A Simple Method Suitable to Test the Ultimate Biodegradability of Environmentally Degradable Polymers

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Summary: A straightforward experimental set-up derived from the Biometer Flask previously utilized for experiments of pesticides biodegradation, has been adopted for testing the ultimate biodegradability of natural, synthetic and semi-synthetic polymeric materials on solid substrates such as soil and mature compost. The use of these whole substrates as incubation media in respirometric experiments, may negatively affect the accuracy of the test due to the large amount of carbon dioxide developed from the blanks, especially in the presence of specimen degrading at low or moderate rates. In the present test procedure soil and compost samples are diluted with perlite, a naturally occurring inert aluminum silicate widely utilized in horticultural applications, in order to ensure optimal conditions for the microbial growth while reducing the carbon dioxide emissions from the blanks. The results so far reported clearly indicate that the adopted procedure is extremely valuable and versatile for the appreciation of even subtle differences in the biodegradation rate of different polymeric materials, as well as for long-term degradation experiments.

Keywords: biodegradation; biometer flask; long-term biodegradation; polymers; respirometric test

Introduction

Polymeric materials and relevant items designed to exploiting their entire life-cycle in natural environments are ever more required to meet ultimate biodegradation at the end of their service life. Actually several test methods aimed at defining the potential biodegradability of polymeric materials have been developed and adopted as official procedures by international standards organizations (ISO) and regional organizations such as ASTM (USA), CEN (Europe) and JIS (Japan). Historically, many of these tests, and particularly those adopted for the evaluation of plastic materials in aqueous media, have been derived from the Sturm test, and Semi-continuous Activated Sludge (SCAS) test which were designed for assessing the biodegradation of surfactants through the determination of carbon dioxide evolving from the test materials as a consequence of

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their microbial digestion. Indeed, respirometric determinations of carbon dioxide productions, as well as biological (BOD) and chemical (COD) oxygen demand, represent the basic point in order to directly quantify the metabolization of the organic carbon constituting a test material. Accordingly, CO_2 measurements are at the basis of several official test methods, comprising those suggested for the evaluation of biodegradability under composting conditions (1). Nevertheless, it should be avoided that large amounts of carbon dioxide deriving from the incubation media (soil and mature compost) may affect the accuracy of the determination of CO_2 amount developed by the microbial assimilation of test materials.

A straightforward practice suitable to increase the level of accuracy is represented by the replacement of relatively large amount of natural solid matrix with inert substrates such as perlite and vermiculite, as previously suggested in the set-up of biodegradation respirometric experiments under soil burial and composting conditions, respectively (2,3). Regarding the determination of CO_2 emissions, it has been recognized that the kind of measurements may influence both the feasibility and the confidence of a test procedure (4). According, the utilization of radiolabeled samples accounts for the highest level of confidence in appreciating the CO_2 emission from the test materials with respect to the blank (5). Nevertheless this technique is very much demanding in terms of laboratory equipment and personnel expertise. Instrumental CO_2 detection can be also carried out by infrared spectroscopy and gas-chromatography in on-line assessment of the exhaust gas flowing from the test cultures. However, also this procedure usually requires costly apparatus that may somehow limit the number of samples and replicates to be analyzed in a biodegradation experiment.

One of the most common methodology utilized in the measurement of CO_2 in biodegradation experiments of organic matter is the absorption of the gas in alkaline solutions, usually $Ba(OH)_2$, as driven by flowing CO_2-free air in the test cultures tightly connected with alkaline solution absorbers. In this case, however not all the CO_2 produced may be quantitatively entrapped, leading to underestimation and errors at risk of cumulation in repeated determinations (6).

In the present contribution we wish report on the description of a simple method for the laboratory determination of the ultimate biodegradability propensity of different natural, semi-

synthetic and synthetic polymeric materials buried in solid substrates (soil and mature compost). Thus convenient test procedure can be considered an inspiring adjustment of the biometer flask test methodology introduced for the determination of the biodegradation of low molar mass organic matter (7). In our case, soil and compost matrices were reduced to a minimal required amount by partially replacing them with an inert material from horticultural applications (perlite). Evaluation of biodegradation extent within the time were carried out under equilibrium conditions by absorption in alkaline solutions placed directly inside the test vessels, of the developed CO_2 an its determination by back titration of the excess base. This practice guarantees for precise appreciation of the CO_2 production from the background emissions also in the presence of test materials displaying low-moderate rates of degradation. Moreover the procedure allows for the contemporary testing of many samples and replicates, useful for statistical results validation, and it has been found effective in long term biodegradation experiments.

Biodegradation assessment of typical polymeric specimens (Table 1) is reported as validation of the adopted procedure that we hope might provide valuable element of the discussion in the ongoing more of the EC-Technical Committee 249 on Plastics, that established a working group WG9 aimed at building up a standard for the degradation of plastics in soil media (8).

Experimental

Pine forest soil samples collected at San Rossore natural park in the in the neighborhood of Pisa (Italy) and mature compost samples from green waste, kindly supplied by Geofor s.p.a. (Pontedera-Italy), were utilized as microbial sources in the biodegradation experiments. The tests were carried out in cylindrical glass vessels (Biometer Flask) (500 or 1000 ml capacity) containing a multilayer substrate in which defined amounts of forest sandy soil (10-25 g) or mature compost (5-7 g) samples sieved at 0.6 mm were mixed with 20-25 g perlite and supplemented with 25 ml of 0.1% $(NH4)2HPO4$ solution. Finally the mixtures were sandwiched between two layers consisting of 20 g perlite wetted with 30 ml distilled water. Polymeric test

specimens (Table 1) were placed in the core of soil or mature compost middle layer at 20-70 mg/1000 mg ratios. The vessels were kept in the dark and incubated at room temperature in the case of soil burial tests, and at 55°C when mature compost was used as incubation medium.

The CO_2 evolved from samples and blanks was trapped in each test vessel by means of 40-100 ml of 0.05N KOH solution contained in a beaker set in the test vessel. The absorbing solution was back titrated with 0.1 N HCl every 2-15 days by adding before titration 0.5 N $BaCl_2$ solution in one-tenth proportion with respect to the overall volume of the absorbing alkaline medium. Phenolphthalein was used as indicator.

The biodegradation extent of each test material was evaluated as neat percentage (corrected for the inoculum endogenous emission from blank samples) of the overall theoretical CO_2 production calculated on the basis of the relevant carbon content in the testing sample.

The apparatus for the test method is schematically reported (Figure 1 A) in a comparison with the real situation (Figure 1 B).

Table 1. Polymeric materials utilized in the Biometer Flask biodegradation experiments.

Origin		
Synthetic	Semi-synthetic	Natural
Poly(ε-caprolactone) (PCL)	Acetylated starch (TPS)	Filter paper
Ecoflex SBX7000	PCL/lignin cast blend (PCL/L)	Lignin
Poly(vinyl alcohol) (PVA)	PVA/lignin cast blend (PVA/L)	
Ethylene-vinyl alcohol (EVOH44)[a)]	PCL/lignin graft copolymer (L-*g*-PCL)	
Low density poly(ethylene)-TDPA-(LDPE)[b)]	PVA/lignin graft copolymer (L-*g*-PVA)	

a) Containing 44 mol % vinyl alcohol units
b) LDPE containing Totally Degradable Plastic Additives (TDPA®) as supplied by EPI-Environmental Plastics Inc.,Canada

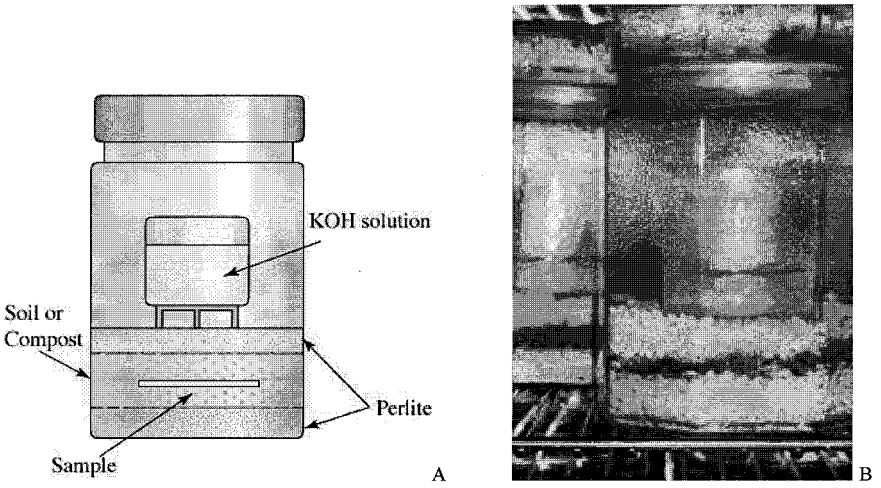

Figure 1. Biometer Respirometric Flask for simulated soil burial and mature compost biodegradation tests on polymeric material specimens. (A) Schematic representation. (B) Real situation with view of two flasks in a thermostat.

Results and Discussion

Biodegradation experiments carried out on typical polymeric specimens biodegrading at relatively high, moderate and low rate, in solid incubation media are described as aimed at demonstrating the simplicity, versatility and general validity of a cost-effective procedure that can be carried out even in laboratories not particularly equipped with expensive apparatus.

The introduction of a chemically inert material, such as perlite, for the partial replacement of natural solid incubation media in the culture substrate, represents the key feature of the adopted test procedure.

The role of perlite, a naturally occurring aluminum silicate, heat expanded, is to reduce the amounts of solid media and hence the carbon dioxide production from the blanks that may negatively affect the validity of the test. Perlite is indeed largely used in horticultural applications as a component of soil-less growing mixes, where it provides aeration and optimum moisture conditions for plant growth. Accordingly perlite was used to ensure satisfactory incubation conditions, whereas soil or compost samples were used mainly as microbial sources. This

arrangement guarantees for the rising of the signal-to-noise ratios, thus improving test accuracy, particularly when limited carbon dioxide emissions are expected from the test samples.

The incubation under static conditions is warning the operators from the utilization of $Ba(OH)_2$ solutions as CO_2 absorbing medium, in contrast with that suggested by ASTM D 5988-96 for determining the degree and rate of biodegradation of plastics in contact with soil (9). The rapid formation of a barium carbonate film on the surface solution, which strongly hinders the complete CO_2 diffusion into the alkaline solution (10), is the basic reason for preferring KOH instead of $Ba(OH)_2$ solution as absorbing medium. On the other hand, the KOH solution titration with HCl leads to the contemporary presence of carbonate and hydrogen carbonate, with a consequent buffering effect, thus determining only weak pH variations during the titration. This makes the appreciation of the color change of any suited indicator at the equivalent point rather difficult. Nevertheless, hydrogen carbonate in the presence of OH^- excess is converted to carbonate that can be easily precipitated by the addition of an adequate amount of $BaCl_2$ solution. The formation of the practically insoluble barium carbonate salt, allows for a simpler and more accurate back titration of the alkaline solution with HCl as appreciable by a clear and sharp change of the phenolphthalein used as indicator.

Mature Compost Biodegradation Tests

The biometer flask procedure described in the Experimental section has been utilized for testing the ultimate biodegradability (e.g. mineralization) in the presence of mature compost inoculum of different polymeric materials. The reliability of the adopted test conditions was demonstrated by the fairly high extent of mineralization (60 %) reached by filter paper and PCL after 60 days of incubation, which appeared to be degraded in a comparable extent as found in previous studies (11,12) (Figure 2). The scarce propensity to microbial attack of PVA in solid substrates (13-16), as well as the well known very low mineralization process of lignin, were also confirmed. Negligible biodegradation extents in the presence of compost microorganisms were indeed recorded in the case of ethylene/vinyl alcohol copolymer (EVOH) and BASF polyester Ecoflex synthetic polymers (Figure 2).

The reported test method was shown to be appropriate in appreciating relatively small differences in the mineralization rates of natural-synthetic polymers blends and copolymers. Accordingly, an investigation aimed at defining the biodegradation behaviors of PCL-lignin, PVA-lignin blends and relevant copolymers (17) in the presence of mature compost inoculum, clearly revealed the higher propensity to biodegradation of the hybrid material having the natural component chemically bound to the synthetic polymer matrices with respect to the comparable solution-cast blends. In particular, lignin-g-PVA copolymer approached 40% biodegradation in 90 days incubation, whereas the corresponding solution-cast blend film reached only 10 % mineralization in the same time frame (Figure 3). A similar behavior was observed in the case of PCL-lignin systems, thus recording a fairly higher extent of biodegradation (about 60 %) for the graft copolymer and a lower value (about 40 %) for the corresponding blend (Figure 3).

Interestingly, small but significant differences in the mineralization rates and extents were also appreciated in respirometric experiments carried out in order to assess the effect of the degree of hydrolysis (HD) on the biodegradation of PVA samples having the same degree of polymerization in the presence of mature compost microflora. The effect played by the HD of the analyzed PVA samples was clearly demonstrated (Figure 4), thus recording the highest biodegradation level (25 %) in the case of PVA containing 50 % of acetyl groups. The positive effect of acetyl groups was confirmed by the fairly high extent of mineralization reached by PVA samples having 30 and 75 % degree of hydrolysis, whereas the lower value was recorded for he almost completely hydrolyzed PVA sample (Figure 4).

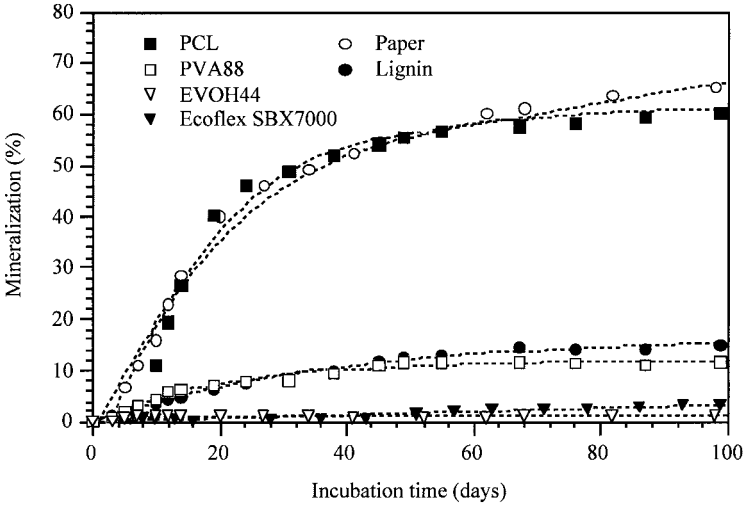

Figure 2. Mineralization profiles of different natural and synthetic polymeric materials as recorded in mature compost biometer flask respirometric tests.

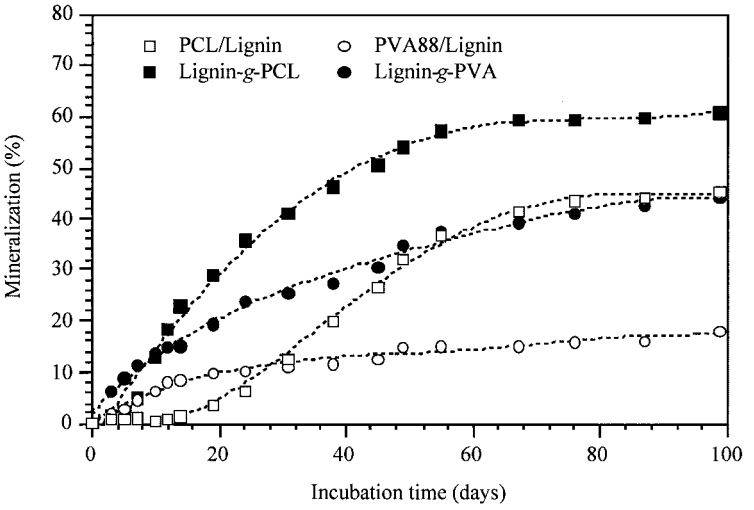

Figure 3. Mineralization profiles of PCL/lignin, PVA/lignin cast blends, and graft copolymers, in mature compost biometer flask respirometric tests.

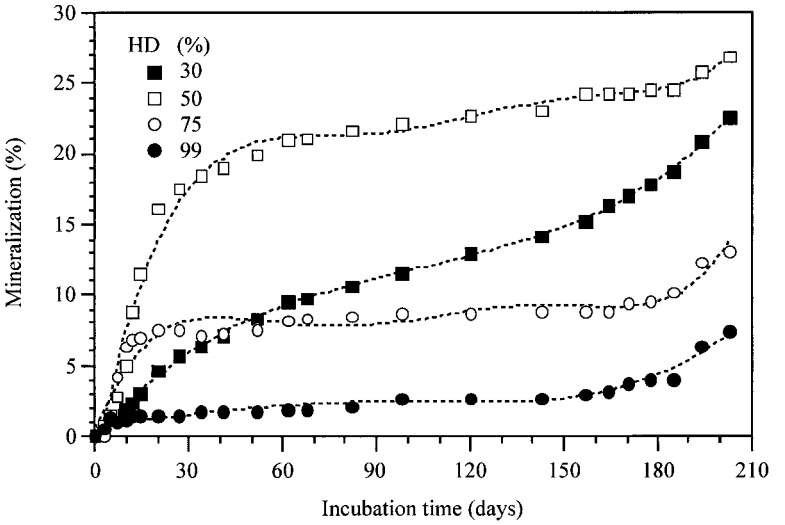

Figure 4. Mineralization profiles of PVA samples having the same DPn and different degree of hydrolysis, in mature compost biometer flask respirometric tests.

Soil Burial Respirometric Tests

In a first experiment, the ultimate biodegradation of different natural, semysynthetic and synthetic polymers was ascertained (Figure 5). Also in this case the accuracy and reliability of the test results were confirmed by the large extent of mineralization approached by filter paper (above 60 % after 120 days of incubation). Similar results were obtained in the presence of partially acetylated starch sample (TPS). The low propensity to biological attack of PVA in solid substrate (13-16), EVOH44 and Ecoflex was also ascertained (Figure 5).

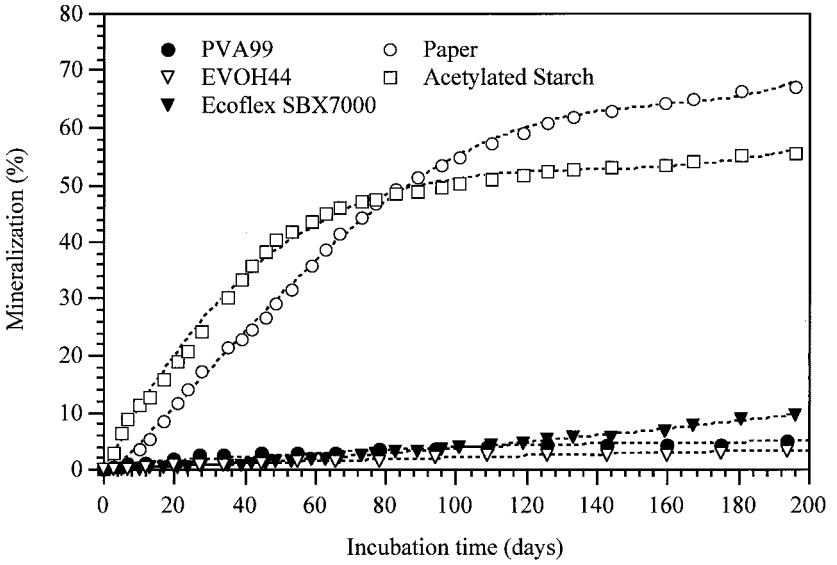

Figure 5. Mineralization profiles of natural, semi-synthetic, and synthetic polymeric materials as recorded in soil burial respirometric tests.

A soil burial test carried out by using the described procedure was also performed in order to evaluate the ultimate biodegradation of thermally-oxidized low density poly(ethylene) (LDPE) sample containing totally degradable plastic additives (TDPA®) as supplied by EPI-Environmental Plastics Inc. Canada. Test materials were loaded in the test vessels at two different proportions with respect to the soil: 22 and 43 mg/1000 mg(sample/soil). The mineralization profiles of samples recorded during 600 days of incubation are reported in Figure 6 along with the biodegradation curve of a filter paper sample utilized as positive reference material. The incubation condition appeared to be satisfactory in terms of growth conditions of microorganisms also during this experiment, as revealed by the high level of CO_2 production from the positive control (filter paper) after 80 days of incubation. The biodegradation process of LDPE (thermally oxidized) samples appeared to start without an apparent lag-phase, with setting however of a plateau at about 4 % that almost lasted for five months (Figure 6). Afterwards a marked exponential phase in the biodegradation profile took place for both samples even though with a

significant difference between the two in the overall degradation extent, as well as in the rate of mineralization. In particular the lowest amount (22 mg/1000 mg soil) approached the highest extent of biodegradation (58.8 %) after 81 weeks of incubation, whereas a lower value (46.8 %) was reached by test replicate, which was added with less favorable inoculum/sample ratio. The profile of biodegradation curves was however characterized in both cases by a positive slope yet at almost 600 days of incubation (Figure 6).

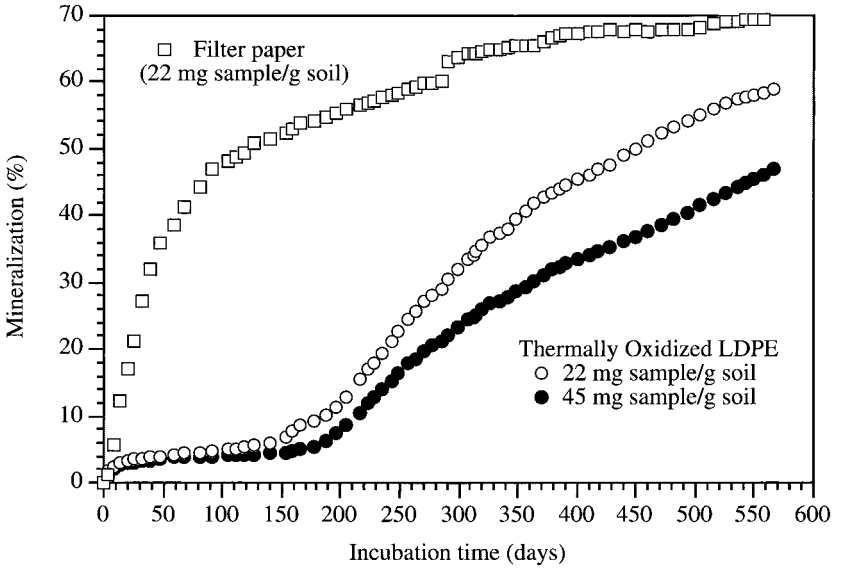

Figure 6. Mineralization profiles of thermally oxidized LDPE, containing TDPA® additives, in soil burial respirometric tests.

The overall level of mineralization reached by polyolefin samples was quite below that comparably reached by filter paper. Hence, LDPE samples were degraded in a substantial minor extent with respect to filter paper. It is however worth mentioning that the formed oxidation number of the carbon atoms in the glucosidic moiety of cellulose is zero whereas that in polyethylene is -2. In the first instance, it is obvious that significant differences in the oxygen uptake are required to reach equivalent amount of carbon dioxide production (e.g. mineralization)

(Table 2). The higher O_2 uptake combined with an higher activation energy of the initial steps of the biochemical reactions in low oxidation state hydrophobic substrates, negatively affects the kinetic of the biodegradation processes as well as the overall yield.

In Table 2 a comparison is reported among various polymeric substrates of the oxygen uptake necessary to reach 60 % level of mineralization.

As expected, polyethylene is holding the highest value of oxygen uptake.

Table 2. Carbon content, theoretical CO_2 production and O_2 uptake required for 60 % mineralization of 100 mg analyzed polymeric materials.

Test Sample	Carbon content (%)	Theoretical CO_2 (mg)	O_2 uptake at 60 % mineralization	
			(mg)	(mmol)
Synthetic polymeric materials				
PCL	63.3	232.1	101.3	3.17
Ecoflex	59.7	218.9	115.8	3.62
PVA88	51.2	187.7	81.9	2.56
EVOH44	70.9	259.9	113.4	3.55
LDPE	76.2	279.4	121.9	3.81
Semi-synthetic polymeric materials				
TPS	41.7	152.9	66.7	2.08
PCL/L (30% L)	62.6	229.5	100.2	3.13
PVA/L (30% L)	50.5	185.2	80.8	2.53
L-*g*-PCL (LCL0)	56.5	207.2	90.4	2.82
L-*g*-PVA (LVAH)	35.3	129.4	56.5	1.76
Natural				
Filter paper	43.4	159.1	69.5	2.17
Lignin	60.9	223.3	97.4	3.04

Moreover it is well known that the respiratory activity of microbial cells is largely dependent upon the chemical structure of the substrate. In particular, the metabolization of *n*-alkanes, whose chemical structure is closely related to that of polyethylene, produce a fairly large amounts of cell biomass (about 400 g/mole substrate in the case of n-hexadecane ($C_{16}H_{34}$), whereas glucose ($C_6H_{12}O_6$, cellulose monomeric component) gives only 190 g of cell biomass/mole (18) which is compensated by a larger CO_2 production.

From the above considerations it is understandable how for a better validation of the adopted procedure and in general for a more direct comparison of structurally different polymeric substrates in biodegradation experiments, it is necessary to proceed with an accurate overall mass balancetaking into account also the different productions of cell biomass.

Trial tests are at present ongoing as aimed at implementing the described procedure with a cell biomass balance.

Conclusions

The reported results of biodegradation experiments carried out on different polymeric substrates by using a simple and versatile procedure designed for mineralization processes mediated by solid inocula (soil and mature compost), suggests the following conclusions.

The adopted biometer flask procedure appears to be suitable to run accurate tests on the ultimate mineralization of different types of polymeric materials both under aerobic composting and soil burial conditions, by using simple and inexpensive laboratory equipments.

The adopted test condition, appear to be satisfactory in terms of both microbial physiology and CO_2 measurements. The reduction of the background noise and the CO_2 measurements under equilibrium conditions account for the detection of even very small (a few mg/week) differences in CO_2 evolution between test and blank samples.

The results obtained from the investigation of the ultimate biodegradability of thermally oxidized poly(ethylene), the recalcitrance to the microbial attack in the conventional formulations is well documented, may help to stimulate a careful revision of the official standard methods in terms of both time duration and reference (positive control) materials. Indeed, CEN standard for the evaluation of the biodegradation of synthetic polymers under composting conditions (EN 13432)

(19) state that at least 90% of the carbon content has to be converted in CO_2 and biomass within 6 months. This time frame does not meet the mineralization process of a natural biodegradable material "by definition" such as lignin, thus excluding hybrid materials containing lignin, whose utilization may enjoy undoubtable environmental beneficial effects. Also oxo-biodegradable polyolefins, whose eco-efficiency has been demonstrated (20,21), could not pass this test, thus eventually discriminating their possible utilization in applications where a fairly long time for disintegration and further biodegradation may be a useful and valid attribute.

The influence of the load of test material relative to the inoculum in both mineralization rate and extent has been also demonstrated in the biodegradation experiments on thermally oxidized poly(ethylene). Even though within the limit of only two experiments, the obtained data clearly suggest that the relative test material load should be large enough for a better appreciation of respirometric activity, but not so high to impede the optimal contact between the test sample and soil or compost particles (22). It is our opinion that the weight ratio between the inoculum and test material (6/1) as suggested in standard like ISO 14855 is too low.

It appears that most of the official standard methods have been developed on the basis of the biodegradation processes applied to polymeric materials susceptible to relatively fast enzymatic hydrolysis, such as aliphatic polyesters and modified polysaccharides, whereas polymeric substrates of both synthetic (polyolefins) and natural origin (lignin) have been repeatedly found to undergo oxidative attacks at relatively low rate.

Finally, the utilization of cellulose as positive control, does not reflect the biodegradation behavior of such different natural and synthetic polymeric materials, because the large differences in respirometric activity and new biomass production is very much depending upon the formal oxidation level of the carbon atoms in the different substrates. Accordingly, further and proper reference materials should be suggested in keeping with the polymeric materials to be tested as well as an accurate mass balance including the production of cell biomass should taken into account.

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