.

INTRODUCTION

Biodegradation of two molecular weight (MW) samples of polycaprolactone (PCL) has been monitored by a respirometric method. Oxygen consumed by aerobic bacteria has been measured, allowing a kinetic approach of polymer degradation, by microorganisms from a suspension of compost, and by a pure culture of an actinomycete isolated from it. Further characterization includes mass loss evaluation of the polymer, gel permeation chromatographic (GPC) analysis of the residue, and the measurement of the biomass produced by microorganisms.

MATERIALS AND METHODS

Details on materials and methods have been described previously [1].

Polymers

Hydroxy-terminated PCL samples were used (M_n = 4,000 and 37,000). Purified samples and methoxy-terminated PCL4000 were prepared. The polymers were used as cut pieces of films (about 50 μm thick) for PCL37000 and as small film fragments for pure and methylated PCL4000 at a concentration of 0.2% (w/v) after sterilization. Crystallinity of the samples was determined by DSC using the value of 135.6 J/g for the melting enthalpy of a 100% crystalline sample [2].

Inoculum

Microorganisms from a suspension of an industrial compost of household refuse and a pure culture an actinomycete isolated from it were used.

Measurement of O_2 Consumption

The method consists in the manometric measurement of O_2 depletion from a closed bottle containing the polymer as carbon source and an inoculum of microorganisms in an inorganic basal medium at 35°C and without light (see Fig. 1).

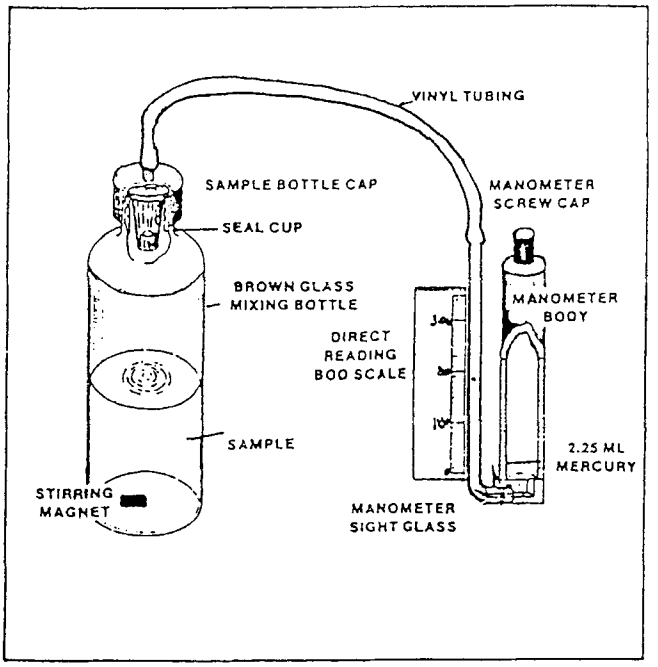

FIG. 1. Manometric system (the polycaprolactone samples used in our tests are not soluble in the inorganic basal medium).

Released CO_2 is trapped by NaOH pellets in the seal cup of the bottle. A blank is run that contains only the inoculum and the inorganic basal medium. The O_2 uptake is subtracted from the value obtained with the test bottle. The difference is assigned to degradation of the carbon source [3]. O_2 consumption is expressed in percent of the theoretical value required to transform quantitatively the substrate in CO_2 and H_2O.

Further Analysis

The experiment was stopped at the plateau of O_2 consumption. The solution was filtered through a 5-μm Teflon filter. The residual polymer and the formed biomass are retained on the filter. Residual polymer is separated from biomass by quantitative extraction with CH_2Cl_2. Undegraded polymer, and hence weight loss, is determined after evaporation of the solvent. The filter is then dried and the biomass weight is measured. The residual polymer is analyzed by a GPC Waters 150C model with Ultrasyragel columns and compared with initial PCL samples, the same quantity being injected on the top of the columns in all cases; a calibration has been achieved with PCL standards.

RESULTS

Typical results are given in Table 1 and Fig. 2 for the first inoculum. Duplicates are reported in Table 1 and Fig. 3 for the actinomycete strain.

Microorganisms from a Suspension of Compost

A small amount of yeast extract (0.01%) was necessary to achieve biodegradation [1]. High O_2 consumption values and nearly complete degradation of the polymer samples were then obtained. More time was required, however, to reach the plateau for PCL37000. Biomass amounts are similar, but relatively low.

Pure Culture of the Actinomycete

Samples of purified PCL4000 and PCL37000 and of methylated PCL4000 were used without any addition of another C source. High values of O_2 consumption rates were registered for all of the tested samples, as well as nearly complete disappearance of the polymers. However, the polymer biodegradation requires different quantities of O_2 at the plateau and results in different amounts of biomass; PCL4000 and PCL37000 samples are differentiated through the O_2 consumption rates.

GPC Analyses

Residual PCL37000 of each test series (Fig. 4), as well as methoxy- or hydroxy-terminated PCL4000 (not shown) of the pure culture, were analyzed by GPC. In all cases, biodegradation of the polymers results in the disappearance or decrease of the high MW peak without any shift, but two small peaks appeared in

TABLE 1. Biodegradation of Polycaprolactone

Inoculum	Substrate[a]	Substrate cristallinity, %	Oxygen consumption,[b] %	Weight loss, %	Biomass, mg	Carbon conversion to biomass,[d] %
Microorganisms from a suspension of compost	PCL 4000 + YE[c] (1)	76	53.8	89.4	17 ± 1	17 ± 1
	PCL 4000 + YE (2)		66.3	93.5		
	PCL 37000 + YE	55	78.0	93.9	16	16
Pure culture of actinomycete	PCL 4000(purified)	77	51.8	99.6	43 ± 4	39 ± 3
	PCL 4000(purified)		55.5	99.0		
	PCL 4000(methylated)	65	57.7	97.9	31 ± 10	29 ± 9
	PCL 4000(methylated)		61.7	93.9		
	PCL 37000(purified)	63	50.5	96.7	49 ± 3	44 ± 4
	PCL 37000(purified)		45.5	97.5		

[a]0.2% (w/v) in the inorganic basal medium.

[b]In percent of the theoretical value of O_2 consumption for complete transformation of the substrate into H_2O and CO_2.

[c]YE: yeast extract (Difco) 0.01% in the inorganic basal medium.

[d]Expressed as C biomass produced (mg)/C substrate used (mg) (C content in the biomass is estimated by the general formula $CH_{1.8}O_{0.5}NO_2$, which is a good average for many microorganisms) [4].

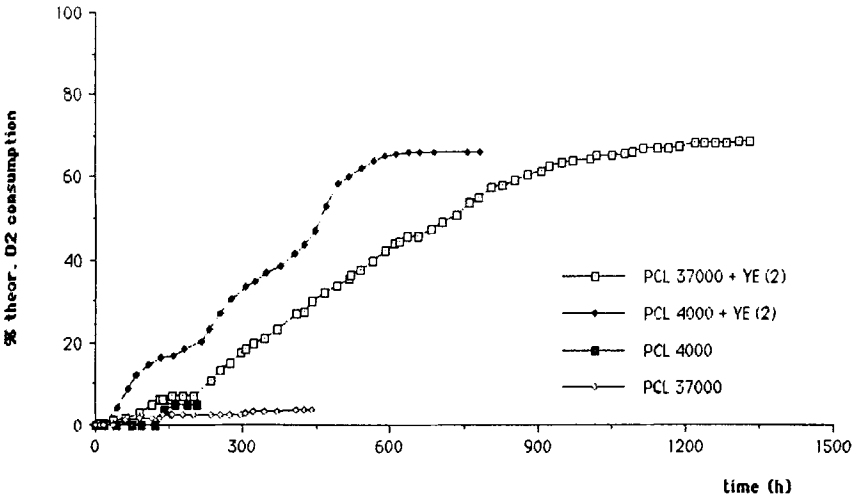

FIG. 2. O_2 consumption by a suspension of microorganisms from compost growing on polycaprolactone 0.2% in the basal medium. YE: yeast extract (0.01%) (Difco) in the basal medium.

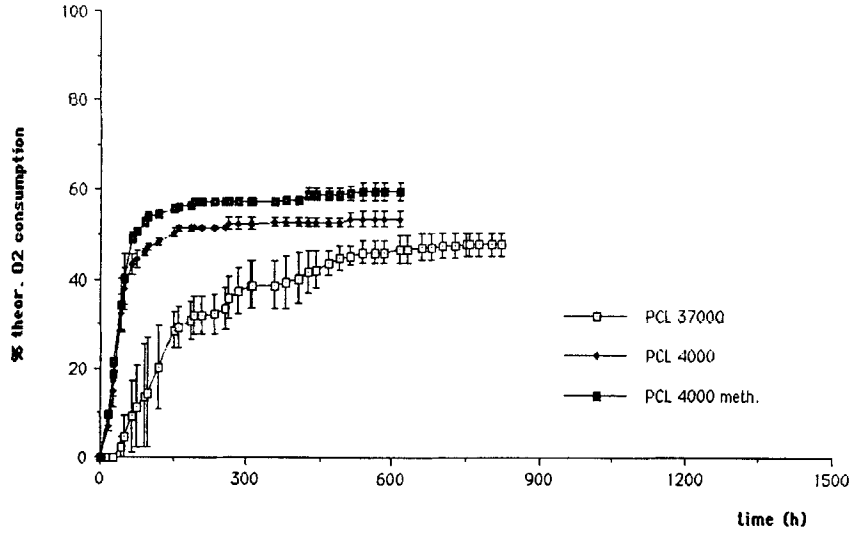

FIG. 3. O_2 consumption by a pure strain of an actinomycete isolated from compost growing on polycaprolactone 0.2% in the basal medium. The points shown are the mean values of the data from Table 1.

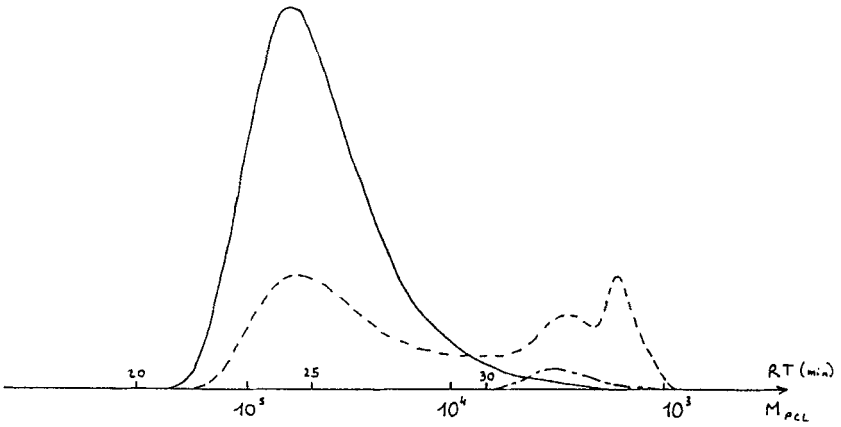

FIG. 4. GPC diagram of PCL37000. (—) Initial polymer. (- - -) Residual polymer after degradation by microorganisms from compost (6.1% residue). (- - -) Residual polymer after degradation by a pure culture of actinomycete (3.3% residue).

the low MW area; they correspond to oligomers of M_n = 2930 and 1430. These oligomers are present in very small amounts, since residual polymer is only a few percent of the initial quantity. They are supposed to be degradation intermediates that would finally be completely degraded.

DISCUSSION

Oxygen Consumption and Degradation Mechanisms

For both kinds of inocula, the rate of O_2 consumption by high molecular weight polymer is always lower, and more time is required to reach the plateau. This leads us to consider the degradation mechanism as an end-initiated unzipping process (see Ref. 1 for further details). This is not incompatible with the same O_2 consumption rates observed for HO- and CH_3O-terminated PCL4000. Indeed, the methoxy-termination is at least five CH_2 units away from ester bonds, so it should not inhibit the binding of the hydrolase enzyme to these bonds. Crystallinity does not seem to retard biodegradation, the O_2 consumption rate being higher for the more crystalline sample (PCL4000) than for the less crystalline one (PCL37000).

Oxygen Consumption and Carbon Conversion to Biomass

Upon biodegradation, the carbon source is transformed into biomass, CO_2, and eventually secondary products. Oxidation to CO_2 results mainly from the tricarboxylic acid cycle, typical of aerobic bacteria metabolism. This cycle is coupled with the electron transport chain. It uses O_2 as the terminal electron acceptor and is referred to as oxidative phosphorylation. This allows ATP synthesis, the aerobic bacteria energy source, and uses the main part of consumed O_2. ATP synthesis, O_2 consumption, and oxidation of the substrate to CO_2 are thus closely related. It has

been proven from theoretical calculations and observations [4] that the nature of the substrate has a great influence on the ATP requirement for transformation into biomass. Compounds such as pyruvate or acetate need more ATP than glucose, so that more CO_2, and then less biomass, is produced with the same amount of O_2 [4].

In the case of PCL, examination of the biomass amount data of Table 1 leads to the following conclusions. With the pure culture, more carbon is converted to biomass in the case of PCL37000 than with PCL4000, indicating that more ATP is required for PCL4000 biodegradation, perhaps because of its higher crystallinity. Methlylated PCL4000 biodegradation likewise requires more ATP. The actinomycete is more efficient in carbon conversion than the consortium of microorganisms from the compost, although yeast extract is provided in it.

CONCLUSION

Nearly complete biodegradation of PCL4000 and PCL37000 has been observed with microorganisms from an industrial compost. Small amounts of oligomers have been noticed in all cases. Initial oxygen uptake rates are greater for hydroxy- and methoxy-terminated PCL4000 than for PCL37000. With microorganisms from the pure culture, carbon conversion to biomass is slightly higher for PCL37000 than for hydroxy-terminated PCL4000, but is significantly lower than both of them for methoxy-terminated PCL4000.

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