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Study of the biodegradation in soil of new generation plastics

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1 Study of the biodegradation in soil of new generation plastics

1.1 Introduction

"Plastic" derives from the Greek words *πλαστικός* (*plastikos*) or *πλαστός* (*plastos*) meaning fit for moulding or moulded respectively. It refers to the material malleability, or plasticity during manufacture, that allows it to be cast, pressed, or extruded into a large variety of shapes (films, fibres, tubes, bottles, boxes…).

Humanity first used natural materials with plastic properties (e.g., clay, amber, arabic rubber, caucciù…), then developed chemically modified natural materials (e.g., rubber, nitrocellulose, collagen) and, finally (around 100 years ago), developed the wide range of completely synthetic materials that, nowadays, we recognise as modern plastics.

In the mid-nineteenth century the Parkesine and the polyvinyl chloride (PVC) were invented. The first is the earliest example of synthetic polymer today known as celluloid, the second is the third most widely produced plastic after polyethylene and polypropylene. Nevertheless Leo Baekeland marked a turning point by creating, in 1907, the Bakelite (a condensation product of phenol and formaldehyde) that is considered the first real synthetic man-made plastic.

The first decades of the twentieth century saw the invention and the production of the first plastic packaging material (cellophane) and of the first coloured plastic, obtained combining formaldehyde, carbon dioxide and ammonia with coloured powder. Nylon was first synthesized in 1935; polystyrene, polyethylene, polyethylene-terephthatate and silicones (widely used today) were developed during the second world war period. In 50s nylon and lycra became a major force in the clothing industry and decorative laminates, such as formica, were widely used (www.plasticseurope.org).

Plastic is relatively low cost and easy to be manufactured; it is versatile and colorable and the end products are generally lightweight; it is water resistant and have excellent thermal and electrical insulation properties; plastic materials are resistant to corrosion and microorganisms attacks.

A lot of work was made in order to increase the stability of these materials and now plastics are used in an extensive and expanding range of products. Plastic is widely used in packaging (for food conservation), building and construction (for applications such as window frames or pipes), transportation, electrical and electronic, agriculture (mulching films, greenhouses…), medical and health, sport, leisure. The result is that most of the objects that we use daily are made of plastic. Plastic has become one of the symbols of XX century.

The production of plastic has increased from around 0.5 millions tons in 50s to over 200 million tons today. As a consequence of the economic crisis, the global production decreased from 245 million tonnes in 2008 to 230 million tonnes in 2009, but Europe remained one of the major producing, with about 25 % of the global production (Plastics - the Facts 2010, www.plasticeurope.org).

At the beginning of the "*plastic age*" (40s) Yarsley and Couzens predicted many of the current applications and of the benefits that would derive from the use of plastic, but not the problems associated with the management of plastic waste and debris (Thompson et al., 2009a).
The intense use of plastics contributes to increase the amount of municipal waste. Plastics are durable and degrade very slowly. Till short time ago, at the end of their life they were generally discarded and disposed in landfill, where they can persist for hundreds of years. Plastic debris have accumulated in the environment, from the poles to the equator, in particular in the oceans (Gregory, 2009; Ryan et al., 2009). The incineration is not a solution: in some cases, plastic burning can release toxic emissions (from PVC, for example, dioxins can be created), hazardous for the environment and the human health. Moreover the manufacturing of plastics can create large quantities of chemical pollutants: phthalate, commonly used as plasticizers, can be released in the environment, and, together with bisphenol A, can be detected in aquatic environment, in dust and, because of their volatility, in air (Thompson et al., 2009a).
In order to solve both environmental and human health problems, in the last decades the attention has been focused on the potential solutions, with particular attention for the management of plastic waste. Reduce, reuse, and recycle are the "three Rs" that has been advocated as a solution (Thompson et al., 2009b). However, for some applications, an important alternative to the conventional plastic materials can be found in the use of the new generation materials: the biodegradable polymers.

Biodegradable plastics are seen by many as a solution for the "environmental plastic problem" because they are environmentally-friendly (Tokiwa et al., 2009). They can be produced from fossil materials (for example polycaprolactone or poly(butylene succinate)), or can derived from biomass or renewable resources (starch, poly(lactide) acid or poly(hydroxybutyrate)), but their most important property is their biodegradability. Actually, a plastic material can be defined as biodegradable only if its degradation results from the action of naturally occurring microorganisms such as bacteria, fungi or algae (ASTM D883, 2008 and ISO 472, 1993). For example, polyethylene or nylon are not biodegradable even if they can be produced from biomass or renewable resources (Tokiwa et al., 2009).
Biodegradation is the process by which microorganisms, under aerobic or anaerobic conditions, convert an organic compound into carbon dioxide (and

or methane) water and mineral salts of any other element present (mineralization) plus new biomass. The complete biodegradation reactions can be described as:

$$C_{polymer} + O_2 \rightarrow CO_2 + H_2O + C_{biomass} + C_{residue}$$

$$C_{polymer} \rightarrow CO_2 + CH_4 + C_{biomass} + C_{residue}$$

The process is generally affected by the amounts and types of microorganisms and by their activity rates as well as by environmental parameters such as moisture, temperature, pH, oxygen, nutrients, etc.…(Muller, 2003; Krzan et al., 2006).
Generally, polymers are hydrophobic and high molecular weight materials, so that biodegradation can be considered as a two-step process. Extracellular enzymes (i.e., enzymes released by microbial cells into the surrounding environment) are secreted by the organisms performing the first step of the degradation process. The result is the generation of water-soluble intermediates, that can be transported into the microorganisms and used in the appropriate biochemical pathways (Muller, 2003). The final result is that biodegradable plastics can be naturally recycled by microorganisms to produce useful metabolites (monomers or olygomers) (Gross and Kalra, 2002; Tokiwa et al., 2009).

Biodegradable polymers have been developed since 1990s and in about twenty years the global annual production has reached, in 2007, about 230,000 tons (European Bioplastics, 2008). The use of biodegradable polymers in specific applications and sectors can be an alternative to landfill disposal and can thus reduce the cost of waste management and the accumulation in the environment.
For example, the catering products (trays, cups, plates, cutlery) can simply be composted after the used and the use of compostable waste bags to collect organic waste can improve the performance of the composting process and the compost quality. In agricultural applications the biodegradable mulch films can be left on the field after their use and offer the opportunity to reduce labour and disposal cost.

The industrial development of new biodegradable plastics has been accompanied by parallel development of suitable standard and criteria defining the compostability (ASTM D6400, 2004; ASTM D6868, 2003; EN 13432, 2002), the biodegradability in soil (ASTM D5988, 1996; ISO17556, 2003) or in synthetic aqueous media (ISO 14851, 1999; ISO 14852, 1999).

Generally laboratory test methods are based on the evaluation of the net carbon dioxide production (the amount of CO_2 evolved from the substrate added with the test material minus the amount of CO_2 evolved from the substrate (blank)) or on the measurement of the net oxygen consumption.

Tests in aqueous media are relatively easy to be carried out and include the possibility of establishing a carbon balance measuring the growth of new biomass deriving from the biodegradation of test material (Longieras et al., 2004; Muller, 2003). However, they don't represent the real conditions in which biodegradable plastics are generally disposed, i.e. composting plants. Therefore, the so called controlled composting tests were developed. In these tests, the material is mixed in mature compost, incubated at 58° C and the CO_2 evolution is monitored. Compostability is something more than biodegradability. In particular: the material must disintegrate sufficiently during the composting process and at least 90 % of its organic fraction must be converted into CO_2 within six months. The test material must not have negative effects on the composting process, the compost quality must not be modified and no toxic effects should occur (ISO 13432, 2002). Tests in solid media, even if more representative of natural conditions, involve some problems, related, first of all, to the presence of carbon in the medium (Longieras et al, 2004). The background CO_2 evolution can be strongly affected by presence of the test material (priming effect) and this can decrease the accuracy in the determination of carbon balance (Muller, 2003). To solve this problem the biodegradation is referred to a degradable reference substance (generally cellulose) incubated at the same conditions, or, as an alternative, the compost matrix can be replaced by a synthetic solid medium (vermiculite) enriched by microorganisms extracted from compost (Bellia et al., 1999; Longieras et al., 2004).

With the increasing use of biodegradable plastics in agriculture, the study of the biodegradation in soil has received intense interest, but the standardization of test methods is difficult (Muller, 2003). Soil, in fact, is a complex matrix: its natural properties can be very different in different sites and can not be controlled in nature. In composting tests, on the contrary, parameters such as pH, temperature, moisture and microbial community can be defined in order to obtain the optimal conditions for the process. In soil, the biological activity and the temperatures are lower than in composting, so that biodegradation in soil is slower than in compost. Agricultural soil is also the medium for the production of food for humans and cattle, so the definition of standard test methods and of specific criteria to verify the biodegradability and the absence of eco-toxic effects in soil are required (Degli Innocenti, 2005). In 1996 a standard method was published for determining aerobic biodegradation in soil of plastic materials by measuring the amount of carbon dioxide evolved in a closed respirometer (ASTM D5988, 1996). In 2003, ISO published a standard method for determining the biodegradation rate of plastic material in soil by measuring the oxygen demand or the amount of carbon dioxide evolved in a ventilated respirometer (ISO 17556, 2003). As in composting tests, the biodegradability is evaluated by measuring the CO_2 production. Nevertheless, in order to describe completely the biodegradation process, it is very important to quantify and

identify possible by-products (Bellia et al., 1999) and to determine the amount of carbon linked to the new generated biomass.

The determination of possible by-products or of the material residues is possible by soil extraction with appropriate solvents and by analyzing the extracts. Biomass determination, instead, is still an unsolved problem.

Soil microbial biomass (SMB) is defined as the "mass of intact microbial cells in a given soil" (ISO 14240, 1997). It is influenced by soil physical parameters (temperature, moisture and texture) and by its chemical composition (such as the concentration of carbon substrate). It is responsible for the degradation of organic matter, the stability of aggregates and the cycle of most nutrients in soils, so it is generally quantified in order to assess the soil fertility, the potential ability to degrade added organic materials, and the effects of added materials on the natural microbial population (ISO 14240, 1997).

Literature reports different methods for determining microbial biomass in soil. They are based on the measurement of the biomass ability to mineralize an added carbon source as substrate-induced respiration (SIR) (Wardle and Parkinson, 1990; Lin and Brooks, 1999) or on physiological analysis such as the determination of extractable phospholipids fatty acids (PLFA) (Bailey and al., 2002) or biochemical assays to ATP determination (Contin et al., 2001; Martens 2001). Fumigation incubation method (based on the comparison between respiration before and after the soil sample has been fumigated with chloroform) or extraction methods (that measure the difference between extractable C in fumigated and un-fumigated soil samples) are described by standard methods (ISO 14240, 1997). However, such methods are not able to provide the needed data to estimate the overall carbon balance in biodegradation processes. The interest in soil biodegradation test is, in fact, to quantify all the extra biomass and the organic matter formed as a result of the biodegradation of the test material (independently whether biomass is still intact or not).

1.2 Structure of the research

The experimental work concerned the study of the different aspects of the biodegradation of new generation plastics in soil. Attention was focused at the fate of the possible by-products of biodegradable polymers (the monomers) and at the determination of the biomass generated during the process. Mineralization in soil of a model polyester, appositely synthesised for this study by Novamont S.p.A. (Italy), was studied and an attempt of carbon balance was realised.

In particular:

1. The mineralization of ten monomers, chosen between the most widely used for the synthesis of plastic materials, was tested according to ASTM D5988 (1996). The effects of the soil pH on the mineralization was evaluated by respirometric tests and the experimental data were used to validate a numerical model that could estimate the amount of carbon used by microorganism for biochemical synthesis.
2. The mineralization of the model polyester was investigated by respirometric tests, and cellulose was used as control. The effects of the initial soil pH and of the addition of organic matter were investigated in different soil mixtures.
3. The sensitivity of the combustion method for determining the amount of organic matter and biomass in soil samples was evaluated by adding to a natural and a synthetic soil different amounts of organic matter.
4. In order to describe carbon balance during the biodegradation of the model polyester, biomass production and polyester residues in soil were estimated. Biomass and organic matter, deriving by polyester biodegradation, were studied by combustion of soil samples at 550° C. Polyester residues were estimated by extractions in soxhlet of soil samples (with chloroform). The extracts were also characterized by ^{1}H-NMR and ^{31}P-NMR acquisitions and GPC.

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Web sites:

- www.european-bioplastics.org
- www.plasticeurope.org

2 Mineralization of monomeric components of biodegradable plastics in soil

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ABSTRACT

In the last twenty years, a new generation of materials was developed: the biodegradable plastics. They reduce the accumulation of plastic in the environment and the cost of waste management because they can be fed in composting plants or, if used in agriculture (mulch films), they are applied to the soil and left there. Ten monomers were chosen among the most used in the synthesis of biodegradable polymers (1,2-ethanediol, 1,4- butanediol, 1,6- hexanediol, adipic acid, azelaic acid, sebacic acid, terephthalic acid, glucose, lactic acid and succinic acid) and tested according to ASTM 5988-96 (a standard test method for determining aerobic biodegradation in soil of plastic materials measuring the carbon dioxide evolution). Two agricultural soils, collected in two different sites in Italy, were used to evaluate the mineralization rate of the monomers. Four tests (two replicates each) were carried out for 27-39 days. Experimental data show no relevant differences in the respirations of the two soils and in the carbon dioxide productions of the tested monomers. The final mineralization percent was 42-45 % for glucose, succinic and lactic acid and 50-56 % for the other monomers.

KEYWORDS biodegradable plastic; biopolymer; monomer; mineralization

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2.1 Introduction

Plastic is a general common name given to synthetic, organic and high molecular weight polymers suitable for the manufacture of industrial products. It refers to their malleability or plasticity during manufacture that allows them to be cast, pressed, or extruded into an enormous variety of shapes and objects (films, fibers, plates, tubes, bottles, boxes, and much more) which found wide applications in every aspect of life and industries. Plastic objects are easy to produce, have high performances and generally can not be attacked by microorganisms and so they are not biodegradable. A problem in their use is the accumulation in the environment. To overcome this problem the efforts of academic and industrial worlds have joined to produce a new generation of plastic materials: the biodegradable plastics, often made of polyesters. Besides reducing the risk of accumulation of plastic materials in the environment, the production and use of biodegradable polymers involves considerable reduction in the cost of waste management (Tokiwa et al., 2009). Furthermore, biodegradable plastics can be recycled to useful metabolites (monomers or olygomers) by microorganisms.

In the last twenty years, the increasing development of biodegradable plastics has promoted initiatives to develop formal standards and laboratory test methods to assess the ultimate environmental behaviour of plastics (Chiellini et al., 2007). Several international and national organizations have issued standard test methods to simulate the fate of biodegradable plastics in different environments. In the 1990s most of the work was focused on biodegradation under composting conditions. The reason for this preference was linked to the concurrent trend in solid waste management policy, which aimed at reducing the use of landfill to as least as possible while promoting recycling. Consequently, criteria and standard test methods were needed in order to verify the compatibility of plastics with composting and this stimulated research and standardisation (Degli Innocenti, 2005).

However, several products made with biodegradable polymers, for instance the plastics used in agriculture (mulch films), are not fed to a composting plant at the end of their life, but just applied to soil and left there. Since the agricultural soil is the medium for the production of food for humans and cattle, the absence of negative effects due to the in situ disposal of plastics and of residue build-up are matters of concern. Definition of standard test methods and of specific criteria to verify biodegradability and absence of eco-toxic effects in soil are nowadays required to clarify all these issues and launch the marketing of safe biodegradable polymers in agriculture (Degli Innocenti, 2005). In 1996 a standard method for determining aerobic biodegradation in soil of plastic materials by measuring the amount of carbon dioxide evolved in a closed respirometer based on was published by the American Society for Testing and Materials (ASTM 5988-96). In 2003, a standard method for determining the biodegradation rate of plastic material

in soil by measuring the oxygen demand or the amount of carbon dioxide evolved in a ventilated respirometer was published (ISO 17556, 2003).
The biodegradation of polymers is normally referred to as an attack by microorganisms on non-water soluble polymer-based materials (plastics) (Muller, 2003). It is a complex process in which the carbon of the polymer is converted into carbon dioxide (mineralization) and biomass. Extracellular enzymes (i.e. enzymes released by microbial cells into the surrounding environment) are secreted by the organisms performing the first step of the degradation. In case of polyesters, enzymes are expected to catalyze the hydrolysis of the ester bonds and cut the solid, hydrophobic polymer into olygomers and monomers, which are released in the environment. Due to their low molecular weight and water solubility, these can pass through the cell membrane and be then metabolized by microorganisms (Tokiwa and Suzuky, 1974; Tokiwa and Suzuki, 1977; Herzog et al., 2006; Muller, 2006). The simple depolymerization can actually lead to the build-up of environmental concentrations of olygomers and monomers, and to their transfer from one environmental component to another (Degli Innocenti, 2005).
The aim of this work was to evaluate the mineralization in soil of different monomers which are generally used in the production of biodegradable plastics. Ten monomers were tested according to ASTM 5988-(96), the above mentioned standard method for determining aerobic biodegradation in soil of plastic materials.

2.2 Materials and Methods

2.2.1 The tested substances

Ten monomers, used in the synthesis of potentially biodegradable polymers, were tested by respirometric test in soil: 1,2-ethanediol, 1,4- butanediol, 1,6-hexanediol, adipic acid, azelaic acid, sebacic acid, terephthalic acid, glucose, lactic acid and succinic acid.

1,2-Ethanediol ($C_2H_6O_2$) is an organic compound widely used as automotive antifreeze and as raw material in plastic production. It is an odorless, colorless and sweet-testing liquid produced from the reaction between ethylene oxide and water. The reaction can be catalyzed by either acids or bases, or can occur, at neutral pH, at high temperatures (Rebsdat and Mayer, 2002). The ethanediol is an important monomer used in the synthesis of polyester fibers and resins: polyethylene terephthalate used to make plastic bottles for soft drinks (for example) is prepared from ethylene glycol. It is one of the constituents of Sky Green (made of adipic acid, succinic acid, butanediol and ethylene glycol), a biodegradable polymer produced by SK Chemicals (Korea) (Lee at al., 2002).

1,4-Butanediol ($C_4H_{10}O_2$) is a colorless and viscous liquid derived from butane by placement of alcohol groups at the end of the chain. In its industrial synthesis, 1,4-butanediol is produced with the method of reacting acetylene with formaldehyde (Reppe process) (Küksal et al., 2002), or, with an alternative bio-based process, from corn-derived glucose. Glucose is fermented to succinic acid which is then purified and reduced catalytically to 1,4-butanediol (Cooper and Vigon, 2001). It is a constituent of biodegradable plastics such as Ecoflex (polybutilene adipate and/or polybutylene therefthalate) produced by BASF (Steinbuchel and Doi, 2002) and of the previously mentioned Sky Green.

1,6-Hexanediol ($C_6H_{14}O_2$) is a white solid organic di-alcohol, with two primary terminally located hydroxyl groups. It is a valuable intermediate product for chemical industry and it finds applications in a variety of polymeric systems. Its configuration results in a rapid and simultaneous reaction in the formation of numerous di-substituted products. 1,6-Hexanediol is used in the production of polyesters, coatings, adhesives and polymeric plasticizers. In these end use areas, it contributes significantly to many high performance characteristics such as hydrolytic stability, high flexibility, good adhesion and surface hardness (BASF intermediates description available in internet). It is used in the synthesis of biodegradable high molecular weight aliphatic-aromatic copolyesters (Li et al., 2009).

Adipic acid ($C_6H_{10}O_4$) it is a white crystalline powder not very soluble in water because of its long aliphatic chain. It is a synthetic molecule normally prepared from cyclohexane by two oxidation steps, which can also be produced starting from natural raw materials (Asahi Kasei Kogyo, 1991). Adipic acid is largely used as a monomer for the production of nylon, but also as plasticizer and lubricant component. It is a constituent of biodegradable plastics such as Ecoflex and Sky Green.

Azelaic acid ($C_9H_{16}O_4$) is a saturated dicarboxylic acid naturally present in wheat, rye and barley or produced by *Malassezia furfur* (Ashbee and Evans, 2002), a yeast that lives on normal skin. For its antibacterial properties, azelaic acid is used for the treatment of skin irritations such as acne (Liu et al., 2006). It is also used in the production of plasticizers, polyamides and alkyd resins.

In its pure state, sebacic acid ($C_{10}H_{18}O_4$) is a white flake or powdered crystal natural substance. For industrial purposes, it is derived from castor oil and is typically used for the production of candles as well as of plasticizers, lubricants and cosmetics, besides and bio-based plastics.

Terephthalic acid (C_8H_6O) is an aromatic colorless commodity chemical used mainly as a precursor to the non-biodegradable polyester such as polyethylene terephthalate (PET) (used to make bottles or clothes) or polybutylene terephthalate (PBT). Its aromatic component provides excellent

material properties, so terephthalic acid is frequently used to improve material properties in polyesters such as Ecoflex (Muller et al., 2001).

Glucose ($C_6H_{12}O_6$) is the most widespread monomer in nature; it is a simple sugar produced by photosynthesis and used as energy source by respiration. Cellulose, the most common polymer in nature, derives from the dehydration of glucose and polymers from cellulose are used as biodegradable plastics (e.g., cellophane).

Lactic acid ($C_3H_6O_3$) is an α-hydroxy acid involved in biochemical processes. It can be produced both by chemical synthesis and by fermentation of carbohydrates by *Lactobacillus* (Sotergard and Stolt, 2002). It is used as a monomer for producing polylactic acid (PLA), which has application as a biodegradable plastic.

Succinic acid ($C_4H_{16}O_4$) is a solid colorless and odorless dicarboxylic acid which plays an important metabolic role in the citric acid cycle by which organisms draw energy. It is a constituent of polybutylene succinate or polybutylene succinate-*co*-butylene adipate copolymers, commercially known as Bionolle, produced by Showa Highpolymers (Japan) (Tserki et al., 2006).
The main properties of the tested substances are reported in Table 1.

Table 1: Main properties of the tested monomers.

Monomer	Molecular Formula	Molar mass (g mol^{-1})	Carbon fraction (-)	Physical state at lab conditions
1,2-Ethanediol	$C_2H_6O_2$	62	0.39	Liquid
1,4-Butanediol	$C_4H_{10}O_2$	90	0.53	Liquid
1,6-Hexanediol	$C_6H_{14}O_2$	118	0.61	Liquid
Adipic acid	$C_6H_{10}O_4$	146	0.49	Solid
Azelaic acid	$C_9H_{16}O_4$	188	0.57	Solid
Sebacic acid	$C_{10}H_{18}O_4$	202	0.59	Solid
Terephthalic acid	$C_8H_6O_4$	166	0.58	Solid
Glucose	$C_6H_{12}O_6$	180	0.40	Solid
Lactic acid	$C_3H_6O_3$	90	0.40	Liquid
Succinic acid	$C_4H_6O_4$	118	0.41	Solid

2.2.2 Soil samples

Two different agricultural soils were sampled in two different locations and used to test the monomers. The texture of the first soil (collected in Albenga, Italy) is made of about 70 % sand, 24 % silt and 6 % clay and its pH (in water) is 7.5 to 8. In the second soil (collected in Arborio, Italy) sand is about 55 %, silt 43 %, clay 2 % and pH (in water) 5 to 6.5.

The soil samples were freshly collected, sieved (< 2 mm) and used within a few days for biodegradation tests.

2.2.3 Biodegradation tests

Biodegradation tests were carried out according to ASTM 5988-96 (1996). To increase organic matter, soil was enriched with compost with a ratio of 1 g compost to 25 g soil which corresponds to a typical application of compost in agricultural land (ASTM 5988-96). Mineral salts dissolved in water were added to soil and compost to obtain the correct ratio of nutrients and the ideal moisture, around 50 % of the water holding capacity of each soil. Mineral salts addition was adjusted to provide: 0.2 g KH_2PO_4, 0.1 g $MgSO_4$, 0.4 g $NaNO_3$, 0.4 g NH_4Cl and 0.2 g urea per Kg of soil.

Soil-compost-salt mixtures (500 g) were incubated at room temperature (21±2° C) in the dark, in hermetically closed jars (3 l), with the test substances. Blank jars, with no test substance, were also prepared. Each jar contained a beaker filled with 0.5 M KOH (40 ml), which was regularly titrated with 0.25 or 0.5 M HCl in order to measure the CO_2 production within the jar. The measurement was carried out every 3 days in the first two weeks, during which biodegradation was expected to be faster, and weekly thereafter. When the beakers were taken away from the jars for titration, the jars remained open from 15 to 30 minutes, so that the air was refreshed before replacing fresh potassium hydroxide.

Tests lasted 27 to 39 days, according to the cumulative CO_2 evolution. Only for terephthalic acid the test duration was extended to 140 days because no plateau phase was reached after 40 days. Moisture was not adjusted during the biodegradation tests. In hermetically closed jars, water evaporating from the soil saturates the headspace in a very short time and, consequently, any further water loss is negligible; therefore the soil moisture can be considered as constant during the test period.

Five monomers (adipic acid, succinic acid, sebacic acid, 1,4-butanediol and glucose) were tested both with soil from Albenga and with soil from Arborio. 1,2-Ethanediol, lactic acid, 1,6-eexanediol, azelaic acid and terephthalic acid were tested only with soil from Arborio. On the whole, four tests (two replicates each) were carried out for each monomer and for the blanks.

2.2.4 Chemicals and analytical methods

All chemicals were of analytical grade purity. Adipic acid, azelaic acid, 1,6-hexanediol, sebacic and succinic acid were supplied by Gamma Chimica S.p.a.. 1,4-Butanediol, 1,2-ethanediol, lactic acid, glucose and terephthalic acid were provided by Sigma-Aldrich.

Moisture content and pH of the soil-compost-salt mixture were measured according to ISO 11465 (ISO, 1994) and ISO 10390 (ISO, 2005)

respectively. Titration was carried out according to Standard Methods (APHA, 1998).

2.3 Results

In a first set of experiments, five monomers were tested with the two different soils: Albenga soil and Arborio soil. The respiration curves (cumulative CO_2 production (mg) measured in the jars) of the blanks and of the tested monomers (adipic acid, sebacic acid, succinic acid, 1,4-butanediol and glucose) are shown in Figures 1 and 2 respectively. Black symbols refer to tests in Albenga soil, white symbols to tests in Arborio soil. Experimental data show that CO_2 produced by the respiration in the blank jars is quite the same for the two soils. The same situation can be observed for the tested monomers. The most important difference of the two soils is their natural pH. It is known that, as temperature and soil moisture do, pH affects the final biodegradation in soil of polymeric materials (Shin and Eun, 1999), but the present results show no relevant difference in the respirations of the two soils and in the CO_2 production measured for the tested monomers.

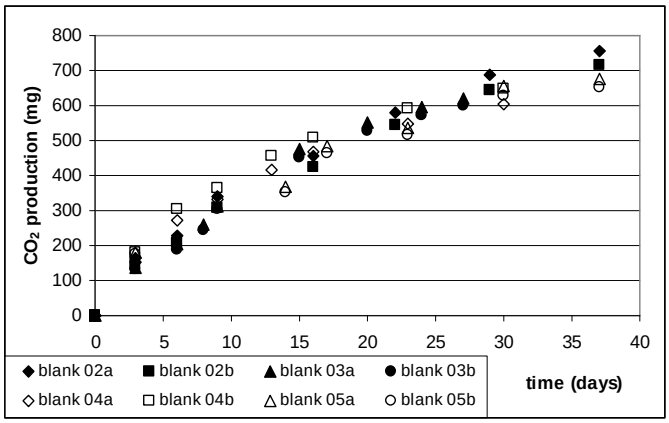

Figure 1: Respiration curves measured for soil Albenga (in black) and Arborio (empty symbols) in the blank jars.

So, the final extent of mineralization was calculated as average from the experimental data obtained with both soils.

Figure 3 reports the mineralization curves for all ten monomers. Each point represents the experimental value obtained in each test, and the broken line represents their best fit. Table 2 reports the average mineralization percent at the end of the tests for each monomer. The reproducibility of the results is good, as shown by the low standard deviations always below 10 %.

The smallest variations in the distribution of data were observed for the three di-alcohols (1,2-ethanediol, 1,4-butanediol and 1,6 hexanediol) and for lactic acid. Their physic state, liquid at room temperature, and their high solubility

in water favour their homogeneous distribution in the liquid phase of soil and their use by microorganisms. This could explain the high reproducibility of the results in the different tests.

Generally, a plateau phase was reached within about three weeks at more than 50 % mineralization. A lag phase of about 3-4 days was observed for sebacic acid, 1,6-hexanediol, azelaic and terephthalic acid whose final mineralization was about the 53 %, 55 %, 54 % and 56 % respectively (as previously mentioned, the final mineralization of terephthalic acid was estimated after 140 days incubation; after 40 days mineralization was below 40 %). For adipic acid the final mineralization was comparable (55 %), but no lag phase was observed. The faster mineralization was observed for glucose, lactic and succinic acid for which the plateau phase was reached in about 10 days but the final mineralization was lower than for other tested monomers (45 % for lactic and succinic acid and 42 % for glucose). For sebacic and azelaic acid the plateau phase seemed to be reached by the end of the tests and the mineralization was about 53 % and 54 %. It is possible that for such monomers longer lasting tests could show better defined plateau and greater mineralization percents, as it was the case for terephthalic acid.

Table 2: Mineralization percentages and standard deviations at the end of mineralization tests.

Monomer	**Mineralization (%)**
1,2-Ethanediol	53.05 ± 3.15
1,4-Butanediol	50.47 ± 3.15
1,6-Hexanediol	55.87 ± 1.88
Adipic acid	54.78 ± 2.26
Azelaic acid	53.86 ± 8.35
Sebacic acid	52.71 ± 2.90
Terephthalic acid	55.94 ± 2.45
Glucose	42.22 ± 0.95
Lactic acid	45.26 ± 5.52
Succinic acid	45.17 ± 3.39

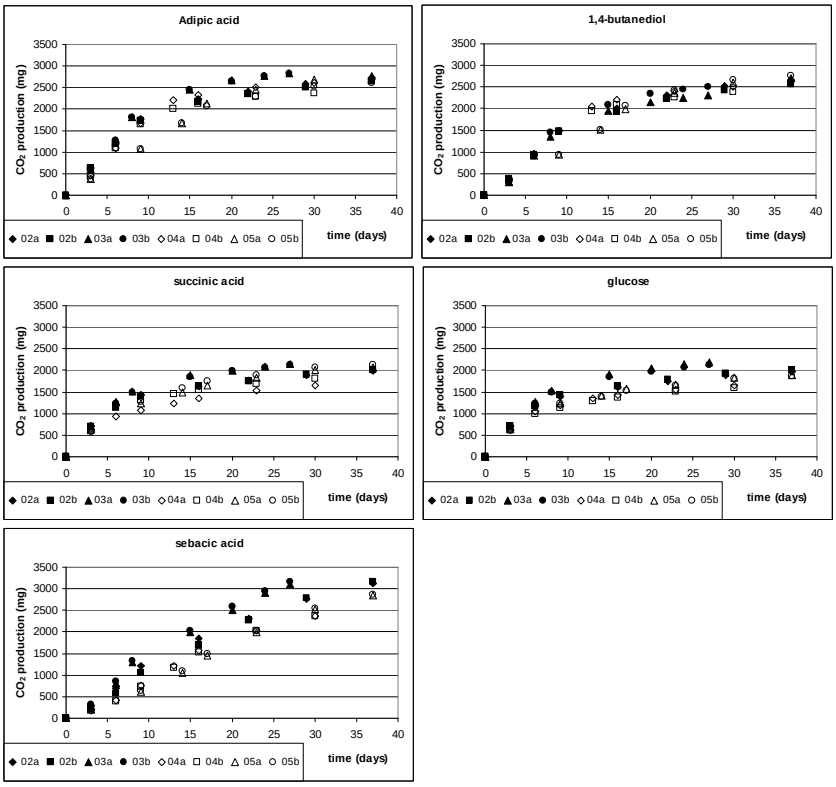

Figure 2: Respiration curves of adipic acid, 1,4-butanediol, succinic acid, glucose and sebacic acid obtained during incubation in Albenga soil (in black) and in Arborio soil (empty symbols).

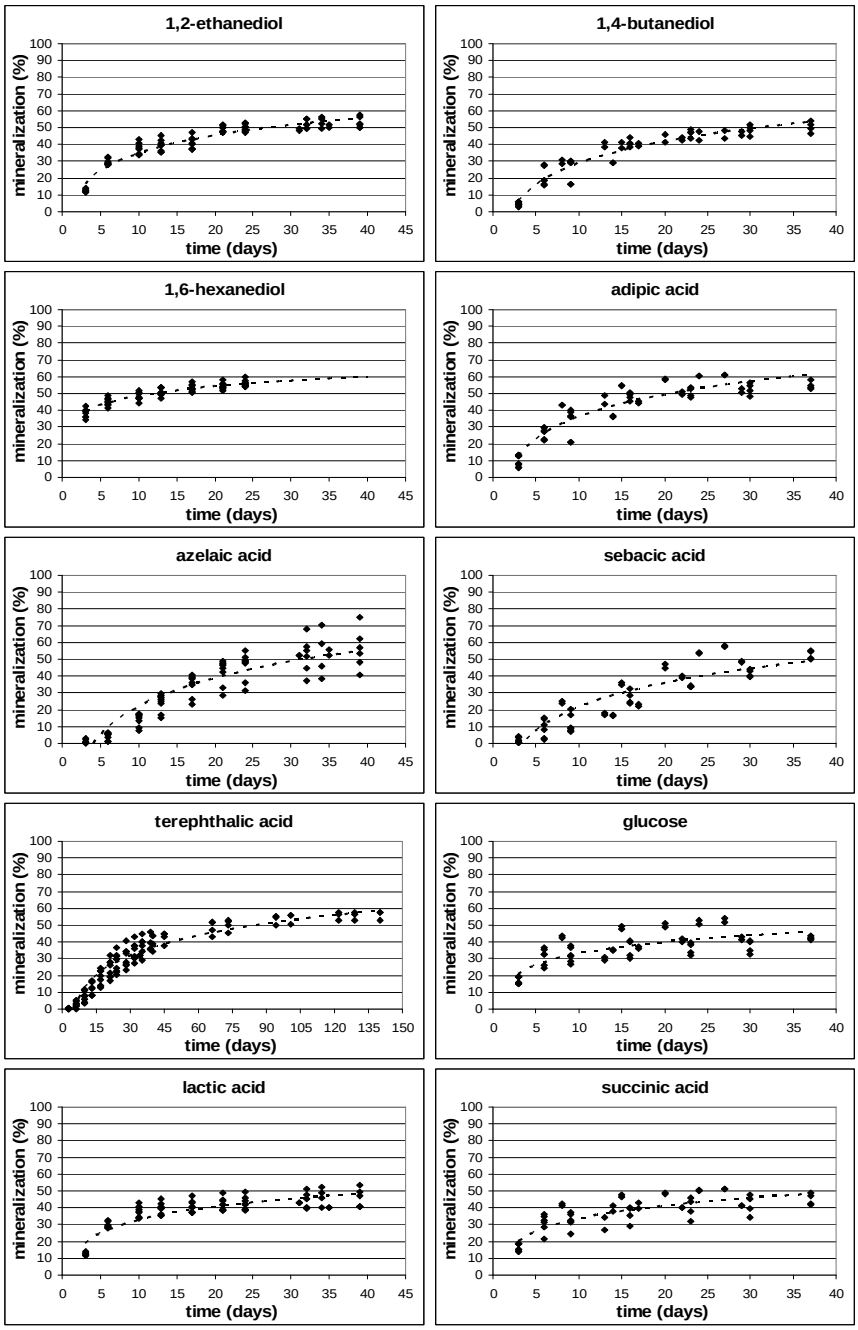

Figure 3: Mineralization curves of the tested substances.

2.4 Discussion and Conclusions

In this kind of research, an important point is the influence of soil and its characteristics on the final results of mineralization. Two different soils were used in this study. The two soils had a different texture and different natural pH: alkaline the first, sub-acid the second.

The results obtained in this work reveal that although the two soils were different, they sustained similar mineralization curves of tested monomers.

The level of mineralization reached by the different substances is not complete. Mineralization percent was 42-45 % for glucose, succinic and lactic acid and 50-56 % for the other monomers. This is expected considering microbial anabolism. The carbon fraction which is not released as CO_2 is incorporated in the cell biomass. A simulation carried out by AQUASIM code estimated that cellular biosynthesis could account for over 50 % for lactic acid, succinic acid and glucose (Siotto et al., submitted for publication).

The availability of data on the biodegradation of monomers in soil is limited, as the attention is generally focused on the biodegradation of polymers. However, the possibility of microorganisms to use the monomers derived from the biodegradation of polymers and the way they use them can have important implications on the one hand in stimulating the overall microbial activity and on the other hand in determining oxygen consumption in soil. Some research studies about microorganisms that use the tested monomers are available in literature. Terephthalic acid, for example, is used by three bacterial strains isolated from soils contaminated with oil and plastic waste (Vamsee-Krishna, 2006). Orchard and Goodfellow (1980) reported strains of *Nocardia* which can grow on adipic and sebacic acid as sole carbon source. Moreover 1,4-butanediol, 1,2-ethanediol and adipic acid are used by *Pseudomonas* strains isolated from soil (Stiegltz and Weimer, 1985).

The comparison of the present experimental data to literature data is quite difficult because, where data are available and experimental protocols are similar, specific aspects such as some soil properties or the time duration of the tests can affect the results. Kim et al. (2001) and Sharabi and Bartha (1993) measured mineralization in soil of some of the monomers studied in present research, and obtained, for some compounds, higher mineralization percent values. However, Kim et al. (2001) used a mixture of forest/agricultural soils and perlite as substrate for incubation and carried out the tests for 32 days. This mixture was used as the core in a multilayer system where the bottom and the top layers were made of perlite. The mineralization percent obtained by Kim et al. (2001) was comparable to that observed in the present study (27-45 days) for adipic acid and 1,4-butanediol, and higher for succinic acid (+19 %) and terephthalic acid (+10 %). Sharabi and Bartha (1993) used a freshly collected sandy loam, buffered with $CaCO_3$ 5 days before the beginning of the tests, and measured the CO_2 evolution over 22 days. Soil moisture was adjusted to 60 % of field capacity

and nutrients were added by a 1% solution of $(NH_4)_2PO_4$. In that case, the mineralization percent was higher for both adipic acid (+40 %) and glucose (+36 %).

Standard respirometric methods are important tools for evaluating the mineralization of the tested compounds in soil, but the evaluation of their results must take carefully into account the specific experimental conditions. Moreover, they cannot fully describe the biodegradation process which includes also biomass incorporation. This component should be measured along the biodegradation tests by specific protocols to provide the needed data for a complete carbon balance.

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3 Kinetics of monomer biodegradation in soil

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ABSTRACT

In modern intensive agriculture, plastics are used in several applications (i.e. mulch films, drip irrigation tubes, string, clips, pots, etc.). Interest towards the application of biodegradable plastics to replace the conventional plastics is growing because they biodegrade in soil after use and do not produce waste to be disposed of. Ten monomers, which can be applied in the synthesis of potentially biodegradable polyesters, were tested according to ASTM 5988-96 (standard respirometric test to evaluate aerobic biodegradation in soil measuring the carbon dioxide evolution): adipic acid, azelaic acid, 1,4-butanediol, 1,2-ethanediol, 1,6-hexanediol, lactic acid, glucose, sebacic acid, succinic acid and terephthalic acid. Eight replicates were carried out for each monomer for 27-45 days. The numerical code AQUASIM was applied to process the CO_2 experimental data in order to estimate values for the parameters describing the different mechanisms occurring to the monomers in soil: i) the first-order solubilization kinetic constant, K_{sol} (d^{-1}); ii) the first-order biodegradation kinetic constant, K_b (d^{-1}); iii) the lag time in biodegradation, t_{lag} (d); and iv) the carbon fraction biodegraded but not transformed into CO_2, Y (-). The following range of values were obtained: [0.006 d^{-1}, 6.9 d^{-1}] for K_{sol}, [0.1 d^{-1}, 1.2 d^{-1}] for K_b, and [0.32 - 0.58] for Y; t_{lag} was observed for azelaic acid, 1,2-ethanediol, and terephthalic acid, with estimated values between 3.0 e 4.9 d.

KEYWORDS. Biodegradable plastic, polyester, soil biodegradation, agricultural use, fate

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3.1 Introduction

In modern intensive agriculture, plastics are successfully used in several applications (i.e. mulch films, drip irrigation tubes, string, clips, pots, etc.): the specific term Plasticulture has been adopted to describe them (Lamont, 1998). A critical issue associated with the application of plastics is represented by their disposal after use. Traditional plastics are expected to be collected and incinerated with energy recovery, or recycled. Uncontrolled incineration or mechanical tillage of plastic residues in the field have high environmental impacts but are unfortunately quite common practices in agriculture. Environmental effects of these practices are air and soil pollution as well as aesthetic disturbance.

In Italy plastic film mulching covers more than 100,000 ha, with an annual consumption of approximately 65,000 t. It is reported that substantial quantity are not recovered but rather abandoned on soil or burnt without control by the farmers The situation is not so different in Europe: Western countries produce approximately 40 million tons of plastics per year, 2.5 % of which are employed in agriculture (1 million tons) and 60 % of that are usually abandoned (BIO-CO-AGRI, 2005). In Australia mulch film usage is approximately 4,000 t a year (Halley et al., 2001).

Interest towards the application of biodegradable plastics to replace the conventional plastics is growing. Using biodegradable mulch films, no waste in practice is produced and needs to be disposed of, because the films are ploughed under after use and are expected to biodegrade in situ. The risk of uncontrolled burning and air pollution is almost suppressed.

Since agricultural soil is the substrate for producing food for humans and farm animals, the absence of negative effects related to the in situ disposal of biodegradable plastics and the absence of residue build-up are matters of concern. The definition of standard test methods and specific criteria to verify the biodegradability and the absence of eco-toxic effects in soil are required to clarify all these issues (Degli Innocenti, 2005). The spreading of biodegradable plastics in soil because of farming practices must be counterbalanced by a continuous removal of the plastics from the soil or, better, by a biodegradation rate that counterbalances the input rate.

A number of polymers designed to biodegrade in the environment belong to the group of polyesters. Biodegradable polyesters are not water soluble and thus not directly bio-available for microorganisms. The biodegradation of polymers normally refers to an attack by microorganisms on non-water soluble polymer-based materials (plastics) (Muller, 2003). Extracellular enzymes (i.e., enzymes released by microbial cells into the surrounding environment) are secreted by the organisms performing the first step of the degradation process. Enzymes catalyze the hydrolysis of the ester bonds and cut the solid, hydrophobic polymer into olygomers and monomers. Due to their low molecular weight and water solubility, these can pass through the cell membrane and are then metabolized by microorganisms (the second

step) (Herzog et al., 2006; Tokiwa and Suzuki, 1977; Tokiwa and Suzuki, 1974 ; Muller, 2006).
The overall degradation process of polyesters under aerobic conditions can, therefore, be described as follows:

$$\text{Polyester} + H_2O \xrightarrow[K_{1deg}]{} \text{monomers} + O_2 \xrightarrow[K_b]{} CO_2 + H_2O + \text{biomass}$$

The first reaction (K_{1deg}) is a hydrolysis mainly mediated by extracellular enzymes while the second reaction (K_b) is the microbial aerobic metabolism.
Laboratory tests currently applied to determine the biodegradation of plastics are based on the measurement of carbon dioxide evolution or of oxygen consumption when the original polymer (e.g., the polyester) is exposed to controlled environmental conditions (e.g., soil, compost, active sludge, etc.). Therefore, such tests cannot provide information on the kinetics of the two steps but only inform on the overall reaction. However, the information about K_{1deg} and K_b is important in order to determine the environmental fate of the polyesters spread into the soil. In fact, if $K_{1deg} >> K_b$ a temporary build-up of monomers is expected. Persistence must be evaluated in terms of complete biodegradation: the simple depolymerization can actually lead to the build-up of environmental concentrations of olygomers and monomers, and to their transfer from one environmental component to another (Degli Innocenti, 2005). In Saponaro et al. (2008), a numerical model was developed and applied to predict the fate and transport of biodegradable polyester residues in soil following successive applications of mulch films. In that model, the values for K_{1deg} and K_b varied within reasonable ranges, but they were not based on experimental measurements. The present work allowed to provide values for K_b of monomers that are or could be used in the synthesis of biodegradable polyesters by polycondensation. These monomers would be released into the soil in case of depolymerization, and finally metabolized. Therefore, the K_b values are relevant information because related to the second step of the overall biodegradation process. The model developed in the present work also allowed to estimate values for the parameters describing other mechanisms occurring to the monomers in soil: i) the first-order solubilization kinetic constant (K_{sol}); ii) the lag time in biodegradation (t_{lag}); and iii) the carbon fraction biodegraded but not transformed into CO_2 (Y).
Other polymers that are used in agriculture are subjected to different degradation mechanisms that need to be studied with other numerical simulation approached (Arnaud et al., 1994).

3.2 Materials and Methods

3.2.1 Tested substances

Ten monomers, which can be applied in the synthesis of potentially biodegradable polyesters, were tested by respirometric tests in soil: adipic acid, azelaic acid, 1,4-butanediol, 1,2-ethanediol, 1,6-hexanediol, lactic acid, glucose, sebacic acid, succinic acid and terephthalic acid.

Adipic acid is a synthetic molecule derived from the oxidation of cyclohexane; historically used for nylon production, it is also used for the production of plastifiers and lubricants. It could also be produced starting from natural resources (Asahi Kasei Kogyo, 1991). It is a constituent of biodegradable plastics such as Ecoflex (polybutilene adipate and/or polybutylene therefthalate) produced by BASF (Steinbuchel and Doi, 2002) and of Sky-Green (made of adipic acid, succinic acid, butanediole and ethylene glycole) produced by SK Chemicals (Korea) (Lee et al., 2002).

Azelaic acid is naturally found in wheat and rye and is used in the production of plasticizers, polyamides and alkyd resins.

1,4-Butanediol is a synthetic organic molecule that can be produced at industrial scale from succinic acid; usually, it is used as a solvent in the production of some kinds of plastics and fibers. It can also be produced starting from renewable raw materials (Cooper and Vigon, 2001). It is used with adipic acid to produce Ecoflex.

1,2-Ethanediol and 1,6-hexanediol are raw materials used for plastics.

Lactic acid can be produced both by chemical way and fermentation of carbohydrates from *Lactobacillus* (Sotergard and Stolt, 2002). It is used as a monomer for producing polylactic acid, which has application as a biodegradable plastic.

Glucose is the most widespread monomer in nature; it is produced by photosynthesis and it is used as energy source by respiration. Polymers derived from cellulose and, as such, based on glucose, are used as biodegradable plastics (e.g., cellophane).

Succinic acid is one of the intermediates in the Krebs cycle, by which organisms draw energy. It is a constituent of polybutylene succinate or polybutylene succinate-*co*-butylene adipate copolymers, commercially known as Bionolle, produced by Showa Highpolymers (Japan) (Tserki et al., 2006).

Sebacic acid derives from castor oil. It is typically used for the production of candles, and employed in the production of bio-based plastics.

Terephthalic acid is a commodity chemical, used as a precursor to the non-biodegradable polyester such as polyethylene terephthalate (PET) or polybutylene terephthalate (PBT). The aromatic component provides excellent material properties, so frequently terephthalic acid is used to improve material properties in aliphatic polyesters such as Ecoflex (Muller et al., 2001).

The main properties of the tested substances are reported in Table 1.

3.2.2 Biodegradation tests

Tests were carried out according to ASTM D5988-96 (1996), a respirometric test based on the measurement of carbon dioxide evolution. A sandy soil (500 g) was enriched with compost (20 g) and mineral salts (200 mg KH_2PO_4 kg^{-1}, 100 mg $MgSO_4$ kg^{-1}, 400 mg $NaNO_3$ kg^{-1}, 200 mg $(NH2)_2CO$ kg^{-1} and 400 mg NH_4Cl kg^{-1}) dissolved in water (40 ml). The soil-compost-salt mixture had 21 ± 1 g kg^{-1} dw total organic carbon, 2164 ± 110 mg kg^{-1} dw total nitrogen, 334 ± 25 mg kg^{-1} dw phosphorus, 15.6 ± 0.4 % w dw^{-1} moisture and pH 7.0 ± 0.3.

The monomer (2.00 ± 0.05 g) was added to the soil-compost-salt mixture and incubated at room temperature (21 ± 2 °C) in the dark, in hermetically closed jars. Blank jars, without monomers, were also prepared. Eight replicates were carried out for each monomer and the blank. Each jar contained a beaker filled with 0.5 N KOH (40 ml), which was titrated with HCl (0.25 N or 0.5 N) and replaced at specific times, in order to measure the CO_2 produced during a time step of the test (Modelli et al., 1999). The measurement was carried out every 2-3 days in the first 10 days of the tests, during which biodegradation was expected to be faster, and weekly thereafter. Test duration varied from 27 to 45 days, according to the cumulative CO_2 evolution. Moisture was not adjusted during the biodegradation tests. In hermetically closed jars, water evaporating from the soil saturates the headspace in a very short time and, consequently, any further water loss is negligible; therefore the soil moisture can be considered as constant during the test period.

The average amount of CO_2 produced in the blank jars was subtracted to the amount of carbon dioxide produced in the monomer jars. The ratio between the net CO_2 production and the theoretical CO_2 production (calculated on the basis of monomer carbon percentage) gives back the biodegradation percent.

Table 1: Physico-chemical properties of the tested substances (U.S.EPA, 2008).

Monomer	Molecular Formula	Molar mass ($g\ mol^{-1}$)	Carbon fraction (-)	Physical state at lab conditions	Water solubility at lab conditions ($mg\ l^{-1}$)	Organic carbon-water partition coefficient ($l\ kg^{-1}$)
Adipic acid	$C_6H_{10}O_4$	146	0.49	Solid	167,300	1.585
Azelaic acid	$C_9H_{16}O_4$	188	0.57	Solid	5,684	10.57
1,4-Butanediol	$C_4H_{10}O_2$	90	0.53	Liquid	636,200	0.4397
1,2-Ethanediol	$C_2H_6O_2$	62	0.39	Liquid	1000,000	0.2239
1,6-Hexanediol	$C_6H_{14}O_2$	118	0.61	Liquid	22,650	3.331
Lactic acid	$C_3H_6O_3$	90	0.40	Liquid	1,000,000	0.2218
Glucose	$C_6H_{12}O_6$	180	0.40	Solid	1000,000	0.01658
Sebacic acid	$C_{10}H_{18}O_4$	202	0.59	Solid	1,420	23.29
Succinic acid	$C_4H_6O_4$	118	0.41	Solid	807,900	0.6751
Terephthalic acid	$C_8H_6O_4$	166	0.58	Solid	1,256	18.28

3.2.3 Chemicals and analytical methods

All chemicals were of analytical grade purity. Adipic acid, azelaic acid, 1,6-hexanediol, sebacic and succinic acid were supplied by Gamma Chimica S.p.a. 1,4-Butanediol, 1,2-ethanediol, lactic acid, glucose and terephthalic acid were provided by Sigma-Aldrich.
Moisture content and pH of the soil-compost-salt mixture were measured by ISO 11465 (ISO, 1994a) and ISO 10390, (ISO, 2005) respectively. Total organic carbon, total nitrogen and phosphorus were measured by ISO 10694 (ISO 1995a), ISO 11261 (ISO, 1995b) and ISO 11263 (ISO, 1994b) methods, respectively. Titration was carried out according to Standard Methods (APHA, 1998).

3.2.4 Modeling and data processing

The "soil column compartment" of the numerical code AQUASIM (Reichert, 1998) was applied to simulate the monomer fate and transport in the jars and to process the experimental data.
Each jar was considered as an isothermal batch system containing a fixed amount (M_T on wet weight basis) of homogeneous soil-compost-salt mixture, with moisture (w) constant with time (t). The amount of soil-compost-salt mixture on dry weight basis was calculated according to:

$$M_S = \frac{M_T}{1+w} \tag{1}$$

and the volume of water (V_W) in the system as:

$$V_W = \frac{M_S \cdot w}{\rho_w} \tag{2}$$

where ρ_w is water density.
The batch system also contained the monomer, which was solubilized into water according to a first order kinetics with respect to the dissolved concentration C_W with constant K_{sol}; solubilization was limited by the monomer solubility in water (S). The following equation was used:

$$\left[\frac{dC_W}{dt}\right]_{solubilization} = \begin{cases} K_{sol} \cdot (S - C_W) & C_W < S \\ 0 & C_W = S \end{cases} \tag{3}$$

The mass of undissolved monomer in the jar, $M_{mon}(t)$, is a function of time according to:

$$\frac{dM_{mon}}{dt} = \begin{cases} -V_W \cdot \left[\frac{dC_W}{dt}\right]_{solubilization} & M_{mon} > 0 \\ 0 & M_{mon} = 0 \end{cases} \quad (4)$$

Two mechanisms are assumed to occur on the monomer dissolved in water:
i) non hysteretic and completely reversible sorption of the dissolved monomer to the soil-compost organic matter, according to a first order kinetics with constant K_s and distribution coefficient at equilibrium conditions K_d (linear isotherm) calculated as:

$$K_d = f_{OC} \cdot K_{OC} \quad (5)$$

where f_{OC} is the soil-compost organic carbon content and K_{OC} is the organic carbon - water partition coefficient for the molecule of concern (Schwarzenbach et al., 1993). The following equations are used:

$$\left[\frac{dC_W}{dt}\right]_{sorption} = -\frac{M_S}{V_W} \cdot \frac{dC_S}{dt} \quad (6)$$

$$\frac{dC_S}{dt} = K_s \cdot \left(K_d \cdot C_W - C_S\right) \quad (7)$$

where C_S is the monomer sorbed concentration;
ii) biodegradation of the dissolved monomer, according to a first order kinetics with respect to the dissolved concentration with constant K_b:

$$\left[\frac{dC_W}{dt}\right]_{biodegradation} = -K_b \cdot C_W \quad (8)$$

In order to allow the model to fit the experimental data, lag time (t_{lag}) was also considered for 1,2-ethanediol, azelaic acid and terephthalic acid.
Evolution over time of the mass of carbon dioxide (M_{CO2}) in the system is described as follows:

$$\frac{dM_{CO_2}}{dt} = V_w \cdot TOC_{mon} \cdot f \cdot (1-Y) \cdot K_b \cdot C_w \quad (9)$$

where TOC_{mon} is the monomer carbon content, f is the carbon dioxide to carbon molar weight ratio and Y is the carbon fraction biodegraded but not transformed into CO_2.
The percentage of monomer biodegraded at time t, B_{mon} (t), was calculated by the following equation:

$$B_{mon}(t) = 100 \cdot \frac{\frac{M_{CO_2}(t)}{f} \cdot \frac{1}{1-Y}}{M_{mon,0} \cdot f_{mon}} \tag{10}$$

where f_{mon} is the carbon fraction on weight basis in the monomer and $M_{mon,0}$ is the amount of monomer put into the jar at the beginning of the test.
The following initial conditions for the variables were applied:

$$\begin{cases} B_{mon}(t=0) = 0 \\ C_W(t=0) = 0 \\ M_{mon}(t=0) = M_{mon,0} \\ C_S(t=0) = 0 \\ M_{CO2}(t=0) = 0 \end{cases} \tag{11}$$

Table 2 summarizes the input data values used to run the model.

Table 2: Input data values used to run the model.

Parameter	Value
f	3.6667
f_{mon}	Carbon fraction from Table 1
f_{OC}	21 ± 1 g kg^{-1} dw
K_b	[a]
K_{OC}	Organic carbon - water partition coefficient from Table 1
K_s	50 d^{-1} [b]
K_{sol}	[a]
M_T	0.560 kg
S	Water solubility from Table 1
t_{lag}	[a]
TOC_{mon}	Carbon percentage from Table 1
w	15.6 ± 0.4 % w dw^{-1}
Y	[a]
ρ_W	1000 kg m^{-3}

[a] Estimated by the code, so as to get the best fit between the measured and the calculated cumulative mass of CO_2 evolved over time, $M_{CO2}(t)$, in each jar.
[b] Value set so as to simulate an instantaneous sorption process.

Model parameters can be estimated by AQUASIM by minimizing the sum of the squares of the weighted deviations between measurements and calculated model results (χ^2); in this work, the objective function was the cumulative mass of CO_2 evolved over time, $M_{CO2}(t)$, in each jar. The user has the choice between two numerical minimization algorithms: the simplex algorithm and

the secant algorithm; both these methods were used in order to verify the agreement between the estimates provided for by the code. Each calculation must be initialized before it can be started, and the user can give the initial state; multiple minima in the domain of the parameters were excluded on the basis of the convergence of the estimates obtained by using different initial values for the parameters within the range [0, 10] d^{-1} for K_b and K_{sol}, [0, 1] for Y and [1, 10] d for t_{lag}.

3.3 Results

The estimation of the parameters of concern carried out by the AQUASIM numerical code on the basis of the experimental data and other input values are shown in Table 3 as mean value ± standard error on replicates (n = 8) for each tested monomer.

K_b ranged between 0.10 and 0.27 d^{-1} for most of the tested monomers but sebacic acid and terephthalic acid displayed higher values (1.2 and 0.7 d^{-1} respectively).

The highest K_{sol} at room temperature was observed for terephthalic acid (6.9 d^{-1}) and the lowest for 1,4-butanediol (0.006 d^{-1}). 1,6-Hexanediol and lactic acid had quite similar values (0.17 and 0.20 d^{-1} respectively), whereas the remaining compounds had K_{sol} between 1.8 and 4.5 d^{-1}. Standard deviation was very high for azelaic acid and lactic acid.

K_{sol} of 1,4-butanediol was much lower than the respective $K_{b,}$ so that the degradation rate could be limited by the solubilization step.

t_{lag} was included in the simulation for 1,2-ethanediol, azelaic acid and terephthalic acid, resulting in values between 3 and 4.9 d, whereas in the other cases a good fit was observed without introducing t_{lag}.

Figure 1 shows as an example the mass of carbon dioxide evolved over time within the jars containing 1,6-hexanediol (a) and 1,2-ethanediol (b) measured with the respirometric tests and the simulated CO_2 production based on the estimated mean values reported in Table 3.

Table 3: Estimation of the parameters provided for by the code, as mean value ± standard error on replicates (n=8).

	Parameter			
	K_{sol}	K_b	t_{lag}	Y
Monomer	(d^{-1})	(d^{-1})	(d)	(-)
Adipic acid	2.4 ± 0.8	0.10 ± 0.03	-	0.34 ± 0.09
Azelaic acid	4.5 ± 3.9	0.27 ± 0.09	4.9 ± 1.4	0.32 ± 0.08
1,4-Butanediol	0.006 ± 0.002	0.21 ± 0.03	-	0.52 ± 0.03
1,2-Ethanediol	2.5 ± 1.5	0.15 ± 0.05	3.0 ± 0.6	0.4 ± 0.1
1,6-Hexanediol	0.17 ± 0.06	0.13 ± 0.01	-	0.42 ± 0.02
Lactic acid	0.2 ± 0.3	0.16 ± 0.01	-	0.56 ± 0.04
Glucose	2.4 ± 1.6	0.21 ± 0.03	-	0.58 ± 0.07
Sebacic acid	1.8 ± 0.9	1.2 ± 0.3	-	0.49 ± 0.05
Succinic acid	3.6 ± 0.7	0.19 ± 0.04	-	0.57 ± 0.06
Terephthalic acid	6.9 ± 1.3	0.7 ± 0.1	4.1 ± 1.5	0.36 ± 0.03

In both cases, the replicates were similar and the tests seemed to have high repeatability. The two curves represent specific but different situations: for 1,6-hexanediol the CO_2 production (i.e. the mineralization) started slowly but immediately after the setup of the test, while for 1,2-ethanediol a 3 days lag phase could be observed. For the latter, the steady state was attained after 20 days, while for the former more than 35 days were necessary.

Figure 2 shows the biodegradation curves obtained for the tested monomers, based on equation (10) for $B_{mon}(t)$, which also accounts for carbon not transformed into CO_2 (byproducts, biomass, etc.); each point is the mean value of replicates, and the error bars represent the standard error on replicates. Measurements of the remaining monomers in soil could not be performed due to the difficulties related to the extraction procedure and the analytical method to apply. In some cases, values over 100% were calculated, but the overestimation was always within 10% and ascribable to the analytical uncertainties.

For terephthalic acid and sebacic acid, a plateau was not reached within the test duration, but at the end of the test sebacic acid was completely biodegraded. The estimated biodegradation of terephthalic acid was 63 %, with a lag phase of 3 days. The longest lag phase (4.9 days) was observed for azelaic acid, whose estimated average biodegradation at the end of the tests was 79 %. The behavior of glucose, succinic acid and lactic acid was similar, with very fast biodegradation, no lag time and steady state reached after about 15 days. As expected, the final biodegradation of these molecules was complete (101 % for glucose and lactic acid and 103 % for succinic acid).

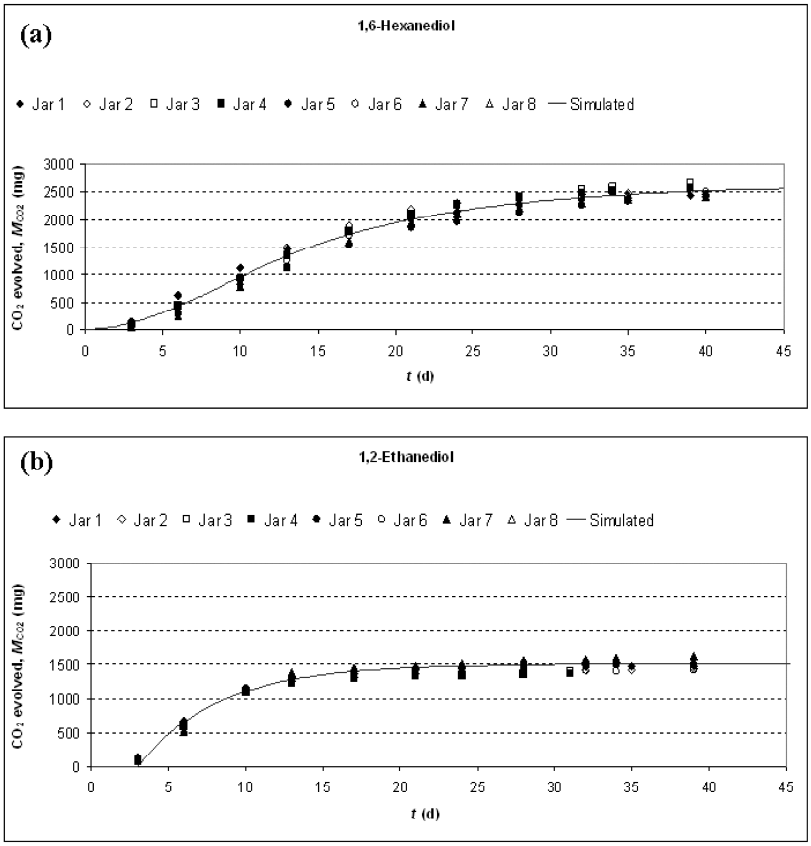

Figure 1: Experimental mass of carbon dioxide evolved over time (all replicates) for 1,6-hexanediol (a) and 1,2-ethanediol (b) and data obtained by simulation on the basis of values in Table 3.

The biodegradation curve for 1,4-butanediol is similar: the steady state was reached after 20 days and biodegradation was complete. For 1,6-hexanediol the biodegradation curve is different: the CO_2 release was lower at the beginning of the test and increased gradually without reaching a defined plateau at the end of the test. In spite of that, at the end of the test the biodegradation was 95 %.

Biodegradation of adipic acid and 1,2-ethanediol was 84 % and 85 % respectively; in both cases the steady state was reached after about 20 days, but 1,2-ethanediol biodegradation curve had a lag phase of 3 days.

On the basis of the experimental data, the applied code estimated that cellular biosynthesis (Y in Table 2 and in the APPENDIX) could account for 32 % to 58 % (Table 3), with values over 50 % for 1,4-butanediol, lactic acid, succinic acid and glucose.

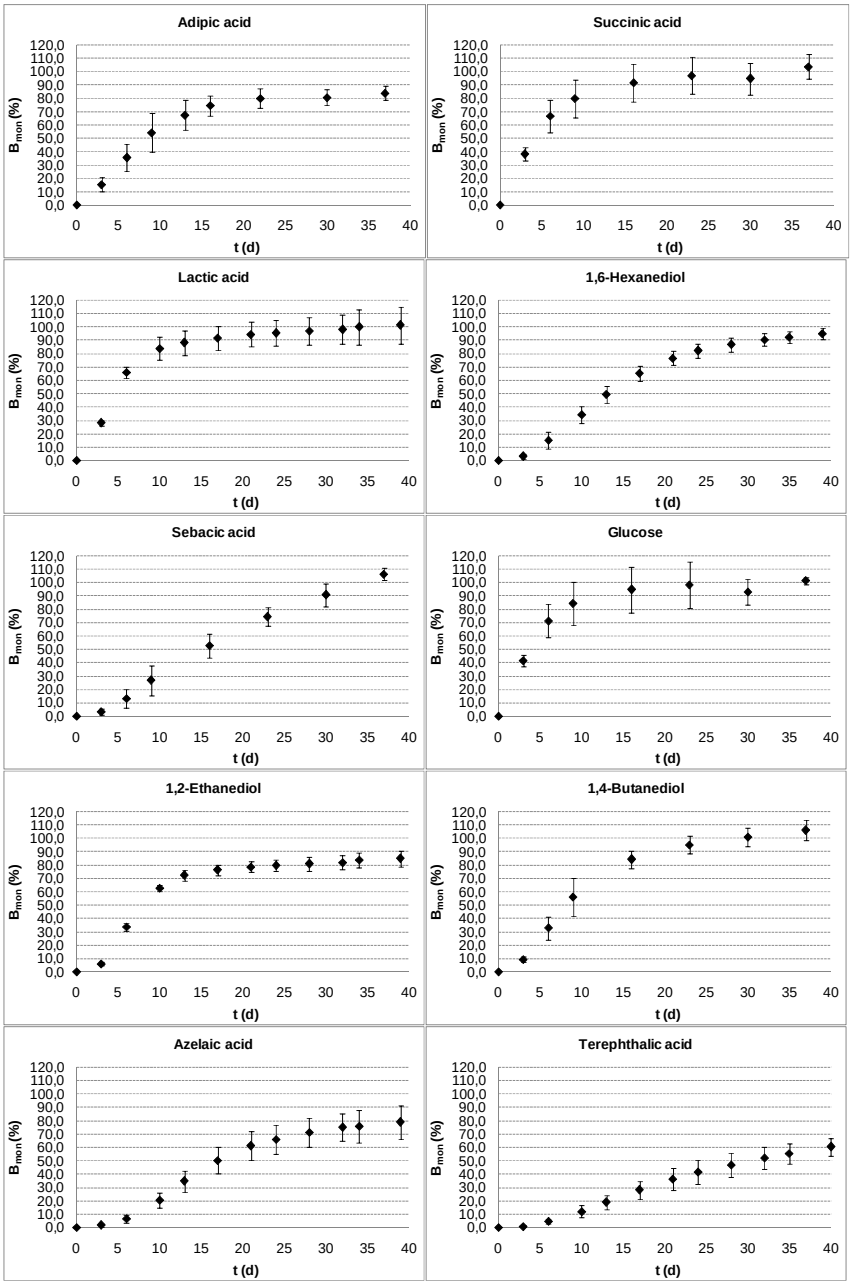

Figure 2: Biodegradation curves calculated for the tested monomers (B_{mon}, based on Equation 10 and Y values from Table 3). Error bars refer to the experimental standard deviation in the CO_2 measurements and do not account for the uncertainty in the AQUASIM estimates.

3.4 Discussion

On the basis of results of the AQUASIM simulations, the main points to discuss are the influence of solubility and the contribution of cell biosynthesis to the overall consumption of the carbon added with the monomers.

The tested monomers had quite different characteristics and, in particular, their solubility varied within a wide range. However, no relation was found between solubility and biodegradation: the least soluble compounds are sebacic acid and terephthalic acid, whose biodegradation was very different (complete for sebacic acid and 60 % for terephthalic acid). Azelaic acid had also a quite low solubility, even if higher than the previous monomers, and its biodegradation was 79 %.

Some of the tested monomers (e.g., glucose, lactic acid, and succinic acid) are known to be readily biodegradable and to play an important metabolic role within the cells. According to the results of AQUASIM, a considerable fraction of the carbon in the tested compound was converted to biomass. The overall biodegradation was complete but conversion to biomass accounted for 58, 56 and 57 % for glucose, lactic acid and succinic acid respectively. Terephthalic acid, which showed the lowest biodegradation curve in the tested series, had also the lowest percent of carbon converted to biomass. The same consideration applies to 1,2-ethanediol whose biodegradation was 85 %, with 36 % carbon converted to biomass.

A comparison of the data found in this study with available literature is not straightforward. The availability of data on biodegradation of monomers in soil is limited. Even though the experimental protocols can be similar, soil composition or room temperature or time duration of the tests or other local factors can affect results in terms of both mineralization percentage and lag time. Moreover, often the variability of the reported average data is not expressed as standard deviation or standard error. Figure 3 shows the maximum mineralization measured during the present research for some monomers compared to the mineralization measured for the same monomers by Kim et al. (2001) and Sharabi and Bartha (1993). Kim et al. (2001) carried out the tests for 32 days, adding the monomers to a mixture of forest/agricultural soils and perlite. This mixture was used as the core in a multilayer system where the bottom and the top layers were perlite. The mineralization percentage obtained by Kim et al. (2001) was comparable to that observed in the present study (27-45 days) for adipic acid and 1,4-butanediol, and higher for succinic acid (+19 %) and terephthalic acid (+10 %). Sharabi and Bartha (1993) used a freshly collected sandy loam, buffered with $CaCO_3$ at least 5 days before the beginning of the tests, and measured the CO_2 evolution over 22 days. Soil moisture were adjusted to 60 % of field capacity and nutrients were added by a 1% solution of $(NH_4)_2PO_4$. In that case, the mineralization percent was higher for both adipic acid (+40 %) and glucose (+36 %).

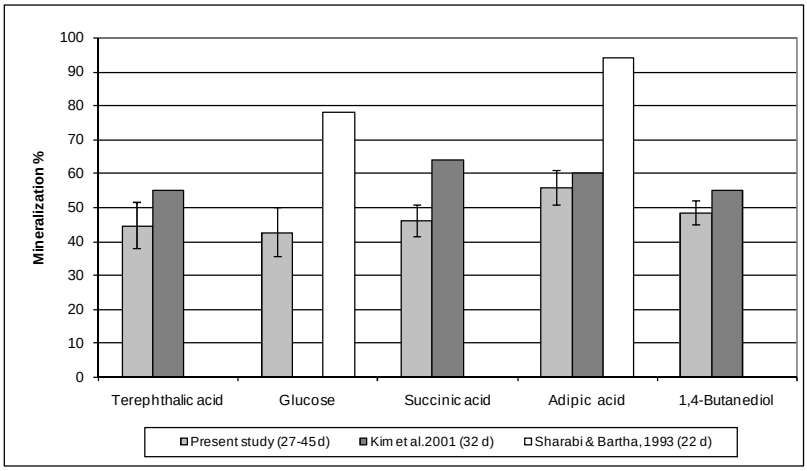

Figure 3: Maximum mineralization percentages reported by other authors, compared with the values measured in the present research.

3.5 Conclusions

In this study, the mineralization kinetics in soil was measured for several monomers applied in biodegradable polyesters, by using a standard respirometric test method. The experimental data were processed with the numerical code AQUASIM, in order to estimate values for the parameters related to the mechanisms occurring to the monomers in soil (solubilization, sorption, mineralization, biomass or by-products production). Some of these data are useful in predicting the fate and transport of biodegradable polyesters in soil as described in Saponaro et al. (2008).

As tests were carried out in a composite medium, including natural soil, compost and nutrient salts, and no selected bacterial strain was inoculated, it is likely the results are representative for the rates and extent of biodegradation in natural environments, even with a possible polyauxic behavior of microbial population. However, some differences between the experimental data and the in-field behavior of the tested monomers could occur, especially if a different substrate/soil ratio (quite high in the test conditions) would lead to a different metabolic hierarchy on the basis of substrate affinity.

On the basis of the results obtained, the applied code estimated that cellular biosynthesis could account for 32 % to 58 %, with values over 50 % for 1,4-butanediol, lactic acid, succinic acid and glucose, which were completely biodegraded.

APPENDIX

Notation

B_{mon} Percentage of monomer biodegraded (%)

C_S Monomer sorbed concentration (kg monomer kg^{-1} dry matter)

C_W Monomer dissolved concentration (kg monomer m^{-3} water)

f Carbon dioxide to carbon molar weight ratio (kg CO_2 kg^{-1} C)

f_{mon} Carbon fraction on weight basis in the monomer (kg C kg^{-1} monomer)

f_{OC} Soil organic carbon content (kg organic carbon kg^{-1} dry matter)

K_b Monomer biodegradation kinetic constant (d^{-1})

K_d Monomer distribution coefficient (m^3 water kg^{-1} dry matter)

K_{OC} Monomer organic carbon - water partition coefficient (m^3 water kg^{-1} organic carbon)

K_s Monomer sorption kinetic constant (d^{-1})

K_{sol} Monomer solubilization kinetic constant (d^{-1})

$M_{CO2}(t)$ Mass of carbon dioxide evolved at time t (kg CO_2)

$M_{mon}(t)$ Mass of monomer at time t (kg monomer)

M_S Mass of dry soil-compost-salt mixture (kg dry matter)

M_T Mass of wet soil-compost-salt mixture (kg)

S Monomer solubility in water (kg monomer m^{-3} water)

t Time (d)

t_{lag} Lag time in monomer biodegradation (d)

TOC_{mon} Monomer carbon content (kg C kg^{-1} monomer)

V_W Water volume in wet soil-compost-salt mixture (m^3 water)

w Soil-compost-salt mixture moisture (kg water kg^{-1} dry matter)

Y Carbon fraction biodegraded but not transformed into CO_2 (-)

ρ_W Water density (kg water m^{-3} water)

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4 Influence of compost and pH on the mineralization of a model aliphatic polyester in soil

ABSTRACT

The use of biodegradable polymers in some applications and sectors (compostable waste bags, catering products, mulch films) can be an alternative to the disposal of plastic in landfill, can reduce the cost of wastes management and the accumulation in the environment. The most acceptable disposal method for biodegradable polymers is composting, however for wastes deriving from the agriculture (in particular mulch films) the biodegradation in soil is a preferable option. The biodegradability is influenced by environmental properties of the medium in which a material is biodegraded: moisture, pH, nutrient availability, presence of active microorganisms, for example, affect the process in soil. The influence of starting soil pH and organic matter concentration on the mineralization of a model polyester was investigated using an agricultural sandy soil. Soil mixtures with different starting pH and organic matter content were prepared and polyester mineralization was evaluated according to ASTM D5988-96; cellulose was used as reference material. Cellulose mineralization resulted not influence by soil pH or organic matter content (maybe because of its wide natural distribution), the model polyester, instead, has shown to be actively mineralized but the mineralization rate was affected by environmental conditions. The best results have been obtained for initial pH values close to neutrality: in this situation mineralization was comparable to the one obtained for cellulose (about 60 %). On the contrary, no clear evidence have been found about the role of compost, even if its presence in the test soil seems to affect positively the mineralization.

KEYWORDS soil pH, compost, biodegradation, polyester

Work in progress

4.1 Introduction

Clay, amber, arabic rubber and caucciù are examples of natural plastic materials and "plastic" refers to their property to maintain the shape after manufacturing. Nowadays, plastic is a common name used to indicate synthetic, organic and high molecular weight polymers. Synthetic polymers have been developed from the beginning of 1900s, when Leo H. Baekeland invented the first cheap synthesis method to obtain a polymer and produced the bakelite. Many different polymers (PVC, nylon, PE, PET…) have been synthesized since then, they have spread worldwide and have become one of the symbols of the twentieth century. Synthetic polymers have been developed to be enduring and resistant to all forms of degradation. Their versatility allows them to be used in many applications: packaging (which used about the half of the plastic production), building and construction, automotive design, electrical and electronic products, agriculture. The use of plastics in agriculture is growing. An estimated amount of 2-3 tons of plastics (in particular polyethylene) are used each year in agricultural applications and almost half of them is used to protect crops (greenhouses, mulching, temporary coverings of structures for fruit trees…) (Kyrikou and Briassoulis, 2007).

Plastics contributes to increase the amount of municipal wastes to be disposed and, additionally, produces urban litter (Andrady and Neal, 2009). For packaging materials, for example, the rate of recycling has increased but is still low and their contamination during use often makes recycling uneconomic if compared with landfill disposal (Song et al., 2009). One possibility to reduce the accumulation of plastics in the environment and the cost of their management and disposal is the use of biodegradable polymers to replace traditional plastics wherever technically possible and economically feasible.

Biodegradation is a complex process in which a polymer is completely converted by microorganisms to carbon dioxide or biogas (in aerobic or anaerobic condition respectively), water, mineral salts and new biomass. The most acceptable disposal method for biodegradable polymers is composting. However, composting requires infrastructures, including collection system and treatment facilities, and is not always the best practice. For wastes deriving from the agricultural use of plastics, for instance, biodegradation in soil is a greatly preferable option to cope with economical and environmental aspects (Kyrikou and Briassoulis, 2007).

Different kinds of biodegradable polymers are available on the market for agricultural applications: Mater-Bi® (Novamont, Italy), Ecoflex® (BASF, Germany), Bio-Flex ® (FKUR, Germany) are used to produce flowerpots, controlled-release fertilisers, plant clips and mulch films. Mulch films, in particular, at the end of their life, are not fed to a composting plant but just applied to soil and left there. The agricultural soil is the medium for the production of food for humans and cattle, so the absence of negative effects

due to the in situ disposal of plastics and of residue build-up are matters of concern. The definition of standard test methods and of specific criteria to verify biodegradability and absence of eco-toxic effects in soil are nowadays required to clarify all these issues and launch the marketing of safe biodegradable polymers in agriculture (Degli Innocenti, 2005). In 1996 the American Society for Testing and Materials (ASTM D5988) published a standard method for determining aerobic biodegradation in soil of plastic materials by measuring the amount of carbon dioxide evolved in a closed respirometer. In 2003, ISO published a standard method for determining biodegradation rate of plastic material in soil by measuring the oxygen demand or the amount of carbon dioxide evolved in a ventilated respirometer (ISO 17556, 2003).
Biodegradation of new generation plastics is affected not only by chemical and physical properties of the polymers, but also by the environment in which they are biodegraded. Environmental properties such as soil moisture, porosity, pH, oxygen and nutrient availability, the presence of active microorganisms, and the concentration of plastics and, eventually, of other contaminants affect the biodegradation process in soil.
The aim of this work was to study the effects of soil pH and of the addition of compost on the biodegradation in an agricultural soil of an experimental aliphatic polyester used as model.

4.2 Materials and Methods

4.2.1 The tested substances

The experimental work was carried out using a polybutilen-sebacate ($(C_{14}H_{24}O_4)_n$) expressly synthesised by Novamont S.p.A. and use as model polyester in this study. It is a linear chain aliphatic polyester made of butanediol and sebacic acid (commonly used to synthesize plastics) and obtained by standard techniques of monomers condensation.
Cellulose ($(C_6H_{10}O_5)_n$) was supplied by Merck and used as reference material.
The two polymers were incubated in soil as powder.

4.2.2 Soil

The agricultural soil used in this work was collected in Arborio, Italy. The texture is made of about 55 % sand, 43 % silt and 2 % clay and its pH in water is 5 to 6.5. Soil samples were freshly collected, sieved (< 2 mm) and used within a few days for biodegradation tests.

4.2.3 Biodegradation tests

Biodegradation tests were carried out according to ASTM D5988 (1996). For each test, 500 g of soil mixture were incubated at room temperature

(21±2° C) in the dark, in hermetically closed jars (3 l), with test substances (2 g). Blank jars, without test substances, were also prepared. Each jar contained a beaker filled with 0.5 M KOH (40 ml), which was regularly titrated with 0.25 M HCl in order to measure the CO_2 production within the jar. The measurement was carried out every 3-4 days in the first two weeks, during which biodegradation was expected to be faster, and every 7-10 days thereafter. When beakers were taken away from jars to titration, the jars remained open from 15 to 30 minutes, so that the air was refreshed before replacing fresh potassium hydroxide. Soil moisture was not adjusted during the biodegradation tests. In hermetically closed jars, water evaporating from the soil saturates the headspace in a very short time and, consequently, any further water loss is negligible; therefore the soil moisture can be considered as constant during the test period.

4.2.4 Analytical methods

Moisture content and pH of the soil mixtures were measured according to ISO 11465 (ISO, 1994) and ISO 10390 (ISO, 2005) respectively.
Titration was carried out according to Standard Methods (APHA, 1998).
Organic matter at the beginning of the test was estimated by combustion of soil samples at 550° C.

4.2.5 Experimental plan

Four different soil mixtures were prepared to investigate biodegradation of cellulose and polyester in the same soil with different starting pH (from sub-acid to neutral values) and organic matter content. The soil mixtures can be described as follow:

enriched soil without buffer: soil was enriched with compost and mineral salts to obtain a soil-compost-salt mixture. Compost, according to ASTM 5988-96, was added to the soil with a typical application in agricultural land (4 % in weight); mineral salts (0.2 g KH_2PO_4, 0.1 g $MgSO_4$, 0.4 g $NaNO_3$, 0.4 g NH_4Cl and 0.2 g urea per Kg of soil) were dissolved in water and added to soil and compost to obtain the correct ratio of nutrients and the ideal moisture (around 50 % of the water holding capacity);

not enriched soil without buffer: soil was used in its natural condition and only moisture was adjusted before the test starts;

not enriched soil with buffer: soil was used without adding compost and mineral salts, but $CaCO_3$ (2 % in weight) was added as a buffer;

enriched soil with buffer: a soil-compost-salt mixture was obtained as previously described and $CaCO_3$ was used to increase the natural soil pH and to buffer any eventual decrease.

The buffered soil mixtures were prepared, left at room temperature and periodically mixed at least 3-5 days before the beginning of the tests to avoid

that the CO_2 released by neutralization could be considered as deriving from the biodegradation process.
Each test condition was tested twice. In the first part of the experimental work, the biodegradation of the test substances was investigated with the four soil mixtures (see Table 1: first group). In this part of experimental work, the soil was collected from the same site in different seasons. In the second phase (see Table 1: second group) , the same soil mixtures were tested at the same time with soil collected in a single sampling.
The tests carried out in the first group lasted from 171 to 251 days, according to the cumulative CO_2 evolution. In the second group, biodegradation was monitored for three months.
On the whole, eight tests (two replicates each) were carried out for polymer, for cellulose and for the blanks.
The name of the tests, the soil mixture, soil pH and organic matter at the beginning of each test are reported in Table 1.
Soil pH was measured during the test period. When pH decreased from the starting values, as in tests 1 and 2, soil was buffered by adding $CaCO_3$ (2 % in weight) in each jar. As expected the effect was an increase of CO_2 production in all cases. As 10 g $CaCO_3$ were added, the maximum amount of CO_2 released should has been 4.4 g per jar. Most of it was probably released when the soil was mixed. Anyway, as CO_2 increase was measured in all jars, the blank ones included, the biodegradation rate of the tested polymers, calculated on the basis of the difference between CO_2 produced in jars with molecules minus CO_2 produced in the blanks, should not have been overestimated.

Table 1: Name of the test, soil mixture and starting soil pH in each test.

	Test name	**Soil mixture**	**starting pH**	**starting O.M. (%)**
First group	test 1	enriched soil without buffer	6.20	6.81
	test 2	not enriched soil without buffer	6.19	6.21
	test 3	not enriched soil with buffer	7.14	6.21
	test 4	enriched soil with buffer	7.53	6.84
Second group	test A	enriched soil without buffer	6.20	7.04
	test B	not enriched soil without buffer	5.24	6.04
	test C	not enriched soil with buffer	7.33	6.25
	test D	enriched soil with buffer	7.25	7.19

4.3 Results

4.3.1 Tests of the first group

Figure 1 shows mineralization curves obtained for the tested polymers in the four tests of the first group. Each point represents the values obtained from experimental data in the jars prepared for the two tested polymers. The reproducibility of results is very good: measures in similar jars of the same test are, in practice, the same. Only for cellulose in test 1 a difference around 10 % between replicates can be seen in the plateau phase.

Cellulose mineralization process shows similar patterns in the four tests. The lag phase is very short (3-4 days), except for the tests based on not enriched and not buffered soil (test 2) where mineralization starts 7-8 days after incubation. The biodegradation phase, in which mineralization is fast, lasts 18-20 days so that in one month about half of the final mineralization percentage is reached in each test. Final mineralization is 56 %, 64 %, 59 % and 58 % in test 1, 2, 3 and 4 respectively. When $CaCO_3$ was added to the soil of test 1 and 2 (after 53 and 96 days respectively), the plateau phases were already reached, and no significant change can be observed in the mineralization curves.

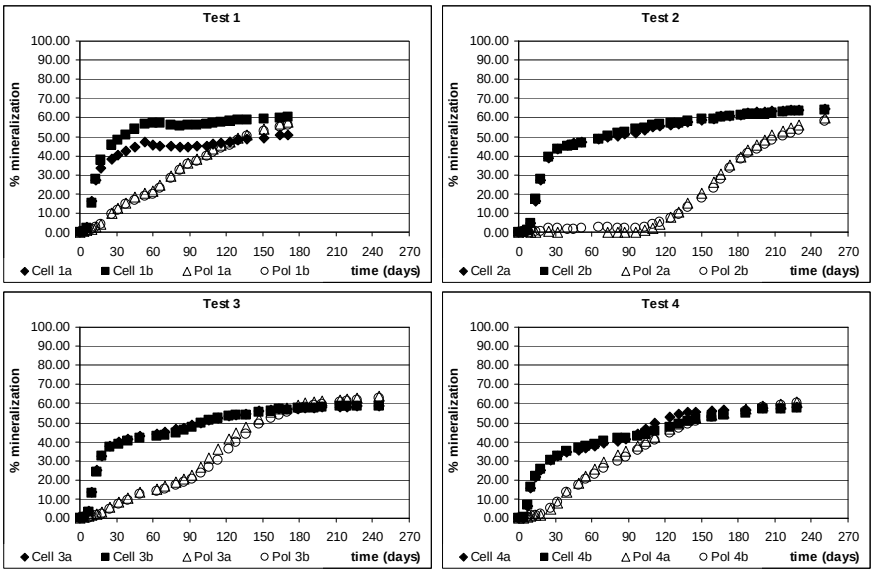

Figure 1: Mineralization curves of test polymers in the four tests of the first part of experimental work. Black points refer to cellulose, white points refer to polyester.

The pattern of the mineralization process is different for polyester. For tests 1, 3 and 4, the experimental curves show a lag phase around 10 days after which mineralization starts and proceeds regularly until the plateau phase is

reached, at the end of the tests. Final values are about 56 %, 63 % and 60 % for test 1, 3 and 4 respectively, comparable to the cellulose ones.
In test 1, after about two months (53 days), the polymer mineralization seemed to be low. The soil pH in the jars was measured and the result (pH 5.6-5.9) showed a little decrease from the starting value (6.20). $CaCO_3$ was added to each jar in order to buffer acidity, the soil was mixed and air refreshed. pH increased and stabilized at about 7.4. The result was an increase of CO_2 production in all the jars. CO_2 measured in jars with soil amended with the polyester was slightly higher than in the blanks, so that the result seemed to be a little increase of the polyester mineralization rate. Nevertheless it was impossible to assess if the increased CO_2 release after the addition of $CaCO_3$ depended on a greater degradation activity, or if it was caused by soil buffering.
Situation in test 2 is different: after about 90 days of incubation polyester mineralization was not started. Soil pH was measured in each jar and the results (pH 5 in the jars with polyester, pH 5.3-5.5 in the other jars) show a consistent decrease from the starting value (pH 6.19). $CaCO_3$ was added, pH increased and stabilized at 6.9 in polyester jars and at about 7 in the others. Also in this situation an increase of CO_2 production was measured in all jars (Figure 2). The increase measured in jars with cellulose (dotted line blacks) was quite the same of the blank jars (unbroken line), but, where polyester was incubated, the increase of CO_2 production (line with white dots) was significantly higher than in the blanks. The mineralization process started and it proceeded as in the other soil mixtures, reaching a similar final mineralization percentage (59 %).
In all the tests, the polymer mineralization, calculated by comparison to the reference substance, can be considered at 100 %.

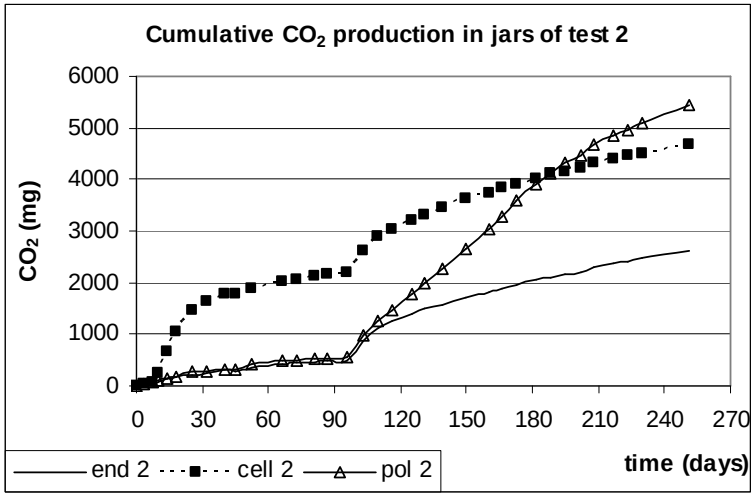

Figure 2: Cumulative CO_2 production (mg) in the jars of test 2. Unbroken line refers to blank jars, black points to cellulose and white points to polyester.

4.3.2 Test of the second group

As in the first part of the work, in the second group of biodegradation tests the reproducibility of experimental data was very good. Figure 3 reports the experimental curves for cellulose and polyester obtained in the four tests. Black symbols refer to cellulose, white symbols to polyester.

Cellulose mineralization is different in the different soil mixtures. In soil enriched and not buffered (test A) lag and biodegradation phases are about 7 and 18 days respectively and mineralization percentage is 50 % at the end of the test. In not enriched and not buffered soil (test B) lag phase is longer than in test A (10-12 days) and mineralization is slower. After 90 days of incubation only 35 % of mineralization is reached. In test C and D the lag phase duration is the same (3-4 days), the biodegradation phase lasts 18-20 days but final mineralization values are different: 55 % and 34 % in test C and D respectively.

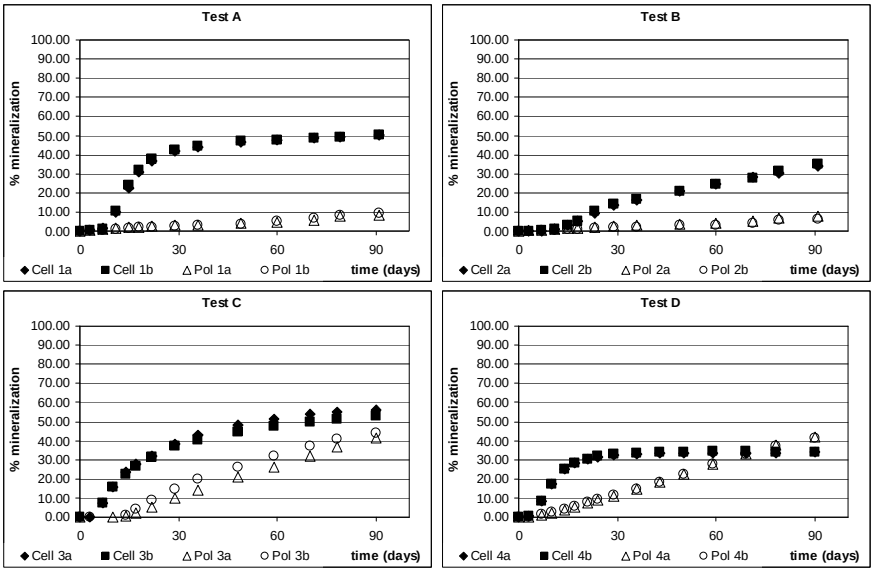

Figure 3: Mineralization curves of test polymers in the four tests of the second part of experimental work. Black points refer to cellulose, white points refer to polyester.

The results obtained for the polyester are different. Mineralization curve of test A is similar to the curve of test B. In the two tests in not buffered soil, mineralization reaches only 9 % in test A and 7 % in test B. No lag phase or biodegradation phase can be recognized. Where soil was buffered (tests C and D), after 10 days from the beginning of the tests, mineralization starts and reaches, at the end of the experimental observations, about 42 % (higher than cellulose mineralization percentage in test D).

After 3 months incubation the mineralization is lower for the polyester than for the cellulose. It can not be excluded that in buffered soil with or without

compost (test D and C respectively) polyester can reach higher mineralization rates in longer time.

4.4 Discussion

4.4.1 The cellulose

The results obtained in the first part of the experimental work suggest that the mineralization of cellulose is not affected by the initial soil content of organic matter. The four mineralization curves of cellulose are presented in Figure 4. Because of the great precision of the experimental data, mineralization curves are represented with the mean values of the two replicates of each test. Error bars, which are generally shorter than symbols, are not reported.

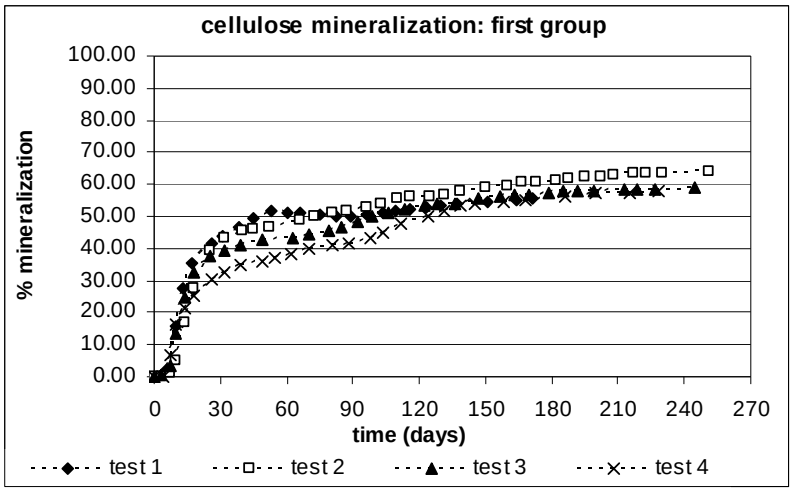

Figure 4: Mineralization curves of cellulose obtained with tests of the first group.

Mineralization curves are about the same in all the tested soil mixtures. Between the 25th and 120th day, mineralization values are slightly different, but no relationship with presence of compost or with soil pH at the beginning of the tests can be recognized. The only noticeable effect, but negligible in overall result, is a slightly longer lag phase (7-8 days) in not enriched and not buffered soil (test 2) than in the other soil mixtures (3-4 days).

Cellulose is the most common organic compound on the earth. It is biodegraded by cellulolytic microorganisms that have an important role in carbon biogeochemical cycle. All organisms known to degrade cellulose efficiently produce an enzyme system (commonly knows as cellulase) with different specificities, which act together in synergism (Béguin and Aubert, 1994). It is known that microbial enzymes have an optimum pH activity and

that soil pH strongly affects the biodegradation of organic molecules and mineralization processes. Moreover, specific enzymes catalyzing the same reaction, but produced by different microorganisms, have different pH optimum for activity (Niemi and Vepsäläinen, 2005; Deng and Tabatabai, 1994). For example, Deng and Tabatabai (1994) observed that the optimal pH for cellulase activity in soil is 5.5. Moreover, they observed that data available in literature indicate for cellulase isolated from *Trichoderma viride* an optimal pH of 6.5, whereas the optimal pH for cellulase isolated from *Polyporus versicolor* is 5.0. Shin and Jung (1999) studied the biodegradation of cellulose in soils with pH 4.1 and 6.95 and, after 120 days of incubation, they observed, in both cases, 54 % of mineralization. Similar results were obtained in the first part of this work, where cellulose was incubated in soil with initial pH 6.20 and 7.53.

The results obtained in the second part of the experimental work (Figure 5), are less clear than those obtained in the first part. Mineralization curves obtained in tests A and C (enriched and not enriched soil respectively, without buffer) are quite the same as those obtained in similar tests of the first part (test 1 and 3). In test B, where the starting soil pH was 5.24, close to the optimal value indicated by Deng and Tabatabai (1994), mineralization was slower than the ones measured in the other test conditions even if the plateau phase was not reached after 90 days. The situation is completely different, and cannot be easily explained, in test D (soil enriched and buffered): mineralization process reached plateau phase after one month of incubation at percent value around 35 %.

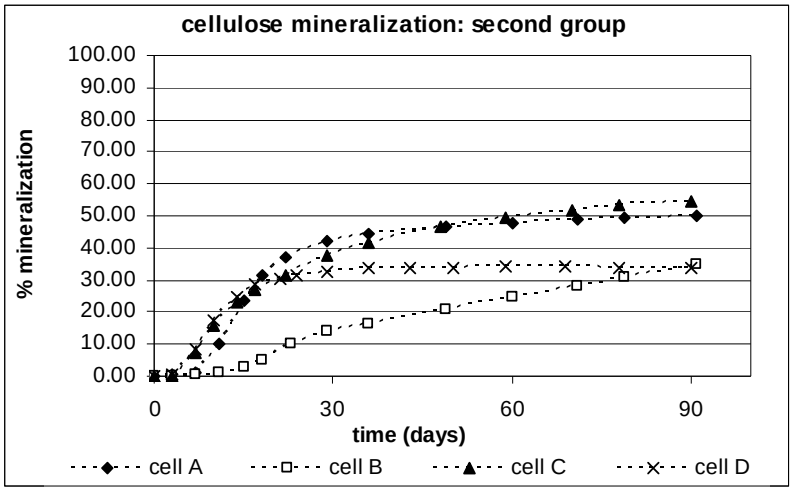

Figure 5: Cellulose mineralization curves obtained with tests of the second group.

4.4.2 The model polyester

Mineralization curves obtained in the two parts of the experimental work are presented in Figures 6 and 7. Each curve shows the mean values obtained from experimental data in each test; error bars are not reported because generally smaller than symbols.

Taking into account test 1, 3 and 4 of the first group, during the first 120 days of incubation, the mineralization of the polyester in soil with compost (test 1 and 4) results higher than in test where soil was not enriched (test 3), even if the final values were the same in the three tests, so that compost seems to improve the process at the beginning of the incubation.

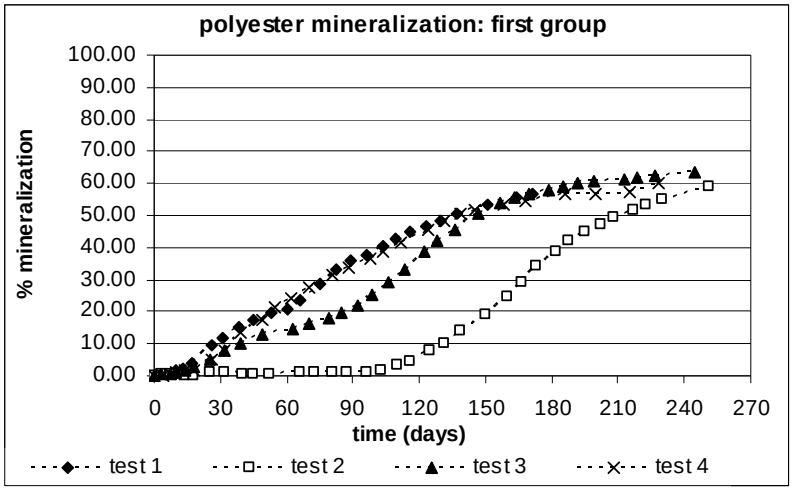

Figure 6: Mineralization curves of polyester obtained with tests of the first group.

When $CaCO_3$ was added to the soil of test 1, mineralization did not increase significantly (curve of test 1 is the same of the one obtained in test 4), but the result obtained in test 2 was very different. Mineralization of polyester in acid soil without compost did not start until $CaCO_3$ was added to the soil (90th day) and then, in about four months, reached the same final value as in the other tests.

The results obtained in the second part of the work suggest that the soil pH seems to have a more important role than compost. In fact, in the two tests where the initial soil pH was > 7 (without or with compost in test C and D respectively) mineralization occurred at a rate comparable to the one obtained in tests 1 and 4 of the first group, while it was very slow in the soils not buffered (tests A and B). It is important to remember that results of this group of tests refer only to three months of incubation, and that the cellulose mineralization (see Figure 5), in particular in test D where only the 35 % was obtained, reaches values slower than previous ones.

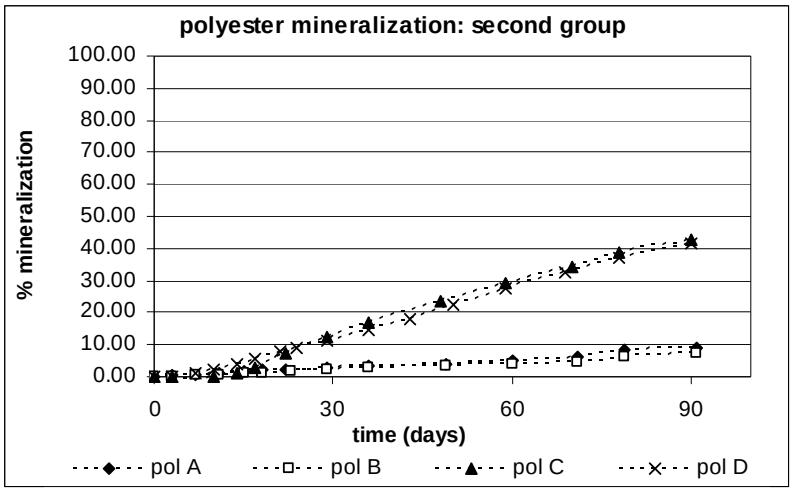

Figure 7: Polyester mineralization curves obtained with tests of the second group.

The tested polymer is a polyester, and its biodegradation involves hydrolytic enzymes whose activity is known to be affected by pH (Turner, 2010; Margesin et al, 2002; Alvarez et al, 1999). Turner (2010) observed that optimal pH of enzymes in soil depends on their origin, i.e. on the organism producing them (plant, bacteria, fungi), on their position in soil (in free solution or associated to organic matter or clays). Similar results were obtained by Kyun and Jung (1999) who tested biodegradation in soil of a commercial polyester and obtained that neutral soil pH was the best condition for the process.

4.5 Conclusions

Two polymers were tested for biodegradation in different soil mixtures, with different organic matter and different pH at the beginning of the tests. The results showed that cellulose biodegradation was not influence by the two factors. This can be related to the wide natural distribution of cellulose and to the consequently large population of microorganisms able to degrade it.
The tested polyester has shown to be actively biodegraded but the biodegradation rate was affected by environmental conditions. The best results have been obtained for initial pH values close to neutrality: in this situation mineralization was generally comparable to the one obtained for cellulose.
The role of compost have been found no so evident, even if its presence in the test soil seems to affect positively the biodegradation process.

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5 Sensitivity of soil combustion to determine organic matter in soil samples. Preliminary results

ABSTRACT

The increasing use of new generation plastics in agricultural stimulate the study of the biodegradation in soil in order to obtain reliable standard test method. Generally test methods measured the mineralization degree of the polymers, but in biodegradation process the biomass production could be an important product in the carbon balance.

The polymers mineralization is evaluated subtracting the respiration of the background (soil without test material) by the respiration of the soil amended with tested substances; the challenge is to evaluated the extra organic matter produced in the soil with the tested substances, resulting subtracting the organic matter of the background by the organic matter of soil with the tested substances. Organic matter in soil samples is generally measured by combustion at 550° C. The aim of the work was to evaluate the sensitivity and the applicability of the method to distinguish low differences of organic matter between soil samples. A natural and a synthetic soil samples were used. Different sources of organic matter (starch, cellulose, an aliphatic polyester and bacteria) were used to increase the original soil organic matter with different low percentages (from about 0.1 % to 1.5 % in natural soil and from 0.1 % to 0.5 % in the synthetic soil). The expected linear relationship between measure values and organic matter percentages added to the soil was obtained (with R^2 from 0.85 to 0.99) but accuracy and precision have to be improved. Results obtained in synthetic soil samples seem to be better than the results obtain in natural soil, maybe because the original organic matter is lower.

KEYWORDS biodegradation test, organic matter

Work in progress

5.1 Introduction

The increasing development of biodegradable plastics since the late 1980s has promoted initiatives to develop formal standards and laboratory test methods to assess the ultimate environmental behaviour of plastics (Chiellini et al., 2007). In the 1990s most of the work was focused on biodegradation under composting conditions because of the new trend in solid waste management policy, which aimed at reducing the use of landfilling to a minimum by the promotion of recycling. Consequently, criteria and standard test methods were needed in order to verify the compatibility of plastics with composting and this stimulated research and standardisation (Degli Innocenti, 2005). However, several products made with biodegradable polymers, for instance the plastics used in agriculture, are not fed to a composting plant, but directly applied to soil.

Generally, current standard test methods measure the biodegradation of a test material, under aerobic condition, on the basis of the amount of CO_2 released as a consequence of the microbial attack to carbon substrate (mineralization degree). However, CO_2 is one of the products of biodegradation due to the metabolism of heterotrophic soil microbial community, the others being living biomass and, through chemo-enzymatic reactions, humic substances. It is generally accepted that in the relatively short-term, 50 % of carbon content of most organic substances is converted to CO_2, the remaining part being assimilated as biomass or converted to humus (Chiellini et al., 2007).

We suppose, for simplicity, the test material made only by carbon, hydrogen and oxygen. This for most biopolymers is actually the case. Under aerobic or anaerobic condition, the complete biodegradation reactions are:

$$C_{\text{polymer}} + O_2 \rightarrow CO_2 + H_2O + C_{\text{residue}} + C_{\text{biomass}}$$

$$C_{\text{polymer}} \rightarrow CO_2 + CH_4 + C_{\text{residue}} + C_{\text{biomass}}$$

To make a complete balance of carbon in a biodegradation test is important to determine not only CO_2 production, but also the amount of polymer residues and of carbon incorporated by biomass. If the first and the second points are currently relatively simple to estimate, the determination of biomass is still an unsolved point.

The test methods used to determine soil biodegradation are respirometric test where the test material is mixed to soil and the total carbon dioxide evolution measured. The total CO_2 evolution is the sum of the CO_2 released by the mineralization of the test material and of the CO_2 evolved by the soil respiration. The "net" test material mineralization is obtained by running blank soil controls in parallel and subtracting the background respiration from the total respiration.

The method should also provide the amount of extra carbon which is incorporated in the soil biomass. A simple measurement of "net" organic carbon (as a difference between the total organic carbon in the reactor containing the test material and in the blank reactor) is not possible, because

most analytical methods cannot distinguish the biomass organic carbon from the residual polymer organic carbon.
The challenge is therefore to quantify the extra organic carbon due to biomass formation, eliminating the interference from the non-biomass residual polymer.
Most approaches to reach this objective have been based on the application of methods developed in soil science.
Soil Microbial Biomass (SMB) is defined as "mass of intact microbial cells in a given soil"; it is responsible for the degradation of organic matter, the stability of aggregates and the cycle of most nutrients in soils. So, often, soil microbial biomass is quantified in order to assess the maintenance of soil fertility, the potential ability to degrade added organic materials, and the effects of added materials on the natural microbial population (ISO 14240, 1997).
The literature proposed various methods for the measurement of soil microbial biomass. Between them, the substrate-induced respiration (SIR) is based on the activity of the SMB after the addition of a readily available substrate (Wardle and Parkinson, 1990; Lin and Brooks, 1999); the chloroform fumigation with incubation (CFI) (based on the comparison between respiration before and after the soil sample has been fumigated with chloroform) (ISO 14240, 1997) or extraction method (CFE) based on the different amount of organic carbon extractable from soil samples which were fumigated or not (Vance et al., 1987). Physiological analysis such as extractable phospholipids fatty acids (PLFA) (Bailey et al., 2002) or biochemical assays to ATP determination (Contin et al., 2001; Martens 2001) were also proposed: PLFA or ATP present in soil sample are, in fact, closely correlated with the amount of microorganism in soil and are proposed for soil microbial biomass determination. However a simple transfer of this approach to the biodegradation test methods is not possible.
The interest in soil biodegradation tests is to quantify the extra biomass formed as a result of the biodegradation of the test material (independently whether biomass is still intact or not) and any organic substances synthesised by microorganisms starting from the test material. So the interest is to find a simple, rapid and reliable method to measure the whole organic matter in soil samples.
Historically, soil organic matter concentrations are estimated from dry combustion, that is, from heating soil for 180 min at 550° C (thermogravimetry) (Schlichting et al., 1995). Organic carbon can be directly and reliably determined from volatile solids converting the organic matter content to organic carbon. For soils, a conversion factor of 1.724 (i.e., g organic matter / 1.724 = g organic carbon) is generally adopted, based on assumption that organic matter contains 58 % organic C (Nelson and Sommers, 1996; Siewert, 2004).

The aim of this work was to evaluate the sensitivity of the measurement of weight loss at 550° C to estimate the variations of organic matter in soil samples during biodegradation tests.

5.2 Materials and Methods

5.2.1 Soil and organic matter sources

A natural and a synthetic soil were used in order to evaluate the sensitivity of the thermogravimetric method to determine organic matter in soil samples. The first was an agricultural soil collected in Arborio (Italy). Its texture is made of about 55 % sand, 43 % silt and 2 % clay, and its volatile solids are generally 5.5 % to 6.5 %.

The synthetic soil was prepared as suggested by ISO / DIS 17556 (currently under revision), using sand (70 %), kaolin (10 %), soil (16 %) and compost (4 %). Its volatile solids are about 3 %.

Starch, cellulose, a synthetic aliphatic polyester and cultured bacterial biomass were used as organic matter sources, and added to the soils to provide samples with different organic matter content.

The polyester (obtained by condensation of butanediol and sebacic acid) is completely combusted at 550° C; its water content is negligible (0.3 %) so that all the amount added to the soil contributes to volatile solids.

Starch and cellulose are completed combusted too, and the percentage added was evaluated on final dry weight (soil plus molecule) taking into account their water content (about 12 % and 4.5 % respectively).

Lysogeny Broth (LB), a nutritionally rich medium, was used for culturing bacteria that were incubated at 37° C for 48 h. In order to remove LB (that contains inorganic components that doesn't combusted at 550° C) after bacterial growth, the medium was centrifuged for 30 minutes at 3000 rpm; the pellet was washed with deionized water by suspending and centrifuging. Then it was oven-dried at 105° C for at least 24 h and dried cells were added to the dry synthetic soil. In order to verify the total combustion of bacteria, they were put at 550° C but variable amounts of residual ashes (from 2 % to 10 %) remained in the crucibles, maybe because inorganic components of the growth medium were not completely and uniformly removed from bacteria.

5.2.2 Volatile solids determination

The organic matter in soil samples was estimated by volatile solids determinations.

Generally, weighed aliquots of soil samples (natural or synthetic) were placed in ceramic crucibles, enriched by a measured addition of organic matter (polyester, starch, cellulose or bacteria) and then oven-dried at 105° C in order to remove water. After 24 h, samples were cooled in a desiccator,

weighted and combusted at 550° C in a muffle furnace. After 8 h samples were cooled and weighted. The volatile solids were calculated using the following equation:

VS (%) = (oven dry soil weight – soil weight after combustion) / oven dry soil weight * 100.

Three replicates were made for each addition of organic matter.

5.2.3 Experimental set up

All the organic matter sources were weighed and added to the soil in order to increase the original organic matter content (background), with different organic matter percentages (theoretical extra organic matter).
The addition of the model polyester was tested both in the natural soil and in the synthetic soil.
For the experiment in natural soil, 75 g of moist soil (water content about 13 %) were weighed and used to determine the original organic matter by volatile solids measurements.
0.050 g, 0.100 g, 0.200 g, 0.400 g and 1.000 g synthetic polyester were weighed and added to 75 g of moist soil in order to obtain different percentages of organic matter (from about 0.08 % to 1.5 % on dry matter basis).
After 24 h at 105° C, the soil samples were combusted and their volatile solids were calculated.
The measured extra organic matter was calculated by subtracting the background from the organic matter evaluated in the different soil samples.
For tests in synthetic soil the same procedure was adopted and the same experimental procedure was used.
For the experiments in synthetic soil, 50 g of moist soil (water content from about 12 % to 15 %) were used and similar amount of polyester were tested. Cellulose, starch and bacteria were also used. 0.050 g, 0.100 g and 0.200 g organic matter were added to the synthetic soil to obtain low percentages of theoretical extra organic matter (from 0.1 % to 0.5 %).

5.3 Results and Discussion

The experimental data obtained in the test with polyester and natural soil are reported in Table 1. For each soil sample the volatile solids, the measured extra organic matter and the theoretical organic matter were calculated (Table 2).
Adding the polyester to the natural soil, the expected linear relationship between volatile solids and percent of the added molecule was confirmed by experimental data (R^2 = 0.87), as shown in Figure 1. Volatile solids (see Table 2) were measured with high precision (standard deviation was generally very low); differences among samples were statistically significant (except for the measure obtained for 0.05 % concentration) but accuracy was

not enough. The measured values, in fact, generally overestimated the expected values, in particular for the lowest organic matter concentrations (see Table 2: the ratio between measured and theoretical extra organic matter).

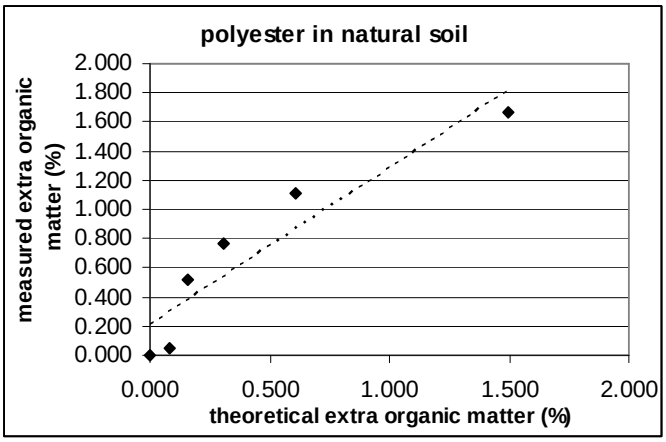

Figure 1: Relationship between the measured extra organic matter and the theoretical one in experiment with polyester in natural soil.

Maybe the organic matter content in the tested soil samples was too high to allow accurate estimates for so low differences. To reduce the background, tests were repeated using synthetic soil.
The experimental weights measured for the determination of the organic matter in the synthetic soil (six replicates) are reported in Table 3.

Table 1: Amount of polyester in natural soil samples and weights after 24h at 105° C and after combustion.

Sample name	Amount of polyester in soil samples (g)			Weight of the soil samples (g) after 24 h at 105° C			Weight of the soil samples (g) after 8 h at 550° C		
	Replicates			Replicates			Replicates		
	I	II	III	I	II	III	I	II	III
background	0.000	0.000	0.000	66.436	65.395	58.000	62.498	61.524	54.619
n. soil+0.050 p	0.056	0.051	0.048	65.484	65.190	65.144	61.727	61.299	61.166
n. soil+0.100 p	0.101	0.099	0.092	65.499	65.534	58.532	61.277	61.308	54.833
n. soil+0.200 p	0.203	0.199	0.200	65.563	65.438	65.507	61.177	61.063	61.185
n. soil+0.400 p	0.404	0.401	0.398	65.783	66.009	65.716	61.226	61.368	61.076
n. soil+1.000 p	0.998	0.997	0.996	66.636	66.608	66.651	61.597	61.602	61.586

Table 2: Measured volatile solids, measured and theoretical organic matter of the different soil samples, ratio between measured and theoretical extra organic matter.

Sample name	Measured V.S. (%) (mean values± s.d.)	Measured extra organic matter (%)	Theoretical extra organic matter (%)	Measured / theoretical extra organic matter
background	5.892±0.055	0.000	0.000	-
n. soil+0.050 p	5.937±0.187	0.045	0.079	0.57
n. soil+0.100 p	6.405±0.074	0.513	0.154	3.33
n. soil+0.200 p	6.658±0.052	0.766	0.306	2.50
n. soil+0.400 p	7.006±0.070	1.114	0.609	1.83
n. soil+1.000 p	7.559±0.042	1.667	1.496	1.11

Table 3: Dry weights before and after combustion of the synthetic soil used for experiment with polyester and starch.

Dry weight of the synthetic soil (g) after 24 h at 105° C						**Dry weight of the synthetic soil (g) after 24 h at 105° C**					
Replicates						**Replicates**					
I	II	III	IV	V	VI	I	II	III	IV	V	VI
43.722	45.390	45.433	43.288	46.460	44.319	42.348	44.052	44.067	41.860	44.995	42.907

Table 4: Amount of polyester in synthetic soil samples and weights after 24h at 105° C and after combustion.

Sample name	**Amount of polyester in synthetic soil samples (g)**			**Weight of the synthetic soil samples (g) after 24 h at 105° C**			**Weight of the synthetic soil samples (g) after 8 h at 550° C**		
	Replicates			**Replicates**			**Replicates**		
	I	II	III	I	II	III	I	II	III
s. soil+0.050 p	0.047	0.051	0.076	44.082	44.072	44.984	42.607	42.644	43.595
s. soil+0.100 p	0.107	0.098	0.106	43.999	43.945	44.278	42.609	42.425	42.748
s. soil+0.200 p	0.190	0.201	0.214	44.255	44.342	44.529	42.645	42.842	42.919
s. soil+0.400 p	0.395	0.403	0.398	43.509	45.676	44.188	41.804	43.784	42.340

Table 5: Measured volatile solids, measured and theoretical organic matter of the different soil samples, ratio between measured and theoretical extra organic matter.

Sample name	Measured V.S. (%) (mean values± s.d.)	Measured extra organic matter (%)	Theoretical extra organic matter (%)	Measured / theoretical extra organic matter
background	3.123±0.127	0.000	0.000	-
s. soil+0.050 p	3.225±0.130	0.102	0.108	0.94
s. soil+0.100 p	3.358±0.172	0.235	0.235	1.00
s. soil+0.200 p	3.545±0.141	0.423	0.454	0.93
s. soil+0.400 p	4.081±0.142	0.959	0.897	1.07

Table 4 reports the amount of polyester added to the synthetic soil, and the experimental weights before and after combustion. Table 5 reports the measured volatile solids, the measured and theoretical extra organic matter content and their ratio. The relationship between measured and theoretical extra organic matter content is showed in Figure 2.

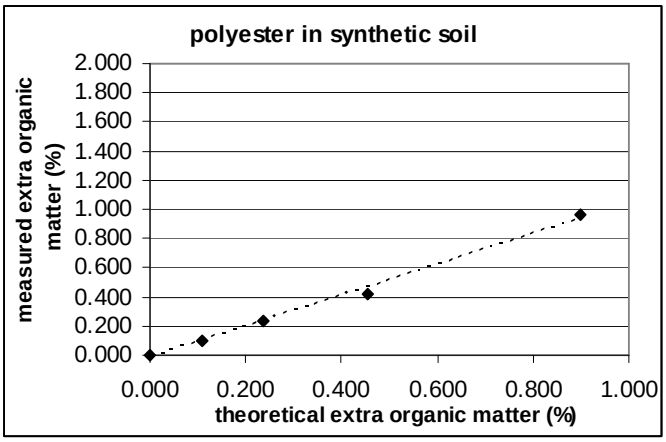

Figure 2: Relationship between the measured extra organic matter and the theoretical one in experiment with polyester in synthetic soil.

A good linear relationship was obtained (R^2 = 0.99). The accuracy of the measure improves (the ratio between the measured and the theoretical is about 1, see Table 5), but the results are less precise than those obtained in natural soil. The standard deviation of the single measures is too high, and the measured values were statistically different from the background only for polyester additions over 0.4 %.

To verify the accuracy obtained using synthetic soil, different sources of organic matter were used: starch, cellulose and bacteria. Experimental data of the amount of organic matter sources and of the dry weights before and after combustion are reported for starch in Table 6. The measured volatile solids, measured and theoretical extra organic matter and their ratio are presented in Table 7.

At the same way experimental data and results are reported for cellulose and bacteria in Tables 8-9-10 and 11-12 respectively.

Table 6: Amount of starch in synthetic soil samples and weights after 24h at 105° C and after combustion.

Sample name	Amount of starch in synthetic soil samples (g)			Weight of the synthetic soil samples (g) after 24 h at 105° C			Weight of the synthetic soil samples (g) after 8 h at 550° C		
	Replicates			Replicates			Replicates		
	I	II	III	I	II	III	I	II	III
s. soil+0.050 s	0.044	0.040	0.050	44.311	44.394	44.341	42.882	42.953	42.940
s. soil+0.100 s	0.090	0.094	0.089	44.147	44.534	44.311	42.548	43.096	42.903
s. soil+0.200 s	0.178	0.178	0.183	44.392	44.740	44.433	42.830	43.117	42.780

Table 7: Measured volatile solids, measured and theoretical organic matter of the different soil samples, ratio between measured and theoretical extra organic matter in tests with starch in synthetic soil.

Sample name	Measured V.S. (%) (mean values± s.d.)	Measured extra organic matter (%)	Theoretical extra organic matter (%)	Measured / theoretical extra organic matter
background	3.123±0.127	0.000	0.000	-
s. soil+0.050 s	3.210±0.045	0.088	0.101	0.87
s. soil+0.100 s	3.343±0.243	0.220	0.205	1.07
s. soil+0.200 s	3.622±0.101	0.500	0.403	1.24

Table8: Experimental data for background determination of synthetic soil used in tests with cellulose.

Dry weight of the synthetic soil (g) after 24 h at 105° C						**Dry weight of the synthetic soil (g) after 8 h at 550° C**					
Replicates						**Replicates**					
I	II	III	IV	V	VI	I	II	III	IV	V	VI
48.108	48.708	44.607	29.622	28.306	29.514	46.713	47.310	43.359	28.735	27.478	28.645

Table 9: Amount of cellulose in synthetic soil samples and weights after 24h at 105° C and after combustion.

Sample name	**Amount of starch in synthetic soil samples (g)**			**Weight of the synthetic soil samples (g) after 24 h at 105° C**			**Weight of the synthetic soil samples (g) after 8 h at 550° C**		
	Replicates			**Replicates**			**Replicates**		
	I	II	III	I	II	III	I	II	III
s. soil+0.050 c	0.049	0.053	0.053	42.996	43.008	42.869	41.640	41.667	41.525
s. soil+0.100 c	0.098	0.097	0.104	43.833	43.030	42.912	41.495	41.714	41.482
s. soil+0.200 c	0.195	0.187	0.194	43.052	43.050	42.974	41.642	41.623	41.559

Table 10: Measured volatile solids, measured and theoretical organic matter of the different soil samples, ratio between measured and theoretical extra organic matter in tests with cellulose in synthetic soil.

Sample name	Measured V.S. (%) (mean values± s.d.)	Measured extra organic matter (%)	Theoretical extra organic matter (%)	Measured / theoretical extra organic matter
background	2.905±0.067	0.000	0.000	-
s. soil+0.050 c	3.136±0.018	0.230	0.120	1.92
s. soil+0.100 c	3.171±0.143	0.266	0.233	1.14
s. soil+0.200 c	3.294±0.020	0.389	0.446	0.84

Table 11: Amount of bacteria in synthetic soil samples and weights after 24h at 105° C and after combustion.

Sample name	Amount of starch in synthetic soil samples (g)			Weight of the synthetic soil samples (g) after 24 h at 105° C			Weight of the synthetic soil samples (g) after 8 h at 550° C		
	Replicates			Replicates			Replicates		
	I	II	III	I	II	III	I	II	III
background	0.000	0.000	0.000	29.622	28.306	29.514	28.735	27.478	28.645
s. soil+0.050 b	0.055	0.052	0.044	42.476	43.144	37.133	41.095	41.854	35.133
s. soil+0.100 b	0.100	0.101	0.090	42.662	42.544	37.420	41.329	41.188	36.288
s. soil+0.200 b	0.201	0.208	0.197	42.899	43.002	42.545	41.374	41.477	41.091

Table 12: Measured volatile solids, measured and theoretical organic matter of the different soil samples, ratio between measured and theoretical extra organic matter in tests with bacteria in synthetic soil.

Sample name	Measured V.S. (%) (mean values± s.d.)	Measured extra organic matter (%)	Theoretical extra organic matter (%)	Measured / theoretical extra organic matter
background	2.955±0.036	0.000	0.000	-
s. soil+0.050 b	3.204±0.054	0.249	0.123	2.02
s. soil+0.100 b	3.112±0.082	0.158	0.237	0.67
s. soil+0.200 b	3.506±0.077	0.552	0.472	1.17

The relationship between the measured extra organic matter and the theoretical extra organic matter are shown in Figure 3, 4 and 5 for test with starch, cellulose and bacteria respectively.
For starch (Figure 3), volatile solids closely correlated (R^2=0.99) with the amounts added to the soil and only in one case (0.2 % addition) a large standard deviation was obtained (the highest generally obtained) (see Table 7). As results obtained with polyester, only when starch addition was 0.4 % the measured values were statistically different from the initial one.

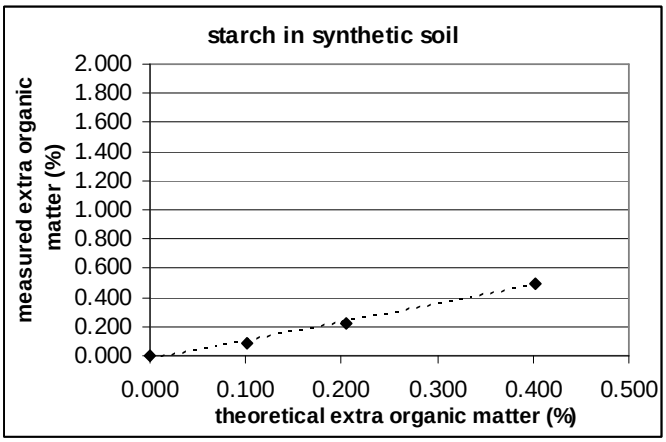

Figure 3: Relationship between the measured extra organic matter and the theoretical one in experiment with starch in synthetic soil.

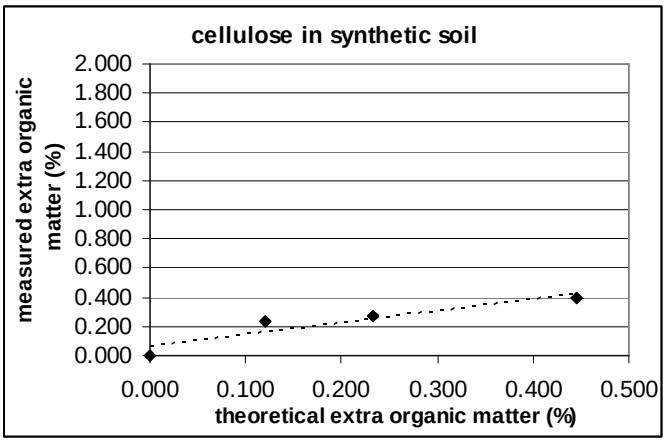

Figure 4: Relationship between the measured extra organic matter and the theoretical one in experiment with cellulose in synthetic soil.

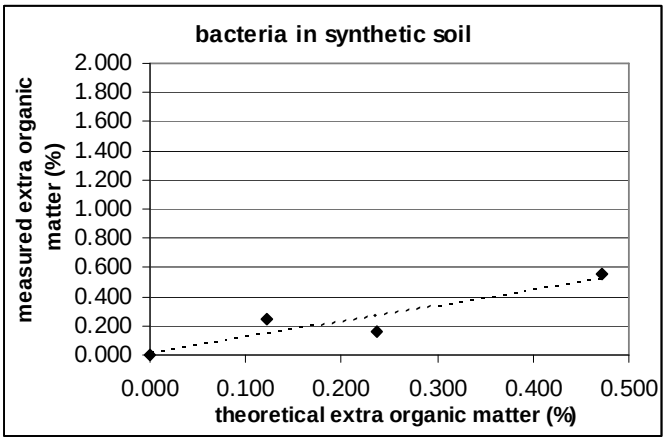

Figure 5: Relationship between the measured extra organic matter and the theoretical one in experiment with bacteria in synthetic soil.

The results obtained with cellulose and bacteria (Figure 4 and 5) show lower correlation (R^2= 0.87 and 0.85 respectively) and variable standard deviations (Table 10 and 12). This could be explained by a lower homogeneity of the synthetic soil sample. Moreover, for bacteria, another source of error can be the incomplete separation of the inorganic components of the growth medium from bacteria.

5.4 Conclusions

The aim of the work was to investigate the sensitivity of combustion at 550° C to measure low differences of organic matter content in soil samples. The preliminary results show a good relationship between measured extra organic matter determined by volatile solids and the amount of the added organic matter. Standard deviations are generally low (< 5 %), but too high to measure small differences in the organic matter content of the tested samples.

So, at present, this method can not be considered completely reliable in the studied range.

Anyway, the confirmed linear relationship suggests that the method can be useful but the accuracy and precision of the measurements have to be improved. The results obtained in synthetic soil samples seem to be better than those obtained in natural soil, maybe because the starting content of organic matter is lower. To reduce errors, particular attention must be paid to the preparation of homogeneous samples and more replicates could be made.

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6 Biodegradation in soil of a model polyester: process description and carbon balance

ABSTRACT

The increasing use of new generation plastics has been accompanied by development of standard methods for studying their biodegradability. Generally, test methods are based on the measure of the CO_2 production, i.e. the mineralization degree of the tested materials. Nevertheless, in order to obtain a complete description and quantification of the biodegradation process, the determination of biomass production and of the amount of material remained in the environment is very important. In this work the biodegradation in soil of a model polyester was studied by quantification of carbon balance at different time of the mineralization test. CO_2 production was measured by respirometric test, biomass developed during incubation was determined by soil combustion, and polyester still present in soil was retrieved by solvent extraction. Nuclear Magnetic Resonance was used in order to identify the structure of possible by-products. The results confirm the precision of the mineralization tests for the CO_2 determination. Carbon balance was obtained after 78 and 140 days of incubation (when polyester mineralization was about 21 % and 37 %) and at the end (245 days, with 63 % of mineralization, 100 % if referred to cellulose used as control). The carbon balance results possible during the test, even if it is overestimated (120 % and 110 % after 78 and 140 days of incubation respectively), so that the precision of the method have to be improved, in particular for biomass determination by soil combustion. NMR analysis of the soil extracts suggest that no polyester by-products remain in soil: the polyester concentration decreases but its structure remains the same during the process. At the end of the test, the biomass in soil amended with polyester results not different from the blank. Moreover no polyester can be extracted from the soil and NMR acquisitions and GPC analysis of the extracts suggest that in soil there is no polyester. The final carbon balance is represented only by the mineralization rate, but everything suggests that polyester was completely biodegraded.

KEYWORDS polyester biodegradation, carbon balance, NMR acquisition

Work in progress

6.1 Introduction

In the last twenty years, biodegradable polymers have received attention because of their possible use in a large number of applications, taking the place of traditional plastics.

From the beginning of 90s several new materials have been developed. The industrial development of new biodegradable plastics has been accompanied by parallel development of suitable standard and criteria defining compostability (ASTM D6400, ASTM D6868, EN 13432) or biodegradability in soil (ASTM D5988, 1996; ISO 17556, 2003). Generally test methods are based on the evaluation of the net carbon dioxide production, i.e. the CO_2 evolved from the substrate added with the test material minus CO_2 evolved from the substrate (blank).

The biodegradation reaction, under aerobic conditions, is:

$$C_{\text{polymer}} + O_2 \rightarrow CO_2 + H_2O + C_{\text{residue}} + C_{\text{biomass}}$$

In order to completely describe biodegradation of a material is very important to quantify and identify possible by-products (Bellia et al., 1999) and determine the carbon linked to the new generated biomass. In biodegradation tests based on complex organic matrix, such as compost or soil (that well simulate real conditions) the presence of carbon in the medium makes carbon balance difficult to perform (Longieras et al., 2004).

Carbon present in the tested substance is generally known and CO_2 production can be accurately measured by respirometric tests; C linked to the residues can be estimated by extractions with solvent. Therefore biomass production could be determined subtracting the carbon mineralized into CO_2 and remained linked to the residues from the initial carbon of the tested substance. Nevertheless, this is an indirect estimation of the biomass production. A measure of the biomass is important for the carbon balance, in particular where extraction of material from the medium results difficult or requires the use of toxic or dangerous organic solvent.

The aim of this work was to describe biodegradation in soil of a model polyester by quantification of the carbon balance at different times. CO_2 production was measured by respirometric test, biomass developed during incubation was determined by soil combustion, and polyester still present in soil was retrieved by solvent extraction. Nuclear Magnetic Resonance was used in order to identify the structure of possible by-products.

6.2 Materials and Methods

6.2.1 The tested substances

The experimental work was carried out using a model polyester expressly synthesized by Novamont S.p.A.. It is a polybutilen-sebacate ($(C_{14}H_{24}O_4)_n$, C content 65.4 %) with a linear aliphatic chain made by butanediol and sebacic acid; it was obtained by standard techniques of monomers

condensation. The relative molecular weights of the polyester were measured by gel permeation chromatography (GPC) and its structure was resolved by ^{1}H-NMR and ^{31}P-NMR before incubation in soil.
Cellulose ($(C_6H_{10}O_5)_n$, C content 44.4 %) was supplied by Merck and used as reference material in biodegradation test.
The two polymers were incubated in soil as powder.

6.2.2 Biodegradation test

An agricultural soil sample, collected in Arborio (Italy), was used for the respirometric test that was carried out according to ASTM 5988-96. Soil was freshly collected, sieved (< 2 mm) and used within a few days. Moisture (measured as weight loss of soil samples after 24 h at 105° C) and pH (measured in deionized water with a soil-to-solution ratio of 1:2.5 (w:v)) were adjusted before the test starts. $CaCO_3$ (2 % in weight) was added to the soil to increase its natural pH from 6.19 to 7.14. The buffered soil was prepared, left at room temperature and periodically mixed for 3 days to let release of CO_2 produced during neutralization.
500 g of moist soil (water content 16.2 %) were incubated at room temperature (21±2° C) in the dark, in hermetically closed jars (3 l), with test substances (2 g, accurately weighted). Blank jars, without test substances, were also prepared. Each jar contained a beaker filled with 0.5 M KOH (40 ml), which was regularly titrated with 0.25 M HCl in order to measure the CO_2 production within the jar. The measurement was carried out every 3 days in the first two weeks and weekly thereafter. When beakers were taken away from jars to titration, the jars remained open from 15 to 30 minutes, so that the air was refreshed before replacing fresh potassium hydroxide. Soil moisture was not adjusted during the test. In hermetically closed jars, water evaporating from the soil saturates the headspace in a very short time and, consequently, any further water loss is negligible; therefore the soil moisture can be considered as constant during the test period.
Mineralization percentage is obtained according to the following equation:

$$(\text{mg } CO_{2\ \text{molecule}} - \text{mg } CO_{2\ \text{blank}}) / \text{sample weight} * \%C * 3.6667 * 0.01$$

where:
mg $CO_{2\ \text{molecule}}$ is the amount of CO_2 measured in the test jars;
mg $CO_{2\ \text{blank is}}$ the amount of CO_2 produced in the blank jars;
3.6667 is the ratio between CO_2 molecular weight and C atomic weight.

Table 1: Experimental set-up.

Reactor (n°)	Amount of soil (dry weight, g)	Test material	Amount of test material (dry weight, mg)	Carbon content of the test material (%)	Theoretical CO_2 that can evolved from test material (mg)	Theoretical CO_2 (mg g^{-1} $_{dry\ soil}$)
R1	419	blank	-	-	-	
R2	419	blank	-	-	-	
R3	419	blank	-	-	-	
R4	419	blank	-	-	-	
R5	419	cellulose	1980.0	44.4	3223.4	7.69
R6	419	cellulose	1981.5	44.4	3225.9	7.70
R7	419	cellulose	1981.3	44.4	3225.5	7.70
R8	419	cellulose	1980.8	44.4	3224.7	7.70
R9	419	polyester	2000.6	65.4	4799.6	11.45
R10	419	polyester	2001.2	65.4	4801.1	11.46
R11	419	polyester	2005.2	65.4	4810.7	11.48
R12	419	polyester	2005.1	65.4	4810.4	11.48

Overall, twelve jars were prepared: four for blanks and four for each tested polymer (see Table 1 for the experiment set-up). After 78 and 140 days of incubation, three jars (one for cellulose and polyester and one blank) were used in order to analyse the soil. At the end of the test (245 days), soil of the remained jars was analysed.

6.2.3 Organic matter determination

Organic matter concentration in soil samples was estimated by dry combustion, heating soil at 550° C. About 50 g of soil samples were placed in ceramic crucibles and oven-dried at 105° C in order to remove water. After 24 h, samples were cooled in a desiccator, weighed and combusted at 550° C in a muffle furnace. After 8 h, samples were cooled and weighed. The volatile solids were calculated using the following equation:

VS = (oven dry soil weight – soil weight after combustion) / oven dry soil weight

Each soil sample was measured in triplicates and results were expressed as mg $g^{-1}_{\text{dry soil}}$.
Organic carbon in soil was indirectly estimated from volatile solids using a conversion factor of 1.724 (i.e., g organic matter / 1.724 = g organic carbon), based on assumption that organic matter contains 58 % organic C (Nelson and Sommers, 1996).

6.2.4 Soil extraction

In order to characterise and quantify residues of the tested polyester, 150 g of moist soil samples (soil amended with polyester and blanks) were oven-dried at 105° C to remove water. The dried soils were extracted in soxhlet with pure chloroform for 8 h. After chloroform was completely removed, the extracts were weighted and analysed by GPC and NMR.

6.2.5 GPC measurement

Gel permeation chromatography (GPC) is the most widely used technique for analyzing polymer samples in order to determine their molecular weights and weight distributions.
10 mg of sample were completely dissolved in 10 ml of chloroform. Suspension was filtered through a polytetrafluoroethylene (PTFE) filter (Ø = 0.2 µm) and 500 µl of the filtrate were used for the GPC measurement (model Agilent 1100). The instrument was equipped with a refractive index detector (RI) and three PLgel 5µm columns in series (10E4, 10E3 and 550 Å). Calibration curve was obtained using polystyrene (from 696500 to 580 Dalton) as standard. The measurement was carried out at 40° C, with pure chloroform as eluant, at a flow rate of 1 ml / min.

6.2.6 NMR acquisition

Nuclear magnetic resonance is used to study chemical structures. It is a simple one-dimensional technique and it finds applications in several areas of science. In this work it was applied in order to identify the structure of the model polyester and to identify the chemical nature of potential residues extracted from soil. Samples were analysed by traditional proton and innovative phosphorus-31 based nuclear magnetic resonance spectroscopy (^{1}H-NMR and ^{31}P-NMR). ^{31}P-NMR is a sensitive technique used for the determination of different phenolic and other lignin functionalities by Argyropoulos and co-authors (King et al, 2009). It has also been used for studying biological system such as lipid metabolites (DeSilva et al., 2009; Oostendorp et al., 2006) with hydroxyl and carboxyl groups or to quantify fatty acids and glycerides in olive oil for quality control purposes (Spyros and Dais, 2000; Dais and Spyros, 2007).

The ^{31}P containing reagent, 2-chloro-4,4,5,5-tetramethyldioxaphospholane (CTMDP), was used as phosphitylation reagent to derivatize the sample (Figure 1). The derivatized functionalities were then detected with enhanced resolution using ^{31}P-NMR. The different investigations previously mentioned report CTMDP to be an excellent derivatizing reagent for molecules with hydroxyl, carboxyl or aldehyde functional groups. This derivatization approach is considered relatively fast and simple; the method requires only modest amount of sample preparation and can be used to identify lipophilic functionalities without the use of chromatography (DeSilva et al., 2009).

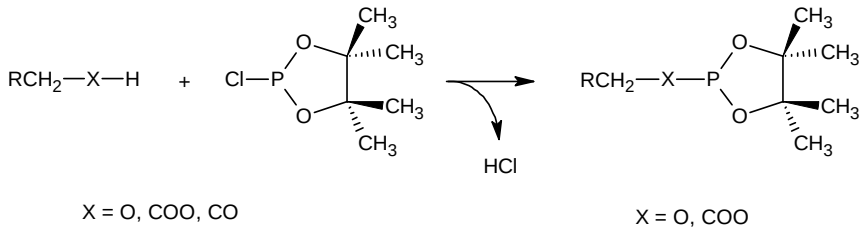

Figure 1: Scheme of sample derivatization using CTMDP (DeSilva, 2009).

^{1}H-NMR

The samples were prepared by dissolving 20 mg of sample in 1 ml of deuterated chloroform ($CDCl_3$) on 5 mm NMR tubes. ^{1}H-NMR spectra were collected using a Bruker-300 spectrometer (operating at 300 MHz). The total number of scans for all experiments was 64 with an acquisition time of 1.60 s.

^{31}P-NMR

20 mg of sample were dissolved in 800 µl of a mixture of pyridine and $CDCl_3$ in 1:1 volume ratio containing chromium acetylacetonate, $Cr(acac)_3$. Respectively 100 µl of the Internal Standard (IS N-hydroxynaphtalimmide)

and 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane (CTMDP) were added (Granata and Argyropoulos, 1995). The obtained solutions was placed in a 5 mm NMR tube and the ^{31}P-NMR spectra were obtained on a Bruker AMX 500 spectrometer operating at 202.2 MHz for the phosphorus-31 nucleus. The probe temperature was 25° C.

6.3 Results and Discussion

6.3.1 Model polyester characterization

Figure 2 shows the powder of the model polyester used in this study. The polyester was analysed by GPC and NMR (^{1}H-NMR and ^{31}P-NMR) before biodegradation test starts.

Figure 2: Polyester as it appears before incubation in soil.

GPC chromatogram (Figure 3) reveals a Gaussian distribution of the molecular weights. The number average molecular weight (M_n) is 54741 g mol^{-1} and the weight average molar mass (M_w) is 141260 g mol^{-1}; the polydispersity index is 2.58.

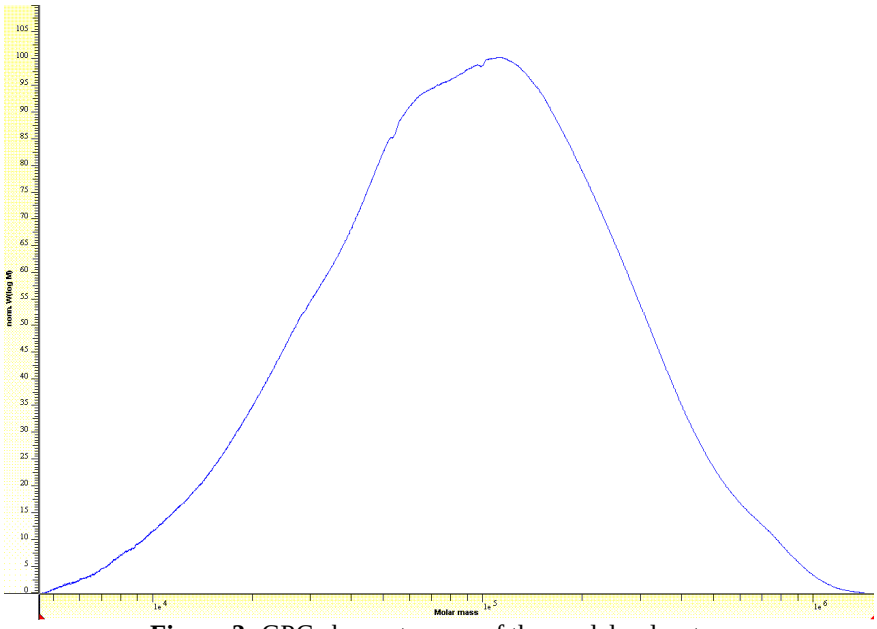

Figure 3: GPC chromatogram of the model polyester.

Figure 4 shows the model polyester chain with letters that indicate the methylene groups. It is a polybutilen-sebacate and its ^{1}H-NMR spectrum (Figure 5) shows five well resolved peaks in chloroform solution. Table 2 lists the observed peaks, their chemical shifts, their integrations and their resonance assignments based on the literature reports (Oostendorp et al., 2006) or on database available on line (SDBS, Spectral Database for Organic Compound, web site).

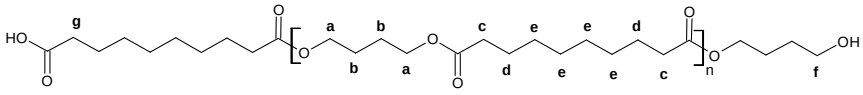

Figure 4: The polybutilen-sabacate chain.

Table 2: Chemical shift, integration and resonance assignments for signals identified.

Peak ID	Chemical shift (δ) (ppm)	Integration	Assignment
a	4.02	4	-C**H**$_2$-O-
b	1.63	4	-C**H**$_2$-CH$_2$-O-
c	2.22	4	-C**H**$_2$-C(O)O-
d	1.54	4	-C**H**$_2$-CH$_2$-
e	1.23	8	-C**H**$_2$-

Terminal methylene groups of butanediol (f in Figure 4 and 5) can be recognized at about 3.64 ppm. Terminal methylene groups of sebacic acid (g in Figure 4) should be at 2.30 ppm, but they are covered by peaks c. The amount of terminal methylene groups results negligible if compared to the internals, confirming that the model polyester chain has an high number of monomeric units.

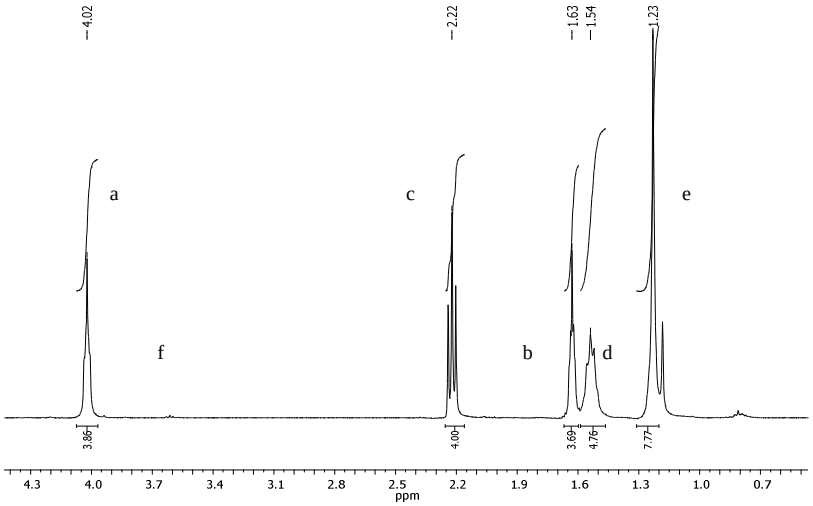

Figure 5: ^{1}H-NMR spectrum of model polyester.

Hydroxyl and carboxylic functionalities were identified by ^{31}P-NMR acquisition after derivatization using CTMDP. Two derivatized species were resolved (Figure 6): butanediol terminal hydroxyl groups appear at 147.15 ppm, sebacic acid carboxylic groups appear at 134.77 ppm. The area of peak related to hydroxyl group is twice the area of carboxylic, and this suggests that butanediol terminals are double than sebacic acid ones. The ^{31}P-NMR permits also an absolute quantification of the groups present in the polymer: 0.05 mmol/g for –OH and 0.03 mmol/g for –COOH.

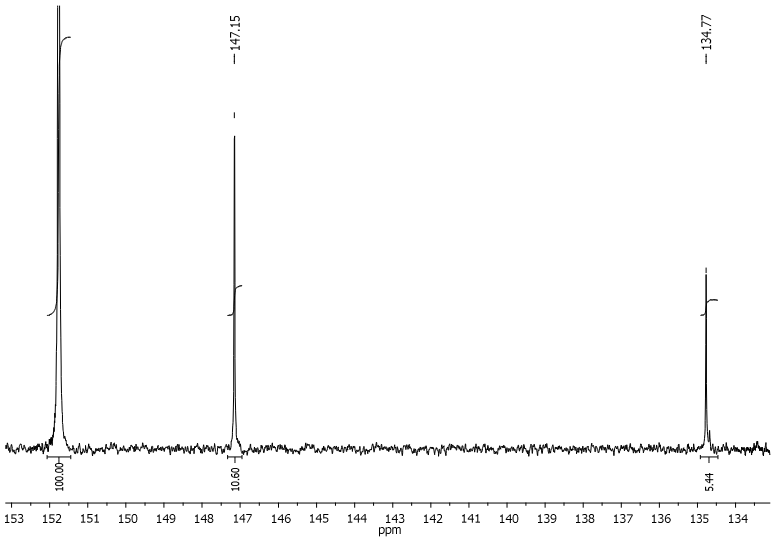

Figure 6: ^{31}P-NMR spectrum of model polyester.

6.3.2 Mineralization, organic matter determination and analytical analysis

At the beginning of the test volatile solids of the soil were measured and results 63.83±0.69 mg $g^{-1}_{dry\ soil}$. Cellulose and polyester were accurately weighed, mixed to the soil and incubated.

Figure 7 shows mineralization curves obtained for the two polymers. Each point represents values obtained from the experimental data of the replicates. Black symbols refer to cellulose, white symbols to polyester. The reproducibility of results is very good: measures in replicate jars are, in practice, the same.

Polyester experimental curves show a lag phase of about 10 days, then mineralization starts and continues steadily until the plateau phase, reached at the end of the test. The final value is 63 %.

For cellulose, lag phase is of 3-4 days; the biodegradation phase, in which mineralization is fast, lasts 18-20 days so that in one month about the half of the final mineralization (59 %) is reached.

If compared to the cellulose (the reference substance), the relative polyester mineralization is 100 %.

At different time intervals, the soil of one reactor out of three replicates (one blank, one in which polyester was incubated and one in which cellulose was incubated) was used for the determination of organic matter and for the extraction of possible biodegradation by-products.

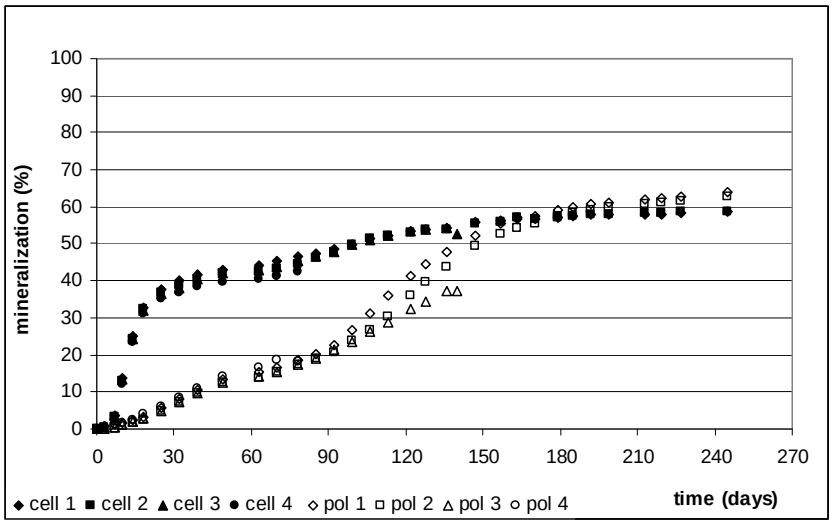

Figure 7: Mineralization curves of cellulose and polyester; black symbols refers to cellulose, white symbols refers to polyester.

After 78 days of incubation, the soil of three reactor (R4, R8 and R12) was used for the analysis. 1245.8 mg, 2652.1 mg and 2283.1 mg of CO_2 were measured in blank and in the jars with cellulose and polyester respectively (mineralization results 42.5 % for cellulose and 21.56 % for polyester).
Volatile solids were measured: (59.77±3.10) mg $g^{-1}_{dry\ soil}$, (60.63±1.95) mg $g^{-1}_{dry\ soil}$, (64.97±1.21) mg $g^{-1}_{dry\ soil}$ were obtained for blank, cellulose and polyester respectively.
Blank and polyester were extracted in soxhlet with chloroform. The extracts (0.3983 mg $g^{-1}_{dry\ soil}$ and 1.6633 mg $g^{-1}_{dry\ soil}$ for blank and for the polyester) were weighted and used for ^{1}H-NMR and ^{31}P-NMR analysis. The spectra obtained by the analysis of the extract of the soil amended with tested polyester were qualitatively the same obtained for the polyester characterization, so blank was not analysed. In ^{1}H-NMR spectrum (Figure 8) a split of the signal can be observed at 4.16 and 2.33 ppm; ^{31}P-MNR spectrum (Figure 9) show that, after 78 days of incubation, the ratio between hydroxyl and carboxylic functionalities results about 1:1, while in pure polyester the ratio was 2:1. These results suggest an increase of short chains, but the original structure of the model polyester was not significantly changed. In fact also the absolute quantification of the groups indicated no modification in the polymer: 0.05 mmol/g for –OH groups and 0.04 mmol/g for –COOH. The polyester extract was analysed also by GPC. The chromatogram is shown in Figure 10 and confirms that, after 78 days in soil, only limited loss of molecular masses can be observed.

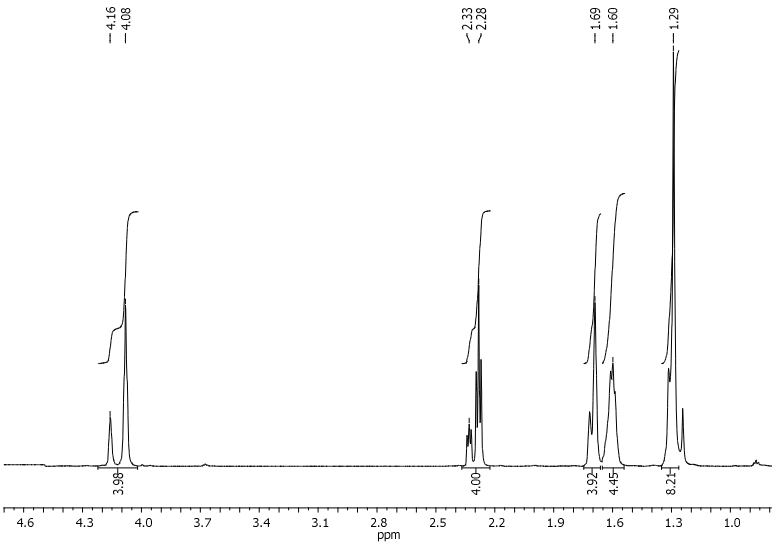

Figure 8: ^{1}H-NMR spectrum of extract of soil in which polymer was incubated from 78 days.

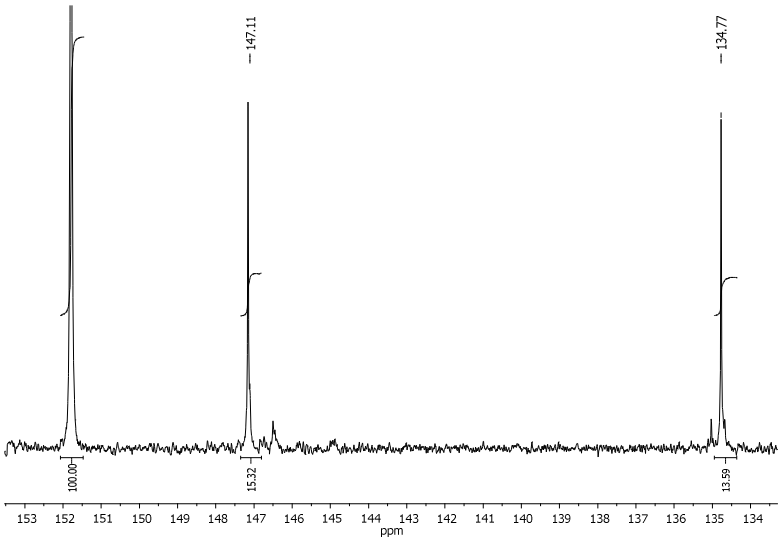

Figure 9: ^{31}P-NMR spectrum of extract of soil in which polymer was incubated from 78 days.

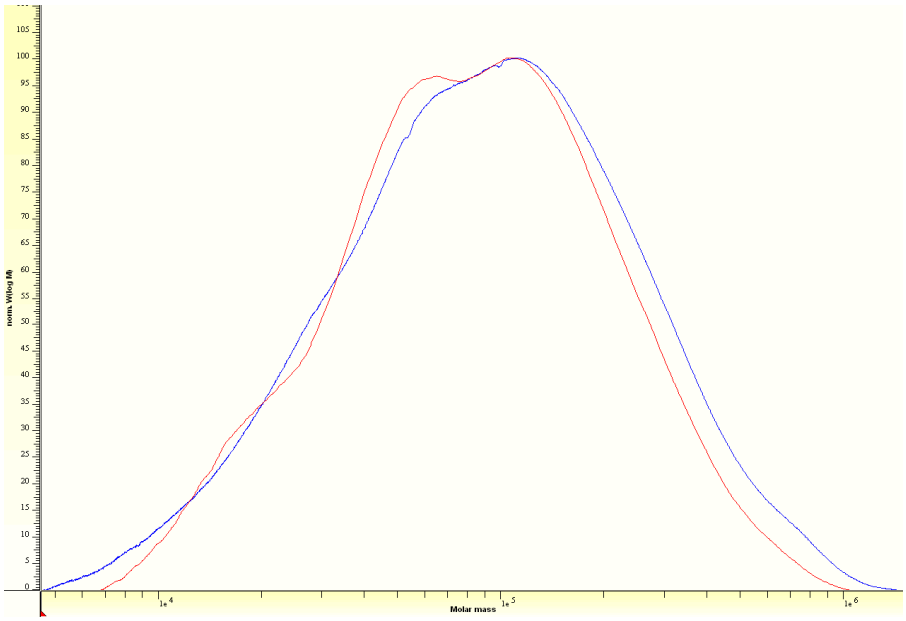

Figure 10: Molecular masses distribution (red line) after 78 days in soil and the original (blue line).

After 140 days of incubation the same analysis were made with other three soil samples (R3, R7, R10). CO_2 evolved from each jars resulted 1787.5 mg, 3479.3 mg, 3580.5 mg for blank, cellulose and polyester respectively and mineralization reaches 54.2 % and 37.1 % for cellulose and for polyester respectively.
Volatile solids result (61.58±0.37) mg $g^{-1}_{dry\ soil}$ for blank, (60.58±0.93) mg $g^{-1}_{dry\ soil}$ for cellulose and (65.42±0.14) mg $g^{-1}_{dry\ soil}$ for polyester.
0.4694 mg $g^{-1}_{dry\ soil}$ and 1.1797 mg $g^{-1}_{dry\ soil}$ were obtained by soxhlet extractions from blank and from polyester respectively. NMR acquisitions (data not showed) were not significantly different from that obtained after 78 days of incubation.

At the end of the biodegradation process (245 days) three of the six remaining jars were immediately analysed (R1, R5, R9).
Final mineralization values of the cellulose and polyester were about 60 %. The final amount of CO_2 measured in the jars was 2316.1 mg for blank, 4180.4 mg for cellulose and 5355.9 mg for polyester.
Volatile solids result (61.94±1.23) mg $g^{-1}_{dry\ soil}$, (59.60±1.38) mg $g^{-1}_{dry\ soil}$, (60.69±1.21) mg $g^{-1}_{dry\ soil}$ for blank, cellulose and polyester respectively.
Blank and polyester were extracted with chloroform and polyester extract weight results lower than blank (0.329 mg $g^{-1}_{dry\ soil}$ for blank and 0.1553 mg $g^{-1}_{dry\ soil}$ for polyester).

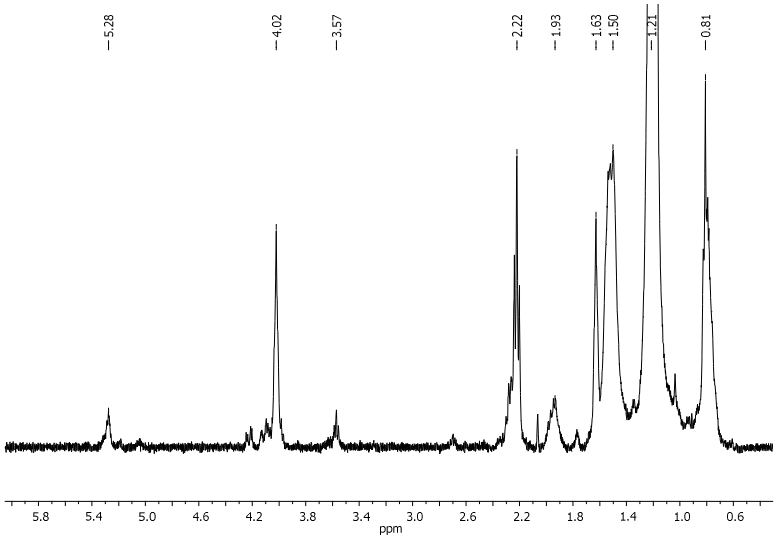

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Figure 11: ^{1}H-NMR spectrum after 245 days of polyester incubation in soil.

After 245 days of incubation in soil, the ^{1}H-NMR spectrum of the extracts of soil amended with polyester (Figure 11) is evidently different from the original one. It is more complex, maybe because background species belonging to the soil overlap same polyester peaks. Peaks refer to the polyester monomeric compounds (chemical shifts 4.02, 1.63, 2.22, 1.50, 1.21 ppm) can be recognized, but others signals (in particular at 0.81 ppm, the typical chemical shift of methyl groups of aliphatic chains) appear on the spectrum, generated maybe by many other molecules, reasonably phospholypids, fatty acid or triglycerols present in soil. Similar peaks can be observed in ^{1}H-NMR spectrum obtained for blank sample (Figure 12).

In the polyester ^{1}H-NMR spectrum, a specific peak at 3.57 ppm can be recognized. Due the fact that this group is absent in the blank soil sample, it can be related only to the polyester (terminal butanediol methylene groups – f in Figure 4 and 5). The ratio between the peak area of the methylene groups of butanediol adjacent to the ester group (a in Figure 4 and 5) and the area of terminal methylene groups (f in Figure 4 and 5) decreases. This suggests that the number average molecular weight (M_n) of model polyester is notably decreased and that only short olygomers remain in soil after 245 days of incubation.

In ^{31}P-NMR spectrum (Figure 13), peaks refer to hydroxyl and carboxylic groups can be recognized. The peak at 134.8 ppm could be related to the acid generated by the hydrolysis of polyester and the acids present in the soil (Figure 12, peak at 2.39 ppm). Moreover, other peaks around 149-145 ppm are detected, highlighting the presence of mono and diacylglycerols from

soil. Also the absolute quantification of the group (0.11 mmol/g for OH and 0.14 mmol/g for -COOH) confirms the polymer degradation.

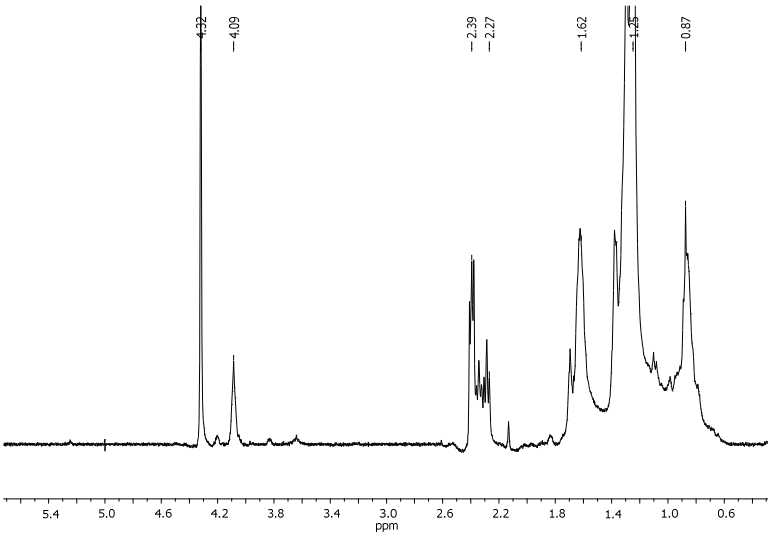

Figure 12: ^{1}H-NMR spectrum of blank extract.

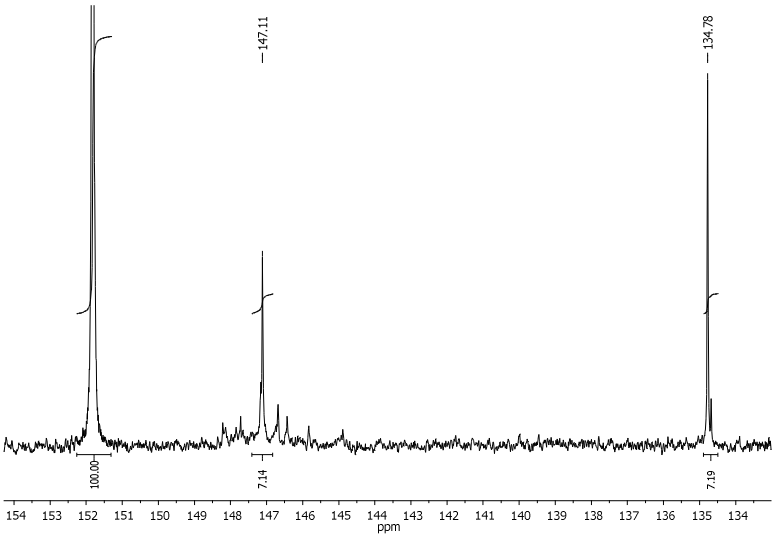

Figure 13: ^{31}P-NMR spectrum after 245 days of polyester incubation in soil

Blank and polyester soil extracts were also analysed by GPC. No differences can be found between the two chromatograms (data not shown). In particular, in the polyester chromatogram, only low intensity and not well

defined signals above 1000 Da (lower bound of columns resolution) can be observed. This confirms the degradation of the polybutilen-sebacate.
The biodegradation of polybutilen-sebacate in soil can also be observed by the aspects of soil extracts obtained in the different time of the test (Figure 14). Extracts of soil added with polymer are in the first line (a) of the picture, in line b there are extracts of blanks. At the beginning and after 78 or 140 days of incubation, polyester can be recovered from soil as a plastic film. At the end of the test the aspect of the extracts of blank and of polyester are very similar and this suggests that polyester is completely biodegraded, or, if some short residues are still present, they are closely mixed to the soil.

a

b

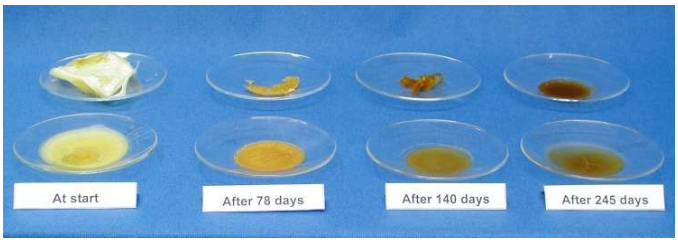

Figure 14: Soil extracts at different time of biodegradation in soil. Down extracts from blanks and up extracts from sample in which polyester was incubated.

6.3.3 Carbon balance

The complete biodegradation reaction of a simple polymer, made only by carbon, hydrogen and oxygen, under aerobic condition, can be described by the following reaction:

$$C_{polymer} + O_2 \rightarrow CO_2 + H_2O + C_{biomass} + C_{residue}$$

At any time of the test, polymer carbon ($C_{polymer}$) is converted by microorganisms into carbon dioxide, water and new biomass ($C_{biomass}$) and part can remain linked to polyester residues ($C_{residue}$).
The three components of carbon balance were obtained as following summarized:

- **$C\text{-}CO_2$:** carbon dioxide production is measured by standard respirometric test and then converted in carbon;
- **$C_{residue}$**: carbon linked to polyester residues can be obtained by extraction of soil samples (soil with polyester and blank) with polymer solvent. The net weight of the extract (weight of extract of soil with polyester – weight of the extract of the blank) is adjusted based on extraction efficiency (80 %) and then converted in $C_{residue}$ based on the carbon content of the tested polyester (65.4 %);
- **$C_{biomass}$:** measure of carbon linked to biomass is a delicate point. Because volatile solids of soil samples can be considered an estimation of organic matter and biomass present in soil, the net volatile solids (volatile solids of soil with tested substance minus volatile solids of blank) can be considered

linked to the biomass or organic matter deriving from polymer biodegradation. This term contains also the polyester residue in soil. So, detracting the amount of polymer still present in soil from the net volatile solids, an estimation of organic carbon linked to biomass can be obtained (using 58 % as correction factor).
The different carbon fractions were compared to the carbon of the model polyester added to the soil (3.12 mg C $g^{-1}_{\text{dry soil}}$) at the beginning of the test and expressed as percentage. Table 3 summarized the data referring to the CO_2 production, the measured volatile solids and the weights of the extracts elaborated as previous described in order to obtain the carbon balance of the model polyester. Table 4 shows the carbon balance obtained at different time of the test.

Table 3: CO_2 production, volatile solids and weight of the soil extracts at different time of the test.

Sample	**Measures**	**Time from incubation**		
		78 days	**140 days**	**245 days**
Blank	CO_2 (mg $g^{-1}_{\text{dry soil}}$)	2.97	4.26	5.53
	VS (mg $g^{-1}_{\text{dry soil}}$)	59.77	61.58	61.95
	Extracts (mg $g^{-1}_{\text{dry soil}}$)	0.40	0.47	0.33
Polyester	CO_2 (mg $g^{-1}_{\text{dry soil}}$)	5.355	8.54	12.78
	VS (mg $g^{-1}_{\text{dry soil}}$)	64.97	65.42	60.69
	Extracts (mg $g^{-1}_{\text{dry soil}}$)	1.66	1.18	0.16

Table 4: Carbon balance in different time of biodegradation process

	Days from the start (days)		
	78	**140**	**245**
C-CO_2 (%) (C evolved into CO_2)	21.56	37.1	63.38
C-C_{residue} (%) (C remained in polyester residues)	32.37	18.59	n.m.*
C-C_{biomass} (%) (C linked to biomass and organic matter)	67.95	55.13	n.m.*
Percentage of recovering of carbon (%) (C products / C at the beginning)	121.88	110.82	63.38

* n.m.= not measurable

During the biodegradation process, the three components of carbon balance can be measured. After 78 days of incubation, CO_2 production results about the 21 %, estimated biomass-organic matter production was of about 68 % and in soil remained about the 32 % of carbon linked to the tested polyester. Because CO_2 and recovered polyester represent about 53 % of the balance, the remained 47% was expected as biomass-organic matter production, but result obtained by volatile solids elaboration was higher than the expected

value. Similar consideration can be made for balance after 140 days. The CO_2 production (37 %) was about the double of the previous, and polyester in soil (18 %) was about the half. Together they justify 55 % of carbon. Biomass-organic matter production was expected the 45 %, but, also in this case, it was overestimated (it results 55 %).
The obtained recoveries are more than 100 %. This can derived from an overestimation of the measures of the net volatile solids probably caused by an apparent decreasing of organic matter in blank from the beginning (volatile solids at the beginning resulted 62.83±0.69 mg $g^{-1}_{dry\ soil}$). A decrease of organic matter in blank jars is reasonable taking into account that organic matter of the soil is the only carbon source for microorganisms in blanks, but a decrease could be negligible in the soil amended with polymer, where polymer mineralization prevails on soil organic matter degradation. This could be important when the object of the work is to quantify and distinguish small amount of carbon contained in the tested polymer from the higher carbon of soil. We have also to taking into account that using natural soil for testing the sensitivity of volatile solids method for biomass determination, overestimation of the expected values was always obtained. The use of a synthetic soil to study biodegradation of new generation polymers seems to be interesting to increase the accuracy and reduce the errors which cause overestimation, and to reduce carbon background which is too high compared with the carbon of the polyester.
At the end of the test volatile solids of polyester results lower than volatile solids of the blank, so that no biomass or organic matter can be estimated; the tested polymer was not recovered by extraction as confirmed by NMR and GPC analysis. Polymer mineralization is higher than cellulose mineralization (63 % and 58 % respectively) so that, referring to the control, mineralization is complete. Everything suggests that the model polyester is completely biodegraded and that no final biomass deriving from it can be measured. At the moment, it is not yet possible to indicate how 35 % of $C_{polymer}$ is converted at the end of the test.

6.4 Conclusions

The aim of the work was to determine the carbon balance during the biodegradation in soil of a model polyester. Respirometric test was used in order to obtain the mineralization rate of the polyester, that is the CO_2 production. Carbon linked to the residues of the biodegradation in soil was recovered by solvent extraction of the soil samples, and biomass or organic matter production deriving from the biodegradation was estimated by soil samples combustion.
Results suggests that the three component of carbon balance can be measured during the biodegradation process, even if the precision of the method have to be improved, in particular for volatile solids determinations. The analytical analysis made on soil extracts reveal that polyester

concentration in soil decrease over time, but its structure remain practically the same, so that no by-products can be recovered.
At the end of the test, when the plateau phase was reached and the model polyester mineralization was higher than the cellulose one, the biomass-organic matter in soil amended with polyester results not different from the blank soil. Moreover no polyester can be extracted from soil and NMR acquisitions and GPC analysis of the extract suggest that in soil there is no polyester.
The amount of carbon added to the soil with tested polyester is too small if compared with the organic carbon of the soil. The high carbon content of the background can be source of errors and create difficult for accurate measures of biomass production. As vermiculite can replace compost in biodegradation in composting condition, a synthetic soil could replace natural soil for respirometric tests. In the synthetic soil, organic matter and consequently organic carbon is ten time less than in the natural soil, so the measurement of few mg of carbon of the tested substance can be more accurate and reliable.
The analytical analysis result very important in order to understand and describe the biodegradation process.

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Web sites:

- Spectral Database for Organic Compounds, SDBS. Free web site organized by National Institute of Advanced Industrial Science and Technology (AIST), Japan. (http://riodb01.ibase.aist.go.jp/sdbs)

Acknowledgments

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7 Conclusions

The work was developed in order to describe the biodegradation of new generation plastics in soil. For such purpose, a synthetic aliphatic polyester was used as model polymer. Most components involved in the reaction, including reactants and products, were taken into account (carbon dioxide, biomass production and polymer) in order to improve the carbon balance.
At the end of the test, no extra biomass was detected and no polyester was recovered from soil. This was confirmed with NMR acquisitions and GPC.

As to mineralization, the influence of some soil properties (pH and organic matter content) was taken into account.
The model polyester has shown to be actively biodegraded, with mineralization values comparable to those of cellulose. Its mineralization is affected by environmental conditions. The role of organic matter is not so clear, even if it seems to favour the process. On the contrary, the role of soil pH seems to be more defined. The laboratory tests have shown that the mineralization of the model polyester is faster at neutral initial soil pH than in sub-acid conditions.
Because monomers are the by-products of polyester biodegradation, their mineralization in soil was also investigated. The attention was focused at the influence of soil pH on the mineralization of ten monomers chosen between the most commonly used to synthesize biodegradable polyesters. The results indicate that different pH sustain the same mineralization rate at short term, so that it is reasonable to suppose that no by-products remain in soil during the biodegradation of polyesters.
The fate and the biodegradation of monomers was investigated by processing the experimental data on CO_2 production (from ASTM tests) by a numerical code proposed by Saponaro et al. (2008), based on AQUASIM. The model allows to estimate the values for the parameters related to the mechanisms occurring to the monomers in soil (sorption, biomass or by-products production) as well as to quantify the cell biosynthesis. The biodegradation results are generally over 80% and for monomers such as glucose, succinic and lactic acid, butanediol or sebacic acid more than 50% of the carbon is estimated to be converted into biomass.
Biomass can be a large fraction of the biodegradation products and is therefore an important factor in the carbon balance.
Biomass variations in mineralization tests were estimated by determining the total organic matter through calcination (i.e. amount of volatile solids). The obtained results encourage to proceed the work in order to improve the accuracy of the method, by making, for example, more replicates for each measurement or by using a synthetic mineral soil as substrate for the biodegradation test. As vermiculite can replace compost in biodegradation in composting condition, a synthetic soil could replace natural soil for

respirometric tests. In the synthetic soil, the amount of organic matter and, consequently, of organic carbon is ten times less than in the natural soil, so the measurement of extra biomass formed during biodegradation can be more accurate.

The results obtained in this work must be considered as preliminary, but represent a starting point for the setting up of a standard procedure. They confirm that biodegradation tests based on the measure of the CO_2 production are very accurate and precise. However, as anticipated, they are not sufficient for a complete description of biodegradation in soil because mineralization is only partial, even in case of full biodegradation, because of biomass formation. The determination of biomass development and the characterization of possible by-products are very important for a complete and reliable description of the process and to avoid the underestimation of the real biodegradation rate when only mineralization is taken into account. In particular it seems important to deepen the applicability of the volatile solids methods for the determination of biomass (since that is a very simple method that could be applied to every kind of soil) and the possibility of replacing natural soil with a synthetic one in mineralization tests in order to reduce the amount of carbon of the medium that could be source of errors.

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Annex

Other publications:

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