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Bioplastics in the organic fraction of MSW: influence of composting process conditions on biotic and abiotic degradation and fate of residues

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Ai miei genitori

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Abstract

The worldwide market of bioplastic products, both film (e.g. biobags) and rigid (e.g. cutleries, dishes, coffee capsules), underwent a significative increase in the last decade. Bioplastics have been considered a suitable alternative to conventional plastics for their cleaner life cycle. They can derive from renewable resources and be treated with the organic waste, to become compost and enter in the chain of food and agriculture. Thus, they are posed as virtuous example of circular economy. Compostable bioplastics, meaning those materials certified to be treatable in aerobic and anaerobic biological processes, should be collected with the organic waste and conferred to aerobic composting possibly preceded by anaerobic digestion. However, some issues have risen in the last years about bioplastic waste management in the industrial treatments for the organic waste. In fact, the conditions set during the tests for standard certifications can largely differ from those of the biological industrial aerobic and anaerobic plants, referring to temperature, humidity and times of the processes.

This research aims to stress the effect of the operative conditions on bioplastics degradation, particularly in aerobic composting. It started from the observation of the industrial composting plants in Italy and Europe to carry out lab scale tests with various operative conditions, and developed a synergic approach of methodologies to monitor their effect on the degradation of Mater-Bi®, polybutylene adipate terephthalate, polylactic acid and polyethylene. The study considered different aspect of the degradation, including chemical and physical analyses to assess the level of deterioration at different times of the tests, and microbiological analyses to investigate how the bacterial community can become more specialized in degrading polymers.

The obtained results disclosed that some critical operative conditions cannot ensure a complete degradation of the complex bioplastics, raising the issue of microplastics release as part of compost in soil and definitively in water environments. Therefore, lab scale tests were carried out in soil, sand, fresh and saltwater, to explore the behaviour of bioplastic residues derived from incomplete degradation in composting. Unfortunately, it was demonstrated that the outside conditions are not favourable for the degradation process to continue. Finally, it was stressed an important question related to microplastics monitoring in compost and sludge, derived from both aerobic and anaerobic biological treatments. The current legislation set a threshold size of 2 mm for plastics quantification in these matrices, basically for a lack of a standardized protocol allowing to identify and recover items smaller than this size. Therefore, an extraction method was tested on microplastics derived from both conventional plastics and bioplastics, with the objective to make a step more towards the capability to properly monitor and characterize compost and sludge quality.

Il mercato mondiale delle bioplastiche, sia prodotti in film (es. sacchetti per la raccolta dei rifiuti) che rigidi (es. posate, piatti, capsule per il caffè), ha subito un significativo aumento nell'ultimo decennio. Le bioplastiche sono infatti considerate un'alternativa più sostenibile rispetto alle plastiche tradizionali in quanto possono essere prodotte da risorse rinnovabili e trattate con i rifiuti organici, per diventare poi compost ed entrare nella filiera agroalimentare. In questo modo si pongono come un esempio virtuoso di economia circolare. In particolare, le bioplastiche compostabili, ovvero quei materiali certificati come degradabili nei trattamenti biologici aerobici e anaerobici, dovrebbero essere raccolti con i rifiuti organici e conferiti a compostaggio o digestione anaerobica. Tuttavia, negli ultimi anni sono sorte alcune problematiche sulla gestione dei rifiuti da bioplastica i nei trattamenti industriali. Infatti, le condizioni dei test per le certificazioni standard di compostabilità possono differire ampiamente da quelle degli impianti industriali aerobici e anaerobici, in termini di temperatura, umidità e tempi di processo.

La ricerca esposta nella tesi ha lo scopo di investigare l'effetto sulla degradazione delle bioplastiche delle condizioni operative tipiche degli impianti industriali, in particolare del compostaggio aerobico. Si è sviluppata a partire dall'osservazione degli impianti di compostaggio industriale in Italia e in Europa, per effettuare prove su scala di laboratorio con diverse condizioni operative, e ha impostato un approccio sinergico di metodologie per monitorarne l'effetto sulla degradazione di: Mater-Bi®, polibutilene adipato tereftalato, acido polilattico e polietilene. Lo studio ha considerato diversi aspetti della degradazione comprese analisi chimiche e fisiche per valutare il livello di deterioramento in diversi momenti dei test, e analisi microbiologiche per indagare su come la comunità batterica può specializzarsi nella degradazione dei polimeri.

I risultati ottenuti hanno rivelato che alcune condizioni operative più critiche non possono garantire una completa degradazione delle bioplastiche complesse, sollevando il problema delle microplastiche presenti nel compost e del loro percorso dal suolo agricolo agli ambienti acquatici. Pertanto, sono stati effettuati test su scala di laboratorio con suolo, sabbia, acqua dolce e salata, per esplorare il comportamento dei residui di bioplastica derivati dalla degradazione incompleta nel compostaggio. I test hanno dimostrato che le condizioni esterne non sono favorevoli al proseguimento del processo di degradazione. Infine, è stata affrontata un'importante questione relativa al monitoraggio delle microplastiche in compost e fanghi, derivati da trattamenti biologici sia aerobici che anaerobici. La normativa vigente ha fissato una soglia non inferiore 2 mm per la quantificazione della plastica in queste matrici, in quanto non esiste un protocollo standardizzato che consenta di identificare e recuperare frammenti di dimensioni inferiori a questa. Pertanto, è stato testato un metodo di estrazione su microplastiche derivate sia da plastiche tradizionale che da bioplastiche, con l'obiettivo di perseguire un miglioramento della qualità del compost e dei fanghi.

Le marché mondial des bioplastiques, qu'il s'agisse de films (par exemple sacs pour la collecte des déchets) et de produits rigides (par exemple couverts, assiettes, capsules de café), a connu une augmentation significative au cours de la dernière décennie. Les bioplastiques sont en effet considérés comme une alternative plus durable aux plastiques traditionnels car ils peuvent être produits à partir de ressources renouvelables et traités avec des déchets organiques, pour ensuite devenir du compost et entrer dans la chaîne agroalimentaire. De cette manière, ils constituent un exemple vertueux d'économie circulaire. En particulier, les bioplastiques compostables, c'est-à-dire les matériaux certifiés dégradables dans les traitements biologiques aérobies et anaérobies, devraient être collectés avec les déchets organiques et envoyés au compostage ou à la digestion anaérobie. Cependant, ces dernières années, certains problèmes sont apparus concernant la gestion des déchets bioplastiques dans les traitements industriels. En fait, les conditions de test pour les certifications standard de compostabilité peuvent différer largement de celles des installations industrielles aérobies et anaérobies, en termes de température, d'humidité et de temps de traitement.

Les recherches présentées dans la thèse visent à étudier l'effet sur la dégradation des bioplastiques des conditions de fonctionnement typiques des installations industrielles, en particulier du compostage aérobie. Il est développé à partir de l'observation d'usines de compostage industriel en Italie et en Europe, pour réaliser des essais en laboratoire avec différentes conditions de fonctionnement, et a mis en place une approche synergique de méthodologies pour suivre leur effet sur la dégradation de Mater-Bi®, polybutylène adipate téréphtalate, acide polylactique et polyéthylène. L'étude a considéré plusieurs aspects de la dégradation, y compris des analyses chimiques et physiques pour évaluer le niveau de détérioration à différents moments des tests et des analyses microbiologiques pour étudier comment la communauté bactérienne peut se spécialiser dans la dégradation des polymères.

Les résultats obtenus ont révélé que certaines conditions d'exploitation plus critiques ne peuvent garantir une dégradation complète des bioplastiques complexes, soulevant le problème des microplastiques présents dans le compost et de leur cheminement du sol agricole vers les milieux aquatiques. Par conséquent, des tests à l'échelle du laboratoire avec du sol, du sable, de l'eau douce et salée ont été réalisés pour explorer le comportement des résidus bioplastiques résultant d'une dégradation incomplète lors du compostage. Des tests ont montré que les conditions extérieures ne sont pas propices à la poursuite du processus de dégradation. Enfin, une question importante a été abordée concernant la surveillance des microplastiques dans le compost et les boues, issus de traitements biologiques aérobies et anaérobies. La législation actuelle a fixé un seuil d'au moins 2 mm pour la quantification du plastique dans ces matrices, car il n'existe pas de protocole normalisé permettant l'identification et la récupération de fragments plus petits que cela. Par conséquent, une méthode d'extraction a été testée sur des microplastiques dérivés à la fois de plastiques traditionnels et de bioplastiques, dans le but de faire un pas de plus vers l'amélioration de la qualité du compost et des boues.

List of acronyms

ABS	Acrylonitrile butadiene styrene
ASTM	American society for testing and materials
ATR	Attenuated total reflectance
BMP	Biomethane potential
CMR	Cumulative measurement respirometry
DGGE	Denaturing gradient gel electrophoresis
DMR	Direct measurement respirometry
EN	European norm
EPS	Expanded Polystyrene
FTIR	Fourier transform infrared
GC	Gas chromatography
GMR	Gravimetric measurement respirometry
GPC	Gel permeation chromatography
HDPE	High density polyethylene
ISO	International organization for standardization
LDPE	Low density polyethylene
MB	Mater-Bi®
MODA	Microbial oxidative degradation analyser
NDIR	Non-dispersive infrared
NIR	Near infrared
OFMSW	Organic fraction of municipal solid waste
OTU	Operational taxonomic unit
PBAT	Polybutylene adipate terephthalate
PBS	Polybutylene succinate
PC	Polycarbonate
PCL	Polycaprolactone
PCR	Polymerase chain reaction
PES	Polyester
PET	Polyethylene terephthalate
PHA	Polyhydroxyalkanoate
PHB	Polyhydroxy butyrate
PLA	Polylactic acid
PMMA	Polymethyl methacrylate
PP	Polypropylene
PS	Polystyrene
PU	Polyurethane
PVC	Polyvinyl chloride
RI	Respirometric index
SEM	Scanning electron microscope
TCD	Thermal conductivity
TGA	Thermogravimetric analysis
TS	Total solids
XPS	X-ray photoelectron spectroscopy

1 Background and aim of the PhD activity

Plastic litter (either at macro- or micro-scale) is a major global environmental problem, however the real scale of the problem, and the ultimate fate of plastics, is not yet properly known. Plastics can age and degrade into microplastics, a term which indicates items of size between 5 mm and 10 μm, under various environmental conditions and will affect both waters and soils (Shruti and Kutralam-Muniasamy, 2019; Thompson et al., 2004).

In order to reduce the amount of plastic waste and potential environmental impact of plastics, there is a drive towards using bioplastics. European Bioplastics agency classified bioplastics in three categories: biodegradable and produced form renewable resource, biodegradable and produced from non-renewable resources, non-biodegradable but produced from renewable resources (Narancic et al., 2020). However, the term of biodegradability requires specifications about environmental conditions which ensure a complete deterioration of the material. Generally, biodegradable bioplastics are labelled as compostable in accordance with EN 13432:2000 or other international standards.

Compostable bioplastics were born to be managed and disposed of with the organic fraction of municipal solid waste (OFMSW) in biological aerobic and anaerobic treatments. However, concerning the fate of compostable bioplastics, the present thesis is mainly focused on aerobic composting treatment. Indeed, it was evaluated that in Italy 3.3 Mt of organic was treated in 281 Italian composting plants, in 2019. The amount corresponds to more than 50% of the OFMSW generated (ISPRA, 2019). Moreover, the wide variability of process conditions has risen doubts about the bioplastics degradation in composting. This issue is one of the main driving forces of the present thesis. Finally, in anaerobic digestion bioplastics are preferentially removed in pre-treatments. In fact, they cause hydraulic problems in pumps that lead waste to the digestor. Moreover, their density, lower than sludge, makes them float on digestor surface, preventing degradation to occur. In combined anaerobic and aerobic treatments, bioplastics can be reintroduced in the aerobic composting phase. Therefore, aerobic composting resulted a treatment of major interest to implement experimental studies about bioplastics degradation.

Compost produced at the end of composting process is an organic soil improvement agent (or amendant) that finds a usage in agriculture. Therefore it allows compostable bioplastics to be properly recycled in the perspective of a circular economy and to be part of a sustainable agricultural system (Álvarez-Chávez et al., 2012). However, to provide a safe use of compost, it is necessary to ensure the degradation and stabilization of the organic waste, together with the removal of potential pollutants (Vázquez and Soto, 2017).

To this purpose, three aspects are stressed by standards about bioplastics compostability: (ultimate) biodegradability, disintegration and compost quality (Shah et al. 2008, Lucas et al. 2008). (i) Biodegradability includes those biotic and abiotic conditions leading firstly to hydrolysis of the complex biopolymers and then to assimilation by microorganisms, thus modifying them up to biomass and simple metabolic products. (ii) Disintegration is

the physical falling apart of the material into small fragments (conventionally smaller than 2 mm). (iii) Compost quality, also called safety, provides information about the impact of the tested material on cultivated lands (e.g. reduction of the agronomic value and presence of eco-toxicological effects on the growth of plants).

In accordance with the international standards, these features are tested as follows: (i) biodegradability is determined by measuring the actual metabolic conversion of the compostable material into carbon dioxide. The acceptance level is 90% of conversion, which must be reached in less than 6 months. The fundamental chemical reaction that qualitatively describes the process taking place during aerobic composting is presented below.

$$Bioplastics + O_2 + Nutrients \rightarrow CO_2 + H_2O + New\ Biomass + stabilized\ organic\ matter + NH_3 + Sulfates + \cdots + heat$$

(ii) Disintegrability is measured after sieving residues from a 3 months composting test, with a 2 mm sieve. The residues of test material with dimensions higher than 2 mm are considered as not having disintegrated. This fraction must be less than 10% of the initial mass. The undersieve is considered analogous to compost. (iii) Absence of negative effects on the composting process, low levels of heavy metals (below the predefined maximum values), and absence of negative effects on the quality of the compost are evaluated with a plant growth test (OECD test 208). There must be no statistically significant difference from control compost. Other chemico-physical parameters that must not be different from those of the control compost after the degradation are: pH, salinity, volatile solids, N, P, Mg, K. Each of these requirements must be met simultaneously for a material to be defined as compostable.

The degradation of compostable bioplastics includes several steps and the process can stop at each stage (Pelmont, 2005). The biopolymer is a complex material which cannot directly enter the bacterial cell walls: biodeterioration is the first step occurring, which ensures the fragmentation of the material into a millimetre structure and the appearance of cracks and fissures. Bioplastics present weakness, discoloration, erosion signs and polymer splitting (Guo and Wang, 2019). Then, due to its easier biodegradable potential and its tiny structure, the second step can occur: the depolymerization will generate a mixture of oligomers and monomers. The energy for the scission of the chemical bonds during this step may have different origins. In fact, a combination of abiotic and biotic factors contribute in both biodeterioration and depolymerization steps (Lucas et al., 2008).

The main abiotic factors are mechanical, physical and chemical. Mechanical factors (e.g. stresses, weathering), cause damages, sometimes visible at microscopic level more than macroscopically. As physical factor, temperature can cause partial melting of a material and a change in the disorganization of chains, facilitating the further accessibility to chemical and biological degradation (Iovino et al., 2008). Moreover, among the chemical

factors, oxygen is the most powerful agent to induce degradation of the material by attacking covalent bonds and producing free radicals. Water splits polymers containing hydrolysable covalent bonds into groups of ethers, esters, anhydride and so forth. Oxidative and hydrolytic degradations are more easily performed within disorganised molecular regions (amorphous). Finally, UV irradiation which generally causes different levels of chemical weathering in dependence on the environment (Cai et al., 2018).

Considering the biotic factors, microbial species can adhere to polymer surface due to the secretion of an adhesive glue which is a complex matrix made of polysaccharides and proteins. The bacterial aggregation, which is attached to the polymer surface through this matrix, is visible with SEM and often referred to as a biofilm. It can produce disruption and erosion of the polymeric surface, but also infiltrates into cracks and pores increasing their size and weakening the mechanical strength of the polymer (Bonhomme et al., 2003). An important role in the biotic degradation is played by the enzymes synthetized by microorganisms. They are responsible for the breakdown of specific bonds in a polymer (Pelmont, 2005). In literature some authors adopt a nomenclature with the abbreviate name of the polymer followed by "depolymerase" (Lucas et al., 2008).

The oligomers and monomers generated from the previous steps go through the cellular membranes and are integrated inside the microbial metabolism (made of both assimilative and dissimilative processes) to produce energy and new biomass. Then, simple and complex metabolites may be excreted and reach the extracellular surroundings (Siracusa, 2019).

Therefore, bioplastics degradation is complex and long process, which is largely enhanced by suitable environmental conditions of composting process. International standards provide specific conditions under which degradation tests must be carried out: high temperature (58±2°C) over long-times scales (≥45 days (d)), and humidity not lower than 55-50%.

These conditions unfortunately do not strictly represent the highly variable conditions of industrial composting plants. In these latter, thermophilic phase is expected to last a minimum of 15-20 d at 50-60°C. However, the new EU Regulation 2019/1009 reported that hygienization is ensured providing one of the following alternatives: 70°C for 3 days, 65°C for 5 days, 60°C for 7 days or 55°C for 14 days (The European Parliament and the Council of the European Union et al., 2019). Therefore, it is possible that some industrial plants have a very short thermophilic phase. During compost maturation, the decomposition declines to a slow and steady pace at temperatures <40°C, with synthesis of humic substances (European Bioplastics, 2009). At the end of the treatment, final compost quality must comply with the required values of monitoring parameters (Legislative Decree 75/2010 Annex II) (Consorzio Italiano Compostatori, 2017).

Most of the research studies on bioplastics degradation have been carried out in standard conditions, generally at lab scale (Emadian et al., 2017). Therefore, there is a substantial lack of knowledge about bioplastics fate in variable temperature and humidity conditions of industrial plants.

This issue is of great concern because the market of bioplastics has significantly increased in the last decade, and it is foreseen to furtherly increase of 20% in five years (European Bioplastics, 2019). Consolidated products in bioplastic films, such as biobags for shopping and waste collection, are available in the supermarkets and commonly used by population. In some countries, compostable bioplastic bags have become the main kind of shopper and bags for garbage collection; for example, in Italy biobags are imposed by the Government for organic waste collection since 2017 (Law number 123/2017). Moreover, the strict limitations promised in the near future about the usage of single use plastic products and plastic packaging (European Commission, 2018) are favouring the change towards the usage of compostable bioplastics also for this kind of products (Peelman et al., 2013).

If the degradation process remains incomplete, some bioplastic residues and microplastics are likely to be released into the environment within compost. The fate and the time scale of degradation of microplastics deriving from bioplastics are mostly unknown (Álvarez-Chávez et al. 2012 ; Emadian et al., 2017), thus making them potentially hazardous, as well as microplastics from conventional polymers.

Other sources of microplastics are pre-treatments in anaerobic digestion process. As previously mentioned, bioplastics are preferentially removed before entering digestors. Pre-treatments generally consist of sieving and/or shredding, which can generate microplastics. These items derive both from bioplastic products and from conventional plastic bags, which sometimes continue to be incorrectly conferred into OFMSW. While microplastics from bioplastics have the chance to degrade or partially degrade during digestion, conventional microplastics will directly end up in final sludge.

In the last decade, microplastics in marine and freshwater environments have become of great concern. On the contrary, microplastics in sludge and compost are still poorly investigated, with a consequent lack of standard methods for samplings, identification and quantification in these matrices.

The present thesis aims to improve the background about bioplastics management, from waste degradation with the organic fraction, to residues release into the environment as part of compost.

In particular, it deals with three bioplastic types: Polybutylene Adipate Terephthalate (PBAT), Mater-Bi® and Polylactic Acid (PLA). The materials are widely discussed through the thesis, but it is here provided a chemical structure of each material (Figure 1.1). It is fair to mention that Mater-Bi® is a commercial product made of starch and PBAT, and it has different percentages of the two compounds in accordance with the needs to produce the final items. In this work, it is considered Mater-Bi® material which is employed to produce biobags for waste collection. Indeed, it is the most common use of the material, in particular in the Italian market.

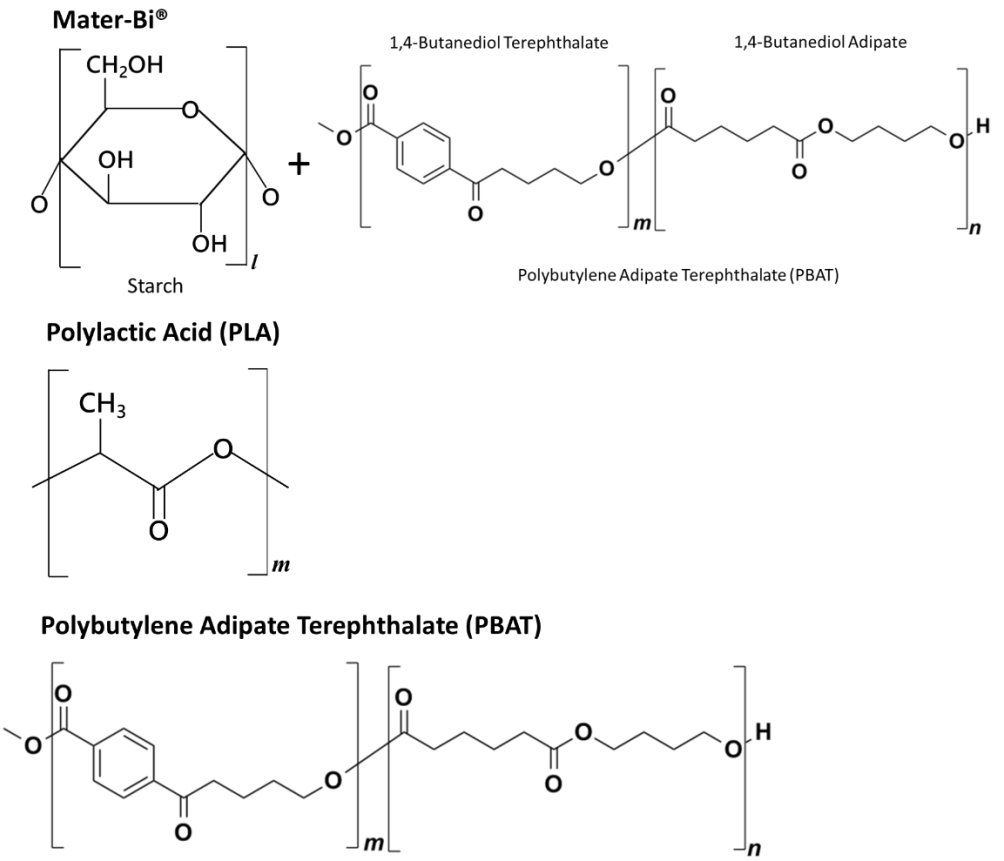

Figure 1.1 Chemical structure of the tested materials considered in this work: Mater-Bi®; PLA; PBAT.

Chapter 2 opens the thesis with a review. The studies of the last two decades dealing with bioplastics degradation are exposed. The aim of the review is to provide a comparison between different studies, in terms of methodologies for degradation monitoring, reference standard tests, as well as thermophilic and mesophilic operative conditions. This literature review rises some important questions. Are the operative conditions required by standard tests for compostability assessment representative of industrial composting? Thus, which is the influence of temperature and length of composting phases on bioplastics degradation? Have bioplastics thickness and typology an influence on degradation, too? Moreover, is it possible to develop a synergic approach of methodologies to broadly monitor many important aspects of degradation?

Chapter 3 starts from these questions to develop experimental studies on both film and rigid bioplastics commonly used in Italy. Composting process is simulated at lab scale, under ten different operative conditions. The experimental research aims: (i) to develop a synergic approach of monitoring methodologies to be applied throughout the thesis. (ii) to study the influence of temperature, humidity and thermophilic phase length, on bioplastics degradation. (iii) to elaborate a kinetic study which allows to foresee the degradation time required under different composting conditions, for both film and rigid bioplastics. However, this chapter leaves some questions open: which role does the

microbial community play in degrading bioplastics? Will degradation continue in the environment, if some residues are released as part of compost?

Chapter 4 expands the experimental research to a wider variety of bioplastics, including throughout the study also a conventional plastic as a benchmark. The operative conditions of the lab scale test are based on: (i) the results of the previous study and (ii) the composting conditions of industrial plants in Europe.

First, the experimental study in this chapter examines the chemical and physical aspects of bioplastics degradation with the previously developed synergic approach of methodologies. Then, it goes in deep about the microbial community colonizing bioplastics in composting. Microbial community structure, studied with 16S rRNA metagenomic analysis, is examined in the different tested plastics and during both thermophilic and maturation phases of composting. The interesting results of the analysis would open new perspective for bioaugmentation in composting of bioplastics degrading species.

Finally, it is tested the degradation in soil and water environments of bioplastic residues incompletely degraded during the composting test. Again, the synergic approach of monitoring methodologies is applied.

Bioplastic residues tested in this chapter have the size of macroplastics. But it is fair to highlight that also microplastics monitoring would be required to preserve the compost quality. Is the current regulation about compost quality too less precautionary about microplastics? Are microplastics, smaller than the threshold of 2 mm, effectively assimilable to compost? The current lack of a standardized method for microplastics recovery in organic fertilizers, both compost and sludge, can be overcome? The last chapters deal with these issues.

Chapter 5 presents a brief review about methodologies to recover and identify microplastics in solid heterogeneous matrices.

Chapter 6 is focused on the fate of microplastics derived from film bioplastics, both in sludge and in compost, aiming to provide further knowledge about their characterization and quantification in these matrices.

A scheme of the thesis structure is reported in Figure 1.2. The scheme shows the University where each experimental part of the thesis was carried out. Indeed, I spent most of my joint-PhD at University of Firenze, Faculty of Engineering, Department of Civil and Environmental Engineering. I stayed 6 months at University of Mons, Faculty of Science, Department of Proteomics and Microbiology, and I had also the opportunity to spend 3 months at Imperial College London, Faculty of Engineering, Department of Materials.

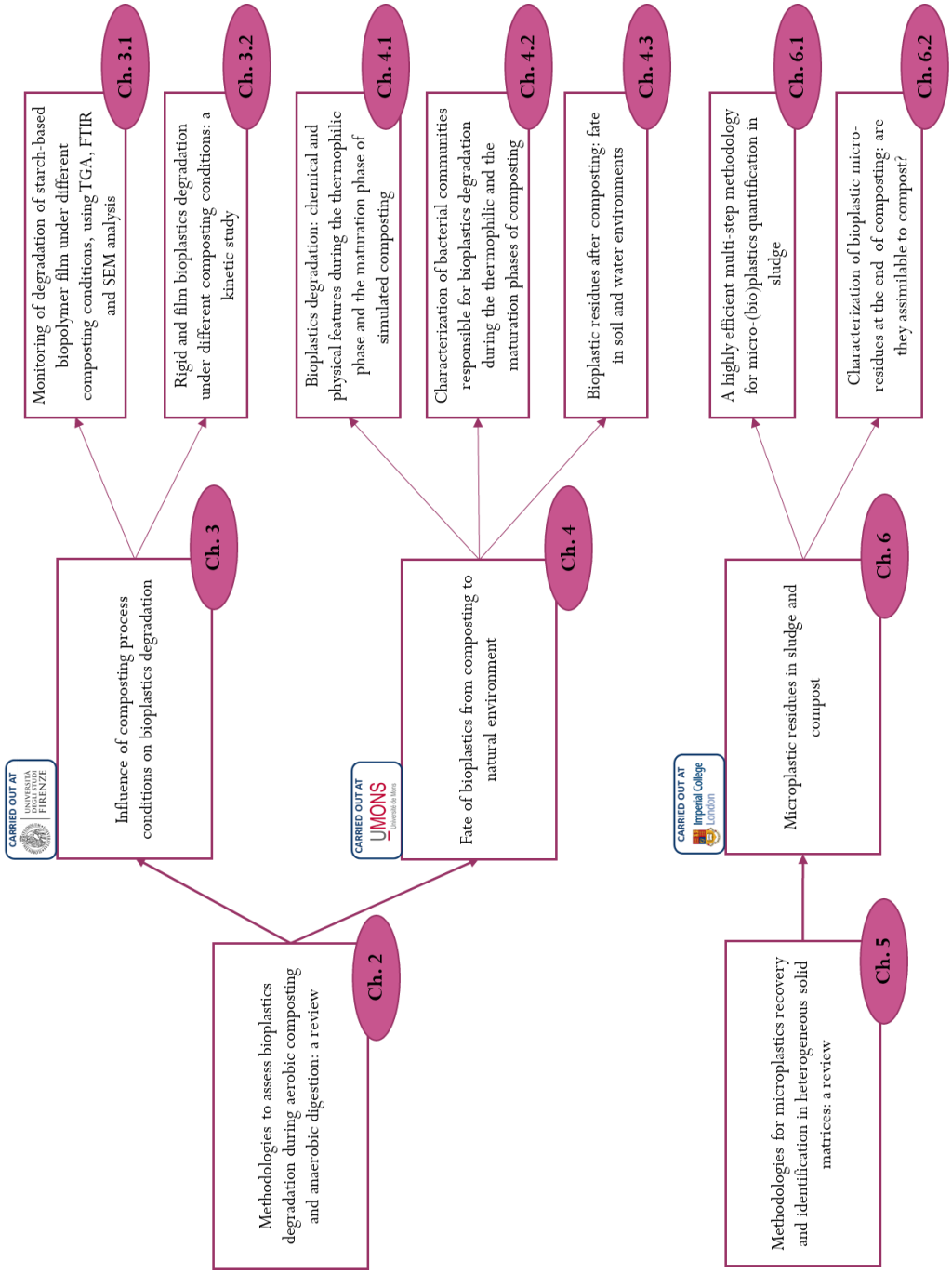

Figure 1.2 Scheme of the activity carried out during PhD at University of Firenze, University of Mons and Imperial College London.

2 Methodologies to assess bioplastics degradation during aerobic composting and anaerobic digestion: a review

Abstract

Bioplastics are emerging on the market as sustainable materials which welcome the challenge to improve the life-cycle of plastics in the perspective of circular economy. The review aims at providing a critical insight of a series of research studies carried out in the last twenty years on the degradation of bioplastics under aerobic composting and anaerobic digestion conditions. It mainly focuses on the various and different methodologies which have been proposed and developed to monitor the process of degradation of several bioplastic materials: CO_2 and CH_4 measurements, mass loss and disintegration degree, spectroscopy, visual analysis and scanning electron microscopy. Moreover, considering a wide range of studies, the process conditions of the experimental setup, such as temperature, test duration and waste composition, often vary from author to author and in accordance with the international standard followed. The different approaches, in terms of process conditions and monitoring methodologies, are pointed out in the review and stressed to find significative correlations between the results obtained and the experimental procedures. These observed correlations allow to reach critical considerations about the efficiency of the methodologies and the influence of the main abiotic factors on the process of bioplastics degradation.

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Introduction

Over the last decades the market assisted to a continuous evolution, due to the role of different kinds of materials within the domains of production. Overcoming glass, wood, ceramic and metal, plastic became a fundamental material to the progression of our man-made environment (Karana, 2012). Plastics assess their predominance on the market with 360 million tonnes produced in 2017 and this amount is forecast to double over the next twenty years. Consequently, in Europe 25.8 million tonnes of plastic waste are generated every year, 59% of which is packaging (Emadian et al., 2017). Nowadays the demand for recycled plastics is very low. Data from Eurostat reported just 6% of recycled plastic demand in Europe, therefore at the end of life plastics are mainly incinerated or landfilled, 31% and 39% respectively ("A European Strategy for Plastics in a Circular Economy," 2018). Plastics disposal has an important impact on the environment, accounting approximately 400 million tonnes of CO_2 per year released into the environment, related to plastic production chain from cradle to end of life. Moreover, up to 4% of global plastics produced ends up in the oceans and seas ("A European Strategy for Plastics in a Circular Economy," 2018), posing a well known risk to the marine environment but also to the safety of animals and humans. The ingestion of plastics by marine animals results both in toxicity risks for them and in bioaccumulation of the material in the food chain (Pathak et al. 2014; Jain and Tiwari 2015; Mostafa and Tayeb 2015). Thus, in the collective imagination severe reactions and criticisms on environmental impacts of plastics have been risen since the beginning of the last three decades (Lindberg et al. 1995). In such a point of global development in which resources are ever more consumed and waste increased worldwide, rethinking plastic production, use and disposal, is a key aspect for sustainable product development (Pracht 2011; Crabbé et al. 2013; Ribeiro et al. 2008). In the perspective of circular economy, wastes and resources could rebalance themselves by recovering wastes as resources after proper treatments. The today challenge in the plastic market is to increase the possibilities for material recovery, thus encouraging not only the demand for recycled plastics, but also considering possible alternatives to plastics, with similar features and uses but a cleaner life-cycle.

Deriving from renewable resources and ending in the organic fraction of municipal solid waste (Brockhaus et al., 2016), bioplastics were created with the objective to welcome this challenge and to emerge on the market as an environmentally-friendly and sustainable material. Considering compostable bioplastics, the following are pretty common: starch-based materials (Re et al., 2013), polylactic acid or polylactide (PLA) (Hottle et al., 2013), polyhydroxyalkanoates (PHA), bioplast, polyhydroxybutyrates (PHB) (Arraiza et al., 2013), starch based Mater-Bi® (MB) (Bastioli, 1998), polycaprolactone (PCL), polybutylene succinate (PBS) (Luzi et al., 2016) and PBAT (Byun and Kim, 2013). Not to be confused with bioplastics, they exist some synthetic polymers containing pro-oxidant compounds and are known with the name of oxo-degradable plastics (Sivan, 2011). These plastics have been found to offer no proven environmental advantage over

conventional plastics, while they just fragment into microplastics without biological degradation.

Common products in bioplastic films, such as biobags for shopping and waste collection, are available in the supermarkets and largely adopted by population. In addition, new perspective for rigid bioplastics are appearing on the market, accounting food-packaging, single use dishes and cutleries, stationary products (Peelman et al., 2013).

The review presents a summary of research at lab and pilot scales carried out to assess the fate of bioplastics during aerobic composting and anaerobic digestion treatments. Concerning these specific environmental conditions, it is provided an overview of the methodologies developed and used by different authors to monitor and measure bioplastics degradation. Generally, in the same study two or more methodologies are applied to measure bioplastics degradation, and several authors use the same methodologies but under different process conditions. To this purpose, the review aims on the one hand at comparing the results and the efficiency of the different methodologies, and on the other at providing a critical discussion of the factors which mainly affect the degradation of bioplastic materials under aerobic composting and anaerobic digestion conditions.

Degradation of bioplastics under aerobic composting and anaerobic digestion conditions

The increasing production of bioplastics and their promising uses on the market, have moved researchers to a scientific interest towards the evaluation of bioplastics degradation under process conditions used during the treatments for the organic fraction of municipal solid waste. Studies were carried out mostly in accordance with standards (e.g. American Society for Testing and Materials (ASTM) International Organization for Standardization, (ISO) European Standards (EN)), which provide indications about environmental conditions, timings and scales of the tests. The result is a series of analyses mainly at lab scale, which consider different durations of simulation for waste treatments, several types of bioplastics, various thicknesses (mostly are films), and finally different temperatures. The combination of these conditions greatly influences the result of bioplastics degradation.

Aerobic composting conditions

In aerobic composting, which consists on oxygen consumption and gaseous H_2O and CO_2 released (Awashti et al., 2014), aeration is required to provide a sufficient amount of O_2, for the oxidation of organic material (Chen et al., 2015), and to evaporate excess humidity (Petric and Selimba˘, 2008). To ensure an adequate aeration of compost feedstock, different turning regimes were used by different authors under forced or natural aeration (Tatàno et al. 2015; Onwosi et al. 2017). Moreover, conventional

composting parameters (temperature, humidity, pH, C/N, volatile solids) (Huang and Wong 2004; Hachicha et al. 2009; Xiu-lan et al. 2016), were monitored during the process (Getahun et al., 2012). In the same way, to simulate composting and to comply with the standard requirements for aeration and monitoring parameters, generally the authors reported the values of degradation obtained during the tests on bioplastics, either at lab scale or pilot scale. To test the degradation of bioplastics under aerobic composting conditions, different standardized equipment have been used in research studies. In Table 2.1. International standards which deal with plastics and bioplastics degradation are summarized. On the one hand, the scope of ISO 20200, ISO 16929, EN 14806 and EN 14045 is the determination of the disintegration degree during composting. On the other hand, ISO 14855-1 and ISO 14855-2, and ASTM D5338 aim at determining also the ultimate aerobic biodegradability of the tested material in composting. Moreover EN ISO 14851 is used to assess aerobic biodegradability in an aqueous medium with an inoculum from activated sludge or compost; so far this latter aspect has been investigated much less with respect to the degradation during aerobic composting. Some authors carried out research following the procedures and analyses provided by these standards, while others do not comply with any specific protocol. The main differences in the conditions required by the above mentioned standards to carry out the tests, involve temperature, duration and scale of the simulation, and composition of the matrix to which the test material is exposed. Keeping an almost constant humidity of 50-55%, tests present either a single constant thermophilic phase, with a range of temperature of 58-65°C, or two phases: thermophilic and second mesophilic. During the maturation phase under mesophilic conditions, the composting matrix progressively cools down to ambient temperature. Moreover, the tests cover a minimum of 30 d, but also a longer period, from 90 to 180 d, even though none of the considered studies exceeds 130 d of simulation. In addition to these variables, the scale of the test can contribute to a substantial variation of the results. Lab scale tests cover a range of size from a hundred ml bottles (Massardier-Nageotte et al., 2006) to few litres (l) flasks. Pilot scale starts from a 35 l reactor as in ISO 16929, up to 140 l in EN 14045: however, without complying with a specific standard, Mohee et al. (2008) developed a simulation in a composting vessel of 200 l (Mohee et al., 2008), while Kale et al. (2007) used piles of 3 m height (Kale et al., 2007). Finally, the matrix of the test can be composed by wet synthetic waste, meaning a mixture of compost, rabbit food, sugar, urea, corn oil and sawdust, by mature compost two or three months old, in a 6:1 ratio with bioplastic material, or by real food and green waste. In Table 2.2, it is presented an overview of the studies considered in the review, reporting the conditions of each test. As it will be described in the next paragraph, the conditions of the reported studies can have a great influence on degradation of the test material.

Table 2.1 Standards to assess bioplastics degradation under aerobic composting conditions.

Standard	Brief description and aim	Environmental conditions
ISO 20200	Test method to determine the disintegration degree of plastic materials in composting. Test requires the use of a standard and homogeneous synthetic solid waste (3 l reactor). The test does not aim at determining the biodegradability.	Two series phases. Thermophilic phase: 58±2°C for a maximum of 90 d. Mesophilic period: 25±2°C for a maximum of 90 d. Minimum test period 45 d.
ISO 16929	Test method used to determine the degree of disintegration of plastic materials in a pilot scale (min. 35 l) aerobic composting test. It cannot be used to determine the aerobic biodegradability of a test material.	Natural self-heating of the spontaneously composted test up to 65°C during the thermophilic phase. Test lasts for 12 weeks.
ISO 14855	Test method to determine the ultimate aerobic biodegradability of plastics and the degree of disintegration under controlled composting conditions. Part 1 is the general method, and part 2 consists on the gravimetric measurement of CO_2 in lab scale test. The method yields the percentage conversion of organic carbon in the test material to evolved carbon dioxide.	58±2°C for a period not exceeding 6 months.
EN ISO 14851	Test method to determine the aerobic biodegradability of plastic materials by measuring the oxygen demand in a closed respirometer. The test material is exposed in an aqueous medium to an inoculum from activated sludge, compost or soil.	Between 20 and 25°C for a period not exceeding 6 months.
EN 14806	Test method to determine the disintegration degree of plastic materials in composting. Test requires the use of a standard and homogeneous synthetic solid waste (3 l reactor). The test doesn't aim at determining the biodegradability.	Two series phases. Thermophilic phase: 58±2°C for 30 d. After the 30th day the range of temperature allowed is from 21 to 58 °C for 60 d. Minimum test period 45 d.
EN 14045	Test method to evaluate the disintegration of packaging materials in a pilot scale (140 l) aerobic composting test. Test material is mixed with biowaste and spontaneously composted in practical oriented composting conditions.	Natural self-heating of the spontaneously composted test up to 65°C during the thermophilic phase. Test lasts for 12 weeks.
EN 13432	Test method to determine the compostability and the anaerobic treatability of packaging material by addressing: biodegradability, disintegration during biological treatment, effect on the biological treatment process and effect on the quality of resulting compost.	Biodegradability test is technically identical with ISO 14855. Disintegration test is technically identical with EN 14045.
ASTM D5338	Test method to determine the ultimate aerobic biodegradability of plastics and the degree of disintegration under controlled composting conditions. The test material is exposed to an inoculum derived from compost. The method yields the percentage conversion of organic carbon in the test material to evolved carbon dioxide.	58±2°C for a period not exceeding 6 months.

Table 2.2 reference conditions of tests carried out to assess bioplastics degradation in aerobic composting.

Standard	Test material	d	T (°C)	Degradation stated (%)	Ref.
ISO 20200	PLA	90	58±2	90	(Luzi et al., 2015)
	PLA	35	58±2	90	(Arrieta et al., 2014)
	PHB	35	58±2	90	
	PLA	90	58±2	90	(Fortunati et al., 2014)
ISO 16929	PHB	84	S.C.*	99	(Weng et al., 2010)
ISO 14855	PHB	39	58±2	81	(Weng et al., 2010)
	PHA	30	58±2	90	(Weng et al., 2011)
	PLA	110	50	70	(Cadar et al., 2012)
	PLA	80	58±2	70	(Petinakis et al., 2010)
	Starch-based	55	58±2	70	(Du et al., 2008)
	Starch-based	90	58±2	87	(Iovino et al., 2008)
	PLA	60	58±2	80	(Kale et al., 2007)
ISO 14851	Mater-Bi®	28	35±2	43	(Massardier-Nageotte et al., 2006)
	PLA	28	35±2	4	
	PCL	28	35±2	38	
EN 14806	Starch-based	90	58±2	85	(Javierre et al., 2015)
	PLA	90	58±2	80	(Sarasa et al., 2009)
EN 14045	Mater-Bi®	55	S.C.	80	(Lavagnolo et al., 2017)
EN 13432	PLA	90	S.C.	5	(Song et al., 2009)
	Mater-Bi®	90	S.C.	5	
	PLA	130	58±2	90	(Balaguer et al., 2016)
ASTM D5338	PHA	115	S.C.	30	(Gómez and Michel, 2013)
	Plastarch	115	S.C.	70	
	PHB	30	55	70	(Tabasi and Ajji, 2015)
	PLA	30	55	70	
	PLA	45	58±2	90	(Pradhan et al., 2010)
No reference	Mater-Bi®	72	35±2	28	(Mohee et al., 2008)
	PLA	30	65	95	(Kale et al., 2007)
	Mater-Bi®	90	25	43	(Accinelli et al., 2012)
	PHA	30	37	20	(Bhatt et al., 2008)

*Simulated Composting (S.C.) in two stages temperature: thermophilic phase 65-58°C and maturation phase 40-25°C.

Anaerobic conditions

Anaerobic digestion, generally applied to the organic fraction of municipal solid waste both alone and mixed with sludge from wastewater treatment plants, involves a complex ecosystem of anaerobic bacteria and methanogenic archaea (Ren et al., 2018). Microbes convert various types of biomass and organic waste into biogas (60-70% methane, 30-40% carbon dioxide, traces of hydrogen and hydrogen sulphide) leaving a nutrient-rich digestate for land application (Sheets et al., 2015). Anaerobic digestion may be carried out either in single-phase or in a two-phase system. In the two-phase system, hydrolysis and acidogenesis react in first reactor, while the utilization of those acids during methanogenesis takes place in the second reactor (Kondusamy and Kalamdhad, 2014). However, it is reported that in Europe almost 95% of anaerobic digestion plants for

organic waste are single-phase system (Forster-Carneiro et al., 2008). Also in the simulations at lab scale for assessing bioplastics degradation it is generally used a single-phase system. The main parameters subjected to monitoring are temperature, pH, ammonia, VFA (Xiao et al. 2017; Yirong et al. 2017), production and composition of biogas (Novais et al., 2018). In Table 2.3, international standards which deal with plastics and bioplastics degradation in anaerobic conditions are summarized. In the mentioned standards the temperature required in simulated anaerobic digestion is 35-37°C, except for ASTM D5511-02 and ISO 13975 which implies thermophilic conditions for the test. In Table 2.4 are reported also many studies that do not refer to a particular standard. Among them, two tests were carried out at 55°C (Yagi et al. 2010, Yagi et al. 2013). As it will be discussed in the next paragraph, temperature seems to be the discriminatory variable in the degradation of bioplastics both under aerobic and anaerobic conditions.

Table 2.3 Standards to assess bioplastics degradation under anaerobic conditions.

Standard	Brief description and aim	Environmental conditions
ISO 14853	Test method to determine the anaerobic biodegradability of plastic materials by anaerobic microorganisms. The test material is exposed in an aqueous medium to sludge.	35°C for a maximum of 90 d.
ISO 13975	Test method to evaluate biodegradability of plastic materials in a controlled anaerobic digestion system. It yields the percentage of conversion of organic carbon in CO_2 and CH_4.	55±5°C (in order to simulate thermophilic digestion) or 35±3°C (in order to simulate mesophilic digestion) for a maximum of 90 d.
EN 13432	Test method to determine the compostability and the anaerobic treatability of packaging material by addressing: biodegradability, disintegration during biological treatment, effect on the biological treatment process and effect on the quality of resulting compost.	Biodegradability test is technically identical with ISO 14853.
ASTM D5526-94d	Test method to determine the degree of anaerobic biodegradability of plastic materials in an accelerated-landfill test environment. The test material is exposed to a methanogenic inoculum derived from anaerobic digesters. The test method yields a percentage of conversion of organic carbon in the sample to carbon in the gaseous form.	35±2°C for a period of at least 7 d.
ASTM D5511-02	Test method to determine the degree of anaerobic degradation of plastic materials in high-solids anaerobic conditions. The test materials are exposed to a methanogenic inoculum derived from anaerobic digesters. The test method yields a percentage of conversion of carbon in the sample to carbon in the gaseous form.	52±2°C for a period of at least 30 d.

Table 2.4 Reference conditions of anaerobic tests of bioplastics degradation.

Standard	Test material	d	T (°C)	Degradation stated (%)	Ref.
ISO 14853	Mater-Bi®	28	35±2	25	(Massardier-Nageotte et al., 2006)
	PCL	28	35±2	Not significative	
	PLA	28	35±2	Not significative	
ISO 13975	PCL	60	55	70	(Yagi et al., 2010)
	PLA	60	55	70	
	PCL	50	55	80	(Yagi et al., 2013)
	PLA	75	55	75	
	PHB	14	55	90	
EN 13432	Starch-based	147	37	8	(W. Zhang et al., 2018)
	PLA	147	37	57	
ASTM D5526-94d	Mater-Bi®	32	35±2	-	(Mohee et al., 2008)
ASTM D5511-02	PLA	50	37	25	(Gómez and Michel, 2013)
	PHA	50	37	25	
No reference	PHB	42	37	-	(Cecily et al., 2013)

Methodologies to monitor bioplastics degradation

The present paragraph proposes an overview of the main methodologies used by authors to monitor the process of degradation of bioplastic materials under aerobic and anaerobic conditions (Table 2.5). The methodologies are summarized in Figure 2.1, divided into four main groups in accordance with their general operation. More specifically, in each group are presented three or four methods of degradation analysis. The pie charts point out which are the most used methodologies among the studies reported in this review (19 for CO_2 measurements methodologies, 19 for mass loss methodologies, 14 for the spectroscopy and 19 for the visual analysis).

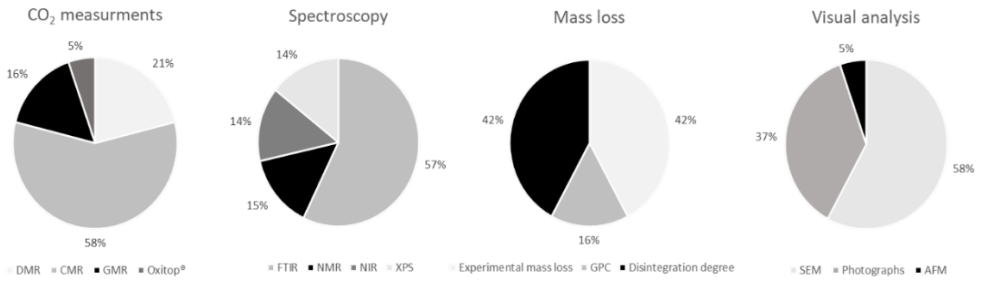


Figure 2.1 Details of methodologies reviewed to monitor bioplastic degradation in biological treatments for organic waste. Refer to the abbreviations list for the meaning of the acronyms.

Table 2.5 Methodologies to assess degradation of bioplastic materials.

Material	Group	Methodology	Ref.
Cellulose-based PLA Starch-based	*CH_4 measurements* *Mass loss*	BMP Exp. mass loss	(W. Zhang et al., 2018)
Cellulose-based Starch-based	*CO_2 measurements* *Mass loss*	DMR Exp. mass loss	(Castro-Aguirre et al., 2017)
Cellulose-based Mater-Bi® PCL PLA	*CO_2 measurements* *Mass loss* *Spectroscopy*	Oxitop® Exp. mass loss FTIR, NMR	(Massardier-Nageotte et al., 2006)
Mater-Bi®	*CO_2 measurements* *Mass loss*	CMR Exp. mass loss	(Mohee et al., 2008)
Mater-Bi® Starch-based	*Mass loss*	Exp. mass loss	(Accinelli et al., 2012)
Mater-Bi® PCL PLA Starch-based	*Mass loss* *Visual analysis*	Exp. mass loss Photographs	(Song et al., 2009)
Mater-Bi®	*Mass loss* *Spectroscopy* *Visual analysis*	Exp. mass loss FTIR Photographs	(Lavagnolo et al., 2017)
Mater-Bi®	*Spectroscopy* *Visual Analysis*	FTIR SEM	(Elfehri Borchani et al., 2015)
PBAT	*CO_2 measurements* *Mass loss* *Spectroscopy*	DMR GPC FTIR, NMR	(Kijchavengkul et al., 2010)
PBAT	*CO_2 measurements* *Spectroscopy*	CMR NIR	(Touchaleaume et al., 2016)
PHA Starch-based	*CO_2 measurements*	DMR	(Gómez and Michel, 2013)
PHA	*Mass loss*	Exp. mass loss	(Bhatt et al., 2008)
PHA	*Spectroscopy* *Visual analysis*	FTIR SEM, photographs	(Weng et al., 2011)
PHA	*Visual analysis*	SEM	(Shah et al., 2008)
PHA PHB	*Visual analysis*	AFM	(Pradhan et al., 2010)
PHB PCL	*CO_2 measurements* *Mass loss*	CMR Disint. degree	(Yagi et al., 2013)
PHB PLA PBAT	*CO_2 measurements* *Spectroscopy* *Visual analysis*	GMR FTIR SEM	(Tabasi and Ajji, 2015)
PHB	*CO_2 measurements*	CMR	(Cecily et al., 2013)
PHB	*Mass loss* *Spectroscopy* *Visual analysis*	Disint. degree FTIR, XPS SEM, photographs	(Weng et al., 2010)
PLA	*CO_2 measurements* *Visual analysis*	DMR SEM	(Petinakis et al., 2010)

PHB PLA	*Mass loss*	Disint. degree	(Arrieta et al., 2014)
	Spectroscopy	FTIR	
	Visual analysis	SEM	
PHB	*Spectroscopy*	XPS	(Correa et al., 2008)
	Visual analysis	SEM	
PHB	*Visual analysis*	SEM	(Eubeler et al., 2010)
PCL	*CO_2 measurements*	CMR	(Yagi et al., 2010)
PLA	*CO_2 measurements*	CMR	(Pradhan et al., 2010)
	Mass loss	GPC	
PLA	*CO_2 measurements*	CMR	(Cadar et al., 2012)
PLA	*CO_2 measurements*	CMR	(Boardman et al., 2017)
PLA	*CO_2 measurements*	CMR	(Balaguer et al., 2016)
	Mass loss	Disint. degree	
	Visual analysis	Photographs	
	Compost quality	Germination index	
PLA	*CO_2 measurements*	GMR	(Kale et al., 2007)
	Mass loss	GPC	
	Visual analysis	Photographs	
PLA	*CO_2 measurements*	GMR	(Tayommai and Aht-ong, 2010)
PLA	*Mass loss*	Disint. degree	(Luzi et al., 2015)
	Visual analysis	SEM	
PLA	*Mass loss*	Disint. degree	(Fortunati et al., 2014)
	Visual analysis	SEM, photographs	
PLA	*Mass loss*	Disint. degree	(Sarasa et al., 2009)
PLA	*Spectroscopy*	NIR	(Ahn et al., 2011)
PLA	*Compost quality*	Germination index	(Tuominen, J. et al., 2002)
Starch-based	*CO_2 measurements*	CMR	(Du et al., 2008)
Starch-based	*CO_2 measurements*	CMR	(Iovino et al., 2008)
Starch-based	*Mass loss*	Disint. degree	(Javierre et al., 2015)

CO_2 and CH_4 measurements

The methodologies of this category provide a percentage value of material degradation through the measurement of the organic carbon transformed to gaseous carbon dioxide and methane, this latter only under anaerobic conditions. Referring to aerobic conditions, the cumulative volume of CO_2 produced during composting is used as an index of microbial assimilation and organic fraction mineralization (Balaguer et al. 2016, Balaguer et al. 2015). CO_2 produced by bioplastics during degradation is compared with a blank, composed by mature compost plus a positive material, generally cellulose (Tabasi and Ajji, 2015), for which biodegradability has already been assessed (Sakimoto et al. 2017; Zhao et al. 2016) and supposed to be complete (Y. Zhao et al., 2017). The measure of CO_2 in gaseous emissions from the process can be carried out with different equipment: the cumulative measurement respirometry (CMR) and the gravimetric measurement respirometry (GMR) (Kijchavengkul and Auras, 2008). GMR was applied in accordance with ISO 18455-2 (Tayommai and Aht-ong, 2010), and also with modified GMR

instruments, as the microbial oxidative degradation analyser (MODA) (Kunioka et al., 2006; Kale et al., 2007). The direct measurement respirometry (DMR) is equipped with a non-dispersive infrared (NDIR) sensor or a gas chromatograph (GC) coupled with a thermal conductivity (TCD) detector to analyse the amount of evolved CO_2 in the output gas (Kijchavengkul and Auras 2008; Castro-Aguirre 2013). The graph in Figure 2.2 presents the results obtained by authors indicated in Table 2.5 approaching the biodegradability analysis of the tested material with one of the above described methodologies. It is fair to notice that when the temperature is below 37 °C the effective degradation during the tests remains below 43% regardless the test duration (Massardier-Nageotte et al. 2006; Mohee et al. 2008, Gómez and Michel 2013). On the contrary, at temperatures in the range 58-65°C, the bioplastic materials tested reaches a percentage of degradation between 70 and 90%.

In anaerobic conditions, the degradation can be assessed through the measurement of biogas production, which is mainly composed by CH_4 and CO_2 (Yang et al., 2018), as provided by ASTM D5526-94d. The test method yields a percentage of conversion of organic carbon in the sample to carbon in the gaseous form. A further equipment to assess the anaerobic biodegradability is the biochemical methane potential (BMP) (W. Zhang et al., 2018), based on the specific methane yield of the test material. In Figure 2.3, they are summarized the results of studies reported in Table 2.5, obtained for bioplastics degradation under anaerobic conditions for different temperatures and duration. The graph shows that no significative results were found under mesophilic conditions, independently on the duration of the test (Massardier-Nageotte et al. 2006; Gómez and Michel 2013; Zhang et al. 2018). However, rising the temperature up to 55°C, higher percentages were gained (Yagi et al. 2010; Yagi et al. 2013).

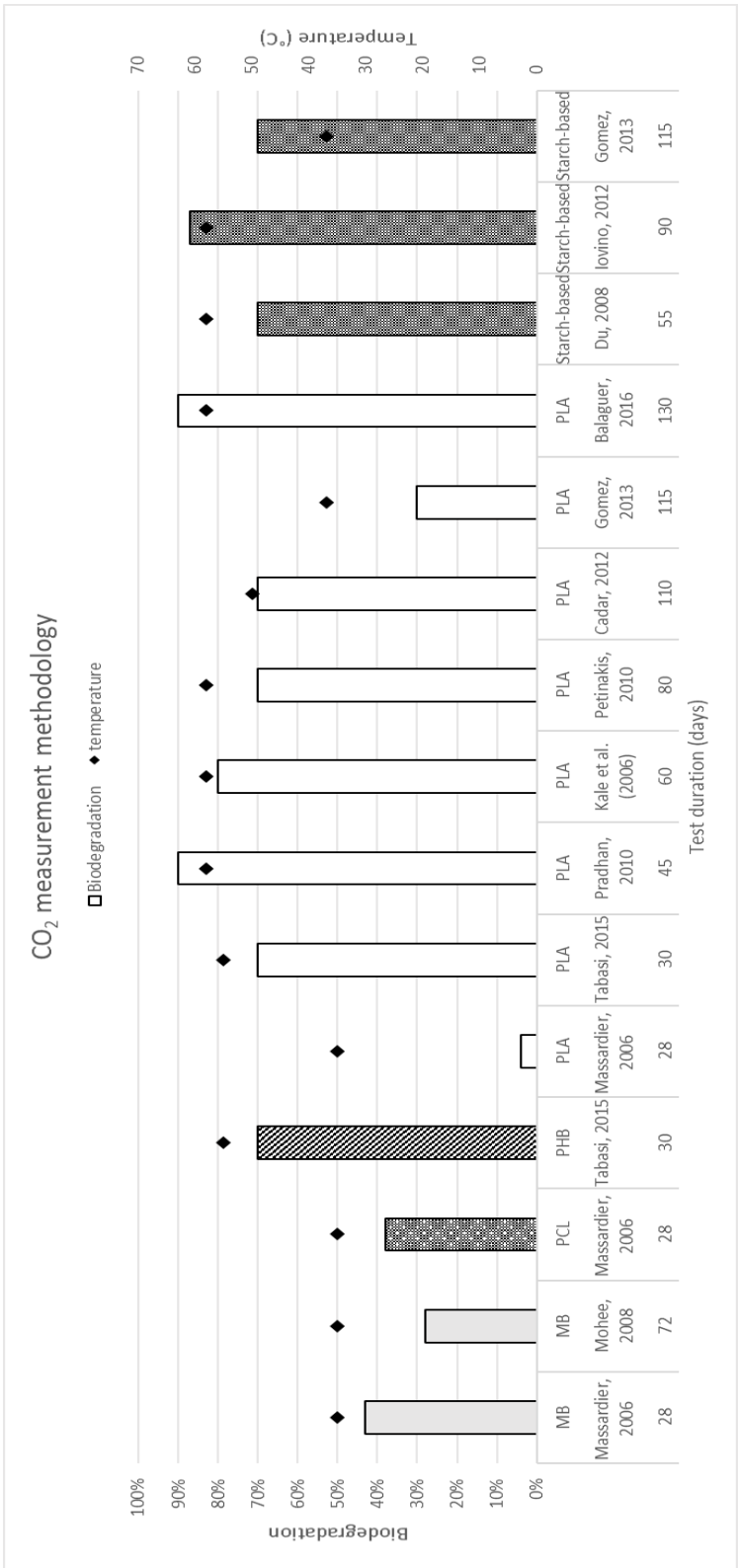
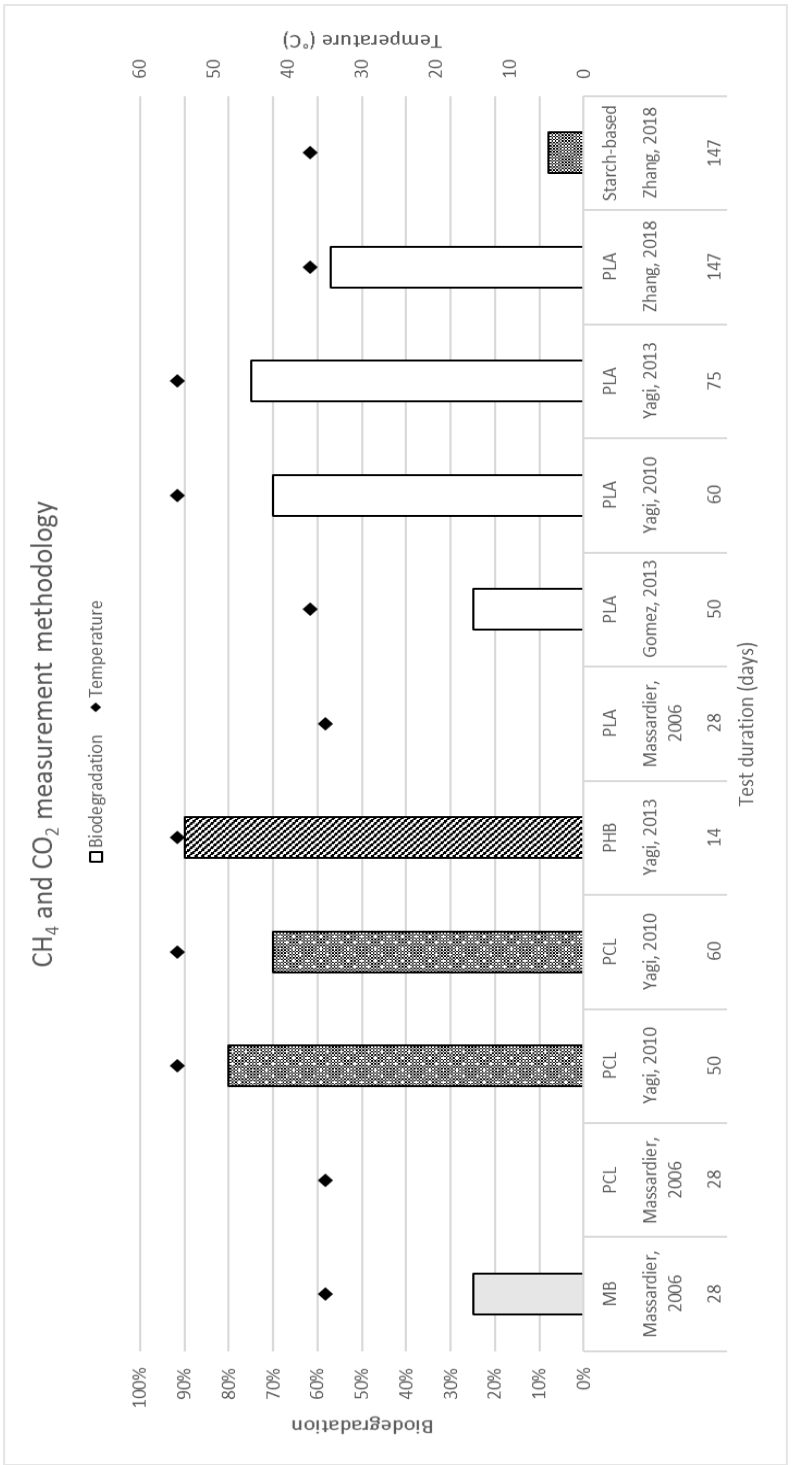


Figure 2.2 Degradation of different bioplastic materials under aerobic composting conditions. Conversion of organic carbon into CO_2 is measured and monitored during the test.



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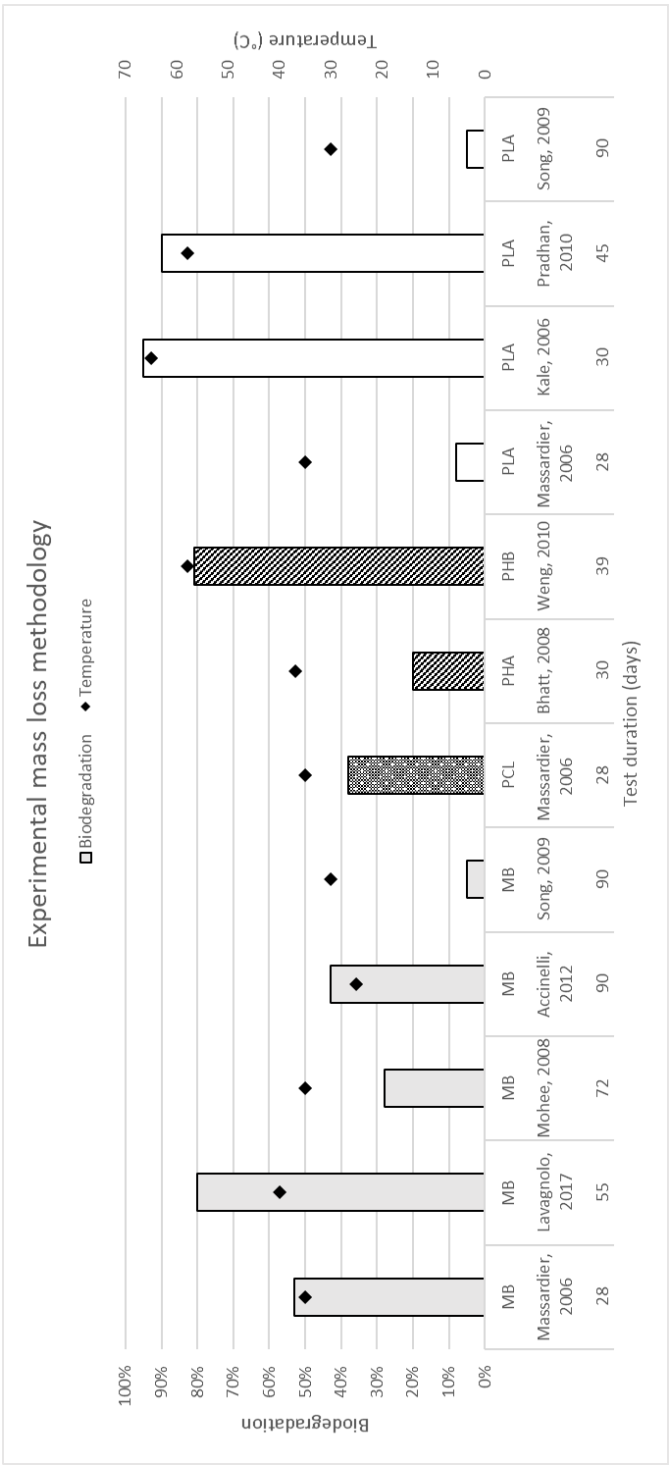

Figure 2.4 Degradation of different bioplastic materials under aerobic composting conditions measured with mass loss methodology.

Spectroscopy

This technique is used to assess the degradation process through the changes in the spectrum of bioplastics during the process. Infrared (IR) spectroscopy means the absorption of IR radiation generally used in the wavenumber range to 4000-400 cm^{-1}. The IR absorption bands have two characteristics: frequency and magnitude. The former is signed in the horizontal axis and it corresponds to the absorbed IR wavenumbers. The latter corresponds simply to the amount of IR, and it is visible in the spectrum through the peaks. Many types of spectroscopic analysis are available. Nuclear magnetic resonance (NMR) is the spectroscopy which gives the sequence of active nuclei, generally expressed on the basis of C, H and O. Moreover, the spectra of the materials can be detected through attenuated total reflectance spectroscopy (ATR-FTIR) and near infrared (NIR). Massardier-Nageotte et al. (2006) used NMR and FTIR for monitoring the degradation of Mater-Bi® and PCL both under aerobic and anaerobic conditions for 28 d at 37 °C (Massardier-Nageotte et al., 2006). The almost unchanged spectra before and after the testing period confirm the low degradation already observed by authors using respirometric analysis.

Some significative results have been outlined by other authors, with a modification of the spectra reliable to degradation process. Using ATR-FTIR, PLA and PHB were observed by Tabasi and Ajji (2015) (Tabasi and Ajji, 2015) for a period of 10 d under composting conditions, resulting in the individuation of a dissociation of the peak 1450 cm^{-1} in two peaks 1454 and 1447 cm^{-1} correlated to the CH_3 functional group. The increase of peaks related to simple bounds was explained by authors with the initial break down, during hydrolysis, of the complex polymers in oligomers or monomers, which are readily biodegradable by microorganisms active in aerobic and anaerobic conditions. The analysis revealed also the increase of wavenumber 1745 cm^{-1} correspondent to the carbonyl group (Tabasi and Ajji, 2015). The same peaks were outlined by Arrieta et al. (2014) on PLA and PHB during a period of 21 d in simulated composting (Arrieta et al., 2014). In addition, the spectral analysis also revealed rising peaks between 3200 and 3600 cm^{-1}, which identifies the hydroxyl group.

If the period is enlarged up to 40 d, it was observed that no significant changes occur in the spectra after 26 d (Weng et al. 2010; Lavagnolo et al. 2017). In Weng et al. the results of ATR-FTIR on PHB underlined that the band at 1725-1724 cm^{-1} assigned to C=O stretching vibration became wider after 30 d of degradation. Moreover, the CH_3 deformation vibrations were found at 1458-1455 cm^{-1} and 1384-1379 cm^{-1}, C-O-C stretching vibration at 1282-1280 cm^{-1} and C-O-H characteristic absorption at 1184-1178 cm^{-1}.

Ahn et al. (2011) applied the NIR on PLA: unlike FTIR, NIR displays the wavenumber range 10000-400 cm^{-1} and provides mainly a qualitative behaviour of the spectra during 60 d of simulated composting, without peaks evaluation (Ahn et al., 2011). X-ray photoelectron spectroscopy (XPS) is another methodology involving spectroscopy: peaks of elements composing the tested material are identified within a certain binding energy (eV) range. In particular, a study of Correa et al. (2008), applied to PHB, followed the

peaks evolution of the spectra for five months in composting. 285.0 eV (used as a binding energy reference) corresponds to C-C and/or C-H, the component at 286.5-287 eV to C-O, and the one at approximately 289 eV to C=O, the component at 532.1-533.1 eV to C-O and the one at higher binding energies to -OH. In accordance with the previous cited studies, the resultant observation for the first month underlined the hydrolysis of the complex polymers. After 2 months no significant changes occurred anymore in the spectra (Correa et al., 2008). Finally, a further methodology to follow the evolution of the spectrum of the tested material is the pyrolysis-gas chromatography-isotope ratio mass spectrometry, up to now used to identified particular additives in PLA (Llana-ruíz-cabello et al. 2016; Llana-ruíz-cabello et al. 2017) through the isotope ratio typical of each material and to identify PE in blends and carrier bags made on biodegradable polymers, PBAT and PLA (Rizzarelli et al., 2016).

Mass loss

Mass loss is considered as an index of degradation. It has been studied by authors by measurement of molecular weight decrease, experimental mass loss, or by assessing the disintegration degree. For the measurement of molecular weight, gel permeation chromatography (GPC) was used in previous studies, in particular on PLA (Pradhan et al. 2010; Kale et al. 2007). Much more used is the measurement of experimental mass loss for pieces extracted during the testing period. The measurement follows a general standardized procedure: sample screening through a series of sieves up to 2 mm size, washing of pieces with distilled water, drying to constant mass, and final weighting. In Figure 2.4, they are reported many studies carried out under different temperature conditions and for periods of variable length (citied also in Table 2.2) by measuring the experimental mass loss. It is observable that for temperatures lower than 37°C the percentage of degradation does not exceed 45%, independently on the test duration (Massardier-Nageotte et al. 2006; Bhatt et al. 2008). On the contrary, at temperatures equal or higher than 58°C the bioplastic materials tested reach a percentage between 80 and 95%.

Considering then the disintegration degree, it is generally measured following the standards normalizing the compostability of bioplastics (aerobic conditions only) in accordance with the percentage of particles which are retained on a sieve of 2 mm. The standards are ISO 14806 and ISO 20200. After 90 d in a lab scale test under aerobic conditions, samples are dried to constant mass and sieved with the objective of separating the remaining plastic pieces larger than 2 mm. The recovered pieces must be washed with distilled water, dried at 40±2°C and weighted for calculating the corresponding disintegration degree (D). It's fair to notice that the equation substantially represents the loss of mass in the conditions set by the test (Equation 2.1).

Equation 2.1

$$D = (M_i - M_f)/M_i * 100$$

Where M_i corresponds to the initial dry mass of bioplastics and M_f represents the dry mass of the recovered bioplastic pieces after 2 mm sieving. In order to validate the disintegration degree, the volatile solids decreasing degree (R) must by higher or equal than 30% (Equation 2.2).

Equation 2.2

$$R = \frac{[m_i(DM_i)(VS_i)] - [m_f(M_f)(VS_f)]}{[m_i(DM_i)(VS_i)]} * 100$$

Where m_i is the initial mass of wet waste matrix, DM_i is the dry mass of waste matrix and VS_i the initial volatile solids of wet waste matrix. With the pedix f the final mass, dry mass and total solids are indicated. DM and VS are expressed as percentage divided by 100. Figure 2.5 shows a comparison between many studies: even though the duration of the test sensitively varies, temperature was kept constant at 58°C by all the reported authors, in accordance with the international standards. It is observable a significantly high degree of disintegration, up to 99%. It is fair to conclude with an additional consideration on bioplastic sieving: the pieces recovered from samples during the period of the test can be sifted with sieves of different sizes, in a range from 10 to 2 mm according with the standards (e.g. EN 13432). After weighting each retained fraction of bioplastic pieces, a granulometric curve can be assessed, and a model of the disintegration process in time can be elaborated (Lavagnolo et al., 2017).

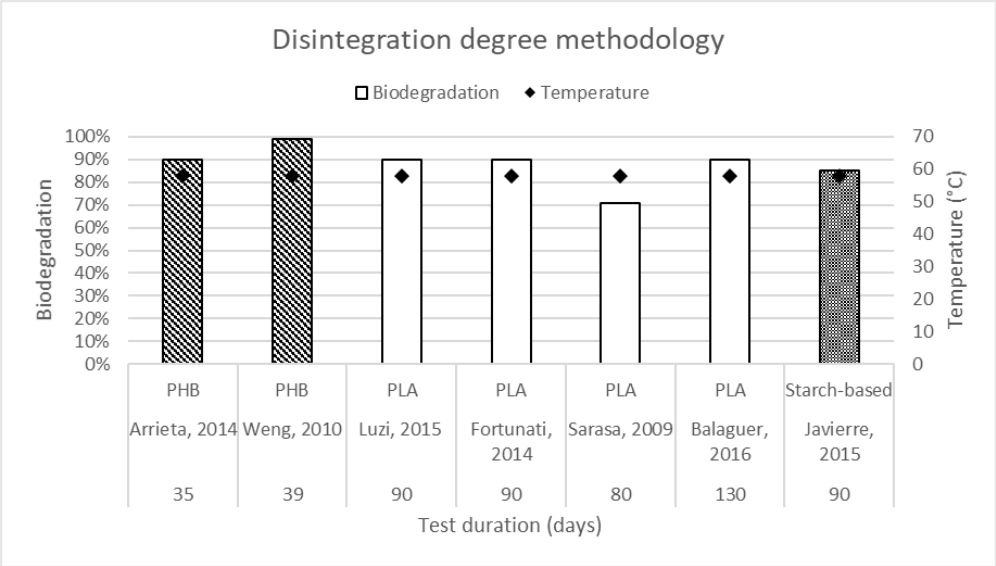

Figure 2.5 Degradation of different bioplastic materials under aerobic composting conditions measured with disintegration degree methodology.

Under anaerobic conditions, in addition to the previous mentioned methods, it was applied by Zhang et al. (2018) a modified version of CEN/TC BT 151 WI: the fraction retained on the sieve was washed with tap water and tokens were recovered by hand. They were then air dried for 3-4 d, counted and weighted. The count of pieces was then used to obtain an empirical pseudo first order decay reaction to provide an estimate of

bioplastics destruction in compost. The five studies carried out on different bioplastic materials and at temperature lower than 37 °C, show a degradation not higher than 35% (Zhang et al., 2018).

Visual analysis

The inspections of surface material changes are indicated as not mandatory analyses in the European standards for packaging biodegradability and compostability, such as EN 14045. However, many authors use visual analysis to confirm the results obtained with one or more of the previous cited methodologies (Bhatt et al., 2008). Visual assessment criteria are generally the distribution of particle size of remaining bioplastic pieces and signs of microbial colonisation. A sample of few pieces can be selected with the intention to provide an impression of all visible degradation phenomena: consistency, thickness, discolouring, erosion of the material (holes, tunnels, etc.), signs of local disintegration and ease of discovery. With the scanning electron microscope (SEM), crack formation, surface roughness and corrosive degradation are investigated. These phenomena were observed on PLA just after 10 d of composting, relating them to the hydrolysis of the longer polymeric chains (Petinakis et al., 2010; Luzi et al., 2015). An interesting comparison between a lab and a pilot scale was carried out by Weng et al. (2010) on PHB. Similar results were observed for both the scales: after 20 d the bioplastic material extracted from the lab scale test presented cavities and surface erosion, while before degradation the surface was smooth (Weng et al., 2010) This fact suggested a first biotic breakdown on the surface of the material (Pradhan et al., 2010). On the pieces extracted from the pilot scale test the cavities are still observed, but this phenomenon followed the formation of filament-like residues which then degrade in many cavities. Within 39 d also the cavities disappeared and PHB films were considered completely biodegraded (Weng et al., 2010). A similar sequence of phenomena was observed by Numata et al. (2008) on PHB and PHA crystals using the atomic force microscope (AFM) which enables the characterization of crystal surface nanostructure in a buffer solution (Numata et al., 2008). To conclude with visual analysis, many studies used photographs to report colour, size, roughness and peculiar features of bioplastics during the test period until they are assimilable to compost. A significative example of photographic report is in Kale et al. (2007) who took photos of PLA bottles every day, observing that while until the 9th day just the shape of the bottles was distorted, from the 15th and the 30th the material totally disintegrated up to disappeared from the photographs (Kale et al., 2007). This result was similarly observed by Arrieta et al. (2014) (Arrieta et al., 2014) and Balaguer et al. (2016), who found out that the pieces became assimilable to compost and smaller than 5 mm after 30 d (Balaguer et al., 2016). Weng et al. (2010, 2011) reported pictures of PHA and PHB films recovered from the composted matrix. Their research reveals that until the 15th day the bioplastic pieces were almost entirely, with little erosion visible more in the margins. From the 20th to the 40th day the number of pieces recovered strongly decreased, and after the 40th day they reached a size smaller than 3 mm. Thus, they were more difficult to be identified and extracted. In parallel, the SEM pictures of

both bioplastic materials confirmed the increasing erosion of the margins of the pieces in the last weeks of the test, and the presence of pores and cavities, which encouraged the disintegration process (Weng et al., 2010; Weng et al., 2011).

Compost quality

The safety of compost is a fundamental requirement to provide a product free of substances which could be a source of pollution for the environment and a threat for the small fauna living in soil and water. The first investigation to assess compost quality is the analysis of physico-chemical parameters of the resulting compost, as well as chemical compositions in terms of: C/N, N-NH_4, P, Mg, K and heavy metals (Balaguer et al., 2016). The bases for the determination of phytotoxicity are in the OECD Guideline for testing of chemicals 208, Terrestrial Plants, Growth Test ("Basis for the determination is the OECD Guideline for testing of chemicals 208 'Terrestrial Plants, Growth Test'.," 1984), which provides the procedure to monitor germination rate of plants in compost within degraded bioplastics. However, in accordance with the certifications for assessing bioplastics compostability, this test is not mandatory. A modified version of the test was applied on PLA by Tuominen et al. (2002), measuring the germination and growth of cress, radish and barely (Tuominen, J. Kylma; J. Kapanen et al., 2002). Balaguer et al. (2016) determined in their research on PLA film the weight of dry biomass in the plants and visual phytotoxicity aspects (chlorosis, necrosis, wilting, leaf and stem deformation), on cress seeds. Within 14 d the authors did not observe significant differences between blank and samples with tested materials, nor mortality of plants. Moreover, they provided the germination index, which is considered a strong measure of the level of phytotoxicity of compost (Karak et al., 2014). Wang et al. (2013) (X. Wang et al., 2013) attributed *GI* to the compost maturity in relation to the germination of the seeds. In Cesaro et al. (2015) (Cesaro et al., 2015) it is reported the equation for the calculation of the *GI* (Equation 2.3):

Equation 2.3

$$GI = \frac{\%G * \%L}{10000} \text{ with } \%G = \frac{G_t}{G_c} * 100 \text{ and } \%L = \frac{L_t}{L_c} * 100$$

where *%G* is the percent germination, G_t is average number of seeds which germinated with the tested material, G_c is the average number of seeds which germinated in the blank. %L is the percentage radicle length, L_t is the average radicle strength and L_c the average radicle length in the blank. In Anjeena et al. (2016) (Anjeena et al., 2017), using this equation, the values of GI reported indicate a phytotoxic-free compost, in fact all the samples exceed a value of 0.5 (Yang et al., 2013). The germination, besides the monitoring of the plant growth, was considered also by Martin-Closas et al. (2014) for measuring compost safety of bioplastic mulching films based on adipic, succinic and lactic acids (Martin-Closas et al., 2014). They did an in vitro crop plant ecotoxicity test, using

lettuce and tomato; the germination of seeds did not present significant differences with the control.

Several bioplastic mulching films for agriculture have been designed for applications ending up in or on soil: a recent example is given by mulches made on Mater-Bi® (Agrobiofilm, 2014). These products have been developed to disappear in situ at the end of their useful life: to this purpose it is important to test such bioplastic materials in accordance with ISO 17556. The test method is used to determine the ultimate aerobic degradation in soil at 25±2°C and it is based on the determination of the carbon dioxide evolved in a period of 4 to 6 months.

To conclude, Jayasekara et al. (2003) (Jayasekara et al., 2003) carried out a test on a starch-based bioplastic, in accordance with ASTM E1976-97, exposing to degraded bioplastic in compost the earthworm *Eisenia fetida*. Juveniles, weight and pathologies of the earthworm were observed for a period of 14 d in comparison with a blank, and the test proved the effective compost safety.

Even though not many studies were carried out on this aspect of bioplastics degradation, all of them resulted in a positive match with standards requirements. However, further developments of ecotoxicity tests are probably to be observed in the next future, especially considering the increasing amount of bioplastic on the market, and the consequent increase in bioplastic concentration within the organic waste. In fact, a higher concentration of bioplastics in waste matrix could be a relevant issue in ecotoxicity and a key aspect to keep under control.

Conclusions

The topic of bioplastics degradation during biological processes, both under aerobic and anaerobic conditions, is nowadays largely developed in the research, involving different methods to face with its complexity. Generally, in the same research two or more of the methodologies stressed in the previous paragraphs are applied. A synergic approach using different methodologies should be provided when dealing with bioplastics degradations. In fact, it would allow to identify possible discrepancies among the results obtained, or otherwise to confirm the observations captured with different methods. Moreover, when more than one author applies the same methodology but under different conditions, comparison between the results allow to make hypotheses on the role of process conditions which influence the fate of bioplastics during biological treatments.

The need to work with two or more monitoring methodologies is for instance highlighted by the results obtained with CO_2 production measurement and mass loss under aerobic composting processes. It is fair to notice that the highest values of degradation observed with the first method range from 70 to 90%, while with the second from 80 to 95%. This difference is supposed to be related to the fact that the mass loss methodology is applied only on bioplastic pieces recovered from sample and generally not smaller than 2 mm. CO_2 production instead is measured on the whole sample of bioplastic material, which can include microplastics smaller than 2 mm and not completely degraded.

Even though quantitative methodologies result in numerical measurements of degradation, it is advisable to support them with qualitative methods as SEM, infrared analysis and visual inspection. From the case studies debated in the previous paragraphs, it was found that infrared analyses have generally confirmed the degradation level revealed with CO_2 production and mass loss. In fact, in Massardier-Nageotte et al. (2006) the almost unchanged spectra before and after the testing period are considered as a proof of the low degradation already observed with CO_2 measurement (Massardier-Nageotte et al., 2006). Conversely, in other studies the strong changes of polymeric structure and features appear as a confirmation of an almost complete degradation of the tested bioplastics (Petinakis et al., 2010; Weng et al., 2010).

Regarding the environmental conditions influencing the degradation process, it has already been discussed the relevance of temperature in both aerobic composting and anaerobic conditions. In particular, the initial thermophilic phase seems to play a fundamental role in making the process start by hydrolysing the complex molecules into more readily biodegradable oligomers, assimilable by mesophilic microorganisms (Emadian et al., 2017). Moreover, observing the degree of disintegration, even though the duration of the tests were variable, temperature was never lower than 58°C. The positive results obtained on the tested material (≥90%) outline that the heat plays an effective role as abiotic condition in the fragmentation of the material. It is then observable that, in addition to a broken down into pieces, thermo-oxidation brings the material to a loss of mass, even though not for sure to a mineralization in the main (Sivan, 2011). The observations related to the environmental conditions outline the importance to dispose bioplastics neither in ordinary bins nor with conventional plastics, but with the organic fraction of MSW: industrial composting plants and anaerobic digestors may exploit research results to provide indeed the best conditions of temperature, humidity and time required to ensure bioplastics degradation.

Beside this, it is fair to underline that the research carried out and previously stressed, assess bioplastics disintegration until millimetric sizes. In accordance with the requirements of standards (e.g. EN 13432), the disappearance of particles bigger than 2 mm ascertains the total degradation of bioplastics in compost. To this purpose, it would be highly recommendable to develop further implementations in disintegration analyses to monitor the eventual presence of microplastics smaller than the size accounted as a safe threshold by standards. In fact, when bioplastics are released into the environment as part of compost, the environmental biotic and abiotic conditions are different than during the waste treatments. The issue of bioplastics degradation in fields and aquatic environments is still strongly debated without certain results. So, if microparticles, derived from bioplastics, smaller than a millimetric size are present in compost used for field fertilization, a fast and complete degradation may not be ensured.

3 Influence of composting process conditions on bioplastics degradation

3.1 Monitoring of degradation of starch-based biopolymer film under different composting conditions, using TGA, FTIR and SEM analysis

Abstract

This study presents the results of a composting lab-scale test carried out on Mater-Bi® (MB) film, a starch-based biopolymer. The test material is composed of starch, additives and PBAT. The test lasted for 45 days and was developed in three replicates under different temperature and humidity conditions, with the aim to assess the influence on Mater-Bi® degradation of less favourable composting conditions as short thermophilic phase, absence of moistening, and a combination of the two factors. The chemical nature and the morphology of the material and of its single components have been investigated before, during and at the end of the composting process, by means of different analytical techniques. Thermogravimetric analysis allowed to obtain activation energy and weight loss; Fourier transform infrared spectroscopy and scanning electron microscopy were used to study changes in the polymeric and morphological structure, and visual analysis provided information on the size of the Mater-Bi® particles. The results showed that the degradation of PBAT was strongly influenced by the environmental conditions (temperature and humidity). On the contrary, in all the three replicates, both starch and additives were completely biodegraded within the first days of the process.

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Introduction

The present study focuses on the influence of composting process conditions on bioplastics degradation. Few words are spent to describe its main phases. (i) One or more mechanical pre-treatments, to ensure an initial shredding and removal of coarse inorganic materials (glass, metals, plastics). (ii) a lag phase of few days during which the biological process starts, (iii) a thermophilic phase (or high-rate phase) of few weeks with temperature ranging between 55 and 60°C, (iv) a maturation phase (or curing phase; duration between 1 and 2 months) with cooling down to room temperature. (v) a final refining with sieves of millimetric mesh is generally provided to obtain acceptable compost quality. In details, the thermophilic phase is submitted to operations of moistening and turning to ensure a humidity not lower than 60-50% and a good aeration of the organic waste heaps. Aeration system can involve also air blowing from the bottom. While the thermophilic phase is characterized by intensive processes of sanitation and degradation of easily biodegradable organic compounds, the maturation phase leads to the stabilization of the organic matter through enrichment by means of humic acids.

Bioplastic-based items can be treated in composting plants in case the material has proven its compostability according to the harmonised European standard. This study refers to EN 13432:2000 (EN 13432, 2000) (for packaging) and to EN 14995:2006 (for plastic materials not used as packaging) (EN 14995, 2006). The technical content of the two standards is identical, meaning that any plastic material that complies with EN 13432 also complies with EN 14995, and vice versa. These standards are the most important technical references for manufacturers of materials, public authorities, composters, certifying bodies and consumers.

Most of bioplastics (e.g. starch-based bioplastics, PLA, PBAT) have been tested under composting conditions, in accordance with the specific standard requirements (Ruggero et al., 2019; Zhang et al., 2018). Authors have indicated that the entity of the degradation of different bioplastic types in these conditions ranges between 70% to more than 90% (Gómez and Michel, 2013; Weng et al., 2011; Balaguer et al., 2016). On the contrary, other authors who carried out the tests under mesophilic conditions, have observed a degradation level not exceeding 43% (Accinelli et al., 2012; Emadian et al., 2017).

This study carried out a degradation test on Mater-Bi® film, a starch-based biopolymer largely used in Italy for biobags production. However, the test differs from the previously mentioned studies because the samples of the tested material were placed under three different conditions (temperature and humidity). By imposing these process conditions, the aim was to simulate variable conditions of composting treatments. They can face with less homogeneous and favourable conditions than those commonly used in the standardized tests. The effect of the abiotic process conditions on the test material as a whole and on each single component of Mater-Bi® bioplastic film (starch, additives and PBAT), was studied by means of TGA, FTIR and SEM. In order to follow the whole degradation process, samples were analysed at different intervals during the composting process.

Materials and methods

Composting test: experimental setup

In the composting test, the waste matrix (initial weight: 5 kg) was composed of food waste and green waste (20% grass, 10% wood chips, 20% vegetables, 30% fruits, 4% tuna, 6% yogurt, 9% cow manure inoculum). The degradation test was carried out in a 12 l vessels made of polypropylene, designed by authors to be used as trapezoidal shaped composters. Each vessel was 20 cm high, 20 cm deep, with top base 30 cm and bottom base 33 cm. Following the indications of the standard EN 14045 (EN 14045, 2003), the vessel was provided with three temperature probes. In order to maintain the thermophilic and mesophilic temperatures of the composting process, each vessel was placed in the shelves of a 250 l ventilated oven model M250-TB manufactured by Tecno-lab (Italy). Then, a drainage system was disposed at the bottom to avoid that compost will flood. It consists on a grain layer, covered with a still net of 1 mm mesh (Figure 3.1). Moreover, a daily moistening and turning during the high-rate phase and every two days during the maturation phase were performed. The turning ensured a proper aeration of the heap. However, also a continuous air flow reached the inner part of the heap thanks to natural convection, entering a perforated polyvinyl chloride tube disposed at the bottom of the bioreactor.

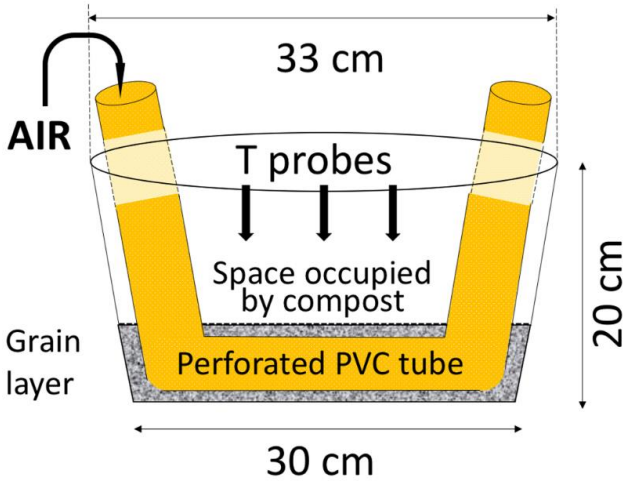


Figure 3.1 Experimental setup of the lab scale composting process.

Analytical methods

The main composting parameters of the waste matrix were measured at the beginning of the test: the values provided in the standard EN 14045 (EN 14045, 2003) for the compost quality required in composting tests were taken as reference. The initial humidity of the matrix was approximately 60%, measured with total solids (TS) content analysis, in accordance with the standard ISO 11465 (ISO 11465, 1993). The measurement of total organic carbon was provided using the Walkley-Black method: this method involved

oxidation of organic matter by potassium dichromate ($K_2Cr_2O_7$) with sulfuric acid (H_2SO_4) to heat the dilution, followed by colorimetric titration (Matus et al., 2009). Total nitrogen and pH were measured in accordance with standard methods ISO 11261 (ISO 11261, 1995) and ISO 10390 (ISO 10390, 2005). Initial values of C/N ratio and pH were of 26 and 6.7, respectively. The values in the reference standard EN 14045 are a humidity not lower than 50%, C/N ratio ranging from 20 to 30 and a pH not lower than 5. The same analyses are done on compost obtained at the end of the composting test and the results are compared with values obtained on a 2 months old stabilized compost provided by the Italian Composting and Biogas Association, named CIC (*Consorzio Italiano Compostatori*).

Tested material and experimental conditions

Regarding the test material, 1 wt% of Mater-Bi® bags was added after having cut them into pieces of 5x5 cm size. To allow an easy recovery and weighting of each sample, each piece was inserted in a tissue net with holes of 1 mm size. The bags, available in Italian supermarkets, have the licence Mater-Bi® and are labelled as compostable by OK compost Vincotte. To give a preliminary overview of the material composition, it is fair to report the study of Elfehri Borchani et al. (2015), who has analytically observed the presence of 20% starch, 10% additives and 70% PBAT in Mater-Bi® biopolymer (Elfehri Borchani et al., 2015).

Three replicates were used for the test (Table 3.1): in the oven, two replicates (A, B) were manually kept in a high-rate phase at 58±2°C for 5 d, followed by a curing phase of 40 d with temperatures at 35±5°C. In replicate A the humidity was manually maintained above 45% for all the test, while in B the humidity went down to 20% during the maturation phase. Finally, replicate C was maintained in a thermophilic phase of 20 d, followed by a maturation phase of 25 d, with moistening conditions equivalent to replicate A.

Table 3.1 Process conditions (temperature, humidity and duration of the phases) of the experimental composting tests.

	Thermophilic phase			**Maturation phase**		
	T (°C)	Humidity (%)	Time (d)	T (°C)	Humidity (%)	Time (d)
A	58±2	55-60	5	35±5	45-50	40
B	58±2	40-50	5	35±5	20-30	40
C	58±2	55-60	20	35±5	45-50	25

Thermogravimetric analysis

Thermogravimetric analysis was performed using a TA Instruments Q-600 (DTA-TG) apparatus using open aluminum pans under nitrogen atmosphere. Measurements were performed in a dry nitrogen flow of 100.0±0.5 cm^3 min^{-1} by increasing the temperature from room temperature up to 500°C at 10°C min^{-1}. 5 mg of material were submitted to

the analysis. The main information provided by the elaboration of TGA, in particular by the first derivative of the TGA curves (DTGA), is related to three aspects. First, from the DTGA curves it is possible to obtain the characteristic temperatures of the peaks associated to the thermal reactions of the components of Mater-Bi®. They are indicated as T_0, T_f and T_{peak} and correspond respectively to the initial, to the final and to the maximum temperature of every peak. Moreover, from DTGA curves, it is possible to obtain both the composition of Mater-Bi® in terms of weight of each single component at a given aging time and the activation energy of every thermal reaction undergone by the components of Mater-Bi®. More in detail, in order to calculate the activation energy, the Friedman equation was applied (Friedman, 1967).

Equation 3.1

$$\ln\left(-\frac{d\alpha}{dt}\right) = \ln A + n\ \ln(1-\alpha) - \frac{E_a}{R\,T}$$

The elaboration of Equation 3.1 was carried out with the Broido method (Mano et al., 2003). E_a is the activation energy [kJ/mol] of the thermal degradation reaction of the polymers constituting Mater-Bi® (PBAT and starch), A is the Arrhenius constant, while α is equal to $(w_0\text{-}w_t)/(w_0\text{-}w_{inf})$, where w_0 is the weight of the sample before the analysis (when $t = T_0$), w_t is the weight at time t, and w_{inf} is the weight of the sample at the end of the conversion (when $t = T_{inf}$). Moreover, *n* is the order of the reaction, R corresponds to 8.314 $\left[\frac{J}{mol\ K}\right]$ and T is the temperature [K]. The T range for data elaboration was chosen in agreement with the value of $\frac{d\alpha}{dt}$ that must be constant. In fact, by considering constant this parameter and $n = 1$ (Mano et al., 2003), Equation 3.1 becomes linear as follows:

Equation 3.2

$$\ln\left(\ln\frac{1}{(1-\alpha)}\right) = -\frac{E_a}{R\,T} + const.$$

Then, by plotting $\ln(-\ln(1-\alpha))$, versus 1000/T, through a linear fitting, it is possible to obtain $-\frac{E_a}{R}$ that corresponds to the slope of the curve. A decrease of the activation energy during the composting process is expected because the degradation implies a simplification of the polymeric structure due to hydrolysis reactions and further transformation of Mater-Bi® in simpler and more stable compounds.

The weight of Mater-Bi® components derived from data analysis of the TGA is expressed through the following equations:

Equation 3.3

$$\int_{T_0}^{T_{inf}} \frac{dw}{dT}\,dT = (PA_i)_j\ (\%)$$

Equation 3.3 is applied separately on starch, additives and PBAT (indicated with j, j=1,2,3), providing the peak area (that is proportional to the amount of the j^{th} substance still present into the degraded Mater-Bi® at time i) of each component at different i-times of the degradation process $(PA_i)_j$. The Equation 3.4 normalizes the peak area over the initial peak area $(PA_0)_j$, providing the fraction (%) of the j^{th} Mater-Bi® component degraded during the composting process at time i.

Equation 3.4

$$100 - \frac{(PA_i)_j}{(PA_0)_j} \; x \; 100 = (WL_i)_j \; (\%)$$

Where $(WL_i)_j$ is the percentage weight loss that corresponds to the amount of the j^{th} component degraded at time i. Then, in accordance with Elfehri Borchani et al.(2015) (Elfehri Borchani et al., 2015), it is finally possible to derive the weight fraction of starch, additives and PBAT present in the sample of Mater-Bi® before the test and at time i, when the degradation is in progress. This allows to make some considerations on the degradation trends of the single components within the biopolymer.

The calculation of the weight fraction is done with Equation 3.5.

Equation 3.5

$$\frac{(PA_i)_j}{\sum_{j=1}^{3}(PA_i)_j} \; x \; 100 = (WF_i)_j \; (\%)$$

Where $(WF_i)_j$ is the weight fraction of the single element j^{th} in the degraded Mater-Bi® at time i.

Moreover, in order to assess the capability of bioplastics to absorb water on their surface as a function of time of the composting process, a gravimetrical determination of the water uptake (WU) was experimentally measured and elaborated in accordance with Equation 3.6:

Equation 3.6

$$\frac{w_h - w_i}{w_{nd}} \; x \; 100 = \; WU \; (\%)$$

Where w_{nd} is the initial weight of the piece before degradation (not degraded) and w_i is its weight at time i during the degradation test. w_h is the weight of the sample after having been removed from the matrix, carefully cleansed with distilled water and superficially dried with a tissue paper.

Fourier transform infrared

FTIR was performed in total reflectance mode with a Shimadzu IRAffinity-1S equipped with a Miracle Pike ATR device. The instrument is supported by LabSolutions IR software. The investigated wavenumber range is 2400-600 cm^{-1}, with resolution 2 cm^{-1} and spectra are collected in absorbance. The analysis was performed on the single pieces, bigger than 1 mm size. The variation of peaks intensity and wavenumbers provides qualitative information about the chemical change of the polymeric structure and about the specific degradation process of starch and PBAT.

Scanning electron microscope

Images of degraded and not degraded Mater-Bi® have been collected with SEM ZEISS EVO NA15 apparatus. The pieces of bioplastics were previously metallized with a 10 nm layer of gold.

Visual inspection

Bioplastics recovered from the waste matrix were reported in photographs to visually define the material in accordance with the following criteria as described by EN 14045 (EN 14045, 2003): distribution of particle size, consistency of the material, discolouring, erosion signs on the surface and lateral erosion signs.

Results and discussion

Thermogravimetric analysis

Characteristic temperatures

Maximum conversion temperature T_{peak} of both starch and PBAT were assessed from TGA analysis at 322°C and 402°C respectively. For starch, T_{peak} decreased already after 5 d of composting down to 301±3°C, likely due to an effective change in the polymeric structure of this material (Weng et al., 2013). Then, temperature fluctuated around this value without a further decrease. On the contrary, T_{peak} of PBAT decreased of few degrees at it stabilized at 397°C in conditions A and C, and at 400°C in B. Concerning the different process conditions of the three replicates, the graphs in Figure 3.2 report the TGA curves (images a, b, c and d) and their corresponding first derivative (DTGA, images e, f, g and h). It is clear that replicate B underwent minor changes during degradation than A and C. Moreover, as peak related to additives disappeared just after 15 d, this component was not included in the data elaboration for the activation energy.

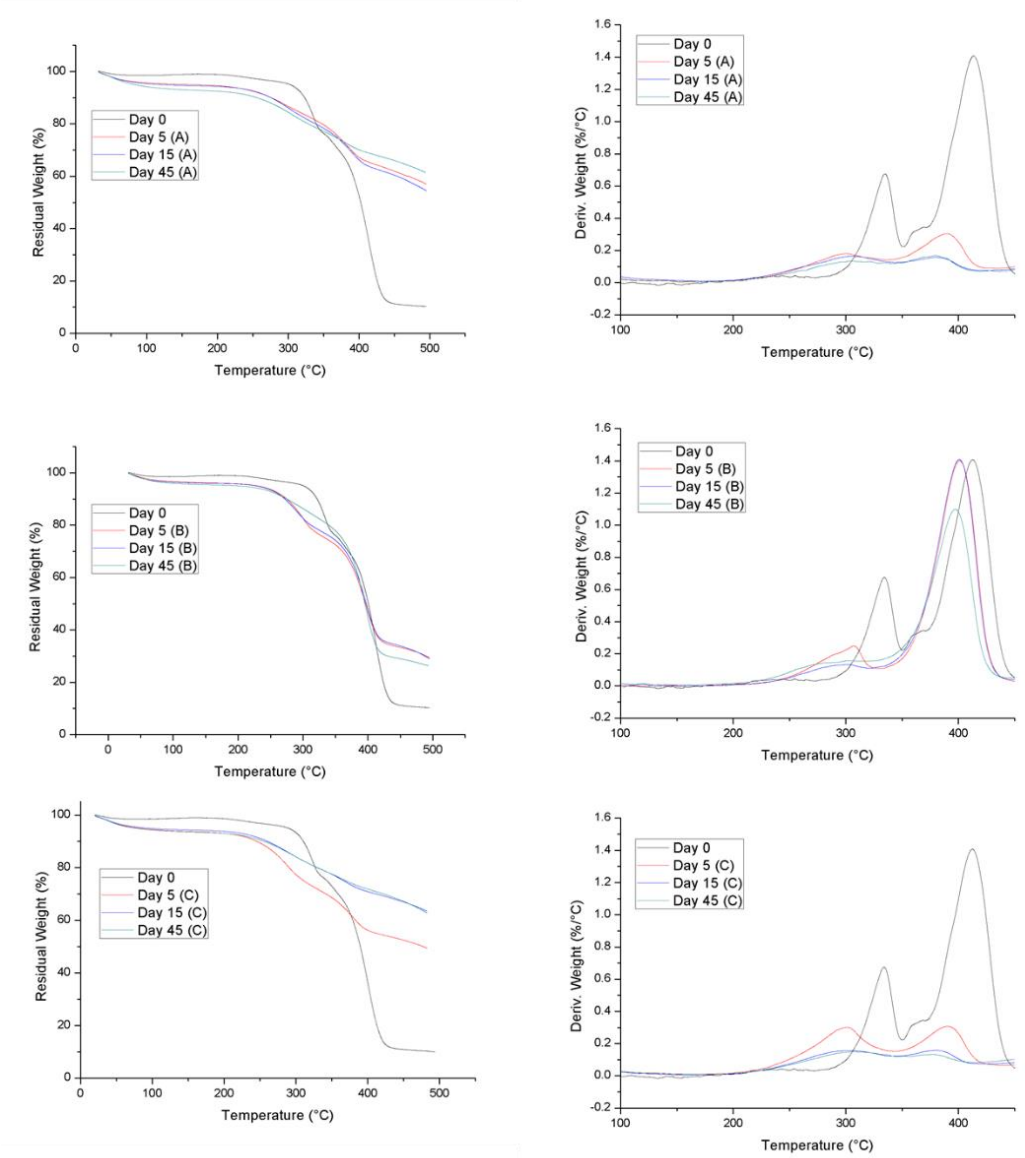


Figure 3.2 Representative examples of TGA and DTGA graphs respectively for samples A, B and C.

Activation energy

The calculation of the activation energy was developed considering a range of temperatures equal to $T_{peak}\pm10°C$. Moreover, in order to evaluate the influence of the choice of the temperature range in the obtained E_a value, two more data elaborations were carried out: in the first, the T range was equal to $T_{peak}\pm7°C$ and in the second to $T_{peak}\pm13°C$. The standard deviation of the E_a values obtained with the three datasets ranges from $\pm0.15\%$ up to $\pm1.8\%$ of the mean value, thus confirming that the choice of the temperature range in the surrounding of T_{peak} does not affect meaningfully the final result. The good quality of data elaborated with Broido method for E_a calculation was confirmed by the R^2 value (~0.995-0,999).

In Table 3.2, they are summarized the activation energies of starch and PBAT for replicates A, B and C. As observed for characteristic temperatures, also the E_a value decreased mainly at the beginning of the composting test (first 5 d). The E_a value of starch dropped from 287.3 kJ/mol, for a not degraded sample (Mano et al., 2003), to 125.3, 142.1 and 117.3 kJ/mol for A, B and C replicate respectively. On the contrary, the E_a value of PBAT underwent a lower decrease, from 251.5 kJ/mol typical of a not degraded Mater-Bi® sample, to 217.5, 231.0 and 210.4 kJ/mol, respectively. In the following 40 days, the activation energies of starch and PBAT decreased much more slowly and settled on values slightly lower than those at the 5th day.

Table 3.2 Mean values of the activation energy calculated with the Broido method for samples A, B and C. The respective standard deviations were calculated with three temperature ranges in the surrounding of T_{peak}.

	E_a (kJ/mol)					
	A		B		C	
Day	Starch	PBAT	Starch	PBAT	Starch	PBAT
0	287.3 ± 3	251.5 ± 4.4	287.3 ± 3	251.5 ± 4.4	287.3 ± 3	251.5 ± 4.4
5	125.3 ± 0.6	217.5 ± 4.3	142.1 ± 1	231.0 ± 2.3	117.3 ± 0.4	210.4 ± 0.7
10	140.2 ± 2.7	221.3 ± 0.6	146.4 ± 2.3	227.8 ± 0.9	125.0 ± 0.6	207.6 ± 1.6
15	136.0 ± 2.4	210.7 ± 0.5	140.9 ± 0.5	227.3 ± 0.7	130.0 ± 3.2	203.7 ± 0.3
30	129.1 ± 1.3	208.0 ± 0.4	134.3 ± 0.6	221.7 ± 1.1	122.6 ± 1.4	201.9 ± 1.6
45	130.4 ± 1.9	208.7 ± 0.8	135.7 ± 1.3	219.5 ± 0.6	123.4±1.2	200.4 ± 1.3

Polymeric weight trend

Considering first the variation of weight for starch, additives and PBAT separately, it was possible to outline some considerations about the influence of humidity and temperature on each single component of Mater-Bi®. The weight loss and the weight fraction, based on Equation 3.4 and 3.5 respectively, are shown in Figure 3.3 for day 0 and 45.

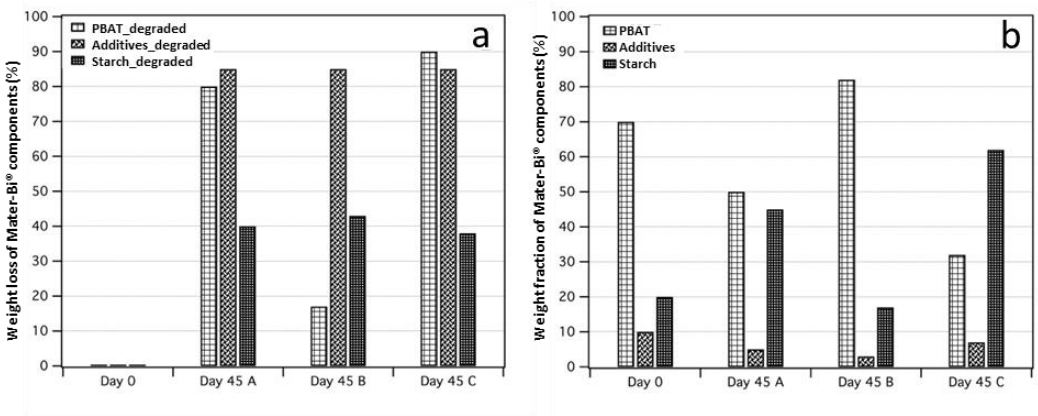


Figure 3.3 The graphs show a) the weight loss *WL* (%) and b) the weight fraction *WF* (%) of each Mater-Bi® component at day 45 for samples A, B and C in comparison with day 0. The calculations are based on Equation 3.4 and 3.5 respectively.

It is fair to notice that starch and additives followed a trend that is almost constant for the three process conditions A, B and C: they presented an average weight loss of 40.5±3% and of 85%, respectively (Figure 3.3a). On the contrary, for PBAT it is necessary to observe the behaviour of the polymer individually in the three replicates: while in case of a low humidity (condition B) the weight loss was only of 17.2%, under a more favourable condition with a humidity between 55% and 45%, PBAT presented a degradation of 79.9% in A (thermophilic phase of 5 d) and 90.4% in C (thermophilic phase of 20 d). Furthermore, in Figure 3.4a it is depicted the weight trend of the whole Mater-Bi® material. Concerning the weight fraction of starch, additives and PBAT, Figure 3.3b reports values at day 0, which comply with the ones observed by Elfehri Borchani et al. (2015), (Elfehri Borchani et al., 2015). Comparing the initial weight fraction of the test material with the composition at the end of the composting process, it is confirmed that for the B replicate the composition had a small variation, with an increase of PBAT percentage due to its much lower degradation with respect to the other components. On the contrary, for samples A and C there was a prevalence of starch at the end of the degradation process. It is fair to conclude that PBAT was the polymeric component of Mater-Bi® more subjected to the influence of humidity and temperature trends. Moreover, being the influence of humidity much more relevant than that of temperature, it is possible to affirm that water played a primary role in the degradation of Mater-Bi®. Water within the waste matrix firstly allowed the exchange of nutrients through the cellular membrane of the microorganisms that concur to the degradation of the bioplastic. Secondly, water was a vehicle for the movement of extracellular enzymes and soluble substrates, and finally was the medium in which chemical reactions take place. Moreover, high water availability allowed the entrance of the microorganisms, present in the matrix, inside of the material, promoting the degradation activity (Alvarez et al., 2006). However, the necessary condition to promote the degradation of the Mater-Bi® is a humidity higher than 40% in the organic waste matrix not only during the period of bio-oxidation, but also during the curing phase. Below this threshold a slowing down of the biological activity was observed, that stopped for humidity values lower than 25%. In these conditions, the degradation process seems stabilized, but previous reports showed that if water is added again, the biological activity is able to restart (Chiumenti et al., 2005). To directly consider the capability of bioplastic pieces to absorb water on their surface, a measure of the water uptake was done in accordance with Equation 3.6. The values of water uptake, as an average of the three replicates, increased until the 30th day and then stabilized around 5% (Figure 3.4b).

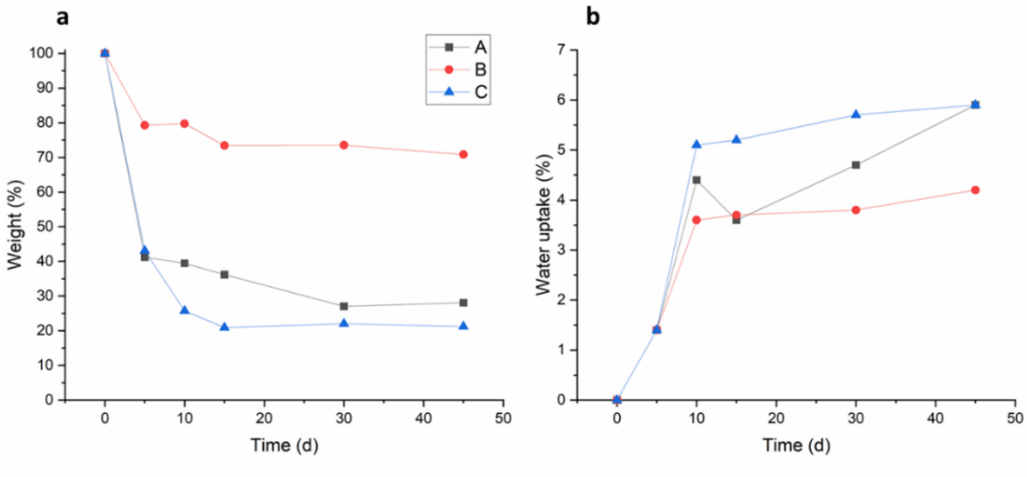


Figure 3.4 Trend of weight experimentally measured (a) and of water uptake (b) in conditions A,B and C of the composting test.

Fourier transform infrared

The ATR-FTIR allowed to detect the chemical composition of Mater-Bi® during the composting process. The most significant spectra are reported in Figure 3.5, with Mater-Bi® at day 0 and samples from A, B and C replicates at day 5, 15 and 45. The figure reports the spectra in the wavenumber range between 1800 and 600 cm^{-1}, where the main diagnostic peaks are present. In Table 3.3, the most important absorption bands of the collected spectra are compared with those identified by other authors for Mater-Bi® (Elfehri Borchani et al., 2015), for starch (Mihaela et al., 2018) and PBAT (Weng et al., 2013; Herrera et al., 2002).

Table 3.3 In the first column of the Table are presented the wavenumbers identified during the study with the FTIR analysis. The absorption bands were compared with those in literature; in the second and third columns are reported the assignment of the molecular bonds.

Wavenumbers (cm^{-1})	Assignment	Material	Reference
1717	C=O	PBAT	(Elfehri Borchani et al., 2015; Weng et al., 2013; Herrera et al., 2002)
1506	benzene	PBAT	(Weng et al., 2013)
1456	phenylene group	PBAT	(Elfehri Borchani et al., 2015)
1409	C-H_2	PBAT	(Weng et al., 2013)
1274	ester linkage	PBAT	(Elfehri Borchani et al., 2015)
1163	CH_2OH	Starch	(Mihaela et al., 2018)
1118, 1081	C-O	Starch	(Elfehri Borchani et al., 2015)
1018	phenyl ring	PBAT	(Weng et al., 2013; Herrera et al., 2002)
726	[-C-H_2-]n≥4	PBAT	(Elfehri Borchani et al., 2015; Weng et al., 2013)

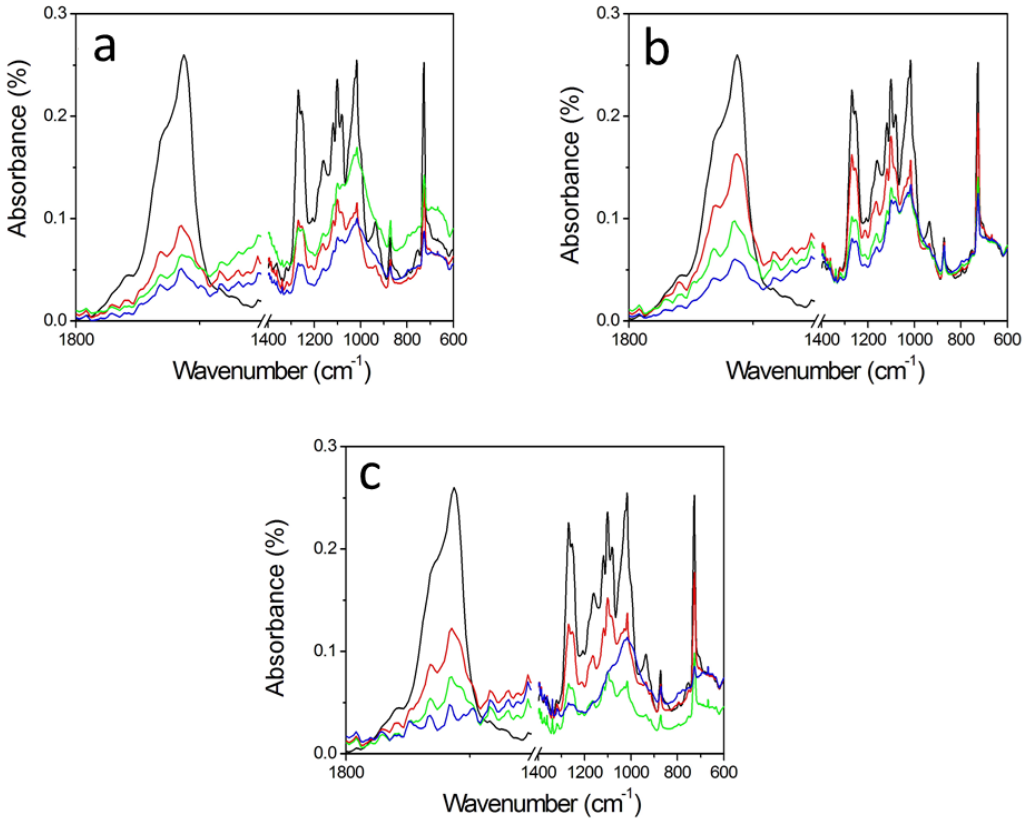


Figure 3.5 Comparison between spectra obtained with FTIR analysis at different days: black corresponds to day 0, red to day 5, green to day 15 and blue to day 45. a) spectra of samples A, b) spectra of samples B, c) spectra of samples C. The range of wavenumber is the most significative for the observations of the main chemical changes.

The peaks attributed to PBAT are the ones at 1717, 1274, 1018 and 726 cm^{-1}. For replicate C an almost complete disappearance of these signals is observed as the degradation process proceeds. Conversely, for sample A and even more for B, they are still present even after 45 d. The observations confirm the results already outlined with the TGA: the degradation of PBAT was strongly influenced by temperature and humidity. Moreover, the spectra reported in Figure 3.5 show that, after 5 days, in the regions between 1650 and 1600 cm^{-1} and between 1550 and 1500 cm^{-1}, there was the appearance of two new peaks attributable to amidic groups of proteinaceous materials. These peaks may be attributed to the compost remained on the surface of bioplastic samples analysed; in fact the spectrum of the compost obtained at the end of the lab scale test confirmed the presence of these two new peaks appearing in the considered range.

Scanning electron microscope

The SEM micrographs of not degraded Mater-Bi® (day 0) are reported in Figure 3.6. The images indicate the presence of a heterogeneous microstructure; the main evidences of the material is the presence of some circular spots which dimensions range in the order of few hundreds of nanometres. These spots are probably composed of starch (Deschamps

et al., 2008; Szymońska et al., 2009), dispersed in a continuous 3D polymeric matrix that is supposed to be composed of PBAT (Muthuraj et al., 2015).

Figure 3.6 shows that, upon degradation, strong changes in the microstructure of Mater-Bi® occurred. With the progress of the composting process, images indicate the progressive disappearance of the circular spots with the formation of small holes (in the order of hundreds of nanometres) in correspondence of the grains that were present before composting. Being that from TGA that the composting process progressively induced a strong degradation of the starch constituting the original Mater-Bi®, it is reasonable to suppose that these circular spots were made mainly by starch.

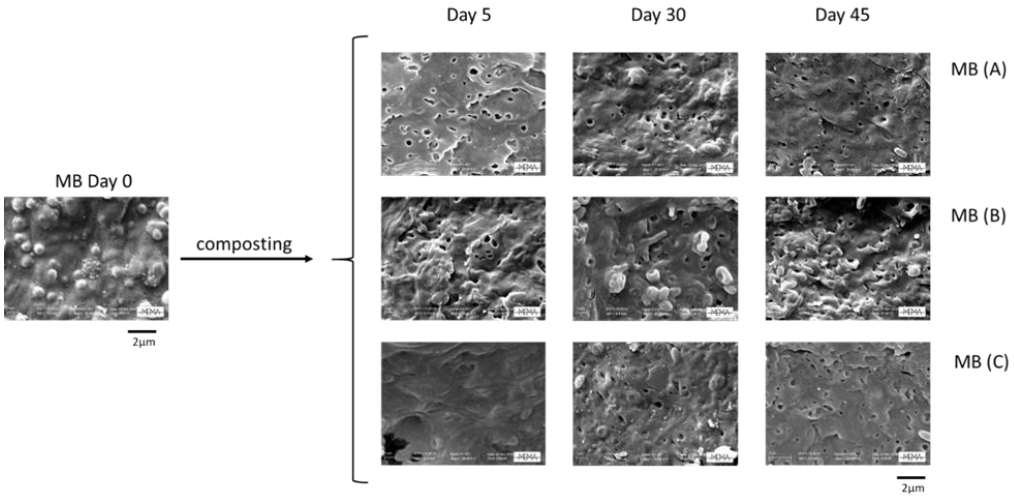


Figure 3.6 SEM micrographs of degraded samples A, B and C during (day 5 and 30) and at the end (day 45) of the composting process, x20000.

Visual inspection

The visual inspection carried out on pieces recovered during the test (Figure 3.7), outlines several interesting considerations. Just after 5 days the material presented signs of erosion, and the aspect was greatly changed due to the contact with the organic waste matrix. Bioplastic pieces presented organic matter deposition on the surface. The organic matter remained strongly attached to bioplastics, thus encouraging the exchange of microorganisms between the materials. Moreover, Figure 3.7 clearly shows the different behaviour of replicates A and B with respect to C. Bioplastics from replicate C were almost completely disintegrated just after 15 days, a trend which is strictly attributable to the temperature that was maintained at 58±2°C. However, it is fair to notice that for the three replicates, after 45 days under the described conditions some micro-pieces were still recoverable. Their aspect, particularly in replicate C, was very similar to compost.

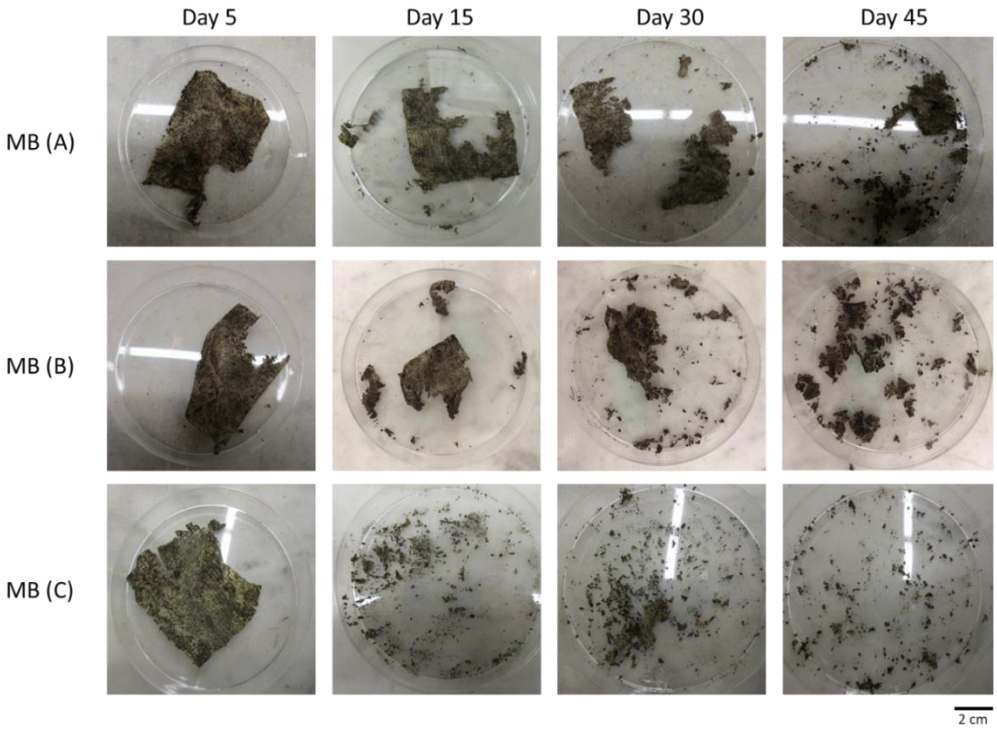


Figure 3.7 Representative pictures, taken during the laboratory tests, of degraded samples A, B and C during (day 5, 15 and 30) and at the end (day 45) of the composting process. MB is used in the figure as abbreviation of Mater-Bi®.

Compost characterization

At the end of the 45 d of composting test, C/N and pH analyses were carried out on mature compost. Compost was previously sieved with 4 mm mesh: in fact, in full scale plants there is generally a final refining with removal of not degraded wood chips and refuses. Moreover, using just few grams of compost for the analysis, the presence of lignin pieces could strongly alter the results, particularly for the total carbon. The refined compost was homogeneous in the three replicates, resulting in C/N and pH equal to 16.1±1.8 and 8.7±0.1 respectively. The same analyses carried out on a 2 months old stabilized compost provided by CIC resulted in C/N 16.2 and pH 8.7. Moreover, compost from the lab tests was analysed through TGA and FTIR; TGA analysis revealed a smooth peak in the T range ~315°C, similar to that of starch T_{peak}. Then, FTIR carried out on compost showed two peaks in the wavenumbers range 1650-1600 cm^{-1} and 1550-1500 cm^{-1}, both due to amidic groups of the proteinaceous materials appertaining to the bacteria acting the degradation. These peaks were present also in the biodegraded bioplastics, due to the residues of these microorganisms not completely detached from the film surface during the cleaning procedure before the FTIR analysis.

Conclusions

The composting of Mater-Bi® inside a heterogeneous organic waste matrix and under different process conditions allowed to study the effect of the environmental conditions

on the degradation of its single polymeric components (starch, additives and PBAT). The methodologies applied in the test displayed that starch and additives underwent a significative degradation already in the first period of composting. The process continues in the following period, even if at a lower rate. Taking into account the different process conditions tested, data indicate that while the degradation of starch and additives were not influenced by the decrease of temperature and humidity occurring as the maturation phase started, the degradation of PBAT was strongly slowed down for replicates A and B during the maturation phase. The most influent parameter for the degradation of PBAT was humidity, that determined the microbial activity and prevented the transformation of the biopolymer into a stable organic matter. This was the case of replicate B, where the humidity passed from 40-50% in the thermophilic phase up to 20-30% in the maturation phase. Moreover, being starch a natural polysaccharide, used as a readily biodegradable source of energy by microorganisms, its degradation rate was faster than the rate of synthetic biodegradable polymers (Wang et al., 2015), as demonstrated by the strong decrease of its E_a. PBAT instead is a synthetic aromatic-aliphatic co-polyester with a molecular structure more complex than starch (Ra et al., 2018). Indeed, it required a longer period to be completely assimilated by microorganisms and transformed into stable products. Moreover, it was much more subjected to process conditions, such as an insufficient humidity.

In conclusion, to carry out an exhaustive analysis of the degradation process, this study highlights the need to use a synergic approach based on different instrumental techniques, giving complementary information. This approach allows a complete analysis of the composting process and shows the influence of the environmental conditions on the degradation of starch, additives and PBAT in Mater-Bi® film bioplastic.

3.2 Rigid and film bioplastics degradation under different composting conditions: a kinetic study

Abstract

The present research implements a kinetic study of bioplastic waste degradation under composting. It aims to delineate the reaction rates characterizing bioplastics degradation under different composting conditions, setting three variables: temperature (37-58°C), humidity (30-60%) and timing of the thermophilic and the maturation phases (15-60 d). The composting tests were carried out following modified guideline ISO 20200:2015 and lasted for 60 days. Bioplastics in the synthetic waste matrix consisted of Mater-Bi® film biobags and PLA/PBAT rigid teaspoons. Beside weight loss measurement, thermogravimetry and infrared analysis were carried out to monitor bioplastics degradation. The kinetic study was performed exploiting the weight loss measurements and outlined faster degradation rates for film bioplastics (pseudo first order kinetics with $k \sim 0.0850\text{-}0.1663\ d^{-1}$) than for rigid ($0.0018\text{-}0.0136\ d^{-1}$). Moreover, films were less affected by the variation of composting conditions, and reached a complete degradation within the 60 days of the test. Concerning rigid products, the only condition which fulfilled the standards for bioplastics compostability was 2 months composting at 58°C and humidity 55-60%. Even though in the other conditions the degradation was not at all prevented to continue, 90% degradation would be achieved in 2-3 years. Finally, in the undersieve of 0.5 mm some microplastics were identified with the ImageJ software, mainly relatable to PLA/PBAT bioplastics. Overall, the results disclosed that the combination of mesophilic temperatures and absence of moistening slowed down both the degradation and the disintegration process of bioplastics.

Introduction

Chapter 3.1 introduced the issue of how different composting conditions affect bioplastic film degradation. The experimental research highlighted the need to improve the basic knowledge about the delicate balance of temperature, humidity and timings, influencing bioplastics degradation. In order to deeper stress this issue in the current chapter, some references have been considered about the variability of composting conditions in the Italian plants.

Thermophilic phase is expected to last a minimum of 20 d at 50-60°C; for hygienization purposes, temperature should remain above 60°C for one week, in order to eliminate pathogenic microorganisms eventually present in the organic waste. During compost maturation, the decomposition declines to a slow and steady pace at temperatures <40°C, with synthesis of humic substances (European Bioplastics, 2009).

Common technologies of industrial composting include windrows composting, aerated static piles, tunnel and in vessel composting, and the most recent technology of bio-oxidation in composting biocells. The diversity of composting technologies entails a variability of the main parameters, such as temperature, humidity and process duration, as well as of aeration, turning and moistening procedures. Considering the Italian composting plants, the aerated windrow composting, largely diffused in the southern part of the country, involves a thermophilic phase in a covered environment, lasting 40, 30 or 20 d (Pergola et al., 2017). It generally follows a maturation phase in an open-air environment, with neither moistening nor aeration. During this phase, compost is organized in piles which are static or weekly turned (Pergola et al., 2018). Similar conditions occur when bio-oxidation is ensured in static aerated platforms instead of channels (European Commission, 2000). Biocells are relatively recent technology: they find a good application for new plants construction and reconversion of old ones. Indeed, biocells are closed systems which prevent odours and litters, and ensure a better control and monitoring of temperature, humidity, oxygen, pH, waste flows, aeration time (Martalò et al., 2020). Industrial composting with biocells has generally a high-rate phase in thermophilic conditions carried out in one cell for 15 d or twice as long in two cells in series. It follows an open-air desiccation phase (European Commission, 2000).

The current research will implement a kinetic study of film and rigid bioplastics degradation under a wide variability of composting conditions. Similar studies have been recently developed to delineate decomposition trend of various organic waste, in particular green waste and sewage sludge (Komilis, 2006; Manu et al., 2016; Abu Qdais and Al-Widyan, 2016).

The study aims to delineate the reaction rates which characterize bioplastics degradation under different temperature, humidity and timings in composting. The results would be a useful reference for bioplastic waste management in the industrial composting plants, enhancing to foresee bioplastics degradation time in given composting conditions.

Materials and methods

Experimental setup and tested materials

The composting test was carried out following the guideline ISO 20200:2015, with temperature and humidity modified. A synthetic solid waste matrix was prepared based on the composition suggested in the guideline (sawdust 40%; rabbit feed 30%; cow manure compost 10%; corn starch 10%, saccharose 5%; corn seed oil 4%; urea 1%). The waste matrix was incubated in 5 l polypropylene vessels covered with a lid.

In order to maintain the chosen temperatures during composting, two 250 l ventilated oven model M250-TB manufactured by Tecnolab (IT) were used to place the vessels. As required by reference standard, turning and moistening were controlled and manually set, daily during the thermophilic phase and weekly during the maturation. Turning procedure ensured to properly aerate the heaps; moreover, in accordance with the guidelines, three holes of 5 mm were made on both the sides of the box to ensure gas exchange between the inner atmosphere and the outside environment.

At the end of the composting test, the mass loss of synthetic waste matrices was monitored by measuring the total solids content, in accordance with the standard ISO 11465 (ISO 11465, 1993). Moreover, the C/N ratio was measured before and at the end of the test. The measurement of total organic carbon was provided using the Walkley-Black method and total nitrogen was measured in accordance with standard methods ISO 11261 (ISO 11261, 1995).

The process conditions of each vessel are graphically summarized in Figure 3.8. Most favourable condition was of 2 months thermophilic phase with humidity not decreasing below 50% (A_H). On the contrary, it is supposed that condition D_L was strictly unfavourable, with thermophilic phase lasting for 15 d and humidity not higher than 30% during the maturation phase.

To allow an easy recovery, bioplastic samples were inserted in tissue nets with holes not larger than 1 mm size. A total of 64 film samples, with 50 μm thickness and weight 0,0573±0.0000063 g, were cut 5x5cm size from biobags purchasable in all Italian supermarkets. Rigid samples were 64 teaspoons (concave part) purchased from Ecozema shop (IT) and easily available also in all Italian supermarkets; samples had 250 μm thickness and weighted 1.0082 ±0.0000136 g.

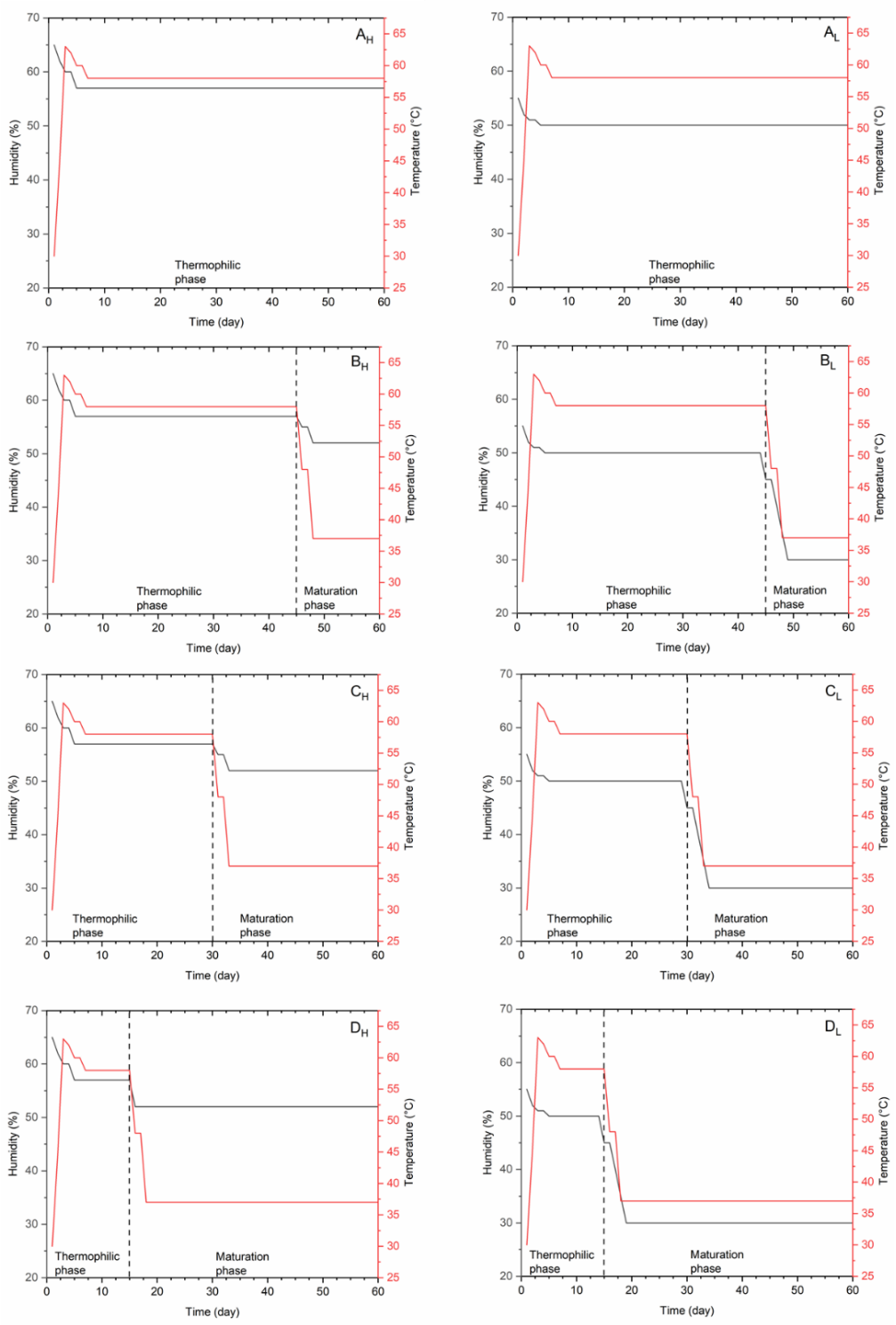


Figure 3.8 Composting process conditions in the vessels of the test

Kinetic study of bioplastics degradation

One rigid and one film bioplastic sample was collected once a week; after tissue net removal, the sample was dried at 40±2°C to constant mass and weighted. Experimental weight loss was calculated based on Equation 3.7.

Equation 3.7

$$\%\, Weight\ loss = \frac{(W_0 - W)}{W_0} * 100$$

Where W_0 and W are the experimental weights before and at the sampling times of the test, respectively.

The experimental data obtained in the present study was fitted in the following pseudo first order kinetic model (Equation 3.8).

Equation 3.8

$$\frac{dW}{dt} = -kW$$

Where k is the degradation rate constant (day^{-1}) and t is the time (d).

Integrating the above equation and letting $W = W_0$ initially when $t = 0$, it gives Equation 3.9:

Equation 3.9

$$ln\left(\frac{W}{W_0}\right) = -kt$$

The reaction rate constant (k) was obtained by plotting $ln\left(\frac{W}{W_0}\right)$ versus time data for film and rigid Mater-Bi® bioplastics under the 8 composting conditions.

Bioplastics characterization

Bioplastic samples were characterized by mean of FTIR and TGA. Before starting the test, the polymeric composition of film and rigid samples was defined; during the test, the analyses were exploited to monitor the degradation process. FTIR and TGA equipment were the same used in the experiments of Chapter 3.1. Therefore, details are given in that chapter.

In addition, the main information used from TGA data was the characteristic temperature of the peaks associated to the thermal reactions of biopolymer components indicated (T_{peak}) and the percentage weight PA_i of each *i* component in the new materials, with Equation 3.10:

Equation 3.10

$$\int_{T_0}^{T_{inf}} \frac{dw}{dT}\, dT = PA_i\ (\%)$$

Where T_0 and T_{inf} correspond respectively to the initial and the final temperature of each peak. Finally, a visual inspection was performed on the collected samples, to observe changes in physical features such as consistency of the material, discolouring, erosion signs on the surface and lateral erosion signs.

Microplastics identification in compost

At the end of 60 d composting test, all samples were collected. The residual matrix was dried out at 100°C±2°C to constant mass and sieved with 2 mm, 1 mm and 0.5 mm meshes. The undersieves were weighted; photographs were taken in order to analyse the undersieves with ImageJ software. Using the function of adjust color threshold, the software allows to set a threshold for microplastics identification within the compost matrix. It provides the total microplastics area. In particular, the function was set with hue 0/255, saturation 0/255 and brightness in the range 232-247/255. The threshold color was B&W with color space HSB in dark background.

Results and discussion

Bioplastics characterization

Spectra of pristine materials are displayed in Figure 3.9a. Concerning film biobags, the main peaks identified the material as Mater-Bi®, composed of starch and PBAT, as widely discussed in previous chapter.

Already after 15 d the main peaks of both starch (1200-1080 cm^{-1}) and PBAT (1717, 1274, 1018, 726 cm^{-1}) were almost disappeared in all the composting conditions. In Figure 3.9b spectra of condition A_H are reported as example.

Concerning the rigid material, FTIR analysis assessed the presence of PLA and PBAT (Figure 3.9a). Peaks referring to PLA were identified in 1748 cm^{-1}, corresponding to C=O group, 1450 cm^{-1} of CH-CH_3 groups, 1181 cm^{-1} relatable to C-O group and finally 1081 cm^{-1} related to C-C stretch in n-alkanes (Arrieta et al., 2014; Fortunati et al., 2014; Correa-pacheco et al., 2020). The PBAT component was assessed through the peaks 1717 cm^{-1}, C-O group, 1274 cm^{-1}, the ester linkage, and 1018 cm^{-1} corresponding to the phenyl ring (Elfehri Borchani et al., 2015; Weng et al., 2013).

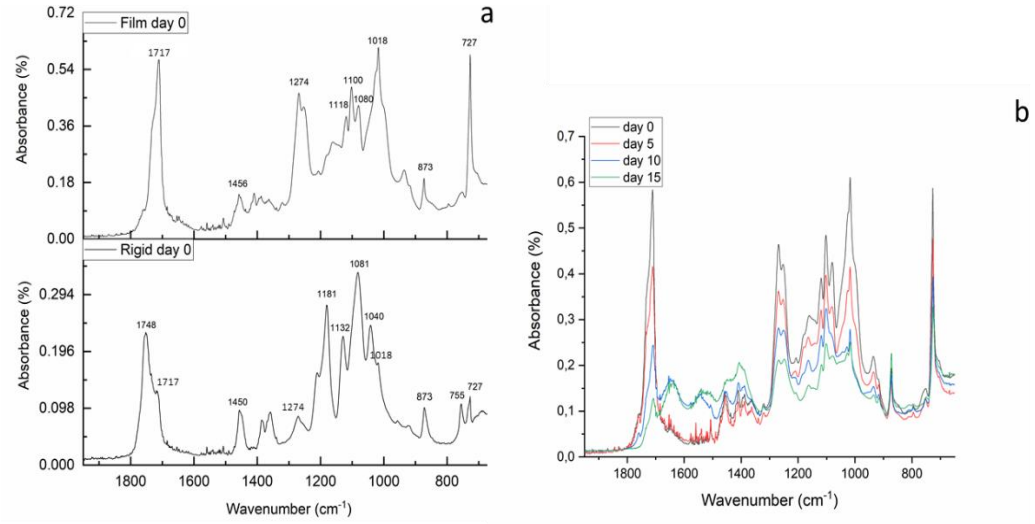


Figure 3.9 Spectra from FTIR analysis of new film and rigid bioplastic samples (a) and degraded film samples (b).

During the composting process, peaks in the spectra of rigid samples from conditions A and B significantly decreased already at the end of the thermophilic phase, in both conditions of low and high humidity (Figure 3.10).

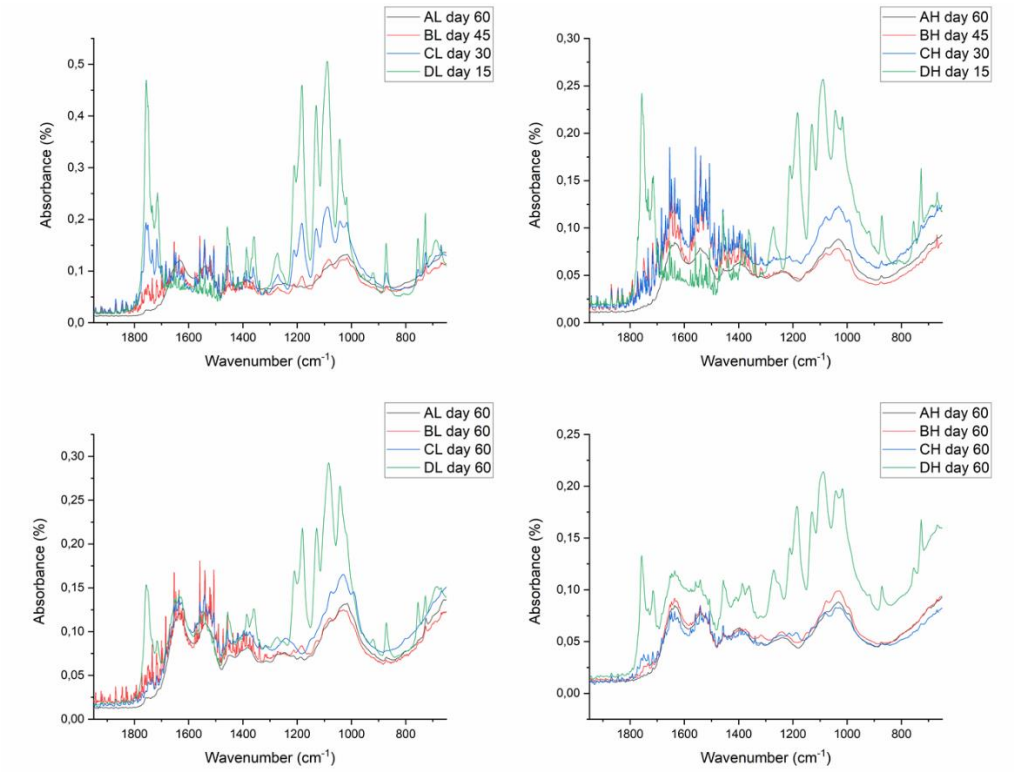


Figure 3.10 Spectra from FTIR analysis of rigid bioplastic samples collected at the end of thermophilic phase and at the end of the test, under the conditions A,B,C and D.

On the contrary, samples in vessels C were submitted to a major degradation in condition of high humidity. Finally, peaks of spectra in condition D depicted an almost constant trend with respect to the spectrum of new material.

The TGA analysis provided a confirmation about the composition of bioplastic teaspoons (Figure 3.11); following the Equation 3.10, composition ratio was 85:15 PLA and PBAT. The result is in line with research development about mixed biopolymers extruded to form film and rigid PLA/PBAT products: different authors showed in their studies TGA and derivatives graphs with the peculiar shoulder of PBAT in a temperature range from 390 to 400°C, depending on the equipment and heating rate (Xiang et al., 2020; Xu et al., 2019; Correa-pacheco et al., 2020).

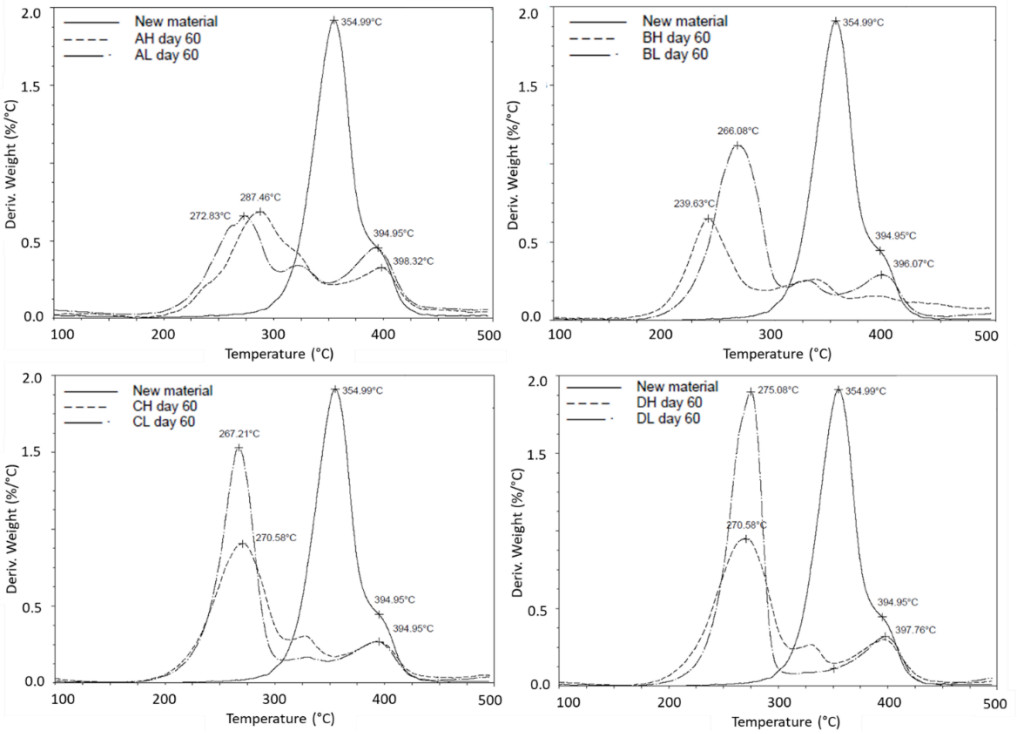


Figure 3.11 Graphs of the derivatives of TGA analysis of rigid bioplastic samples collected at the end of thermophilic phase and at the end of the test, under the conditions A,B,C and D.

In Figure 3.11, it is then reported the behaviour of PLA/PBAT teaspoons at the end of the test. The peak 395°C, related to PBAT, is almost stable: a slight increase occurred in some conditions, but it is basically due to cross-linking or recombination reactions which are typical of degradation (Kale et al., 2007). On the contrary, in the case of PLA, a substantial reduction of the main peak temperature was measured at the end of composting, and it can be considered as an index of degradation (Luzi et al., 2015). This temperature shifted from 355°C to around 270°C in conditions C and D, down to a minimum of 240°C in B_H. A peculiar trend was shown in condition A, where at both low and high humidity the PLA T_{peak} after composting was slightly higher than other conditions. However, it is fair to observe that these peaks are less sharp and less homogeneous, with a tendency to incorporate the small peak which rose at temperature around 320°C. This peak, which appeared in the TGA of day 60 in almost all the conditions, is relatable to the residual organic matter strongly attached to the surface of the material, as observed in previous study (Ruggero et al., 2020a).

Therefore, both A_H and A_L, as well as B_H, underlined a deep degradation. On the contrary, C_H, D_H and D_L showed the lowest degradation, confirming what already assessed with FTIR analysis.

Kinetic study of bioplastics degradation

The degradation of both rigid and film samples met a lag phase lasting for 4 days at the beginning of the composting process, similar to that observed by previous authors for several organic waste matrices (Abu Qdais and Al-Widyan, 2016). After the lag phase, the trend of weight loss followed a significantly different behaviour between film and rigid samples. Film bioplastics completely degraded within the 60 d of composting test; rigid samples degraded up to a maximum of 60% for the most favourable condition (A_H). The curves of weight loss for both film and rigid bioplastics are presented in Figure 3.12.

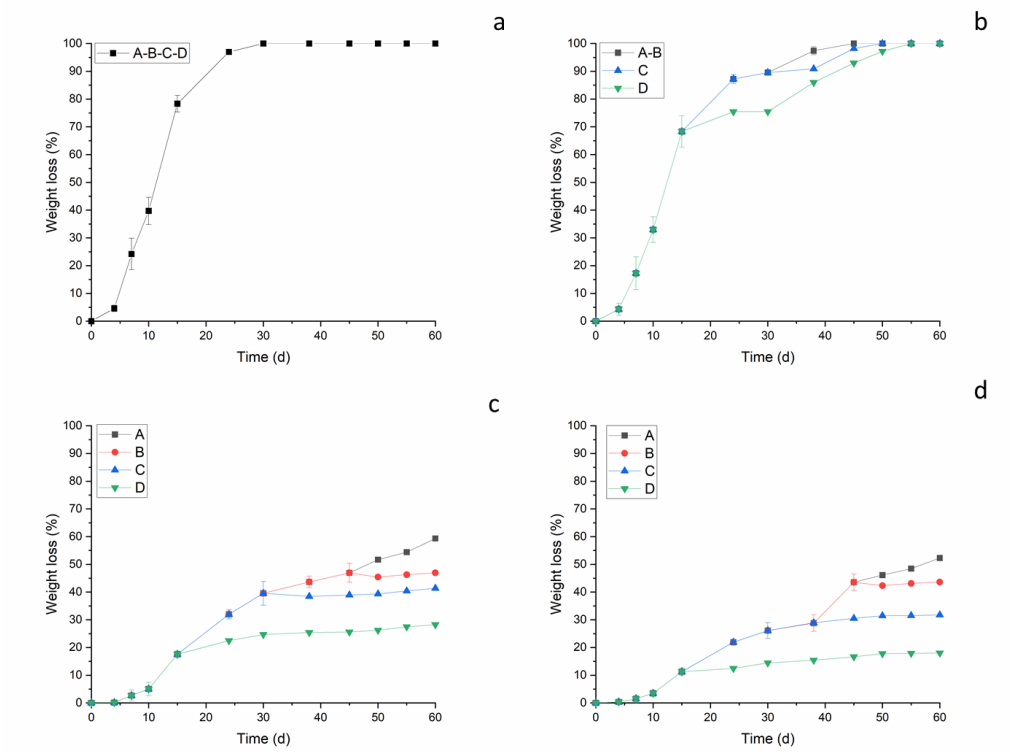


Figure 3.12 Weight loss calculated from experimental data with Equation 3.7: film bioplastics in high humidity conditions (a), film bioplastics in low humidity conditions (b), rigid bioplastics in high humidity conditions (c) and rigid bioplastics in low humidity conditions (d).

Concerning film samples, a humidity not lower than 50% (conditions H) allowed to reach a complete degradation within 30 days. On the contrary, a humidity reduction during the maturation phase (condition L) resulted in the less degradation of the material, which took up to 50-55 days (Figure 3.12a and b).

Considering the rigid samples, the trend of weight loss largely differed from one condition to another. In Figure 3.12c (condition H), it is clearly visible the strong steepness change in correspondence of the maturation phase. Moreover, in the same temperature

conditions, Figure 3.12d shows that slower degradation and sharper steepness variations occurred in the case of a lower humidity of the waste matrix (condition L). This trend confirmed the substantial influence of humidity both during the thermophilic and the maturation phase.

Beside the results related to bioplastics, it is fair to mention some observations about the synthetic waste matrix. TS and weight monitoring of the synthetic waste matrix reported a weight loss ranging from 35% (A and B) to 10-15% (C) and 0-5% (D). From these measurements, it is assumable that the reduction of humidity, jointly with thermophilic phase shorter than one month, affected also the degradation of the synthetic waste. A further index of the influence of unfavourable composting conditions on the synthetic matrix was provided by C/N ratio. The initial value of 27±2 decreased down to a range of 11-18 for the conditions A and B, and to 21-26 for C and D. A value between 10 and 20 is normally expected for a good compost quality (Veneto Agricoltura, 2009).

From a comparison with previous studies on film bioplastics, it emerged that the standardized synthetic matrix seems to encourage the degradation with respect to compost or mixtures of green and food waste (such as in e.g. EN 14855, ASTM D5338, EN 13432). Indeed, degradation of different types of film bioplastics (PLA, PHB, starch-based) in synthetic wastes was found to be not lower than 90% in terms of weight loss (Arrieta et al., 2014; Fortunati et al., 2014; Weng et al., 2010). In the same conditions of temperature and test duration, bioplastics degradation in not synthetic matrices reached up to 70% (Tabasi and Ajji, 2015; Gómez and Michel, 2013), or it required longer composting period to reach 90% (Balaguer et al., 2016). This assumption meets one more evidence in the experimental study of Rutkowska et al. (2004), (Rutkowska et al., 2004) on rigid starch-based bioplastics. After 4 months the degradation was considered complete in synthetic environment, whilst in compost and natural waste it reached a maximum of 20%, in terms of weight loss. The author found that the environment in the synthetic matrix was highly favourable, in terms of pH, matrix homogenization and ammonia loss available for the microorganisms. Therefore, the bioplastics degradation is encouraged.

Starting from weight loss measurement, the kinetic study was developed applying Equation 3.9. The calculation of reaction rate k excluded the period of lag phase and started from day 4th. Moreover, the strong change of temperature and humidity conditions from the thermophilic phase to the maturation phase was considered in the elaboration of the degradation kinetics. Therefore, the kinetic of the thermophilic phase was elaborated with the values obtained while the samples A,B,C and D were at 58±2°C. Then, three different mesophilic kinetics were assessed for B, C and D, starting after the temperature decrease down to 37±2°C. The degradation kinetics for film and rigid bioplastics are displayed in Figure 3.13, differentiated basing on set humidity conditions (L or H). The values of k, intercept and R^2 are reported in Table 3.4. The kinetic data was reasonably fit well to pseudo first order reaction as shown by R^2 (~0,93 – 0,99).

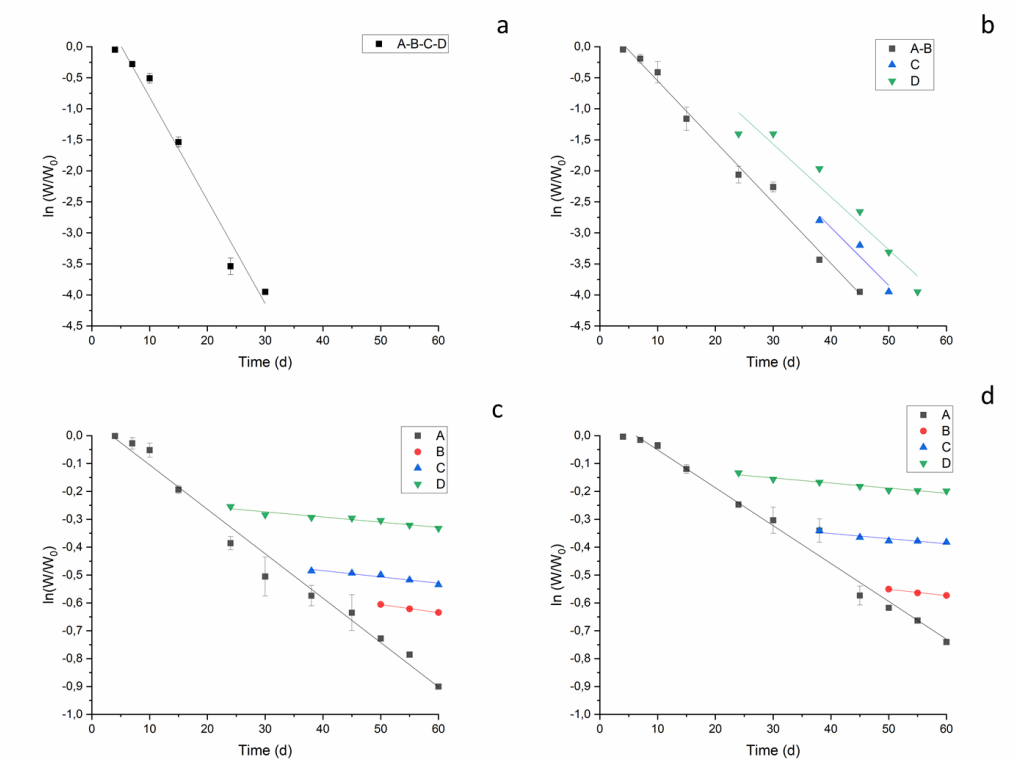


Figure 3.13 Pseudo first order kinetic curves for: film bioplastics in high humidity conditions (a), film bioplastics in low humidity conditions (b), rigid bioplastics in high humidity conditions (c) and rigid bioplastics in low humidity conditions (d).

Table 3.4 Reaction rates of bioplastics degradation in composting, calculate from Equation 3.9.

	Film bioplastics			Rigid bioplastics		
	k (d^{-1})	**Intercept**	**R^2**	**k (d^{-1})**	**Intercept**	**R^2**
A_L	0.0981	0.432	0.9905	0.0136	0.085	0.9307
B_L	0.0981	0.432	0.9905	0.0023	0.439	0.9833
C_L	0.0934	0.822	0.9289	0.0018	0.278	0.9626
D_L	0.0850	0.981	0.9395	0.0018	0.097	0.9433
A_H	0.1663	0.851	0.9759	0.0159	0.055	0.9842
B_H	0.1663	0.851	0.9759	0.0029	0.461	0.9954
C_H	0.1663	0.851	0.9759	0.0023	0.394	0.9298
D_H	0.1663	0.851	0.9759	0.0019	0.217	0.9304

Finally, from the reaction rate it was extrapolate the theoretical time required to complete the degradation process. It corresponds to 8 and 10 months for A_H and A_L, respectively. Times raised for B, C and D due to the reaction rate decrease during the maturation phase at 37±2°C. In temperature and humidity conditions of the maturation phase, B_H would take around 3 years for completely degrading; B_L and C_H not less than 4 years; C_L and D_H around 5 years; finally, D_L would degrade in 6 years.

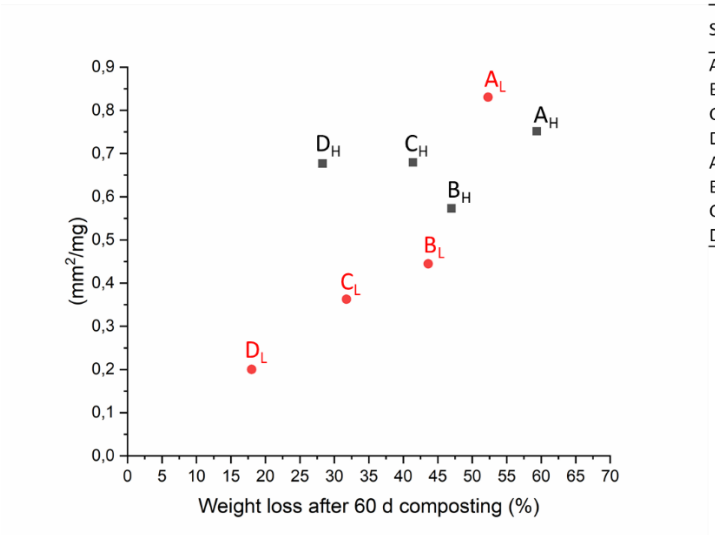
Furthermore, it is fair to evaluate the times to reach 90% degradation, which is the percentage required for bioplastics to comply with the international standard (i.e. EN

13432). A_H and A_L can degrade up to 90% within 5 and 6 months, respectively, thus complying with the international standard (Rutkowska et al., 2004; EN 13432:2000). On the contrary, the operative conditions of the maturation phase slowdown the kinetic, and consequently 90% degradation can be acquired within 2-3 years.

However, bioplastics degradation was not completely prevented during the maturation phase, where it continued at slow kinetics. It is assumable that the degradation was encouraged by mechanical factors, mainly stress and abrasion during turning, and by microorganisms infiltrated into the porous structure of the material. They are protected against desiccation by the slime matrix secreted during microbial adhesion to bioplastic surface and made of polysaccharides and proteins (Lucas et al., 2008). The presence of proteinaceous material found a confirmation in peak 1550 cm^{-1} (Bonhomme et al., 2003), which rose in the FTIR spectra collected after 60 d of degradation.

Microplastics identification in compost

Microplastics detection in the retained matter of 2 mm and 1 mm fractions was negligible, accounting no more than one item in each vessel. Indeed, it was expected that tissue nets retained pieces ≥1 mm size. On the contrary, ImageJ software measured surface areas from 20 to 50 mm^2 in the undersieves <0.5 mm. The graph in Figure 3.14 reports the area/weight ratio of microplastics in each test condition, in relation with the maximum weight loss reached by the respective bioplastic sample after 60 d of composting.



Sample	Area (mm^2)	Undersieve weight (mg)
A_H	40.3	53.6
B_H	48.6	84.8
C_H	50.3	73.6
D_H	30.6	45.2
A_L	46.0	55.4
B_L	33.4	74.2
C_L	28.8	79.4
D_L	20.1	100.2

Figure 3.14 Microplastics in the undersieve of 0.5 mm for each test condition of composting.

In low humidity conditions, it was observable a quite linear trend. At the end of composting, condition D with the shortest thermophilic phase corresponded to the lowest abundance of microplastics in the undersieve of 0.5 mm. On the contrary, the condition A where 58±2°C were maintained throughout all the test, was characterized by the highest microplastics abundance. Therefore, it was confirmed the role played by high

temperatures in disintegrating bioplastics, already assessed by previous studies (Lavagnolo et al., 2020; Weng et al., 2011).

In high humidity conditions, the trend was quite different because samples A,B, C and D did not present strong variation in microplastics abundance in the undersieve of 0.5 mm. Moreover, the area covered by microplastics in each sample (reported again in Figure 3.14) was generally higher in conditions H than L. Thus, beside the temperature, humidity had a role in the disintegration process. A combination of mesophilic temperatures and absence of moistening slowed down both the degradation process and the disintegration of larger pieces into microplastics.

Overall, microplastics identification with ImageJ software was easily applicable thanks to microplastics round shape and light colour, which enhanced threshold setting in dark matrix and dark background (Figure 3.15a and b). Finally, visual identification of microplastics generally required a validation of the items identified as microplastics. Therefore, FITR analysis was randomly carried out on the items (Song et al., 2015). The analysis both confirmed the nature of the fragments and provided information about the type of bioplastics. As expected, microplastics derived from rigid PLA/PBAT teaspoons, which had not been completely degraded within 60 days. Examples of pictures of teaspoons residues collected from the tissue nets at different timings of the test are reported in Figure 3.15c.

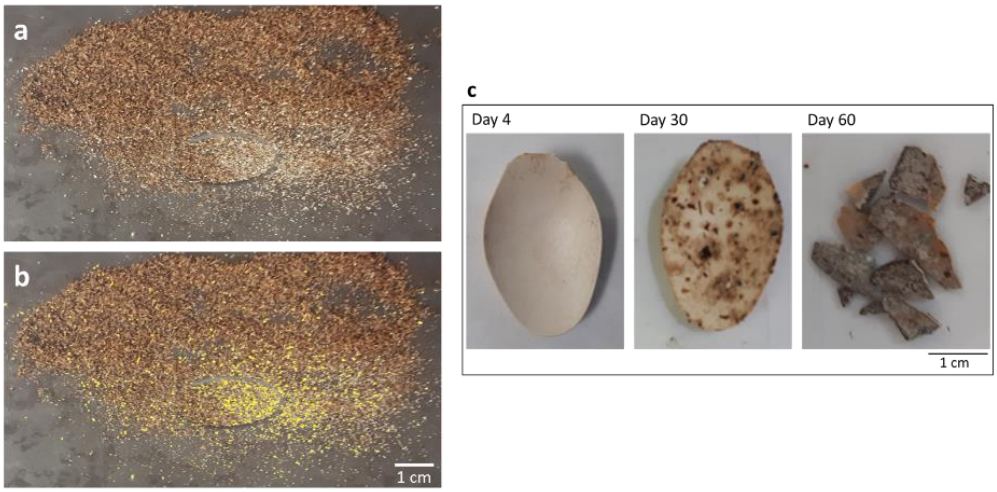


Figure 3.15 Microplastics without (a) and with (b) threshold setting in ImageJ; (c) residues of PLA/PBAT spoon collected in B_H vessel.

Conclusions

The present research provided an improvement of the basic knowledge about the influence of composting conditions on film and rigid bioplastics degradation, which can be useful in the perspective of bioplastic waste management in industrial composting plants. To this purpose, the kinetic study was fundamental to deep analyse the degradation process. Due to the significant variation of conditions between the thermophilic and the maturation phase, the kinetic analysis was separately performed in the two phases.

Even though the kinetic study showed major influence of composting conditions on rigid bioplastic waste than film, the maturation phase always led to a strong slowdown of the degradation rate. Humidity, as well as temperature jump down to mesophilic conditions, were found to significantly affect the degradation process.

Mater-Bi® bioplastic films reached a complete degradation within the 60 days of composting. However, constant moistening to maintain humidity above 50% allowed film bioplastics to degrade within 30 days. On the contrary, in condition L where the matrix was left to dry out, the degradation was not completed before 50-55 days. Concerning rigid bioplastic samples, the degradation rates where much slower and A_H and A_L were the only conditions which would enable to reach 90% degradation within 6 months, as required to certify the compostability. Even though in the other conditions the degradation was not at all prevented to continue, the overall time to 90% degradation ranges from 2 to 3 years.

It is fair to remind that a comparison with previous studies revealed that the synthetic waste matrix provided by the standard, may enhance bioplastics degradation more than natural composting environment. This issue should be accounted also in further studies about compostability assessment in composting.

Finally, microplastics abundance in the undersieve of 0.5 mm was found to generally increase when bioplastics reached higher degradation. Moreover, the results disclosed that beside the temperature, humidity had a role in the disintegration process. Indeed, a combination of mesophilic temperatures and absence of moistening slowed down both the degradation and the disintegration process of bioplastics.

Acknowledgments

The lab scale composting tests of Chapter 3 were carried out in the laboratory of Sanitary Engineering at the Department of Civil and Environmental Engineering (DICEA) of the University of Firenze. The TGA and FTIR analyses were carried out in the laboratories of the CSGI Consortium and of the Department of Chemistry "Ugo Schiff". The SEM analysis was done at MEMA centre in the University of Firenze.

4 Fate of bioplastics from composting to natural environment

4.1 Bioplastics degradation: chemical and physical features during the thermophilic phase and the maturation phase of simulated composting

Abstract

The recent regulations, which impose limits on single use plastics and packaging, are encouraging the development of compostable bioplastics market. However, bioplastic products labelled in accordance with standards for compostability assessment, present a wide variability in type and properties, affecting the composting efficiency. The current work aims to study the degradation mechanisms and efficiency of different marketable bioplastic products under monitored composting process. It follows the guidelines of ISO 14855-2 and stresses novel elements which can strongly influence bioplastics degradation: the simulation of industrial composting conditions and the thickness of bioplastic products, ranging between 50 µm and 500 µm. This research approaches these critical aspects by simulating a composting test with 20 d of thermophilic phase followed by 40 d of maturation phase, on Mater-Bi®, PBAT and PLA. Conventional LDPE was introduced as negative benchmark. An overall study with FTIR, TGA, GPC, SEM and visual inspections was applied. Results highlighted that MB film presented the highest degradation rate in compost, 45±4.7% in terms of weight loss. Both MB and PBAT were subjected to clear changes in their physical and chemical features, while LDPE presented slight degradation signs. The most critical observations have been done for PLA, which is strongly influenced both by thickness and thermophilic phase duration.

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Introduction

The current chapter continues to deviate from the standard test, and it introduces novel elements in the study of bioplastics degradation. It comes closer to the conditions of industrial composting process not only with respect to the operational conditions mentioned in the guidelines, but also considering the effect of bioplastic thickness.

The operational conditions of the test were based on an overview provided by the European Commission (European Commission, 2000), which accounts 17 plants from 6 European countries (Spain, France, Ireland, Italy, Portugal, United Kingdom). The mean length of the thermophilic phase is 2-3 weeks; just two cases differ from the average, presenting a thermophilic phase of one week and one month. On the contrary, the maturation phase has a wider variability among the plants: from a minimum of 2 weeks to a maximum of 6 months. Starting from this overview, it was set the duration of the thermophilic and the maturation phase in the lab scale test at 20 d and 40 d, respectively. Considering the increasing trend of bioplastics usage (European Bioplastics, 2019), the input of the tested material has a concentration slightly higher than in the standard tests. A further element of novelty was related to the selection of the type and the thickness of tested bioplastics. Indeed, they are strictly related to the usage of bioplastic products: Mater-Bi® (MB) is widely employed for biobags production, while PLA is generally used for single use glasses, cutleries and dishes manufacturing. These products are ten times thicker than film bags.

In the degradation monitoring, the key criterion highlighted by the methodologies of the common guidelines, is the weight loss. Though, this parameter has some limitations: (i) the visual recoverability of the entire sample due to the disintegration into micro-pieces, (ii) the variables that influence the weight, such as compost residues on plastic surface and water absorbed during composting, and finally (iii) the complexity of the degradation process which cannot be addressed. Based on these considerations, the current research investigates the degradation process in simulated industrial composting conditions with a wide range of methodologies, enabling to provide an insight into the chemico-physical changes of the tested polymers and to define the key drivers of the degradation steps.

Materials and methods

Tested materials

The bioplastics selected for the experimental tests are available on the European market and are products commonly used by citizens and disposed in the organic fraction of waste. Three bioplastic types have been selected. The first one was MB; as widely discussed in Chapter 3, bags made of this material are commercially available and were bought in Italian supermarket. They were manually cut (thickness =50 μm, size =5x5 cm, melt temperature=64°C, density =1.2 g/cm^3). It is reminded that the material is composed of starch (20%), PBAT (70%) and some additives (10%) (Bastioli, 1998). PBAT seems to be the most critical part of MB, because its degradation is largely influenced by

composting process conditions (especially low humidity and short thermophilic phase) (Ruggero et al., 2020a). Therefore, pure PBAT was separately tested to better investigate its degradation process. PBAT Ecoflex® F Blend C1200 was supplied by BASF (thickness=90 μm, size=5x5 cm, melt temperature=110-120°C, density=1.25-1.27 g/cm^3. It is fair to highlight that PBAT is fossil based and not biobased, but it certificated as compostable bioplastic. The third material was PLA. However, differently from MB and PBAT, it was tested in rigid form, in order to simulate the degradation of some thick products made of PLA and widely diffused on the market, such as single use cutleries, dishes and glasses. PLA was supplied by NatureWorks LLC (USA): PLA98.6/1.4 (L/D-lactide) is a grade for the extrusion of films (D-isomer <1.4%; relative viscosity=3.94; residual monomer=0.14%, melt temperature=210±8°C). Pellets were dried in vacuum oven overnight at 60°C and processed using a press model 4122CE manufactured by Carver, with circle shape (thickness =500 μm, ø =5 cm,).

Conventional LDPE in film form was introduced in the test as a negative benchmark for its low degradation efficiency. Furthermore, as some LDPE garbage bags are incorrectly conferred to industrial composting plants, an improvement of the knowledge about their fate in composting conditions could rise the need to provide stricter limits regarding the use of LDPE bags for organic waste collection. LDPE film ET311350/2 was bought from Goodfellow company (UK) (thickness is=250 μm, size=5x5 cm, melt temperature=116°C, density=0.925 g/cm^3).

Experimental setup

Composting tests were carried out in accordance with modified guidelines ISO 14855-2:2018 (ISO 14855-2, 2018). It was used 10 g of plastic, completely mixed in 350 g of compost (concentration 2.9%). A 3 months stable compost (IR_5 5.6 mg O_2/g TS) from green and agricultural waste was provided by Ipalle industrial composting plant, located in Froyennes (BE), together with the chemico-physical analyses carried out by Liège University (BE). Mature compost shall be used as inoculum of the aerobic biomass to provide sufficient diversity of microorganisms and enhance the degradation process of the tested materials. Humidity, C/N and pH of compost were 47%, 15 and 7.5, respectively.

Composting tests were carried out in three replicates for each material; moreover, three reactors contained only compost as background controls. Glass reactors were cylindrically shaped with a volume of 5 l, manufactured by Pierre E. bvba (Vilvoorde, BE). The reactors were connected with a system provided by Wetlands Biosciences sprl (Louvain-la-Neuve, BE), which allowed the automatic control of the air flow through the reactor. The air flow was humidified by passing through distilled water in a glass bottle, of which each reactor was provided. The 15 reactors were placed into two Binder BD400 incubators in order to maintain the conditions required during the test.

The test was carried out under thermophilic conditions (58±2°C) for 20 d, followed by a maturation phase (37±2°C) for 40 d. Reactors were opened once a week: humidity was checked and manually adjusted to be in the range 50-55% and the sample was mixed to ensure a proper homogenization of the plastic material within the compost.

Some pieces of the material from each reactor were recovered after 20 and 60 d to be submitted to the chemico-physical analyses in 3 replicates. Before being analysed, the samples were gently brushed off with tissue papers to remove compost residue from their surface.

Fourier transform infrared

FTIR spectra were collected in total reflectance mode (ATR) with Bruker Tensor 27 IR. The investigated wavenumber range is 4000-500 cm^{-1} and the resolution is 2 cm^{-1}. Moreover, the analysis was directly performed on the pieces of the tested materials, with a size bigger than 1 mm. The system is coupled with software 6.5 Opus. The spectra were acquired in absorbance.

Experimental weight loss

Compost recovered after 60 d of composting was first sieved (2 mm mesh). Plastic pieces both from the over and the undersieve were extracted with tweezers, as far as it was possible with a naked eye. They all were brushed off and weighted to be compared with the initial weight (10 g). Even after brushing, the plastic pieces presented compost residues attached to the surface. Moreover, part of the tested plastics acquires the capability to absorb water during composting process. These two variables could alter the experimental weight of plastic pieces recovered at the end of the test.

To provide an estimation of these variables and subtract them from the experimental weight, information shown by TGA analysis were exploited. Percentage values of water and residues depicted by TGA were used as the two quantities to be subtracted from the experimental weight, resulting in the following equation:

Equation 4.1

$$Ex.Weight\ loss\ (\%) = \left(100 - \left(\frac{W_{60}}{10}\right) * 100\right) - water\ (\%) - residues(\%)$$

were W_{60} is the experimental weight (g) of the plastic pieces recovered after 60 d of composting, 10 g is the initial weight of the plastic sample, $water\ (\%)$ and $residues(\%)$ are the values depicted by TGA analysis, as explained in next paragraph.

Thermogravimetric analysis

TGA was performed using a TA Instruments Q-500 (MTG-TGA) apparatus using open aluminium pans under a nitrogen atmosphere. Measurements were performed in a dry nitrogen balance purge flow of 40.0±0.5 ml/min and sample purge flow of 60.0±0.5 ml/min by increasing the temperature from room temperature up to 800°C at 10°C/min. 10 mg of samples were submitted to the analysis.

From the first derivative of the TGA curves it is possible to obtain the characteristic temperature associated to the thermal reaction, indicated with T_{peak} and corresponding to the maximum temperature reached during the conversion. Moreover, the weight loss of the materials at given aging time can be acquired from the raw data, exploiting the following equations:

Equation 4.2

$$PA\ (\%) = \int_{T_0}^{T_{inf}} \frac{dw}{dT}\ dT$$

Where T_0 and T_{inf} are the initial and the final temperatures of conversion, readable in the DTGA graph. The integral corresponds to the peak area, which basically is the weight of the material. Knowing the peak area (PA) of the material before (PA_0) and after degradation (PA), it's possible to get the weight loss $(WL\ (\%))$.

Equation 4.3

$$WL\ (\%) = \ 100 - \frac{(PA)}{(PA_0)}\ x\ 100$$

Finally, the TGA provides values of water absorbed by tested material $water\ (\%)$ and of residues of compost attached to the plastics surface $residues(\%)$: the temperatures set for the percentage weight of water and residues are T<105°C and T>500°C, respectively. These two values are exploited in the measurement of the experimental weight loss, as previously discussed.

Gel permeation chromatography

Gel permeation chromatography was applied on bioplastic residues in order to determine the loss of molecular weight during composting. Samples were initially dissolved in chloroform (only MB, PBAT and PLA are dissolvable), then the analysis was carried out with Agilent1200 series GPC equipment. Size exclusion chromatography (SEC) was performed in $CHCl_3$ at 30°C using an Agilent liquid chromatograph equipped with an Agilent degasser, an isocratic HPLC pump (flow rate=1 ml/min), an Agilent autosampler (loop volume=100 µL, solution conc.=1 mg/ml), an Agilent-DRI refractive index detector and three columns: a PL gel 10 µm guard column and two PL gel Mixed-D 10 µm columns (linear columns for separation of MW(PS) ranging from 500 to 107 g/mol). Polystyrene standards were used for calibration.

Scanning electron microscope

Images of degraded and intact plastic pieces were collected with SEM, JSM 7200F JEOL emission field scanning electron microscope apparatus. The pieces of bioplastics were

pre-treated before SEM analysis by submitting them to ethanol baths (one night 70%, 2 baths 90% for 30 min and last bath 100% for 1 hour). After this procedure, the samples were dried passing to the critical point of CO_2. The samples were finally metallized with gold using JEOL device JFC 1100E ion sputter, fine coat. The metallization took place at 10 mA for 3 min.

Visual inspection

Plastic residues recovered from compost were reported in photographs to visually define the macroscopic changes of the material in accordance with the following criteria as described by EN 14045 (EN 14045, 2003): distribution of particle size, consistency of the material, discolouring, erosion signs on the surface and lateral erosion signs.

Results and discussion

Fourier transform infrared

FTIR analysis provided an overview of the chemical bonds which characterize the tested materials, displaying the material through the main peaks (Figure 4.1).
The observed FTIR spectrum of the fresh PLA was similar to that reported previously with the same material (Tabasi and Ajji, 2015; Arrieta et al., 2014): the peak at 1748 cm^{-1} is due to the asymmetric stretching of C=O in lactide; 1450 cm^{-1} relatable to CH_3 in alkanes, 1132 cm^{-1} which corresponds to C-C stretching in alkanes; 1181 cm^{-1} which is the C-O stretching in -CH-O group; 1081 cm^{-1} of C-C in n-alkanes; 755 cm^{-1} that identifies the crystalline phase of PLA (866 cm^{-1} is instead the wavenumber of amorphous PLA).
The FTIR spectrum of fresh LDPE showed specific peaks of this material: 2914 cm^{-1} and 2485 cm^{-1} which correspond to antisymmetric and symmetric CH_2 stretching in n-alkanes, respectively. Then 1463 cm^{-1} is attributable to CH_2 deformation in n-alkanes and 721 cm^{-1} is from methylene rocking. Finally, 1306 and 1351 cm^{-1} were observed in the LDPE spectrum, connected to the twisting and wagging deformation of the CH_3 group (Da Silva and Wiebeck, 2017).

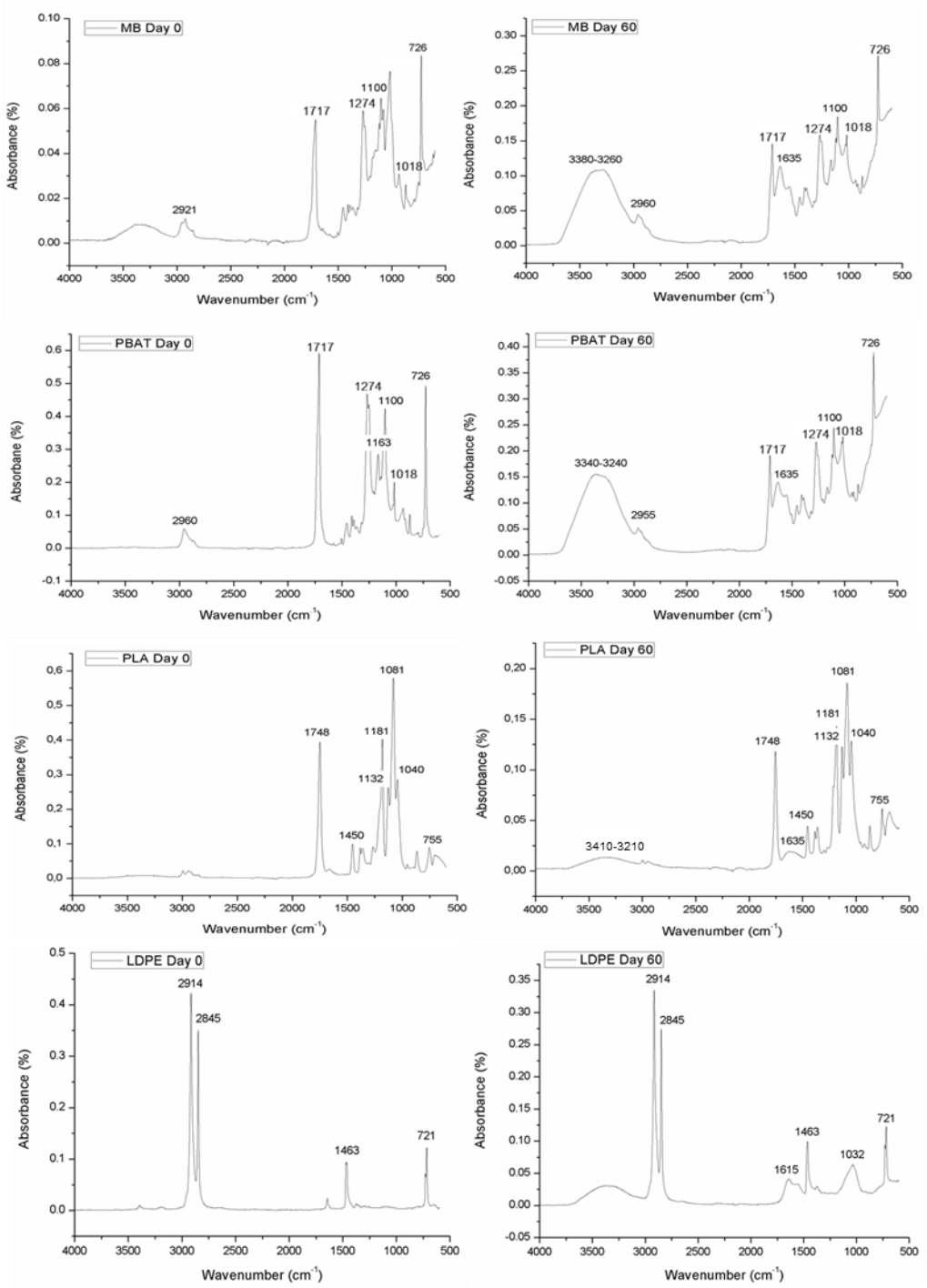

Figure 4.1 Spectra of MB, PBAT, PLA and LDPE resulting from the FTIR of samples analysed before and at the end of the test, day 0 and 60 respectively.

The FTIR spectra collected at the end of the test (day 60) reported some peaks which are specific to all the degraded bioplastics and LDPE. In the range between 3360 cm^{-1} and 3240 cm^{-1}, the band between 3380 cm^{-1} and 3340 cm^{-1} is due to the stretching of the OH bond of the hydration by water molecules, while 3340-3290 cm^{-1} is assigned to NH group of amides. Thus, the broad peak in this range, which was more intense after 60 d, can be attributed to two main phenomena: the hydrolytic degradation of the biomaterial and the presence of proteinaceous substances due to the bacterial activity. In

particular, the peak at 1635 cm^{-1} could be correlated to carboxylate ions ($R\text{-}COO^-$) which formation is due to the activity of microorganisms (Arrieta et al., 2014); moreover, after 60 d, the presence of the peak at 1550 cm^{-1} is attributable to proteinaceous material (Bonhomme et al., 2003).

It is fair to observe that the spectra of 60 d degraded MB and pure PBAT were almost identical: they depicted the same characteristic peaks. This indicates that on the one hand PBAT was subjected to degradation during the composting process both as pure material and as part of MB, and on the other hand that the presence of starch was almost negligible in the degraded MB (as indicated by the strong decrease of the peaks between 1150 cm^{-1} and 950 cm^{-1}). Regarding PLA, this material, after degradation, did not present significant changes with respect to the fresh material, as already observed in previous studies (Massardier-Nageotte et al., 2006). It is interesting to compare this observation with the hydrolytic degradation process of ester bonds, typically occurring in PLA, which always generate oligomers or monomers (Benali et al., 2015). For this reason, it is fair to expect that during the degradation the peaks related to the bonds characterizing PLA do not change.

Finally, in LDPE just a slight sign of degradation was demonstrated by the two peaks at 1615 cm^{-1} and 1032 cm^{-1} which rose after composting; the first, as already observed in a previous study (Delacuvellerie et al., 2019), is reliable to C-O bond, and the second is a CH deformation.

Experimental weight loss

Figure 4.2 reports the experimental weight of the tested materials, expressed in grams of residual weight of both macro and micro (<2 mm) residues. In particular, for MB some micro (<2 mm) and macro residues, with a size between the initial 5x5 cm and 2 mm (Figure 4.2a), are still present at the end of the degradation process. In fact, the weight loss experimentally measured with Equation 4.1 was 45±4.7% (Figure 4.2b). This value is not far from observations of previous studies operating in similar temperature conditions for a test period from 28 to 90 d (Massardier-Nageotte et al., 2006; Mohee et al., 2008; Accinelli et al., 2012): they found that degradation ranges between 30 and 55%. It is already interesting to observe a difference with the weight loss of pure PBAT film, which was 8±1.6%.

Considering the PLA, it was found that the degradation did not exceed 3±0.7%. The observed trend of PLA requires to make a comparison with previous studies which found better degradation results. In a prolongated thermophilic phase (>30 d) , the degradation exceed 90%, in terms of experimental weight (Kale et al., 2007; Pradhan et al., 2010). Conversely, it was found that mesophilic conditions (30-90 d) stop PLA degradation at around 10% (Massardier-Nageotte et al., 2006; Song et al., 2009; Shrestha et al., 2020). However, available studies on PLA were performed only on film material, with thickness ranging from 30 to 50 μm. Thus, the current research disclosed the importance of considering the thickness as significant variable in the degradation test, as well as composting conditions and the complexity of polymeric structure.

Besides MB, only PLA presented microplastic fragments after 60 d of composting; a further discussion of this phenomenon is provided in the next paragraphs.

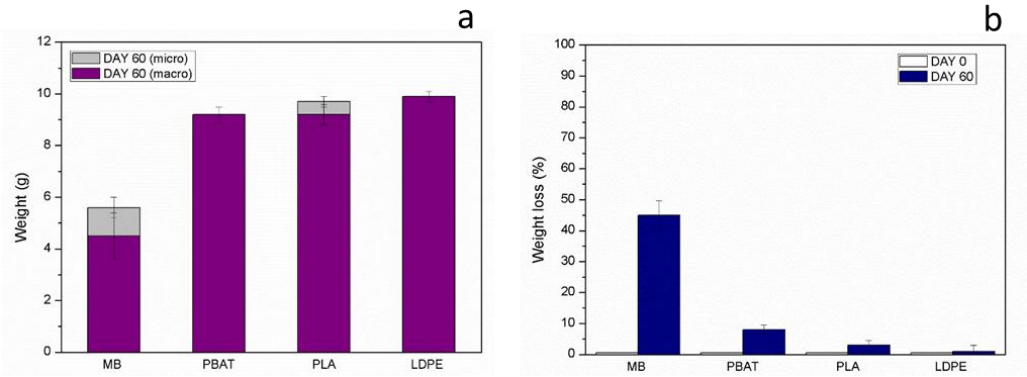

Figure 4.2 Experimental weight of the tested materials, expressed in grams of residual weight of both macro (5 cm – 2 mm) and micro (< 2 mm) residues (a) and in percentage of weight loss (b), elaborated in accordance with Equation 4.1

Thermogravimetric analysis

The results derived from TGA analysis indicate that the major contribution in MB weight loss was given by starch degradation: up to 88±2.9%, as indicated in Figure 4.3, while this value is about 18±1.8% for PBAT (obtained by Equation 4.3). For MB it was also possible to provide an estimation of the global weight loss, considering that starch and PBAT constitute 30% and 70% of polymer, respectively. The result estimated by these proportions was 39±2.1%.

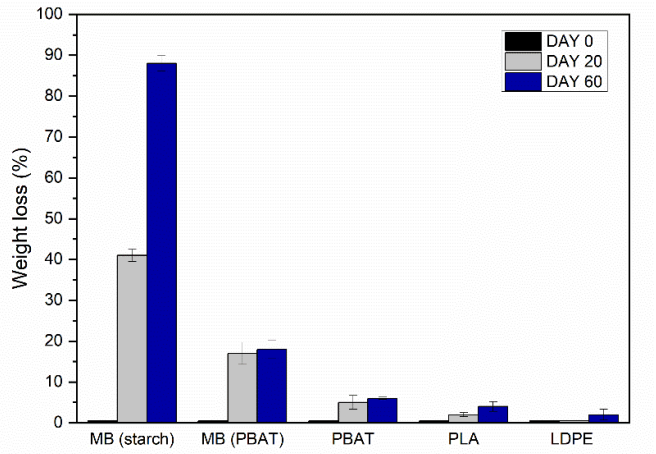

Figure 4.3 Trend of weight loss of the tested materials, derived from TGA analysis in accordance with Equation 4.3.

As expected from the results of the experimental measurements previously described, the weight loss derived from TGA for the other tested materials was not very high: 6±0.3% for pure PBAT, 4±1.2% for rigid PLA and lower than 2% for LDPE.

It is interesting to compare the results obtained by TGA with the weight loss experimentally measured. TGA results obtained for MB and PBAT, which presented the major degradation among the materials, were 20±5% lower than experimental values.

This discrepancy can be attributed to the higher precision of TGA analysis compared to a method based on a first visual recovery. Especially for MB, the experimental weight loss method was highly limited by the naked eye recovery of micro fragments. Therefore, the smallest pieces were likely to remain into the compost matrix and were not accounted in the residues weighted.

The T_{peak} (see Table 4.1) is a further parameter that can describe the degradation level of the investigated polymers. In MB, the strong decrease of T_{peak} of the starch component (from 317°C for the undegraded MB, down to 303°C for the sample of MB collected 20 d after the beginning of the composting process) confirmed its stronger degradation with respect to the PBAT component. A progressive shift of T_{peak} to lower temperatures was found also in PLA: from 361°C for the fresh material, down to 338±4.5°C after 60 d of composting, indicating that a chain degradation occurred for this polymer.

On the contrary, a slight increase of T_{peak} was observed for both PBAT and LDPE: this interesting phenomenon was already observed in earlier studies (Kale et al., 2007) and it is probably due to cross-linking or recombination reactions of the biopolymer chains occurring during the first step of degradation.

Table 4.1 Table reports information derived from TGA analysis on the tested materials.

	Day	T_{peak} (°C)	Weight loss (%)	Water (%)	Residue (%)
MB (starch)	0	317	-	-	-
	20	303±0.8	41±1.5	3±0.9	23±1.2
	60	-	88±2.9	9±2.4	25±1.8
MB (PBAT)	0	395	-	-	-
	20	396±0.6	17±2.9	3±0.9	23±1.2
	60	395±0	18±1.8	9±2.4	23±1.8
PBAT	0	395	-	-	-
	20	399±0.8	5±1.7	1±0	10±1.3
	60	400±0.3	6±0.3	-	10±0.3
PLA	0	361	-	-	-
	20	349±1.6	2±0.5	2±0.5	2±0
	60	338±4.5	4±1.2	2±0.5	1±0.9
LDPE	0	467	-	-	-
	20	469±1.8	-	-	2±1.3
	60	470±0.3	2±1.3	-	2±1.3

Gel permeation chromatography

An important confirmation that biodeterioration and further depolymerization occurred in the selected polymers was also provided by GPC analysis (Figure 4.4).

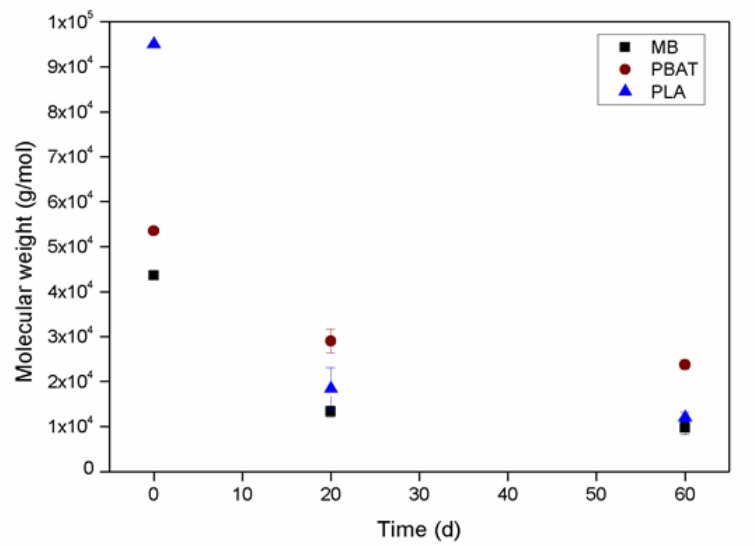

Figure 4.4 Trend of molecular weight loss (g/mol) for the tested bioplastics at the beginning, during (day 20) and at the end (day 60) of the test.

Concerning MB, after 60 d of composting, a decrease of the average molecular weight of 75% was observed; this data suggests the progressive fragmentation of MB into a mixture of oligomers and monomers (Siracusa, 2019).
Concerning PLA, the GPC data indicate that the molecular weight decreased from 9500 g/mol down to 1750 g/mol, which is consistent with the depolymerization process that gives the formation of simpler chains due to the hydrolysis occurrence (Pantaloni et al., 2020).
A peculiar situation is instead related to pure PBAT. While TGA and weight loss did not highlight a strong degradation process, the GPC analysis reported a reduction of the molecular weight close to 50%. This indicates that the depolymerization started. The result seems in contrast with the slight increase of T_{peak}, because both molecular weight and T_{peak} can be considered as indexes of the complexity of the material. It was expected that the decrease of the first index, which suggests a decomposition of the material into smaller polymer or oligomer chains, was accompanied by a decrease of the T_{peak} (Luzi et al., 2015). However, as mentioned in the previous paragraph, in this case, T_{peak} increase can be attributed to cross-linking or recombination reactions occurring in PBAT polymer while degrading.

Scanning electron microscope

Morphological analysis with SEM introduced an overview of the material biodeterioration and allowed to characterize the degraded residues through some interesting aspects.
In Figure 4.5, fresh MB appears as a composite polymer with starch grains embedded in a flat film, which corresponds to PBAT, as confirmed by the micrograph of a flat surface of pure PBAT at day 0. Fresh PLA and LDPE are also characterized by a flat surface.

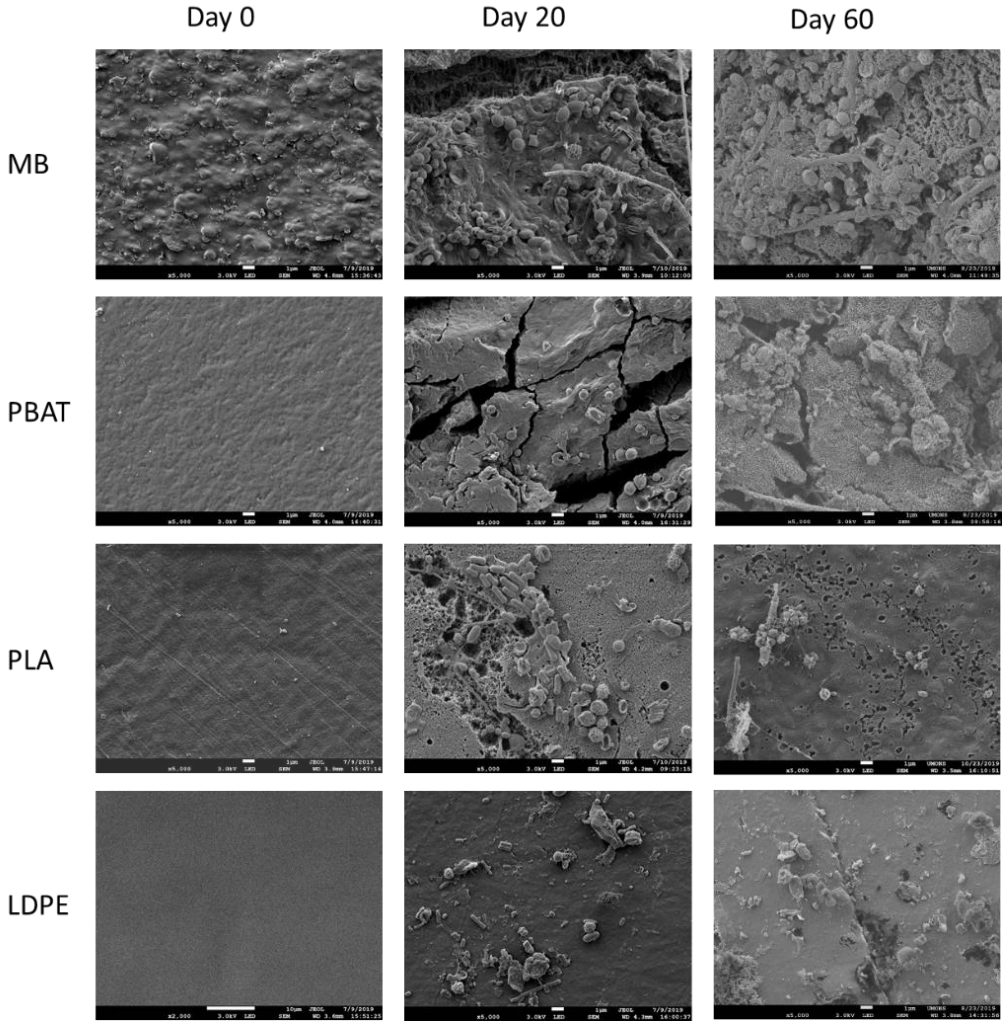

Figure 4.5 SEM micrographs at different timings of the test carried out on the tested materials.

Concerning MB film, the disappearance of the starch grains embedded into the continuous matrix was evident already after the thermophilic phase of composting (day 20). Moreover, during this phase, the surface was fractured and colonized by different microorganisms. Some peculiar long chains distinguishable on the micrographs (Figure 4.6a) were identifiable as fibres in the internal structure of the biopolymer, which constitute the strongest and less degradable part of the material. Thus, these fibres seem to have surfaced due to the progressive erosion of the material, as observed in previous studies dealing with plastic fibres (Pivokonsky et al., 2018; Afzaluddin et al., 2019). A further characteristic of the degraded material was the nanometric light dots which appeared on the surface after 60 d of composting (Figure 4.6b_1). The same nanodots were present on the surface of degraded PBAT (Figure 4.6b_2). Due to the small size, they could not be related directly to microbial organisms, but it was fair to hypothesize that they are residues of bioplastics assimilation.

A further peculiar feature of PBAT was the tendency of the surface to break (Figure 4.6c). This aspect was already observed by previous authors (Weng et al., 2013; Wang et al., 2015) and were related to a physical degradation, whose effects are usually not immediately visible at a macroscopic level (Duval, 2004). The present study confirmed

the physical degradation and considered these surface cracks as a first step of film disintegration that can result in the formation of nanoplastics (dimensions below 10 μm). Moreover, SEM analysis indicated that the pure PBAT was much less subjected to diffuse bacterial colonization than in MB.

Observing the degraded PLA, SEM micrographs illustrated the roughness of the surface and the appearance of pores after composting. Both observations characterize the degradation of the material (Luzi et al., 2015). Further peculiarities were found. First, a porous structure with nanometric holes homogeneously distributed on the surface was observed and this shape was completely different from the flat surface before composting (Figure 4.6d_2). Holes on PLA surface were observed by previous studies (Bijarimi et al., 2013; Valerio et al., 2017), but in the current case this phenomenon was much more extensive. Secondly, as reported in Figure 4.6e, an extensive bacterial colonization occurred on PLA. Conversely, LDPE did not present particular visual changes after 60 d of composting.

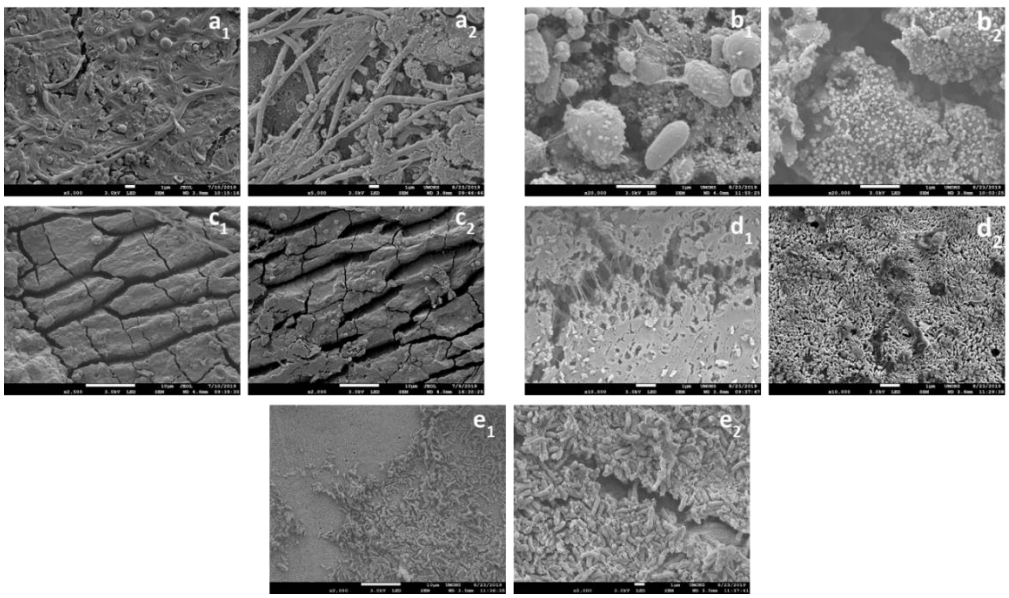

Figure 4.6 Peculiar features observed with SEM analysis. (a) filaments on MB surface (a1 is for day 20 and a2 for day 60). (b) light dots on MB (1) and PBAT (2). (c) PBAT after 20 (c_1) and 60 days (c_2) of composting. (d) Sponge structure of MB (d_1) and PLA (d_2). (e) bacterial colonization on the surface of PLA.

Visual inspection

Photographs of the tested materials showed some macroscopic physical changes occurring during composting (Figure 4.7). The size reduction was visible for all the bioplastics, but especially in MB film. In the first weeks, the phenomenon was mainly related to the folding of the bioplastic sheets and to the increase of its stickiness. This latter was due to the temperature of the thermophilic phase, close to the melting point. Moreover, the disintegration was induced by the increasing fragility of the biomaterial and the concomitant biotic activity. The disintegration effect was widely observed in the residual MB recovered at the end of the test, as reported in Figure 4.2a (around 25% of the residues are microplastics; size below 2 mm). Finally, both macro and micro residues had

colour and consistency similar to compost, turning from transparent or white to uniform or spotted brown.
Also PLA, as already found in earlier studies (Kale et al., 2006; Luzi et al., 2015), showed the tendency to disintegrate. A further observation (Figure 4.7) on this thick bioplastic was the loss of transparency already after one week of composting: this effect can be attributed to a progressive loss of the crystallinity. These aspects were considerable signs of abiotic and biotic activities involved in biodeterioration (Kale et al., 2007).
Finally, LDPE photographs reported an almost integer surface of LDPE (only the presence of compost residues was observable onto the film surface), still transparent and flat even after 60 d of composting.

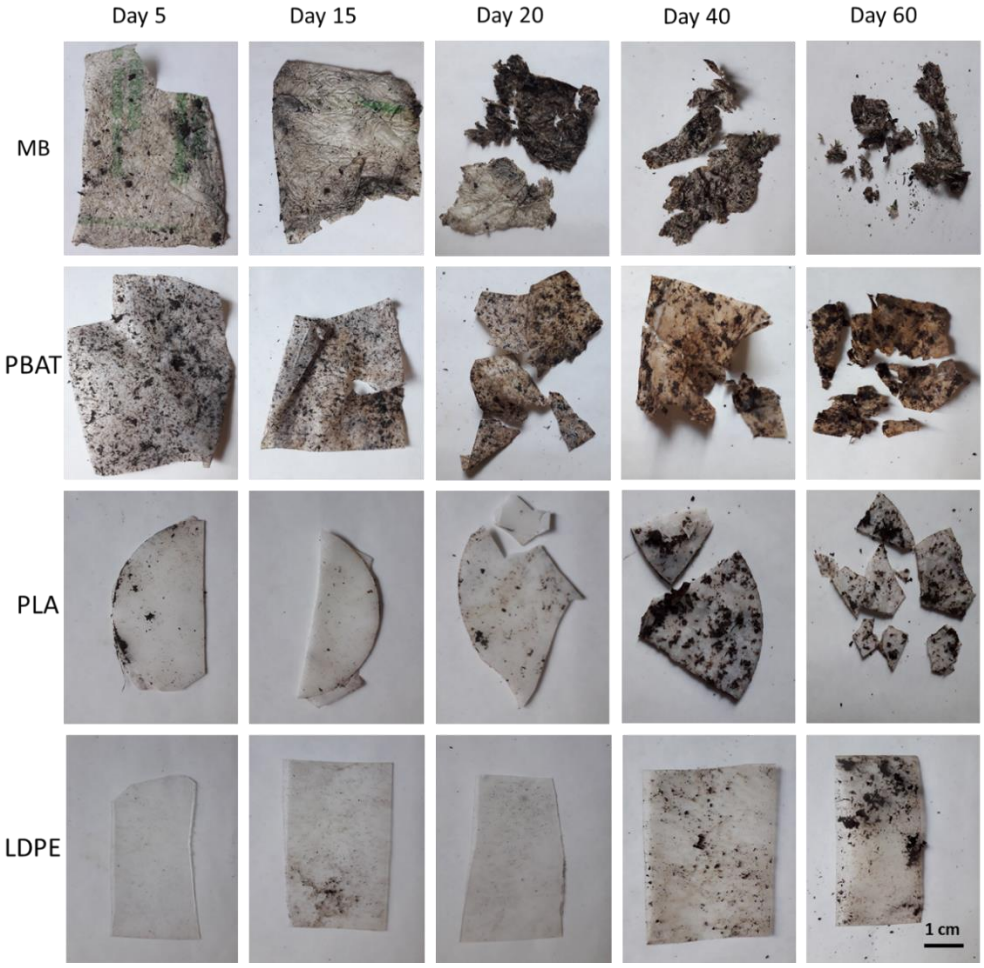

Figure 4.7 Photographs of the tested materials at different timings of the degradation process.

Conclusions

The current research introduced a novelty among the studies related to bioplastics degradation: it investigated the difference of composting process conditions with respect to the guideline methodology and the variability of bioplastics thickness.
The approach combining a variety of methodologies disclosed a wide range of useful information about the degradation process. The implementation of industrial composting

conditions to study the plastic degradation highlighted some interesting points. Signs of degradation were observable in all the tested materials. First, the decrease of molecular weight, particularly accentuated for PLA and MB. Then, the peaks in the spectra assigned to proteinaceous material, relatable to biofilm formation on the surface of the bioplastics. Moreover, there were signs of erosion and disintegration visible from microscopic level (in PBAT) to macroscopic level (PLA and MB), and nanoparticles detected with SEM micrographs on the surface of PBAT and MB residues. Finally, it was assessed the depolymerization into oligomer chains, assimilable by bacteria.

MB and PBAT displayed the major degradation rate, which was faster for the PBAT present in MB than for pure PBAT: in fact, starch grains in MB degraded first, generating cavities which enhanced the degradation of the whole polymer. Despite this, it was noticeable from all the analyses that additional time was required for the complete degradation and therefore assimilability to compost. Furthermore, it emerged the issue of micro and nano fragments detected at the end of the test: further studies are recommended to carefully monitor their fate into the environment and to rethink regulation targets.

Rigid PLA exhibit key features of a progressive degradation, but the study disclosed the importance to include the thickness as fundamental variable in the degradation process. Indeed, it was found that the combination of a 20 d thermophilic phase and the 500 µm thickness of the material prevented a complete degradation within 2 months.

Finally, as reported in the introduction about the overview of the European Commission, the industrial composting plants have a mean thermophilic phase of 14-21 d. The current study found that a thermophilic phase of 20 d is not sufficient to ensure bioplastics degradation. This result is of great concern, because it confirms that the discrepancy between the standard conditions (e.g. EN 13432, ISO 14855) and the industrial composting conditions is an issue to be accounted in the bioplastics management system. If necessary, a proper degradation time in the industrial plants may be acquired by recirculating macro-residues of bioplastics after a refining treatment. To this purpose, a last important issue emerged from the test of LDPE in composting. The conventional plastic showed a minor weight loss and not significative visual changes with respect to bioplastics. Moreover, the presence of LDPE in the retained material of refining treatments prevents the recirculation of the whole oversieve, which is therefore destined to incineration or landfilling. Thus, regulations should force the correct conferment of only bioplastics in the organic waste, to allow recirculation cycles of bioplastics and to improve the performance of the industrial plants.

4.2 Characterization of bacterial communities responsible for bioplastics degradation during the thermophilic phase and the maturation phase of composting

Abstract

The microbial community plays a fundamental role in bioplastics degradation in composting process, as polymers can be used by heterogeneous microorganisms for their growth. The present study aims to investigate bioplastics-associated bacterial communities which are present under thermophilic and maturation phases of composting. Composting process was simulated in a 2 months lab-scale test. Mater-Bi®, PBAT, PLA and conventional LDPE were considered as benchmark. A first qualitative screening was done with Denaturing Gradient Gel Electrophoresis (DGGE), then the bacterial community profile was disclosed with a 16S rRNA amplicon analysis. The bacterial communities developing on bioplastics and LDPE became more specialized in degrading polymers, and depicted distinct bacterial genera in mesophilic and thermophilic conditions. Some peculiar bioplastics-associated bacteria were identified in the current work, in particular *Streptomyces*, *Pseudomonas*, *Aeribacillus*, *Schlegellela*, *Limnobacter*, and *Cohnella* were among most abundant genera. In addition, some bioplastics degrading bacteria were found to be dependent on the plastic nature, but a larger variety of specialists were present on two or more plastic types. Finally, an enrichment method was applied with the objective to investigate the bacterial species using bioplastics as sole carbon source. The method disclosed the possibility to culture some specific bioplastics degrading species, opening the frontiers for bioaugmentation practice in industrial composting.

Introduction

In accordance with the European Bioplastics Agency, bioplastics are divided into three categories: biobased bioplastics, which derived from renewable resources; biodegradable bioplastics, degradable by microorganisms; bioplastics which feat both the characteristics. Compostable bioplastics are biodegradable by microorganisms under composting conditions, and they can be biobased or derived from fossil fuel. Even though compostable bioplastics represent just a small part of the global plastic production, their increase on the market will require further research to improve the management in biological waste treatments. Furthermore, the data from the Italian Composting and Biogas Association, named CIC (*Consorzio Italiano Compostatori*) revealed that part of the plastics arriving to the organic waste treatment plants are not biodegradable (e.g. LDPE). During degradation, the polymer is first biodeteriorated and depolymerized by a synergic action of physical and biological agents; then the smaller monomers are assimilated by microorganisms and mineralized (Lucas et al., 2008). A small portion of the polymer however is expected to be incorporated into biomass, humus and other natural products (Shah et al., 2008). The microbial community plays a fundamental role in plastics degradation, as polymers are potential substrates for heterogeneous microorganisms (Glass and Swift, 1989). About 90 microorganisms, both aerobes and anaerobes, have been identified in previous studies for their capability to assimilate both natural and synthetic plastics: bacteria, fungi, archaebacteria and lower eukaryotes (Emadian et al., 2017; Gu et al., 2003). Fungi and actinobacteria were particularly studied for degrading PLA PHA, LDPE (Karamanlioglu et al., 2017; Lee, 2000). Overall, microbial communities associated to plastics are responsible for the degradation and catabolism with amylolytic, hydrolytic or lipolytic activity thanks to intracellular and extracellular specific enzymes (Kumaravel et al., 2010; Tokiwa and Calabia, 2004). Microorganisms present a broad biodiversity because their growth is strictly related to the matrix and the environmental conditions (Zettler et al., 2013). The recent research about bacterial biodiversity responsible for plastics and bioplastics degradation has been more focused on soil and sediments than on compost (Emadian et al., 2017). But compost should be an environment of great concern because of the matrix heterogenicity and of the variable process conditions (temperature, humidity, pH) which can influence the composition of bacterial communities. To address these limitations, the current research focused on the biological degradation of bioplastics during composting. Therefore, the aim was to determine the impact of bioplastic waste in composting on the bacterial community structure, which could push towards the selection of communities more specialized in polymers degradation. Moreover, the lab scale experiments were carried out by testing various types of materials (bioplastics compared to LDPE) under both thermophilic and maturation phase of composting. This setup allowed to make detailed observation on bacterial community differentiation and specialization. First, Denaturing Gradient Gel Electrophoresis (DGGE) and 16S rRNA amplicon sequencing were used to compare the microbial communities present in the compost matrix to the ones attached to bioplastic pieces. Second, an enrichment method was applied to select bacterial species capable of

forming biofilms on the selected bioplastics. The microbial composition of the enriched biofilm was assessed by 16S rRNA amplicon analysis and plastics degradation after incubation in the enrichment culture was observed with SEM and FTIR.

Materials and methods

Experimental setup

Samples of different bioplastics were submitted to the composting test: starch-based Mater-Bi®, PBAT and PLA. The test was complemented with a background control (no addition of plastics) and a negative control with the addition of conventional LDPE, known for its low degradation rate. The lab scale composting test was carried out for 60 d with a thermophilic phase of 20 d at 58±2°C followed by a maturation phase of 37±2°C. Samples of compost and bioplastic fragments were recovered at the end of the thermophilic phase and at the end of the test, to carry out the analyses of microbial communities. The residues of the tested materials had been submitted to chemical and physical analysis to observe the degradation after composting. Indeed, first the pieces were weighted in order to obtain an experimental measure of the weight loss after the composting test. TGA was performed to observe the characteristic temperatures of the materials before and after the degradation. FTIR allows to monitor the degradation of the chemical structure of the materials, and SEM analysis provides a highly precise overview of the morphological changes. A description of samples, experimental setup and methodologies are provided in the section Materials and methods of Chapter 4.1.

Denaturing gradient gel electrophoresis

The bacterial community structure and diversity were preliminarily studied by DGGE in compost (control and reactors with test material) and bioplastic pieces. Samples of compost (control and reactors with tested materials), of bioplastics and of LDPE in triplicates were recovered and directly processed after the thermophilic phase and at the end of the test. Therefore, a total amount of 57 samples was processed. First, DNA was extracted, both from compost and from bioplastic and LDPE pieces. Before starting the DNA extraction procedure, plastics were rinsed in phosphate-buffered saline (PBS) to remove compost residues from the surface and therefore ensure to focus the extraction on the bacteria attached to the pieces surface. Then, both bioplastics .purified following the manufacturer's instructions. After measuring the DNA concentration with BioSpec-nano manufactured by Shimadzu Biotech, the solution was diluted at 2 ng/µl to allow the further steps of the procedure. Touchdown PCR amplification of 200 bp fragments of the 16S rRNA gene was carried out using the following primers: 518R (5'-ATTACCGCGGCTGCTGG-3') and GM5F-cl2 (5'-CGCCCGCCGCGCCCCGCGCCCGTCCCGCCGCCCCCGCCCGCCT ACGGGAGGCAGC46AG-3') (Gillan et al., 1998; Muyzer et al., 1993; Wawer and Muyzer, 1995). The PCR cycle was as follows: 10 min at 95°C, and then the first cycle was carried out 20 times using a denaturation temperature of 94°C for 30 seconds (sec), a hybridization temperature of 65°C for 45 sec (decreasing by 0.5 °C at each cycle) and an elongation temperature of 72°C for 30 sec. The second cycle was performed 10 times using the same denaturation and elongation temperatures and a hybridization temperature of 55°C for 45 sec. The PCR was ended by heating

at 72°C for 10 minutes. 40 µl of PCR products were analysed by DGGE in a gel containing 25-75% (v/v) denaturants (100% denaturants corresponding to 40% (v/v) formamide and 7 M urea). The gel also contained 0.5% (v/v) TAE buffer (Tris, Acetate, EDTA) and 10% acrylamide (v/v). Finally, 0.2% final (w/v) ammonium persulfate (APS) and 0.1% final (v /v). Temed were used to polymerize the gel. The gradient of the gel was made with a U-tube-type Gradient Former Model 385 Bio-Rad and the ISMATEC® peristaltic pump at 0.170 ml/min. The gel was placed in the Bio-Rad DcodeTM vat and filled with 40 µl PCR products per well. The migration was carried out in 0.5% (v/v) TAE buffer for 16 hours at 60°C and 75 volts. The gel was then stained in a solution containing 0.005% Gel Red (v/v). The gel was visualized and photographed by UV illumination using Gel Doc 2000. A comparison was done for samples on a single gel (difference of composting conditions for one plastic type), and between gels (difference between plastics) thanks to the blank which served as reference among gels. It allowed a preliminary qualitative observation about the presence of eventual common bands and peculiar bands of each sample.

16S rRNA amplicon sequencing

The 16S rRNA amplicon analysis was made by Novogene, with the following procedure (reported in Novogene 16S Analysis Report of X204SC20050178-Z01-F003). Total DNA from samples was extracted using CTAB/SDS method: cetyl trimethylammonium bromide (CTAB) is a cationic detergent that releases the cellular inner components and promotes the separation of proteins and polysaccharides from nucleic acids, while sodium dodecyl sulfate-based (SDS) buffers are used for the lysis step (Barbier et al., 2019). DNA concentration and purity were monitored on 1% agarose gel. According to the concentration, DNA was diluted to 1 ng/µl using sterile water. 16S rRNA genes of distinct regions (16SV4/16SV3/16SV3-V4/16SV4-V5, Arc V4) were amplified using specific primers, e.g. 16S V4: 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACHVHHHTWTCTAAT-3'). All PCR reactions were carried out with Phusion® High-Fidelity PCR Master Mix (New England Biolabs). The PCR cycle was as follows: 30 sec at 98°C, and then the first cycle was carried out 35 times using a denaturation temperature of 98°C for 10 sec, a hybridization temperature of 61°C for 30 sec (decreasing by 0.5°C at each cycle) and an elongation temperature of 72°C for 15 sec. The PCR was ended by heating at 72°C for 7 minutes. The same volume of loading buffer (contained SYBR green) was mixed with PCR products and loaded on 2% agarose gel for detection: samples with a bright distinct band between 400-450 bp were chosen for further experiments.

PCR products of the triplicates were mixed in equidensity ratios. Then, mixture PCR products was purified with Qiagen Gel Extraction Kit (Qiagen, Germany). Sequencing libraries were generated using NEBNext® Ultra™ DNA Library Pre-kit for Illumina, following manufacturer 's recommendations and index codes were added. The library quality was assessed on the Qubit 2.0 Fluorometer (Thermo Scientific) and Agilent Bioanalyzer 2100 system. Finally, the library was sequenced on an Illumina platform and 250 bp paired-end reads were generated.

Sequences analysis was performed by Uparse software. Sequences with ≥97% similarity were assigned to the same Operational Taxonomic Units (OTUs). For each

representative sequence, the Greengene database was used based on Ribosomal Database Project (RDP) classifier algorithm to annotate taxonomic information.

Importantly, in order to study phylogenetic relationship of different OTUs, and the difference of the dominant species in the groups, multiple sequence alignment was conducted using the MUSCLE software (Version 3.8.31).

The sequencing quality was verified using the rarefaction curves using the PAST software (Hammer et al., 2001). OTUs abundance information was normalized using Limma RGui package (15090 read counts). To evaluate the microbial community diversity, Venn diagrams were performed using Venn Diagram RGui package using OTU presence/absence to assess the distribution into the different conditions. (Hanbo and Paul, 2011) Alpha-diversity indices (OTU richness and the Shannon index) were calculated on the rarefied data using PAST3 software (Hammer et al., 2001) and the beta-diversity was assessed using multivariate analysis by the PERMANOVA test (vegan RGui package) (Wang et al., 2012). This analysis tested the factor significance (type, plastic, place) using the Bray-Curtis dissimilarity with 10000 permutations. A heatmap was performed with 34 OTUs for thermophilic conditions and 103 OTUs for the mesophilic conditions, showing that it was significantly affected by the different conditions. These OTUs were defined using a nbGLM (negative binomial distribution and Generalized Linear Model) revised by 1000 resampling iterations of the residual variance. The nbGLM is a deviance analysis performed using the mvabund Rgui package (Dixon, 2003). Two RGs were defined with a cluster dendrogram using the Euclidean distance and an average clustering (vegan RGui package).

Enrichment cultures

Three 2x2 cm pieces of each bioplastic type and of LDPE were recovered, at the end of both thermophilic and maturation phase, with the purpose to isolate bacterial species specialised in plastic degradation. One piece of the material was taken from each reactor (three reactors per tested material) and doubly rinsed in PBS to detached compost and bacteria not strongly adherent to the plastic surface. The plastic samples were added into 50 ml polycarbonate Falcon® tubes filled with 15 ml low carbon source medium (0.2% ammonium sulphate, 0.05% yeast extract and 1% trace elements (0.1% $MgSO_4.7H_2O$, 0.1% $FeSO_4.7H_2O$, 0.01% $ZnSO_4.7H_2O$, 0.01% $CuSO_4.5H_2O$ and 0.01% $MnSO_4.5H_2O$)) in 20 mM (N-morpholino) propane sulfonic acid (MOPS) pH 8; adapted from (Yoshida et al., 2016) and previously described (Delacuvellerie et al., 2019). The enrichment cultures were carried out by an incubation at 58°C, for those pieces taken at the end of the thermophilic phase, and at 37°C, for those collected at the end of the test (after the maturation phase).

Bioplastic and LDPE residues were recovered after 2 months of incubation and were submitted to FTIR and SEM, to observe the degradation process. The equipment used for FTIR and SEM analysis is the same described in Chapter 4.1.

Bacterial isolations were also performed on the recovered pieces. First, bacteria were directly isolated from the plastic surface with the following protocol: plastic pieces were

cut in millimetric fragments and transferred into a 50 ml tube Falcon® with 25 ml of PBS, previously filtered with a 0.2 µm pore size. Then, the steps were as follows: soft sonication on ice for 10 min to ensure a smooth released, vortex for few seconds, horizontal shaking for 20 min and finally vertical sedimentation and room temperature for 10-15 min. Petri dishes were prepared with Lysogeny Broth (LB) medium and 100 µm of diluted solutions (three 1:10 dilutions in water) were plated. Petri dishes were incubated at 37°C and 58°C for a period of 48h (for the first and second dilution) and 72h (for the third dilution).To isolate the bacteria, each individual colony was collected with a bacteriological loop and striped into new LB plates. DNA was extracted from all these individual colonies using the QIAamp DNA Mini kit Qiagen, dedicated to isolation of genomic, mitochondrial, bacterial, parasite or viral DNA. DNA was purified following the manufacturer's instructions. After measuring the DNA concentration with BioSpec-nano manufactured by Shimadzu Biotech, it was diluted at 2 ng/µl to allow the further steps of the procedure. Touchdown PCR amplification of 500 bp fragments of the 16S rRNA gene was carried out using primers: 534R (5'-ATTACCGCGGCTGCTGG-3') and F8 (5'-TTTCATAATATGTGCTACGCAACCTA-3'). The PCR cycle was identical as the one described for the DGGE and PCR products were purified with AmpliClean™ Magnetic Bead PCR Clean-up kit in accordance with the instruction of manufacturer. 16S rRNA amplicon analysis were performed with Beckman Coulter CEQ-8000; the data were elaborated with Sequencing Analysis software and a database search was carried out using BLAST programme (NCBI, Maryland, USA) to identify the microorganisms at species and strains levels.

Results

Bacterial community structure

At the end of composting test, the tested materials displayed different levels of degradation. In particular, while MB showed large bacterial colonization, deep erosion signs and a significative weight loss (45±5%), the other bioplastics displayed less evident signs of degradation, as well as a much lower weight loss (8±1.6% PBAT and 3±0.7% PLA). Finally, LDPE was almost undegraded (2% weight loss) and bacterial colonization on the plastic surface was almost negligible. These preliminary results, widely discussed in Chapter 4.1, are important to delineate a correlation between the activity of the microbial community studied and the effective degradation of the tested materials. The analyses of the bacterial communities were performed on the different samples (blank 'B', plastics 'Pl' namely MB-PBAT-PLA-LDPE and compost 'Co'), and on samples at different times of the composting process: at the launch of the test (Start, 0), at the end of the thermophilic phase (Thermo, 20 d), at the end of the maturation or mesophilic phase (Meso, 60 d). It is fair to mention that the species accumulation boxplot reports enough numerosity of the samples, which ensures good quality results. Moreover, the rarefaction curves of the data set generally tended to an asymptotic plateau, thus the species biodiversity is well represented.

Preliminary observations about the community were extrapolated from the non metric multidimensional scaling (nMDS) profile (Figure 4.8).

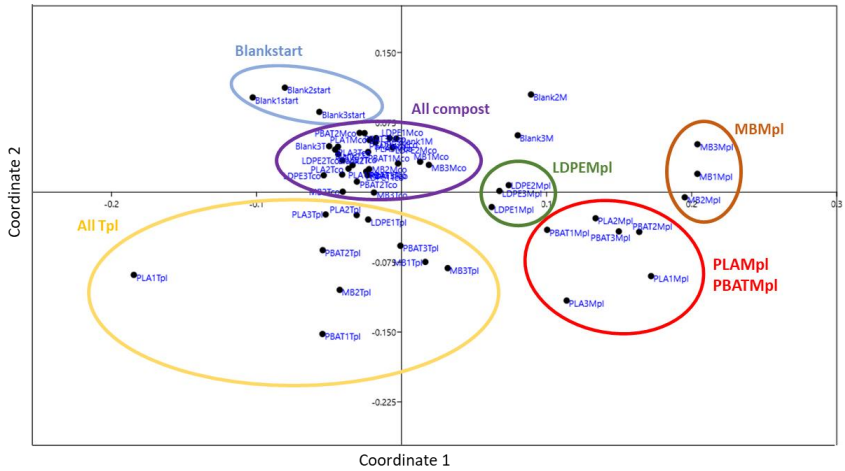

Figure 4.8 nMDS profile of the pairwise community dissimilarity (Bray-Curtis) indices of the 16S rRNA amplicon sequencing. The samples from harvested compost before the test (blank start: blue circle) are well distinguishable; the compost samples from bioreactors during the test are all groupable in the purple circle. Plastics composted under thermophilic conditions stay all in the yellow group, while plastics under mesophilic conditions are groupable in three circles: red contains PLA and PBAT; green LDPE and brown MB.

The nMDS profile discriminated compost from plastics-associated communities, as well as thermophilic from mesophilic communities. The microbial community in harvested compost (blank start) was found to be highly different than after test incubation. The other samples of compost are all grouped together (colour purple). Plastics under mesophilic conditions are divided into different groups, based on the type; on the contrary, plastics under thermophilic conditions are all into the same group (colour yellow).

An equilibrium in the dominance of the species was generally observed from the equitability index (Figure 4.9a and b).

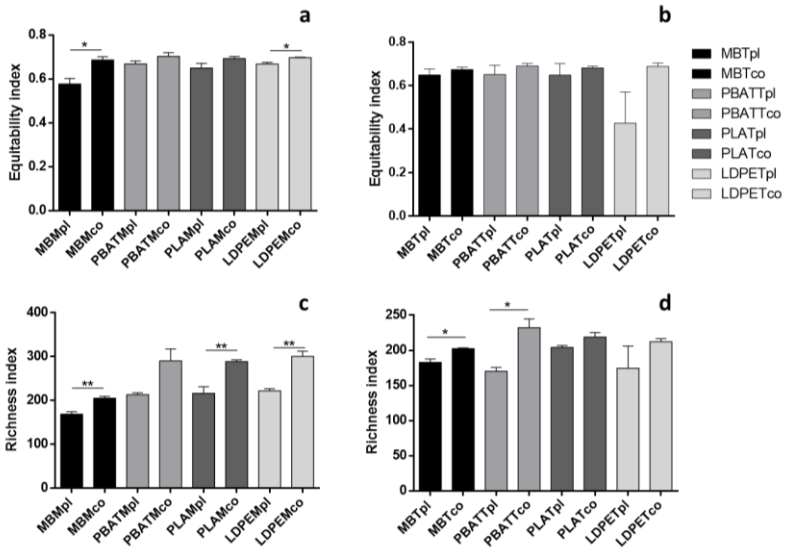

Figure 4.9 (a and b) Equitability index (or Shannon) and (c and d) OTU richness obtained from 16S rRNA amplicon sequencing for samples types (plastics and compost under mesophilic and thermophilic conditions). T-test; *, P <0.05; **, P <0.01; ***, P <0.001.

LDPE under thermophilic conditions is an exception, showing the presence of dominant species in the communities. The communities peculiar of compost are characterized by a higher biodiversity of species than the communities developed on plastic samples, as shown by the richness index. Moreover, from the richness index it emerged that the mesophilic communities (Figure 4.9c) had a higher number of species than the thermophilic communities (Figure 4.9d), both in compost and in plastics.
A common core about 177 (47%) and 195 (57%) OTUs for thermophilic and mesophilic conditions were displayed by the Venn diagrams (Figure 4.10a and b). LDPE and PLA had a larger number of unique OTUs than MB and PBAT. 9.5 and 7.4% of thermophilic OTUs were unique to LDPE and PLA, respectively. The percentages were slightly lower under mesophilic temperature, with 7.3 and 7% of OTUs unique to LDPE and PLA, respectively. 4 and 3.7% of thermophilic OTUs were present uniquely in MB and PBAT. The percentages were lower for mesophilic OTUs, corresponding to 0.6 and 1.7% for MB and PBAT, respectively.

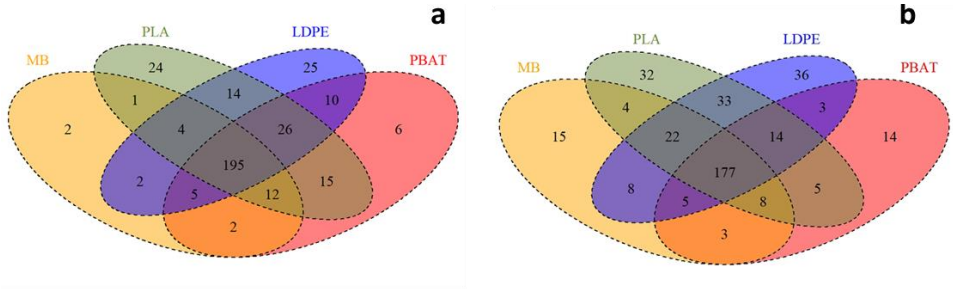

Figure 4.10 Venn diagrams showing overlap of bacterial OTUs for the different tested materials: a) after the maturation phase in mesophilic conditions, b) after the thermophilic phase.

The same common core was found in the preliminary qualitative screening provided by DGGE (Figure 4.11).

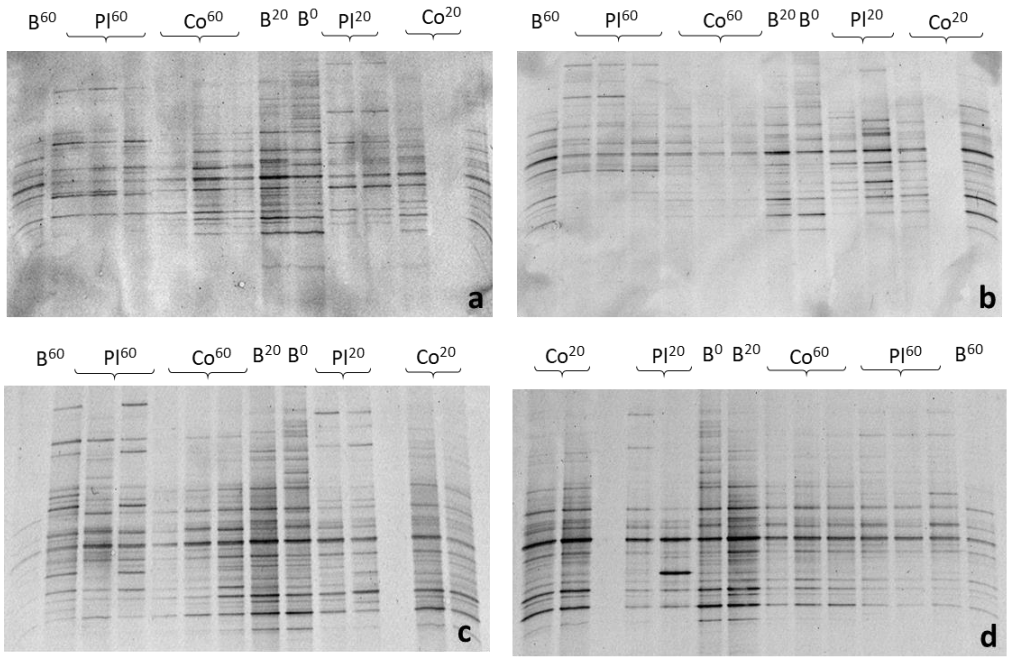

Figure 4.11 DGGE gels: MB in a), PBAT in b), PLA in c) and LDPE in d). In the legend, B: sample from compost in blank bioreactor; Pl: biofilm from the surface of the plastics; Co: compost samples from the same bioreactor of Pl. The apex corresponds to the time of the test, 0: starting point; 20: end of thermophilic phase and 60: end of the test.

The gels depicted the distinctness of the microbial community between compost and bioplastics, as well as between the thermophilic and the maturation phase. The distinct bands (species) were compared within the sample on a single gel (differences of composting conditions for one plastic) and between gels (differences between plastics) thanks to the blank which serves as a reference among gels. A core bacterial consortium was shared among the conditions tested, but the band counts and intensity differed between PLA, PBAT and MB and also between thermophilic and maturation phase, indicating a change in the bacterial community structure according to bioplastics degraded and to the composting temperature/duration.

The 16S rRNA amplicon analysis revealed that the microbial communities were mainly composed of *Firmicutes, Actinobacteria* and *Proteobacteria*, the sum of all three reaching a minimum of 58.8% of the total community (communities on MB, mesophilic conditions). Bar graphs of phyla for each plastic type and compost were elaborated: in Figure 4.12, it is reported the bar graph of the communities peculiar of Mater-Bi®, accounting samples recovered both from compost and from the bioplastic pieces.

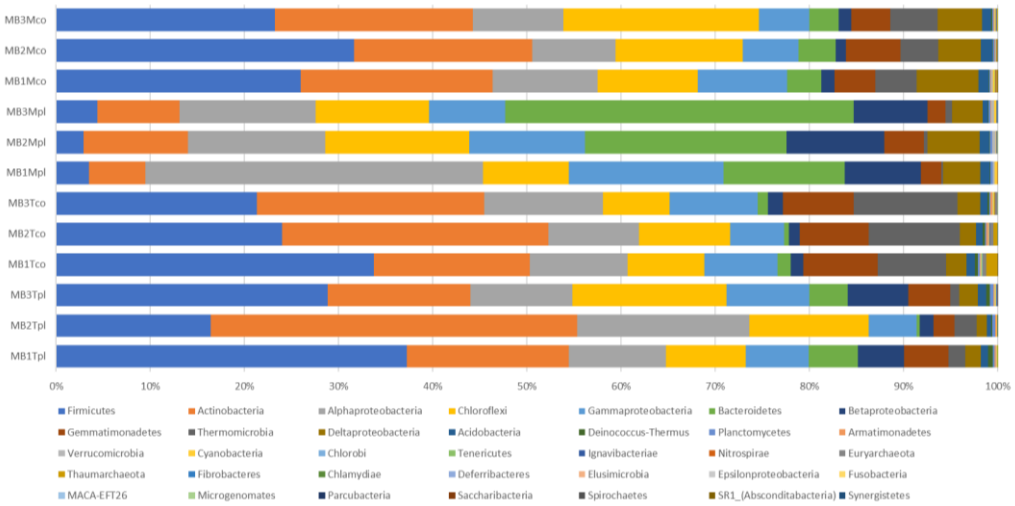

Figure 4.12 Diversity of bacterial communities presented by a bar graph reporting the relative abundance in phylum in all Mater-Bi® samples.

The most abundant phylum in all samples was *Firmicutes*, except for the bioplastics-associated population in mesophilic conditions, where *Proteobacteria* represented 37 to 49% of the population. Compost-associated community had a balanced distribution among the major phyla (*Proteobacteria* 20-24%, *Actinobacteria* 20 %, *Firmicutes* 25-35%), independently of the incubation conditions, except for 'blank meso' which had more *Bacteroidetes* and *Chloroflexi* (respectively 15 and 11%). Conversely, the communities associated to bioplastics were a little more heterogeneous with a marked difference between thermophilic and mesophilic conditions: the community composition displayed a shift with an increase of *Bacteroidetes* abundance, reaching 10 to 23% of the population, and of *Proteobacteria* especially with the mesophilic bioplastics.

The heat maps (Figure 4.13, 4.14 and 4.15) represent the relative abundance of genera in each compost and bioplastic sample, enabling to highlight the most abundant genera and the general trends.

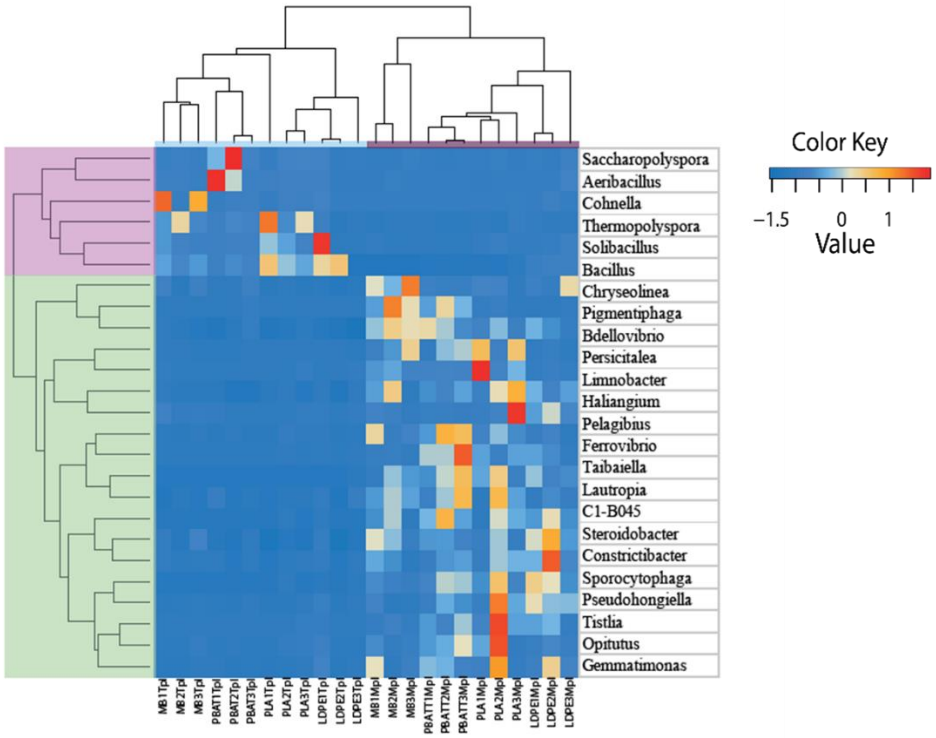

Figure 4.13 Heat map of the relative abundance of the bacteria in genera. It compares the plastics under mesophilic and thermophilic conditions.

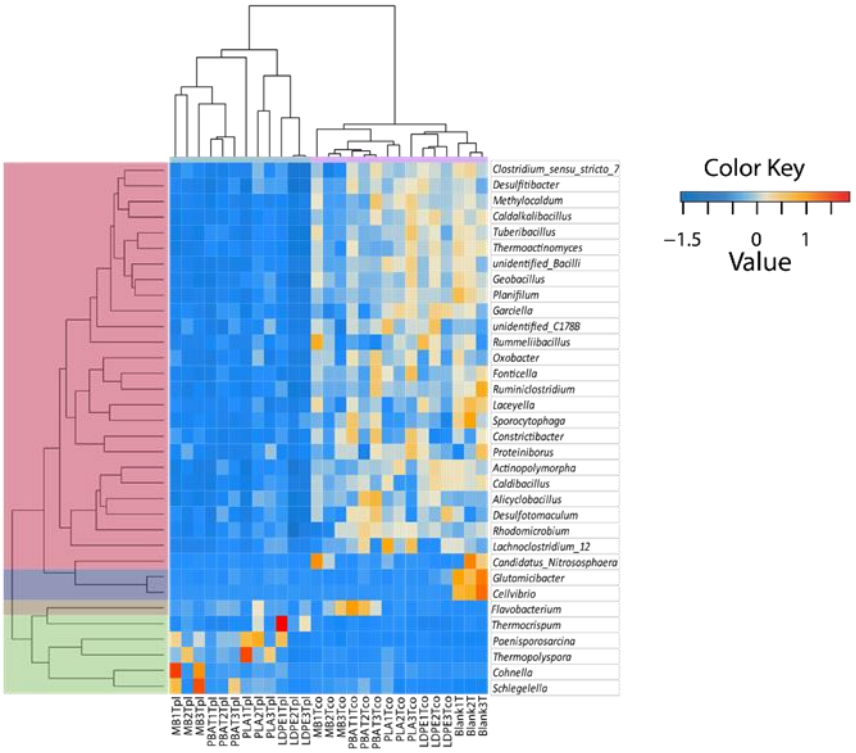

Figure 4.14 Heat map of the relative abundance of the bacteria in genera. It compares all plastic types and compost under thermophilic conditions

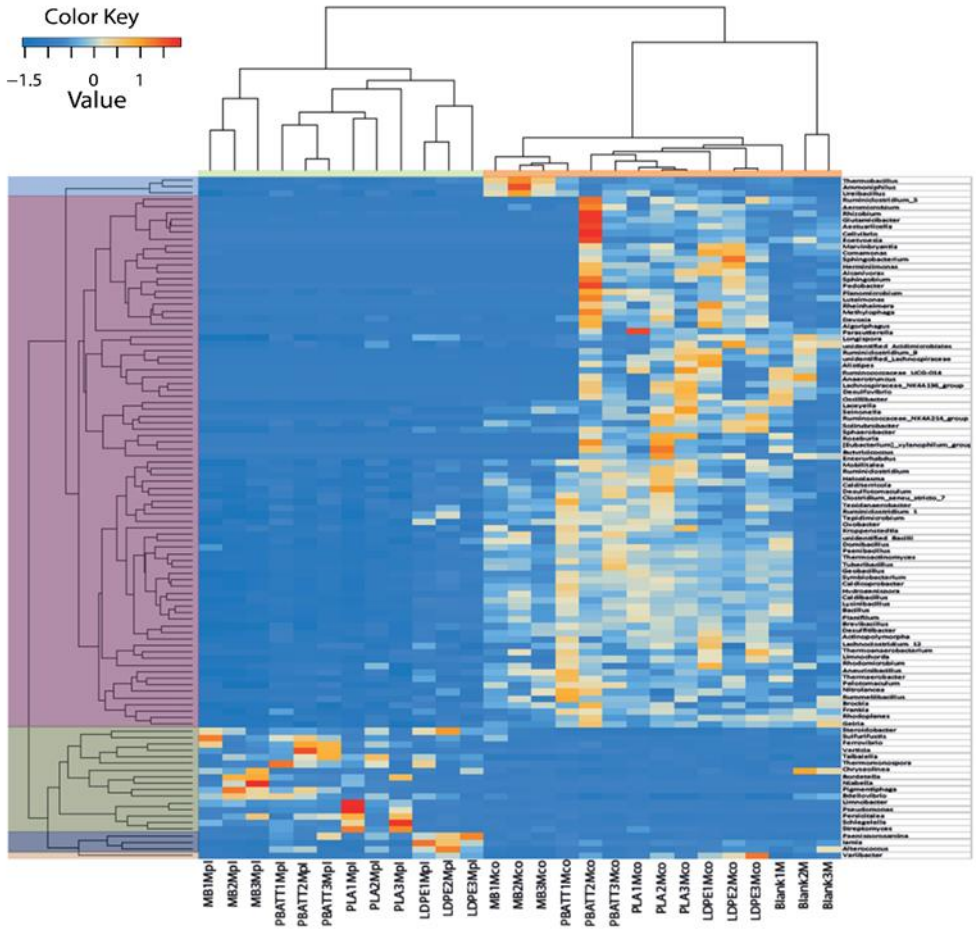

Figure 4.15 Heat map of the relative abundance of the bacteria in genera. It compares all plastic types under mesophilic conditions (maturation phase) and compost blank.

Sphaerobacter, *Hydrogenispora*, *Planifilum* and *Tuberibacillus* were more present in compost, with respectively and average abundance of 4.88%, 2.79%, 1.68% and 1.28% in compost samples (including the blanks). The abundance of specific genera were associated to the composting conditions, i.e. the mesophilic and the thermophilic phases. *Thermopolyspora, Cohnella* and *Thermocrispum* (*Actinobacteria*), *Paenisporosarcina*, *Bacillus* and *Geobacillus* (*Firmicutes*), were found especially in thermophilic communities of bioplastic samples (Figures 4.13 and 4.14). During the maturation phase, the phylum of *Proteobacteria* was largely predominant: in particular, *Pigmetiphaga*, *Steroidobacter*, *Sulfurifustis*, *Ferrovibrio* and *Verticia* were identified associated to bioplastics (Figure 4.15). In addition, *Chryseolinea* and *Taibaiella* (both *Bacterodites*) were also two major genera detected, with respectively 7.06% and 4.01% associated to mesophilic plastics compared to 1.12% and 0.05% with themophilic plastics.

Beside the previously described bacteria, some others appeared to be more specific to one or more bioplastics. In thermophilic conditions, *Firmicutes* were dominant in MB, in particular *Thermobacillus* (2.25%), *Paenibacillus* (1.94%) and *Cohnella* (4.01%). The *Aeribacillus* was also mostly associated to PBAT under thermophilic conditions (6.41%).

During the maturation phase, PBAT was colonized preferentially by *Verticia* (4%) and *Sulfurifustis* (2.88%), the latter one also present on MB (2.65%). The *Proteobacteria*

Limnobacter (3.47%) and *Schlegelella* (7%), as well as *Actinobacteria Pseudomonas* (4.57%), and *Streptomyces* (3.28%) were more specific to PLA even if slightly present also in the other mesophilic plastics. Concerning LDPE, among *Proteobacteria* two thermophilic specialist bacteria were identified: *Cupriavidus* (6.13%) and *Rhizobium* (6.07%).

Enrichment cultures

Characterization

SEM observations did not report pronounced differences between samples incubated at 37°C and at 58°C (Figure 4.16).

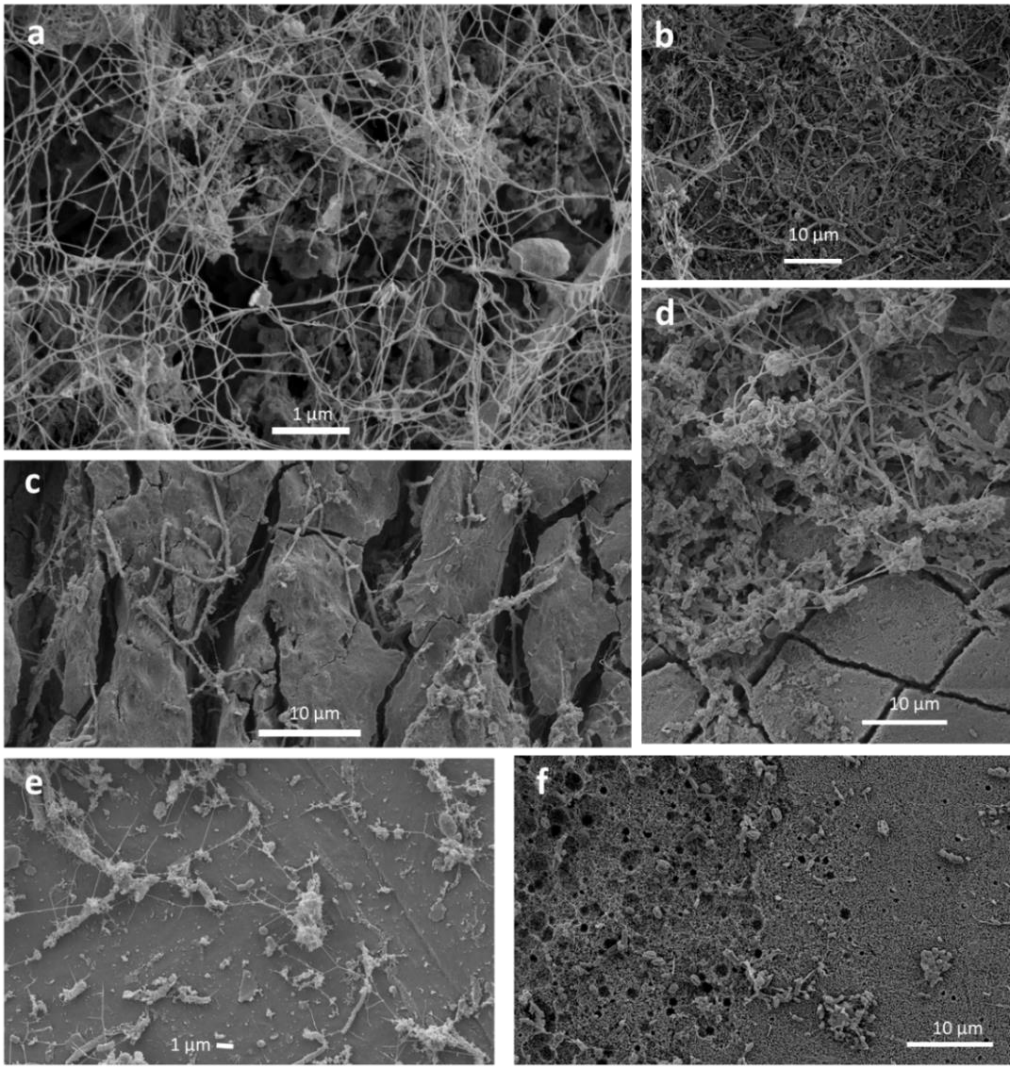

Figure 4.16 SEM images of bioplastics surface after 2 months incubation in different temperature conditions: a) MB at 58°C; b) MB at 37°C; c) PBAT at 58°C, d) PBAT at 37°C, e) LDPE at 37°C and f) PLA at 58°C.

MB and PBAT had a thick layer of biofilm which ensured nutrients exchange between bioplastic surface and bacteria; microorganisms were distributed both on the surface and

inside cracks of bioplastics (Figure 4.16a-d). PLA and LDPE had less diffused and stratified biofilm, but more than before the enrichment cultures (Figure 4.16e-f). From SEM micrographs it was visible that the material structure of all the tested material was subjected to a strong degradation during the 2 months incubation, whatever the incubation conditions were: indeed, the remaining plastic residues did not display a homogeneous surface as at the start of incubation, but rather displayed cavities and cracks providing specific sites for bacterial settlement (particularly for PLA and PBAT). Lumps on LDPE surface were attributed to metabolites of microbial activity or to the tendency of the flat surface to flake off during degradation.

The FTIR spectra reported the presence of the major peaks which can be associated to the microbial communities development during the enrichment cultures: 1450 cm^{-1}, assigned to NH group of amides in the proteinaceous substances of biofilm (Bonhomme et al., 2003), and 1635 cm^{-1}, related to carboxylate ions ($R\text{-}COO^-$) formed due to microorganisms activity (Arrieta et al., 2014). Interestingly, the main peaks of MB and PLA almost disappeared after incubation at 58°C. For MB: 1717 cm^{-1} (C=O bounded to an alkyl group) and 1150-950 cm^{-1} (C-C in n-alkanes of starch). For PLA: 1748 cm^{-1} (C=O in lactide), 1132 cm^{-1} and 1081 cm^{-1} (C-C stretching in alkanes), 1181 cm^{-1} (C-O stretching).

Bacterial communities in the enrichment cultures

The 16S rRNA amplicon analysis of the 42 isolated bacteria was performed at the genus and species level. All the identified bacteria belong to the phylum of *Firmicutes*; they are reported in Table 4.2.

Table 4.2 Bacteria isolated from enrichment cultures under thermophilic and mesophilic conditions

Genus	Species	Temperature conditions	Plastic
Bacillus	*borbori*	Thermophilic	MB
Bacillus	*cereus*	Mesophilic	MB, PBAT, LDPE
Bacillus	*cleronius*	Mesophilic	LDPE
Aeribacillus	*pallidus*	Thermophilic	PBAT, PLA, LDPE
Aneurinibacillus	*migulanus*	Mesophilic	MB, PBAT, PLA, LDPE
Brevibacillus	*borstelensis*	Thermophilic	MB
		Mesophilic	PBAT
Geobacillus	*thermoleovorans*	Thermophilic	PLA, LDPE
Geobacillus	*kaustophilus*	Thermophilic	PLA, LDPE
Parageobacillus	*thermoglucosidasius*	Thermophilic	PBAT, PLA

After incubation under thermophilic conditions, 13 isolates belonging to *Bacillus*, *Geobacillus* and *Parageobacillus* were identified. In particular, *Geobacillus thermodenitrificans*

was found in PBAT and MB, one bacterial isolate associated with each polymer. *Geobacillus thermoleovorans* was isolated on PLA and LDPE, one isolate per plastic, and *Geobacillus kaustophilus* was also isolated on the same plastics but with respectively 1 and 4 isolates on PLA and LDPE. Two *Parageobacillus thermoglucosidasius* were identified, one in the culture of PBAT and one in PLA. *Bacillus borbori* and *Brevibacillus borstelensis* were detected in MB culture. One *Brevibacillus borstelensis* was also isolated in PBAT under mesophilic conditions. Finally, *Aeribacillus pallidus* was detected with almost all the bioplastics: 3, 4 and 1 bacterial species were isolated respectively in PBAT, PLA and LDPE.

After incubation under mesophilic conditions, 20 isolates were obtained belonging to four different species, and therefore a smaller number compared to the thermophilic conditions. Most of them were present in almost all the plastics and therefore was not specific to one material. Beside the already mentioned *Brevibacillus borstelensis* isolated in PBAT, one *Bacillus oleronius* was found in the isolation process with LDPE. The, *Bacillus cereus* was identified with almost all the bioplastics. 3, 2 and 2 bacteria of this species were isolated respectively in MB, PBAT and LDPE. Finally, *Aneurinibacillus migulanus* was identified with all the bioplastics: 2 were found in MB, 2 in PBAT, 5 in PLA and 2 in LDPE.

Discussion

Bacterial community structure

In the present experiment, degradation activity was detected in all the compostable bioplastics tested, but FTIR, SEM and TGA analyses showed that the more complex, and/or thicker, is the material, the longer is the degradation time. While bioplastics presented degradation signs at the end of composting tests, conventional LDPE was still undegraded and did not present a wide bacterial colonization, as seen in Chapter 4.1.

Despite these differences, the amplicon sequencing disclosed that all the tested materials were increasingly colonized by bacteria. Moreover, the highest relative abundance of specialized bacteria was detected in LDPE; this conventional plastic displayed the lowest degradation. LDPE is a conventional, not compostable, plastic: therefore, significative signs of deterioration during composting were not expected. It is fair to assume that the less biodegradable is a compound, the more specialised is the group of bacteria exploiting the compound as carbon source. Indeed, bacteria that may have the capability to produce enzymes useful to degrade the complex structure of the polymer can predominate, as observed from the equitability index (Figure 4.9b). On the contrary, a polymer with more readily biodegradable compounds, such as MB and PLA, can be colonized by a wider group of bacteria without specific metabolism. The present study analysed in deep the bacterial communities developed on the tested materials, having the objective to identify bacteria that potentially degrade bioplastics (MB, PBAT, PLA compared to LDPE) in a composting environment, differentiating mesophilic and thermophilic communities. In the present study, a large variety of bacteria was identified in compost matrix, which have already been identified in previous research carried out in composting environment: e.g.

Geobacillus (Poli et al., 2011), *Tuberibacillus* (Hatayama et al., 2006), *Planifilum* (Han et al., 2013), *Hydrogenispora*. In line with literature, the present research outlined a significant variation of bacteria according to the composting conditions and plastic types (Liu et al., 2020; Wang et al., 2017), as shown by nMDS profile (Figure 4.8). In particular, temperature had an impact on the structure of the bacterial community (Chen et al., 2015), and bacteria in the thermophilic phase needed to face high temperature conditions by, for example, forming heat-resistance endospores, as commonly done by most of *Firmicutes* (Sangeetha et al., 2017) and by *Thermopolyspora* (Goodfellow et al., 2005). Moreover, the bacterial community originally present in the harvested compost, changed significantly during the degradation process, and the abundance of polymers degrading bacteria increased from day 0, to day 20 and finally to day 60 (Zhang et al., 2019), with the progressive appearance of e.g. *Paenibacillus*, *Pseudomonas, Schlegellela*, *Sulfurifustis*, *Pigmetiphaga* (Figure 4.15). This latter one was found specifically in wastewater containing dying agents, for the capacity to use pigments of the plastics (Yoon et al., 2007). However, *Proteobacteria*, *Firmicutes*, *Actinobacteria*, *Bacteroidetes* and *Chloroflexi* always remained the most represented phyla in the bacterial community of both compost and plastics. The same predominance was already assessed in previous studies with compost both during the thermophilic and the maturation phase (Cerda et al., 2018; Zhong et al., 2018; Kong et al., 2020).

These findings led to the following consideration: a core bacterial consortium is maintained in the compost, while specific plastic degraders progressively appeared during the 60 days composting process, as confirmed by the Venn diagrams. This also means that the concentration of bioplastics in compost (2.9%), as well as the duration of the composting phases, were enough to change the bacterial community towards a more specialized in degrading polymers.

Focusing on specific genera associated to plastics degradation in this study, some of them were as well associated to plastics degradation in previous research (Table 4.3).

Table 4.3 Comparison between the microbial genera highlighted by 16S rRNA amplicon sequencing (data from the heatmaps) and found in the literature. * No reference about these genera associated to plastic degradation.

This study			In the literature	
Genus detected by amplicon sequencing	Temperature condition	Tested material	Plastic with the genus	Ref.
Thermopolyspora	Thermophilic	PLA		*
Streptomyces	Mesophilic	PLA	PHA; PHB polyester	(Boyandin et al., 2013; Hsu et al., 2012; Trinh Tan et al., 2008)
Bacillus, Geobacillus,	Thermophilic	All	PHA; PHB PBAT	(Boyandin et al., 2013; Hsu et al., 2012; Zhang et al., 2019)
Paenibacillus	Thermophilic	MB	PBAT	(Jeszeová et al., 2018; Teeraphatpornchai et al., 2003)
Cohnella	Thermophilic	MB		*
Aeribacillus	Thermophilic	PBAT		*

Pigmentiphaga, Sulfurifustis	Mesophilic	MB, PBAT		*
Ferrovibrio, Verticia	Mesophilic	PBAT		*
Pseudomonas	Mesophilic	PLA	PLA; PHA	(Pattanasuttichonlakul et al., 2018; Boyandin et al., 2013)
Schlegellela, Limnobacter	Mesophilic	PLA	PHB	(Elbanna et al., 2003)
Cupriavidus	Thermophilic	LDPE	PHA	(Boyandin et al., 2013)
Rhizobium	Mesophilic	LDPE, PBAT	PET	(Delacuvellerie et al., 2019)

From literature, it was found that some of the specialized bacteria identified in Table 4.3, may be capable to produce enzymes typically involved in hemicellulose, chitin and lignin degradation in composting. Chitinase enzymes degrade the chitin, a fibrous substance consisting of polysaccharides. *Cohnella* was associated in previous studies to the production of chitinases (Aliabadi et al., 2016; Narancic et al., 2020). Like chitin, hemicellulose and cellulose are slower to be degraded in compost than sugars, starch, lipids, acids and proteins, but faster than lignin. These compounds are degraded by xylanase enzymes (Quitadamo et al., 2019). Regarding the bacteria associated in this study to bioplastics degradation, *Thermopolyspora* was found to be able to produce xylanase enzymes in a previous research (Anbarasan et al., 2017). Finally, concerning lignin, scission reactions by specific enzymes are more difficult. The enzymes capable to degrade lignin can interact with low molecular weight that could lead to the formation of free radicals and consequently to oxidise and to cleave complex macromolecular lignin network (Lucas et al., 2008; Peelman et al., 2013). The oxidizing ability of such microorganisms can also contribute to the degradation mechanisms of complex molecular structure of polymers. Previous research about polymeric blends degradation, in particular polyethylene and starch-based, reported evident signs of erosion in presence of lignin degrading bacteria of the genus *Streptomyces* (Lee et al., 1991; Shah et al., 2008). *Streptomyces* was also identified in this study among PLA degrading genera.

Some other bacteria were found to be abundant in the community structure. The following bacterial species were not mentioned in Table 4.3, as they were not directly associated to plastics degradation: *Steroidobacter, Sphaerobacter, Chryseolinea, Taibaiella* and *Iamia*. Though, they can be considered as a part of the core bacterial consortium shared among the tested samples. This core was also displayed in a qualitative way in the DGGE gels (Figure 4.11) and in the Venn diagrams (Figure 4.10). Most of the mentioned bacteria are known from previous research for their abundance in compost and soil (Storey et al., 2015; Xu et al., 2018). In the present study, these species were identified in both plastic and compost samples, and were largely present also in the initial compost samples (Figure 4.13-4.15). These bacteria cannot produce any of the previously described enzymes involved in polymers degradation mechanisms. However, it is fair to report that e.g. *Taibaiella* and *Iamia* have a metabolism capable to easily degrade some shorter acids and sugars which can be intermediate products of plastics degradation (Zhong et al., 2018;

Xu et al., 2018). Thus, the tested materials can indirectly be an additional suitable substrate in compost.

Important references were found in literature about some plastic degrading bacteria, which have not yet been mentioned in the present study. However, an oriented search of these species in the heatmaps, detected some of these bacteria in the community. Their low abundance prevented the association with bioplastics degradation at first screening. In particular, *Brevibacillus* and *Clostridium* in the phylum of *Firmicutes* were associated in a previous study to PLA and PBAT deterioration (Boyandin et al., 2013; Ghosh et al., 2013). In the phylum of P*roteobacteria*, *Alcanivorax* was found to play a fundamental role in the degradation of LDPE (Delacuvellerie et al., 2019), and finally in the phylum of *Actinobacteria*, *Saccharopolyspora* was identified in a previous study for its abundance in soil reached in microplastics (Nakei, 2015).

Enrichment cultures

It was disclosed that *Firmicutes* dominated both the thermophilic and the mesophilic communities in the enrichment cultures. This phylum was commonly recognized as a fermenting group of bacteria (Sangeetha et al., 2017), better heat-resistant than other phyla (Zhang et al., 2017). Considering that LB is generally suitable medium for various enrichment cultures (Yoshida et al., 2016), it is assumable that the predominance of *Firmicutes* was mostly relatable to their much better adaptability to the cultivation conditions. Moreover, *Bacillus* and *Geobacillus*, highly developed in the cultures, were abundant in bioplastic samples already before cultivation.

Geobacillus thermodenitrificans, was found from previous study to be capable to use starch for its metabolism, in particular the strain *BGSC* (Manachini et al., 2000). Indeed, in the current work, this species was identified in the cultivation with MB bioplastic containing starch. It is also interesting to report that *Geobacilli* are generally capable to exploit n-alkanes present in the polymers as substrate, i.e. the species *thermoleovorans* and *kaustophilus* (Nazina et al., 2001), and *thermoglucosidasius* of *Parageobacilli*, the strain *ATCC 43742* (Inoue et al., 2019).

Among the colonizing *Firmicutes*, *Aeribacillus pallidus* was detected in almost all the bioplastics in thermophilic conditions, while before cultivation it was predominant only in MB. Moreover, *Brevibacillus*, one of the plastic degrading bacteria with low abundance in the original community, colonized MB and PBAT in both mesophilic and thermophilic conditions. This finding confirmed what disclosed by previous authors about the capability of *Brevibacillus* to exploit PBAT as sole carbon source (Boyandin et al., 2013). PBAT is also the prevalent component of MB polymeric structure. Both *Brevibacillus borstelensis* and *Aeribacillus pallidus* were furtherly investigated in literature, finding that specific strains can be capable to degrade complex carbonic structure, respectively the strain *DX-4* (Y. Q. Wang et al., 2013) and the strain *C10* (Yildirim et al., 2017). Concerning *Brevibacillus borstelensis*, the strain *707* was studied by Hadad et al., 2005 as specialist in LDPE degradation in soil (Hadad et al., 2005); however, in the LDPE cultures of the present study, *Brevibacillus borstelensis* was not identified.

Conversely, from the isolation process with LDPE it was found *Bacillus oleronius*: it has the capacity to adapt to mesophilic conditions, but is also known for its ability to cause extended lesions in skin and blepharitis (Owusu-Darko et al., 2017; Szkaradkiewicz et al., 2012). *Bacillus cereus* was isolated from LDPE, but also from MB and PBAT. It was found to well adapt to mesophilic conditions; moreover, this species present many strains with different performances and capabilities (Sen et al., 2015). *Bacillus cereus* can be found in soil, water and food, where it is a potential pathogen for humans (Ivanova et al., 2003). In particular, the strain *ATCC 14579* exploits a large number of carbon sources, such as starch and other saccharides, amino acids, dipeptides and glycerol, while other strains of the same bacillus are not able to use these nutrients for their growth (Mols et al., 2007). Finally, *Aneurinibacillus migulanus* was slightly present in the community before the culture, but at the end of the test it was isolated in all the mesophilic cultures. This bacterium cannot produce any specific enzyme involved in polymers degradation and it is generally used against plants diseases for its antibiotic effect (Alenezi et al., 2017; Takagi et al., 1993). Therefore, it is assumable that *Aneurinibacillus migulanus* did not survive in the enrichment cultures for its capability to use polymers as carbon source, but due to an endogenous growth.

After the enrichments culture, bioplastics showed much deeper signs of degradation, as visible from FTIR and SEM. These results, jointly with the peculiar metabolism of the colonizing communities, highlighted that the mentioned bacterial species strongly contributed to degrade bioplastics.

Conclusions

The present study disclosed that 16S rRNA profile in bioplastics associated communities largely differed from that of the initial compost, collected before composting test. After 2 months composting, the tested bioplastics showed deep signs of erosion, in particular Mater-Bi®. On the contrary, conventional LDPE was found not to be subjective to a significative degradation. However, thanks to the 16S rRNA amplicon sequencing, the current research identified some bacteria which have a higher abundance in one of the tested materials with respect to the other samples. Therefore, for each bioplastic and LDPE, peculiar bacteria capable to degrade the material were found. In addition, references from literature report that some of these bioplastics-associated bacteria may have the capability to produce enzymes, which typically degrade complex structure, such as hemicellulose or lignin.

The bacterial communities developing on bioplastics and LDPE hosted distinct bacteria in mesophilic and thermophilic conditions. Some specialists were found to be plastic nature dependent, but a larger variety of bacteria colonized two or more plastic types. This finding is important in the promotion of research studies on bioaugmentation. Bioaugmentation practice can enhance the degradation of bioplastic waste in industrial composting (LDPE is not compostable). However, a wide variety of bioplastics is conferred to composting plants; therefore, wider is the variety of bioplastics degradable by bioaugmented bacteria, more efficient may be the bioaugmentation practice.

With the objective to investigate the possibility to culture some specific bioplastics degrading species, an enrichment method was applied. After 2 months cultures in the chosen medium, *Firmicutes* completely predominated in bioplastics colonization, under both thermophilic and mesophilic conditions. Most of the species identified with the cultivation approach were found to be specialist of one or more tested bioplastics. However, one species, *Aneurinibacillus migulanus*, survived in all the cultures with endogenous growth.

In the present study, some genera capable to specifically degrade bioplastics were identified also among *Proteobacteria* and *Actinobacteria*. Therefore, enrichment methods - enhancing the growth of species from these phyla - may be further investigated.

4.3 Bioplastic residues after composting: fate in soil and water environments

Abstract

The degradation of bioplastics in composting has been deeply studied in previous chapters, highlighting the possibility that some not completely degraded residues are released into the environment as part of compost. Therefore, the current research discloses a new perspective in the monitoring of compostable bioplastics, following their fate after composting in soil, freshwater, saltwater and sand. Residues of Mater-Bi®, PBAT, PLA and LDPE were recovered after composting lab scale test previously carried out. It consisted of 20 d of thermophilic phase, followed by 40 d of maturation phase. LDPE was used as a negative benchmark. The residues were then incubated into the four environmental matrices, in a day-night cycle, for three months. The analyses performed on the remaining fragments at the end of the test, with TGA, FTIR, SEM and visual inspections, broaden the scenario relating to bioplastics degradability in the environment. The results in fact outline that in water environments the degradation of the residues continues, even though with a not negligible slowdown. On the contrary, the degradation in soil and sand is strongly prevented by both physical factors and microbial competition.

Introduction

Environmental pollution from plastic waste and microplastics was widely investigated in the last years of research: however, the predominant fields of interest are marine and fluvial environments (Horton et al., 2017). Therefore, there is a wide gap of knowledge regarding plastic pollution in agricultural soils and terrestrial ecosystems (Huerta Lwanga et al., 2016). Plastic waste entering soil environment comes from some main sources: plastic mulch films, municipal waste (municipal solid waste and compost), biosolids (sewage sludge and anaerobic digestate), plastic-coated fertilizers and atmospheric pollution (Rodríguez-Seijo and Pereira, 2019). However, the most important sources among these are likely to be agricultural films and compost (Hurley and Nizzetto, 2018) (Bläsing and Amelung, 2018). Both these sources refer to conventional plastics, but also to bioplastics. Compost in particular is obtained at the end of industrial composting treatment of organic waste fraction and it is employed in agriculture as source of organic carbon for the improvement of the natural conditions of the cultivated fields (Pergola et al., 2017; Sánchez et al., 2017). It is fair to remind that conventional plastics should not be conferred with the organic waste and their presence is expected to be reduced with an increasing improvement of the separate collection. On the contrary, the fate of bioplastics labelled as compostable is to be treated in biological processes with the organic waste. Despite this, compostable bioplastics require specific conditions of temperature, timing and humidity for their degradation (Rudnik and Briassoulis, 2011), which are not always fulfilled by the industrial composting plants. From this issue, it raises the possibility that some not completely degraded residues can remain at the end of the treatment and be released into the environment as part of compost (Ruggero et al., 2020a).

From the best of my knowledge, in literature there are no studies about the fate of plastics and bioplastics, which are residual from composting treatments and are released as part of compost in soil and water.

If in soil, water and sand the degradation rate of bioplastic residues is slowed down because no proper conditions are met, there is a risk of progressive accumulation (Liu et al., 2014) and macro-fragments disintegration into microplastics (Ramos et al., 2015). In fact, regarding bioplastics, it is fundamental to remark that their degradability is strictly related to the environmental conditions and the material must fulfil specific requirements to be labelled as biodegradable, not only in composting but also in soil or water (Kjeldsen et al., 2019).

The present research intents to provide further knowledge about the progression of bioplastic residues degradation after release in the environment as part of compost. Figure 4.17 shows a simple conceptual model of a potential pathway for bioplastic residues from the source, compost, to environmental targets: soil, water, and sand. The agricultural soil is the more easily reachable target and it is on the pathway to water, due to run off and irrigation. The pieces are transported to the irrigation canals and delivered first to rivers and later to seas. The transport of particles in fresh and saltwater environment is governed by complex mechanisms: they depend on particles physical features (size, density, shape) and on dynamic properties in sinking (Chubarenko et al., 2018).

Therefore, bioplastic residues can float on water surface or deposit in the marine sediments, and part of them can be driven on the beaches due to the waves motion.

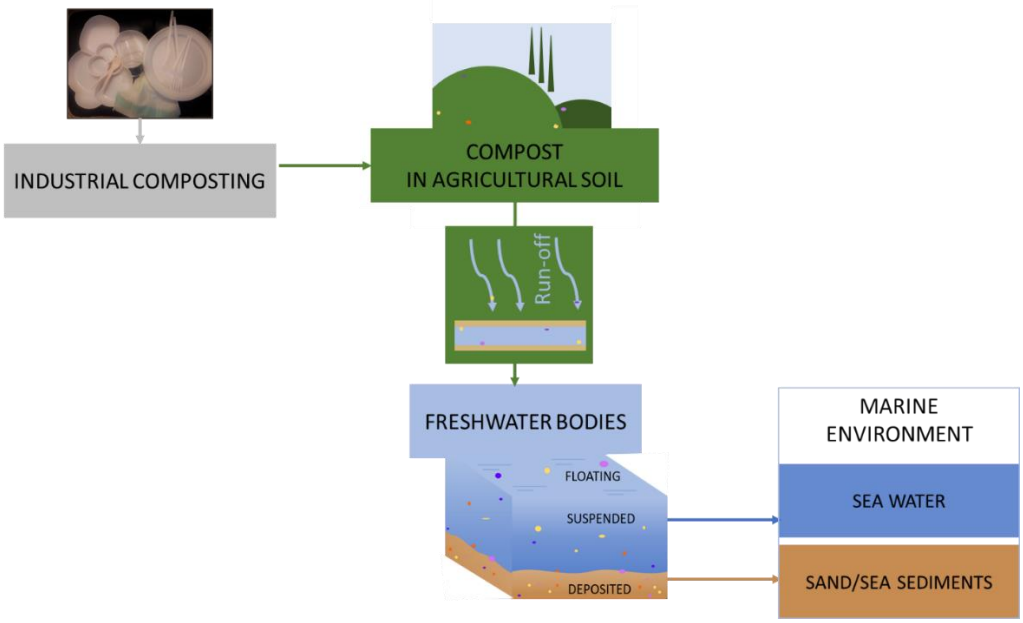

Figure 4.17 Conceptual model of bioplastic residues pathway in the environments after release in agricultural soil as part of compost.

New studies on the fate of bioplastic residues in the environment are needed because many aspects of their impact on water and e terrestrial ecosystems are still unknown (Weithmann et al., 2018). These impacts may be summarised as follows: (i) these materials may act as vectors for other contaminants, such as organic pollutants, heavy metals and human pathogens (Qi et al., 2020). (ii) it is actually unclear the contribution of plastic and bioplastic residues in loss of soil structure, reducing of rainwater infiltration and water loading capacity (Liu et al., 2014), as well as their impact on soil organic C, N and minor nutrients (Liu et al., 2017). (iii) there is an important gap of knowledge regarding the impact on living organisms. More studies are needed to assess the potential for biomagnification in the food chain (Teuten et al., 2009) and the organisms capability to adsorb and desorb pollutants from ingested plastics. Preliminary results on this topic comes from a recent research on microplastics in water, which found that organic pollutants are not readily transferred to the host (Bakir et al., 2016). (iv) finally, the distribution and migration mechanisms from agricultural soils into the environment require further investigation (De Souza Machado et al., 2018; Zhou et al., 2018), even though it has been assessed that size and density are important variables in these transport mechanisms (Nizzetto et al., 2016).

With the effort to enlarge the scenarios of the fate of bioplastic residues in natural environment, the present chapter experimentally tests the degradation of some MB, PBAT, PLA and LDPE samples, derived from composting test exposed in Chapter 4.1.

Materials and methods

Samples preparation

Samples of bioplastics and LDPE were recovered from composting test carried out as described in Chapter 4.1. It is reminded that the test lasted for 60 days with a thermophilic phase of 20 d at 58±2°C followed by a maturation phase under mesophilic conditions, 37±2°C. At the end of the lab scale composting some MB, PLA, PBAT and LDPE micro and macro-residues were still present in the compost matrix, even though showing signs of advanced degradation. Among the recovered residues, pieces not smaller than 2x4 cm were chosen to carry out the current test.

Experimental setup

Fresh and saltwater environments

Fresh water was sampled in the Canal du Centre at Thieu (BE) and salt water came from the North Sea, collected in the coastal city of Oostende (BE). Samples were harvested in July 2019. To the purpose to remove microorganisms living in water, which could be a further variable in the already complex phenomenon of degradation, both salt and fresh waters were filtered with 0.2 μm filter.
Plastic samples were then rinsed in a series of three bakers filled with filtered phosphate-buffered saline (PBS), to remove the compost residues attached to their surface, without compromising the fragile integrity of the degraded samples. Glass tubes of 15 ml capacity to storage water were sterilized for 1 h with UV radiations, then 0.50 g (2-4 pieces) of bioplastics or LDPE were added.
The tubes were placed on a shaker moving at 50 osc/min, to simulate convective motions of water environment.

Soil and sand environments

Sand was collected in the coastal city of Oostende (BE) and soil was harvested from agricultural fields in the city of Ath (BE) in July 2019. Glass flasks of 100 ml were used to storage 50-60 ml of sample: half of the flask was left for the head space and cellulose cap was used as coverage to allow air filtration and avoid water evaporation. For sand, 100 g of matrix were added and humidified with 8 ml of distilled water. For soil, 60 g of matrix were wet with 5 ml of distilled water. To simulate the rain and to avoid that bacteria dry out, once a week 8 ml and 5 ml were vaporized in sand and soil, respectively. Both the tubes of water microenvironments and the flasks were incubated in a 500 l cabinet (SPH-5042.00, Parasonic). The conditions were maintained in a day-night cycle, where the light was provided by 1 UV lamp during the 12 hours simulating the day condition. The temperature had a small gap of 5 degrees from day to night, passing from 30°C to 25°C. The test lasted for a total period of 90 d.

Bioplastics characterization

The samples of bioplastics and LDPE were submitted to characterization: after recovery from 60 d of composting and then after the successive 90 d of incubation in the different environments. Before being analysed, the tokens were just brushed off with tissue papers to remove eventual residues of the matrix from their surface. The bioplastic residues were submitted to TGA, SEM, FTIR. The methodologies and equipment used are described in Chapter 4.1.

Results

Fourier transform infrared

The spectra after 90 d in the microcosms of the four environments were almost at all equal to those after composting. Providing an overview of the spectra, the peaks detected after 60 d of composting were still identifiable: 1717 and 726 cm^{-1} relatable to C=O an $(CH_2)_{n=4}$ in PBAT. In PLA the main peaks were 1748 cm^{-1} of the C=O bond in lactide, 1181 and 1081 cm^{-1} corresponding to C-O in -CH-O group and C-C- in alkanes. LDPE presented the peaks 2914 and 2645 cm^{-1}, both relatable to CH_2.

After incubation in the microenvironments, indexes of bacterial colonization were found in the peaks of proteinaceous material constituting the biofilm: 1635 and 1550 cm^{-1} (Bonhomme et al., 2003). The broad peak in the surrounding of 3300 cm^{-1}, already identified during composting, was still high in all the pieces extracted from water environments. It is attributable to both hydrolytic degradation and microbial activity (Ohtake et al., 1998).

The only new peaks were identified in LDPE, corresponding to 1055 cm^{-1} in water environments and to 1030 cm^{-1} in soil and sand. They are normally assigned to C-O vibration and to in plane CH deformation, respectively. Both depicted that also polyethylene was subjected to physico-chemical alteration while being incubated in water and soil.

Thermogravimetric analysis

TGA analysis allowed to define the percentage weight loss of the tested materials. The weight loss of pieces recovered after the incubation in the microenvironments is compared in Figure 4.18 with the weight loss after 60 d composting. It is interesting to observe that the degradation was still encouraged in water environment. Starch in MB degraded up to 79 and 86% in salt and freshwater respectively. PBAT in MB had a peak of 27% weight loss in freshwater, while pure PBAT rised to 8% degradation in water with respect to 6% when recovered from composting. A surprising result was observed for LDPE in saltwater, as it degraded up to 7%, starting from 2% after composting.

PLA differed from the trends of the other plastics: the highest degradation was reached in sand, with 6% weight loss. Moreover, PLA showed in each microenvironment a fall of

T_{peak} value, particularly emphatic in sand, from 361°C to 285°C (Table 4.4). This feature suggested that a depolymerization process was still occurring in the microenvironments and that the residues after 60 d of composting were not yet oligomers and monomers readily assimilable by bacteria (Lucas et al., 2008; Benali et al., 2015).

Table 4.4 Characteristic parameters from TGA analysis.

	New	Composting	Composting +			
			f. water	s. water	soil	sand
			MB (starch)			
T_{peak} (°C)	317	-	-	-	-	-
water (%)	-	12	48	63	3	3
residue (%)	-	25	14	16	31	22
			MB (PBAT)			
T_{peak} (°C)	395	395	395	396	395	397
water (%)	-	12	48	63	3	3
residue (%)	-	25	14	16	31	22
			PBAT			
T_{peak} (°C)	397	400	398	398	398	397
water (%)	-	1	-	-	1	-
residue (%)	-	10	8	7	11	11
			PLA			
T_{peak} (°C)	361	338	300	302	315	285
water (%)	-	2	2	2	-	4
residue (%)	-	2	1	1	2	1
			LDPE			
T_{peak} (°C)	467	470	467	467	465	466
water (%)	-	-	-	-	-	-
residue (%)	-	-	3	7	1	-

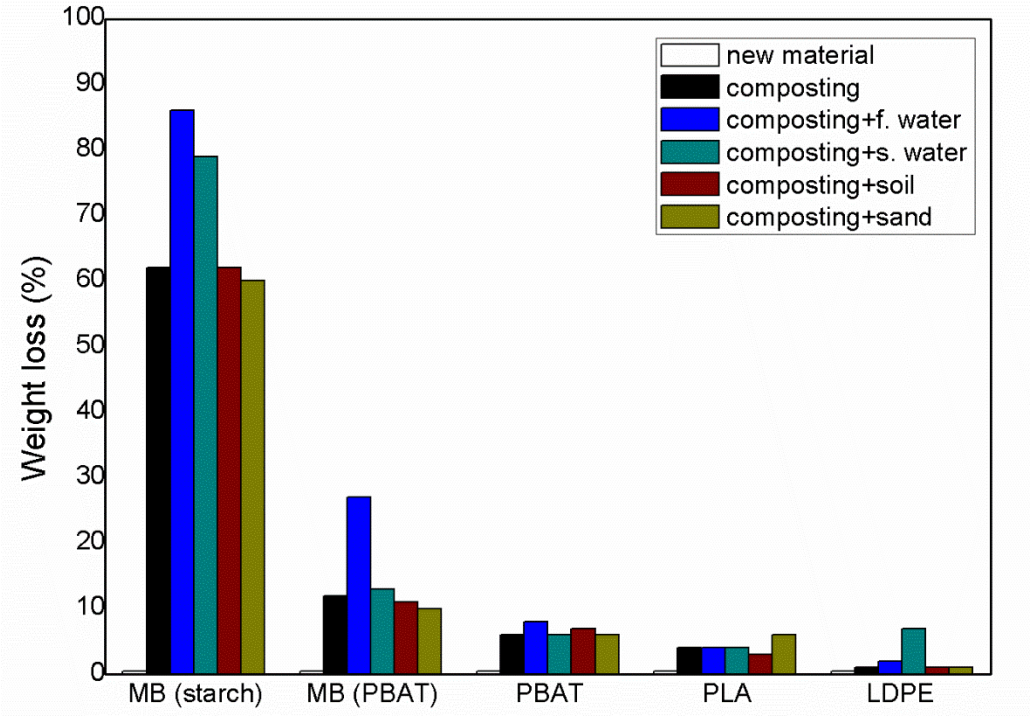

Figure 4.18 Weight loss (%) from DTGA analysis.

Scanning electron microscope

SEM pictures accentuated some features already observed after 60 d degradation in composting (Figure 4.19), such as diffuse biofouling, particularly in water environments. In Figure 4.20 micrographs of bacterial colonization are reported with a higher magnification (x10000). Comparing the fragments recovered from water incubation with residues immediately after composting, it was observable a larger biofilm extension, which embedded bacteria on the surface of bioplastic residues. The three bioplastics presented cracks and fractures, that were widened with respect to composting residues, especially in soil and sand. LDPE reported wide horizontal fractures in the fragment incubated in freshwater, jointly with small but diffuse mechanical cracks on the surface of the piece recovered from saltwater. Residues from soil and sand did not present differences with respect to 60 d of composting. PLA residue after sand incubation presented enlarged pits, which provided favourable locations for oxidation to occur.

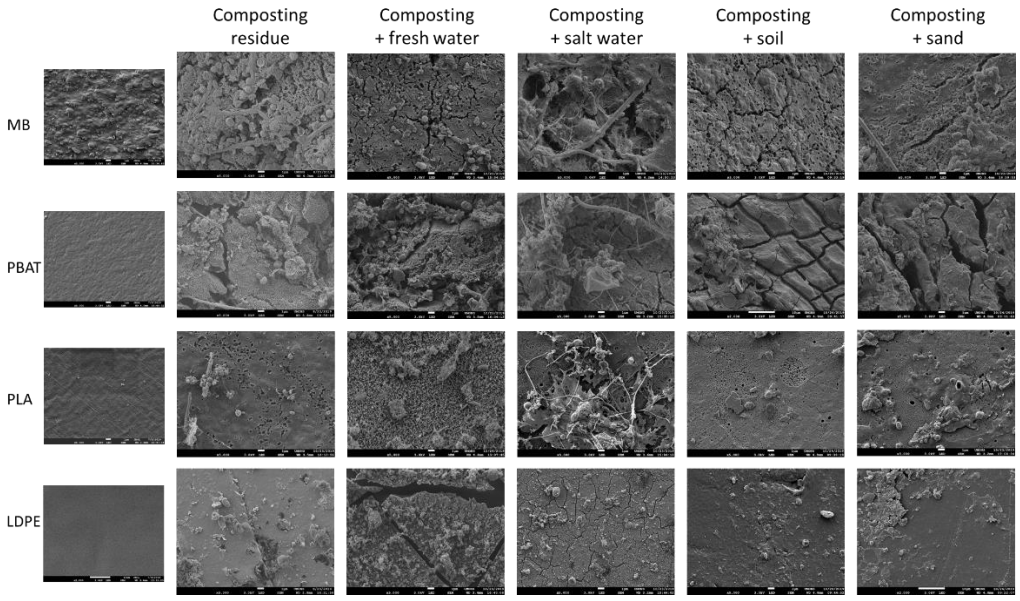

Figure 4.19 SEM micrographs of the tested materials after recovering from composting and incubation in water, soil and sand environments.

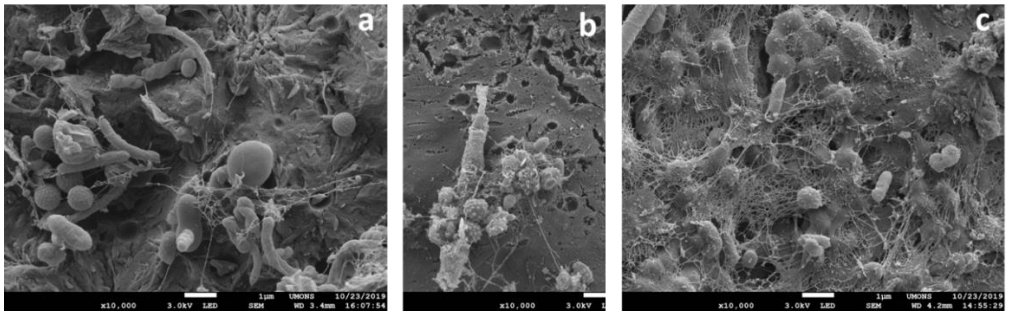

Figure 4.20 SEM micrographs which report the bacterial colonization and biofilm formation on plastic surface in water environment. PLA in pictures (a) and (b), MB in (c).

Visual inspection

Pieces recovered after 60 d of composting presented physical features imputable to a degradation of the material: darker surface colour, lateral and superficial erosion, size reduction and brittleness. After the subsequent period in the microenvironments, these signs were a little more extended (Figure 4.21). The folding and stickiness effects were observed in MB pieces recovered from waters, while superficial erosion was more pronounced in MB and PBAT samples from soil and sand. In bioplastics recovered from these matrices, some soil and sand grains were found attached to the surface.

Finally, it is fair to notice the brittleness of PLA in water environments. Indeed, the pieces immerged in the water environments were fragmented during the incubation.

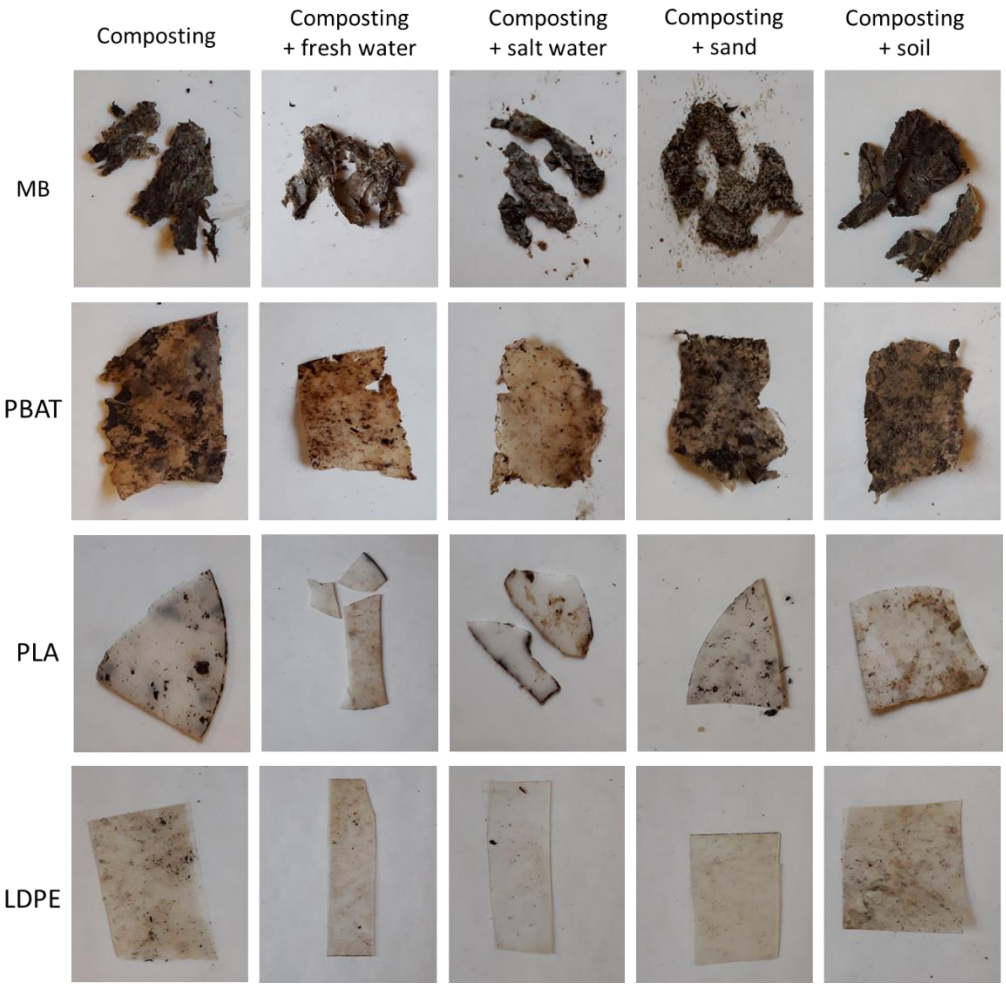

Figure 4.21 Photographs of the degraded materials recovered from composting first and then after further incubation in the microenvironments.

Discussion

Fresh and saltwater environments

From the observations on residues recovered after incubation in water environments, it emerged that bioplastic residues degradation was not prevented at all. The effects of physical weathering and microbial activity were underlined by the analyses and both enhanced the deterioration, which had not yet been completed during composting.

However, it was observed especially from weight loss that the degradation rate in the microenvironments was lower than during the 60 d composting.

It is fair to notice that erosion signs, visible at macroscale, and grooves, pits and fractures, observable in SEM micrographs, were just at superficial level on PBAT, PLA and LDPE. On MB instead, superficial pits and microcracks have already enlarged, generating a deeper erosion. Nearly all the initial breakdown occurs on surface layer, where first the material becomes weaken and brittle and then generates fractures (Andrady, 2011). Therefore, it is assumable that the same process seen in MB could later occur in the other materials tested. It just required longer time due to the major thickness and less available

surface of the materials (especially PLA), jointly with the minor degradability of PLA, PBAT and LDPE residues when incubated in the environments. Moreover, the degradation of MB was enhanced by starch (readily degradable material) in the structure. It is the first to degrade and it left further pits beside those generated by external physical weathering, thus enlarging the surface directly exposed to physical weathering and microbial activity.

Weathering agents, which are likely to influence degradation process, were all reproduced in the fresh and saltwater microenvironments. Temperature decrease, from 37±2°C in maturation phase of composting down to 25-30°C of water, was expected to be an inhibition factor for degradation (Andrady, 2011). Moreover, temperature change between day (30°C) and night (25°C), as well as the high humidity, induced a stress which made the material susceptible of fractures (Ho et al., 1999). UV light, imposed during daily part of cycle, was a fundamental factor for its involvement in the oxidative process of plastic surface. It produced radical hydroperoxide, responsible for diffuse holes and pits (Brine and Thompson, 2010). Furthermore, these pits on bioplastic surface were favourable sites for microbial cells to grow: the intense microbial colonization explored with SEM suggests that the microbial community took part in the degradation via physical and metabolic means (Zettler et al., 2013). It is fair to remind that no indigenous bacteria were present in water. On the one hand, the absence of competition with indigenous bacteria enhanced the bacterial community from composting to survive to environmental conditions change, as well as to continue the degradation process. On the other hand, a further study may include the indigenous community to account the eventual contribution of species naturally present in water and capable of plastic degradation.

Finally, it is interesting to mention the thick biofilm layer formed on bioplastics surface. This phenomenon is positive for nutrients exchange between polymer and bacteria embedded on it. Despite this, a diffuse and thick fouling on the surface can prevent UV light and oxygen from reaching the inner part of the bioplastic piece (Andrady, 2011). This can be an issue especially for rigid products with thickness ≥500 μm, whose degradation can be very slow, as exemplified by PLA in the current work.

Soil and sand environments

In soil and sand, it was observed that the degradation of bioplastic residues was strongly slowed down. The inhibition factors, and the chemico-physical features of degradation, were similar in the two matrices. Consequently, they are together discussed in the current paragraph.

The analyses disclosed that the main evidences of degradation were mechanically related: embrittlement of pieces and digging of the superficial erosion. These signs of mechanical abrasion were relatable to the collision with soil or sand grains (Cooper and Corcoran, 2010).

Despite this phenomenon generated proper sites for oxidation and bacterial cell grow, an almost negligible degradation was observed with TGA and SEM analyses. This suggested that there were inhibitors preventing significative structural changes and microbial

attacks. They were identified in the decrease of temperature and in the not uniform exposure to humidity and UV light, due to different orientation and depth of the piece surface (Sakai et al., 2001). This latter in particular inhibited chemico-physical deteriorations, such as oxidative process and hydrolysis (Brine and Thompson, 2010). Moreover, the lower and not homogeneous humidity on bioplastic surface was one of the conditions which largely affected bacterial colonization (Ohtake et al., 1998). This basic knowledge about humidity effect and the SEM observations done, enabled to roughly say that the bacterial community was more sporadic after incubation in soil and sand than after composting.

The poor microbial activity detected on bioplastics surface may be attribute also to the competition with the microorganisms already present in soil and sand. Indeed, there was a vast pool of microbial competitors, especially in soil, and a wide range of mechanisms that could be responsible for the emergence of dominant microbial populations (Penkhrue et al., 2015; Ishii et al., 2008). Beside this, the same communities which in composting used biopolymers as carbon source, may have found in soil alternative and more readily assimilable substrates (Hibbing et al., 2010; Adhikari et al., 2016).

Conclusions

Whether plastic and bioplastic residues are released into the environment as part of compost, they can interact through different pathways with soil and freshwater, until saltwater and sand. It is of great concern to provide a basic knowledge about the fate of these undegraded residues in the environment.

Considering the great resonance of bioplastics, it is fair to make clear the appropriate disposal of such items and the consequences of inappropriate management. Bioplastic products, such as bags or single use cutleries, that are labelled as biodegradable, are likely to make consumers more relaxed about discarding them via the dedicated pathway of organic fraction of MSW sort collection, but unfortunately also by direct disposal into the environment, right in view of their perceived "biodegradability". But the term of biodegradability is strictly related to the environmental conditions. It means that these items are not supposed to quickly degrade in natural habitats. In particular, compostable bioplastics should be conferred with the organic waste and require proper temperature, humidity and duration of the industrial composting process for their degradation. Therefore, if discarded directly into the environment, they are supposed not to find favourable conditions for a short-term degradation.

Furthermore, the current study outlined that also the degradation of bioplastics derived from composting is strongly slowed down in the natural habitats. In soil and sand, low and not uniform humidity, as well as the competition with the indigenous microbial community, prevented the degradation process to continue. The only factor which contributed to a superficial deterioration of the fragments was the mechanical abrasion with grains. In fresh and saltwater, the degradation process continued thanks to both physical factors (i.e. waves motion, UV light) and microbial activity. Nevertheless, the

slowdown of the degradation process was not negligible, resulting in a long-expected time needed for a complete degradation in these environments.

In conclusion, whether bioplastic products do not meet proper conditions in composting plants for their complete degradation, some residues are likely to be released into the environment. They may accumulate and disintegrate into micro fragments, favouring potential impacts on the environment and living organisms, about which further scientific knowledge should be acquired.

A first prevention of bioplastic fragments release into the environment can be achieved by promoting the optimization and monitoring of composting processes, as well as the implementation of proper refining treatments for an efficient removal of undegraded bioplastics.

Acknowledgements

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5 Methodologies for microplastics recovery and identification in heterogeneous solid matrices: a review

Abstract

The missing link in plastic mass balance between mismanaged plastic waste worldwide and plastic waste effectively detected in marine environments has recently risen the attention on microplastics. In fact, beside primary sources of microplastics such as cosmetic products and textile fires, there are secondary microplastics generated from plastic items due to weathering agents and biological degradation. While the marine and freshwater environments are actually of great concern, ground environments, and matrices related to it, have been less considered in the recent research about microplastics detection. Major attention should be reserved to solid heterogeneous matrices, such as soil, compost, sediments and sludge. Worldwide regulations about compost, which is used as amendant in agricultural fields, have a threshold size ranging from 2 to 15 mm for the requirements related to plastic impurities. Microplastics which pass through the mesh of the threshold sieve are considered assimilable to compost. One of the main lacks that prevents the improvement of these regulation, is a standard protocol for microplastics detection in solid heterogeneous matrices. To this purpose, the current review proposes an outline of methods tested in previous research for microplastics recovery and identification in the matrices of interest.

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Introduction

Preliminary models have been built in the last years to estimate the amount of mismanaged plastic waste whose fate is reaching the fresh and saltwater environments. The numbers obtained range from an overestimation of 25 million tonnes (10% of total plastic waste generated) (Jambeck et al., 2015), to 5-13 million tonnes (European Commission, 2018). Many sampling campaigns in water environments were carried out to provide more precise numbers about plastic waste pollution (Brennholt et al., 2017; Gasperi et al., 2014). However, from a comparison between estimations and samplings, it emerges a difference of several order of magnitudes (Lebreton et al., 2019), a missing link between the amount of plastic waste expected to be found and the samples collected (Thompson et al., 2004). The last years studies effort to provide explanations about this missing link and to overcome the gap (Cressey, 2016). Beside recent developments which highlight that the majority of plastics seems to sink in the deep ocean, where is undetectable during superficial samplings (Eriksen et al., 2014; Woodall et al., 2014), part of the missing plastics can be related to microplastics, pieces smaller than 5 mm very difficult to be recovered from the environment (Thompson et al., 2004). Microplastics come either from primary sources, such as personal care products, cosmetics, textile fibres, as well as raw materials and subproducts in plastic manufacturing (Rocha-Santos and Duarte, 2017), or from disintegration of plastic waste due to weathering agents and biological degradation (Silva et al., 2018).

Microplastics, both primary and secondary, are likely to be found not only in water, but also in ground environments, such as public gardens, agricultural soil and compost, where they can remain for long time even if eventually they will enter the marine environment via inland waterways, wastewater outflows, and transport by wind or tides (Ritchie and Roser, 2018).

While the marine and freshwater environments are actually of great concern, soil environmental matrices have been less considered in the recent research about microplastics detection. Solid heterogeneous matrices, with a high content of organic matter, that should require a higher attention for the presence of microplastics are soil, compost, sediments and sludge. The current review is mainly focused on these environments and includes also some cases studies related to sand and biota, when the techniques developed may be efficient for the matrices of main concern. Sludge could be considered in the boundary between liquid and solid, even though the main recovery techniques which have been found for water, have not the same good applicability for sludge due to the much higher presence of organic matter (Jouraiphy et al., 2005). Among these matrices, sediments have been quite largely studied (Costa et al., 2019), much less soil (Qiu et al., 2016; S. Zhang et al., 2018) and sludge (Li et al., 2018), and almost none compost (Ng et al., 2018). In particular, fertilizers derived from aerobic composting should be submitted to a higher consideration as potential vehicle for the entry of microplastics in agroecosystems (Weithmann et al., 2018). In fact, comparing the compost standards within Europe, America and Australasia, the most precautionary indication requires that plastics >2 mm are <0.5% of compost weight in dry mass. Plastic

pieces which pass the 2 mm mesh are considered assimilable to compost: in some countries the threshold is 10 to 15 mm (e.g. Spain and New Zealand), while in some other European countries and in USA plastic is not mentioned in the requirements for impurities inspection. This observation outlines that the risk of microplastics pollution in terrestrial environments is much less accounted than in water. Moreover, the absence of standard protocols for their extraction and quantification in such solid matrices does not allow to improve compost regulations regarding plastic contamination limits.

With the aim to provide a useful overview of the main techniques to recover and identify microplastics in heterogeneous solid matrices, it is proposed a subdivision of the methods in accordance with the usage. A first paragraph is dedicated to different methodologies applied for microplastics recovery from a bulk sample, including cases studies which exploit the use of combined methods. The second paragraph is focused on the identification and characterization of microplastics, displaying traditional and innovative techniques.

Microplastics recovery

When sampling environmental matrices to investigate the presence of microplastic waste, the bulk sample has a high presence of organic and inorganic materials, from which the plastic particles should be recovered. Previous research studies employed various methodologies which act either to remove the solid matrix (e.g. digestion), or to separate the microplastics exploiting the difference of their properties with respect to the bulk sample (e.g. flotation). In Table 5.1, it is presented an overview of the available techniques, indicating the environmental matrix and the characteristics of the microplastics (polymer type, size and shape). In order to develop more efficient procedures for complex matrices, some current studies have also proposed a combination of two techniques, as displayed later on.

Table 5.1 Overview of the techniques to recover microplastics from different solid heterogeneous matrices.

Group	Technique	Polymer	Size	Shape	Matrix	Ref.
Flotation	(Distilled) Water (1.0 g/cm^3)	PE, PP	50-100 µm	Fibres, fragments	Soil	(S. Zhang et al., 2018)
	NaCl or NaI solution (1.2-1.4 g/cm^3)	PP, PET, PVC	0.5-3 mm	Particles	Sediments	(Nuelle et al., 2014)
		PE, PP, PS	10 µm-5 mm	Fibres, film, microspheres	Sludge	(Li et al., 2018)
		PE, PP, PS, Nylon, PET, PUR, PVC	20 µm-1 mm	Fragments, pelletes, powder	Sand	(Erni-cassola et al., 2017)
	$ZnBr_2$ or $ZnCl_2$ (1.6-1.7 g/cm^3)	PE, PP, PS, PET, PVC	200 µm-1 mm	Fragments	Sediments	(Quinn et al., 2017)
		PC, PE, PP, PS, Nylon, PET, PVC	1-3 mm	Fragments, pellets	Sediments	(Imhof et al., 2012)

	Oil-water separation	Nylon, ABS, EPS, PES, PVC	500 µm-4.75 mm	Fibres, particles	Sediments	(Ellika Crichton et al., 2017)
Elutriation	Water column with sieving separation	Nylon, PVC	60 µm-2 mm	Particles, powder	Sand	(Kedzierski et al., 2016)
	Water elutriation column	PS, PVC	10-300 µm	Fibres, microspeheres	Biota, sediments	(Claessens et al., 2013)
Digestion	Alkali Digestion (KOH or NaOH)	PE, PP, PS, Nylon, PET, PUR, PVC	100 µm-1 mm	Fibres, Fragments	Sediments	(Maes et al., 2017)
		ABS, PC, PE, PP, PET, PUR, PVC, MB, PLA, PHB	5 mm	Foam, fragments, pellets	Sand	(Kühn et al., 2017)
	Acid Digestion (HCl or NHO_3)	LDPE, PP, PS, Nylon, PET, PVC	80-300 µm	Fragments	Biota	(Karami et al., 2017)
		HDPE, PS, Nylon, PVC	5 mm	Films, microspheres pellets	Biota	(Naidoo et al., 2017)
		PE, PS	60-600 µm	microshperes	Marine snow	(S. Zhao et al., 2017)
	Enzymatic Digestion	PE, PP, PS, Nylon, PET, PUR, PVC	100 µm-1 mm	Fibres, Fragments	Sediments	(Maes et al., 2017)
	Oxidizing Agents (H_2O_2 or Fenton)	PS, PVC	10-300 µm	Fibres, microshperes	Biota, sediments	(Claessens et al., 2013)
		PE, PP, PS	10 µm-5 mm	Fibres, film, microshperes	Sludge	(Li et al., 2018)
		PS, PET	0.25-5 mm	microshperes	Sludge	(Lares and Ncibi, 2018)
		LDPE, PP, PS, Nylon, PET, PVC	80-300 µm	Fragments	Biota	(Karami et al., 2017)
			100-300 µm	Fragments	Sand	(Shim et al., 2016)
		-	100 µm-1 mm	Fragments	Sediments	(Cauwenberghe et al., 2013)
Sieving		PS, PET	0.25 – 5 mm	microshperes	Sludge	(Lares and Ncibi, 2018)
		-	0.5-5 mm	Fragments	Sediments	(Qiu et al., 2016)
		PE, PP, EPS	50 µm-1 mm	Fibres, film, fragments	Sand	(Song et al., 2015)

Flotation

It is mainly a technique based on density separation between the microplastics and the environmental matrix in which they are being detected. In Table 5.2 are reported the densities of plastic materials that has a wide use in the common products.

Table 5.2 Densities of widely used plastics

Plastic Class	Acronym	Density (g/cm3)
Expanded Polystyrene	EPS	0.03-0.15
Acrylonitrile butadiene styrene	ABS	0.90-1.53
Polypropylene	PP	0.91-0.95
Low density polyethylene	LDPE	0.92
High density polyethylene	HDPE	0.96
Mater-Bi®	MB	1.1-1.2
Nylon (Polyamide)	Nylon (PA)	1.02-1.06
Polystyrene	PS	1.05
Polymethyl methacrylate	PMMA	1.09-1.20
Polyurethane	PU	1.2
Polycarbonate	PC	1.20-1.22
Polyhydroxy butyrate	PHB	1.25
Polylactic acid	PLA	1.25
Polyvinyl chloride	PVC	1.35-1.39
Polyethylene terephthalate	PET	1.38-1.41
Polyester	PES	-

Enough density difference between matrix and microplastics is provided in a sample of sediments (2.7 g/cm^3). A solution with a density <1.2 g/cm^3 can easily allow the separation of all microplastic types from the bulk sample (Costa et al., 2019). On the contrary, compost and sludge (1.2-1.4 g/cm^3) are more heterogeneous matrices with a much lower density than sediments and close to the density of some polymers. Thus, flotation can work with a solution with a density >1.2 g/cm^3 to avoid the flotation of the environmental matrix together with microplastics.

For soil, flotation can be successfully carried out in water or distilled water; a previous treatment of sonication can be applied to ensure that each particle in the bulk sample deposits or floats in accordance with its own settling velocity (S. Zhang et al., 2018). NaCl solution (1.2 g/cm^3) is one of the most used, as the salt is highly available, cheap and eco-friendly (Nuelle et al., 2014), but it is limited to polymers with lower density (Song et al., 2015). NaBr and NaI (1.4-1.6 g/cm^3) solutions are also able to separate heavier polymers with good recovery rate. In sediments, it has been assessed that a density separation with NaI can provide a recovery rate higher than 90% (Nuelle et al., 2014).

Higher density solutions obtained with $ZnCl_2$ and $ZnBr_2$ (1.6-1.7 g/cm^3) were found to be the most effective ways to separate all polymers from sediments in a single washing,

while generally lower density solutions require up to three passages to obtain a good recovery rate (Quinn et al., 2017).

An alternative method involves the use of oil to enhance plastic separation from the environmental matrix, exploiting the hydrophobicity of polymers. Solution with oil was found to improve the recovery rate by increasing wettability of plastics and reducing the surface tension. The method was tested in a sediment matrix: the addition of pine oil to a $ZnCl_2$ solution significantly improves plastics flotation (Imhof et al., 2012).

Moreover, an oil extraction protocol (named OEP) was defined in a recent study for extraction of Nylon, EBS and PVC microplastics in sediments (Ellika Crichton et al., 2017). The mixture is composed of 100 ml water and 5 ml canola oil with 50 g of bulk sample. The flotation in a separatory funnel requires no more than 2 hours. To allow the analysis of the recovered particles with FTIR or other characterization techniques, the surnatant is washed with ethyl alcohol which removes oil traces.

The most common ways to carry out flotation are 1 l backer and separatory funnel, this one particularly used for separation in water and oil-water solutions. Once the flotation is ended, the surnatant is recovered, filtered and dried, before being submitted to further analyses.

Elutriation

Elutriation is a technique based on density separation and it has been mainly tested in sediments. It is carried out in a column of height in a range of 1 to 2 m, with air or water inflow at the bottom of the column to encourage particles flotation or deposit along in accordance with their density. The flow rate shall be adjusted to achieve a maximum extraction efficiency and minimal contamination of the sample with the matrix. It was experimentally determined that a flow rate of 300 l/h for 15 min is adequate to keep sand in the pipe, while other materials, including microplastics, flowed over the edge (Claessens et al., 2013). The surnatant can be recovered and filtered to be submitted to a further separation treatment or to characterization analysis. However, some authors suggest inserting on the top of the column one or more sieves to separate the particles in accordance with their size (Kedzierski et al., 2018). It has been demonstrated that this step improves the recovery rate of microplastics (Kedzierski et al., 2016). In fact, while without granulometric separation the particle mass controls the flotation-deposition equilibrium, when particles are divided within granulometric fractions, volume stays in a defined range and consequently the equilibrium is a function of the particle density.

Digestion

Digestion, when dealing with a bulk sample mainly composed of organic matter, such as compost and sludge, contributes to remove most of the matrix and to reveal microplastics, which instead are resistant to digestion treatment.

Digestion of organic matter can be acid, alkali and with the use of oxidizing or enzymatic agents. Acid digestion is carried out with HNO_3 or HCl: even though it has been assessed

that higher is the amount of organic matter, stronger is the resistance of microplastics to oxidation, the effects of acids on plastics is of melting, yellowing or total destroy with a heating above 60 °C (Karami et al., 2017; Devriese et al., 2015; Naidoo et al., 2017a). This leads to an underestimation of microplastics in the bulk sample.

Alkali digestion with NaOH and KOH has been confirmed to work for removal of organic matter from sediments and biota after heating at 60°C from 12 to 24 hours (Maes et al., 2017). Nonetheless, it should be taken into account that both the alkali agents can cause discoloration and degradation of the microplastics (Kühn et al., 2017).

Digestion with oxidizing agents, Fenton's agent and H_2O_2, has found a wide usage in recent studies with different application protocols. Three protocols have been assessed for H_2O_2, and validated on PP, PE, PS, PVC and PET. To observe the impact of H_2O_2 on microplastics, the agent was added in a ratio 100:1 to microplastic sample: the impact of the oxidizing agent was considered negligible (Nuelle et al., 2014). In accordance with the first procedure, 35% H_2O_2 is added to bulk sample and left acting for 7 d at room temperature (Karami et al., 2017). The residue is then rinsed with water or with 80% ethanol and filtered. In the second protocol, 30% H_2O_2 acts on the matrix for 7 d at 55°C. The residue is then diluted with water and filtered (Avio et al., 2015). The third protocol was assessed on biota, with 35% H_2O_2 at 60°C for 4 d, reaching almost 100% of organic matter removal (Karami et al., 2017).However, other authors obtained a good removal rate under lower temperatures, from 25 to 50°C (Shim et al., 2016; Cauwenberghe et al., 2013). Finally, enzymatic digestion is less used and generally applied as previous treatment to staining (Maes et al., 2017).

Sieving

Wet or dry sieving of the sample divides both environmental matrix and microplastics into granulometric fractions. In accordance with the size of the particles characterizing the matrix and the microplastics inside it, it is possible that some of the fractions are completely clean or on the contrary are composed almost only by microplastics, which are easily identifiable by a naked eye (Qiu et al., 2016). Moreover, when the research aims to recover only microplastics from the sample, a preliminary sieving is done to remove all the material with a size higher than 5 mm.

Sieving technique can be used also in field to collect samples of plastics smaller than a specific size (e.g. 1 mm (Song et al., 2015))

Combined methodologies

A few series of research studies depict the usage of combined methodologies to ensure extraction of microplastics from the bulk sample. In particular, in most of them digestion of the organic residues follows a previous treatment for microplastics separation. This application was done for sediments samples after flotation with NaCl (Nuelle et al., 2014) and after elutriation in water (Claessens et al., 2013). These matrices in fact allow a

proper separation from plastics due to the high difference in density, but can require a further digestion to remove eventual organic residues which float due to their lower density. In sludge, matrix digestion is combined with a previous sieving treatment (fractions from 60 μm to 2 mm), which provides the removal of most of the sludge but leaving bigger size organic residues (Lares and Ncibi, 2018). Finally, it is fair to report the treatment which couples elutriation to sieving, already discussed with the elutriation methodology, for its high efficiency in sand samples (Kedzierski et al., 2016).

Microplastics identification

Most of the cases studies displayed in the previous paragraph combined recovery methodologies with techniques which allow microplastics identification among the residues remained after separation from the bulk sample (Figure 5.1). Some of the identification methodologies (Table 5. 3) are simple and cost-effective methods (e.g. FTIR and visual inspection), but not easily applicable on the smallest microplastics. In Figure 5.2, it is proposed a comparison of recovery and identification methodologies, grouped in eight main categories, used in accordance with the minimum particle size. Moreover, in the following paragraphs some techniques recently introduced for microplastics identification and characterization are discussed, such as Vis-NIR spectroscopy and methods involving thermal degradation.

	Recovery 1°	Recovery 2°	Identification 1°	Identification 2°	Ref.
Sludge	Digestion		Visual inspection		(Li et al., 2018)
	Sieving	Digestion	Visual inspection		(Lares and Ncibi, 2018)
			Thermal degradation		(Dümichen et al., 2017)
Soil	Flotation		Visual inspection		(Zhang et al., 2018)
			Spectroscopy		(Corradini et al., 2019)
Sediments	Digestion		Visual inspection		(Cauwenberghe et al., 2013)
	Digestion		Staining	Spectroscopy	(Maes et al., 2017)
	Elutriation	Digestion			(Claessens et al., 2013)
	Flotation		Spectroscopy		(Imhof et al., 2012;Quinn et al., 2017; Ellika Crichton et al., 2017)
	Flotation	Digestion	Spectroscopy		(Nuelle et al., 2014)
Sand	Digestion		Spectroscopy		(Zhao et al., 2017)
	Digestion		Staining	Spectroscopy	(Shim et al., 2016)
	Digestion				(Kühn et al., 2017)
	Elutriation	Sieving			(Kedzierski et al., 2016)
	Flotation		Staining		(Erni-cassola et al., 2017)
	Sieving		Spectroscopy		(Song et al., 2015)
	Sieving		Visual inspection		(Song et al., 2015)
			Visual inspection		(Heo et al., 2013)
			Thermal degradation		(Hermabessiere et al., 2018)

Figure 5.1 The graph presents the main studies discussed in the review in accordance with the combination of recovery and identification methodologies applied by authors.

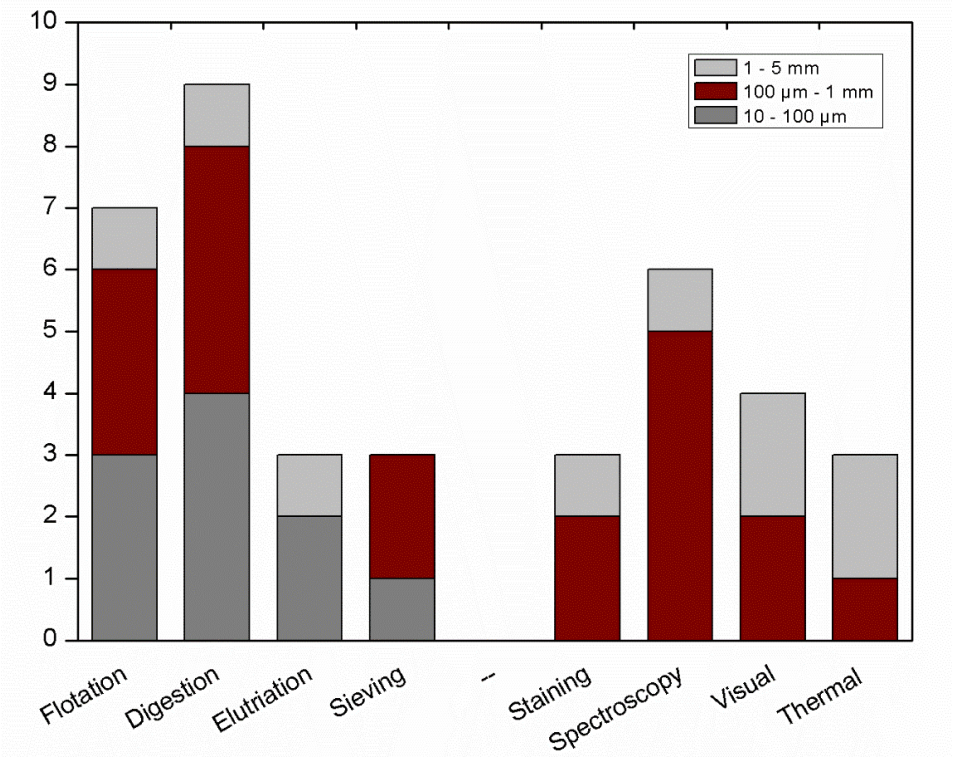

Figure 5.2 The graph displays the usage of recovery and identification methodologies in accordance with microplastics size. The minimum size of the microplastics tested with a specific method is included in one on the following ranges: 10-100 µm, 100 µm-1 mm, 1-5 mm.

Table 5.3 Overview of the techniques to recover microplastics from different solid heterogeneous matrices.

Group	Technique	Polymer	Size	Shape	Matrix	Ref.
Staining and Fluorescence Microscopy	Nile Red in green fluorescence	PE, PP, PS, Nylon, PET, PUR, PVC	20 µm-1 mm	Fragments, pelletes, powder	Sand	(Erni-cassola et al., 2017)
			100-300 µm	Fragments	Sand	(Shim et al., 2016)
	Nile Red in blue fluorescence	PE, PP, PS, Nylon, PET, PUR, PVC	100 µm-1 mm	Fibres, fragments	Sediments	(Maes et al., 2017)
	Fluorescent Withening Agents	PE, PP, PS	1-5 mm	Fibres	Sand	(Qiu et al., 2015)
Thermal Degradation	TED-GC/MS	LDPE	1 mm	Film, fragments	Sludge	(Dümichen et al., 2017)
	Py-GC/MS	LDPE	1 mm	Film, fragments	Sludge	(Dümichen et al., 2017)
		PE, PS, PMMA	100-200 µm	Fibres, microsphere	Sand	(Hermabessiere et al., 2018)
		PP, PET, PVC	0.5-3 mm	Particles	Sediments	(Nuelle et al., 2014)
Spectroscopy	FTIR	PE, PP, EPS	200 µm-1 mm	Fibres, film, fragments	Sand	(Song et al., 2015)
		PE, PP, PS, PET, PVC	200 µm-1 mm	Fragments	Sediments	(Quinn et al., 2017)

		Nylon, ABS, EPS, PES, PVC	0.5-4.75 mm	Fibres, particles	Sediments	(Ellika Crichton et al., 2017)
	Vis-NIR	LDPE, PET, PVC	0.5-1 mm	Powder	Soil	(Corradini et al., 2019)
	AFM-IR	PU	100 nm	Fibres	-	(Dazzi et al., 2015)
	Raman	PE, PP, PS, Nylon, PET, PUR, PVC	100-300 µm	Fragments	Sand	(Shim et al., 2016)
		PE, PS	60-600 µm	Microsphere	Marine snow	(S. Zhao et al., 2017)
		LDPE, PP, PS, Nylon, PET, PVC	80-300 µm	Fragments	Biota	(Karami et al., 2017)
		PC, PE, PP, PS, PET, PVC	1-3 mm	Fragments, pellets	Sediments	(Imhof et al., 2012)
Visual Analysis	Optical Microscopy	PS, PET	0.25-5 mm	Microsphere	Sludge	(Lares and Ncibi, 2018)
		PE, PS	60-600 µm	Microsphere	Marine snow	(S. Zhao et al., 2017)
		PE, PP, EPS	50 µm-1 mm	Fibres, film, fragments	Sand	(Song et al., 2015)
		PE, PP	50-100 µm	Fibres, fragments	Soil	(S. Zhang et al., 2018)
	SEM	LDPE, PP, PS, Nylon, PET, PVC	80-300 µm	Fragments	Biota	(Karami et al., 2017)
		-	100 µm-1 mm	Fragments	Sediments	(Cauwenberghe et al., 2013)
		PE, PP, PS	10 µm-5 mm	Fibres, film, microshpere	Sludge	(Li et al., 2018)
	Visual Inspection	-	2-5 mm	Fragments	Sediments	(Shim et al., 2017)
		-	1-5 mm	Foam, fragments, pellets	Sand	(Heo et al., 2013)
Hot Needle Test		-	200 µm-1 mm	Fibres	Biota	(Devriese et al., 2015)
		-	-	Fibres	Biota	(Griet et al., 2015)

Staining and fluorescence microscopy

Staining of microplastics has found a quite common use in the recent research, in particular since Nile Red has been discovered as a good fluorescent agent for the detection of many polymers (Erni-cassola et al., 2017). The effect of Nile Red agent has been previously tested on different polymers to assess the capability of plastics to fluoresce.

PP, PE, PS, Nylon, and PUR are strongly fluorescent, while the sensitivity is much less for PET and PVC.

The procedure of staining is generally carried out on a PC membrane to provide a not fluorescent background. A solution 1 µg/ml of Nile Red is prepared in methanol, and 2-3 drops are added to the sample which is then covered with a coverslip and maintained 10 min in the dark at 60°C. Fluorescence has been tried in different colours. Green in the emission wavelength 460-525 nm (Erni-cassola et al., 2017; Shim et al., 2016) revealed three advantages with respect to red and orange. First, synthetic polymers are largely more fluorescent; then, organic particles after digestion are not fluorescent in green while they are in red. Finally, the background signal is much less visible. Also fluorescence in blue provided a strong fluorescent index for synthetic polymers (Maes et al., 2017).

Staining is generally preceded by a treatment of digestion with the purpose to remove the organic matter in the sample, because Nile Red marks all the organic particles surrounding the microplastics. Another treatment provided before staining is flotation with water or density modified solution.

In the methodologies implying the fluorescence, they can be included also fluorescent whitening agents (FWAs). Some PP, PE and PS produced in China contain FWAs, a feature which was found to be relevant to identify these microplastics with fluorescence microscopy (Qiu et al., 2015). A risk of overestimation could derivate from the release of FWAs from the plastics and the absorbance on organic matter particles. Thus, as for the use of Nile Red, a treatment for organic removal should be provided before the fluorescence microscopy.

Thermal degradation

The techniques which imply thermal degradation allow identification of microplastics in a bulk sample without a previous treatment, as pyrolysis coupled with gas chromatography mass spectrometry (Py-GC/MS). Moreover other techniques combined TGA with: thermo-extraction and desorption coupled with gas chromatography mass spectroscopy (TED-GC/MS), or thermal desorption gas chromatography mass spectrometry (TDS-GC/MS) (Dümichen et al., 2015). In both the methods, first the test is carried out only on the polymer. It is submitted to thermal degradation in the TGA at temperatures of up to 600°C at a heating rate of 10°C/min. The polymers express some specific degradation products (markers), which must differ from the degradation products of the environmental matrix. These are trapped on the solid phase absorbers and analysis with a GC-MS system identifies them.

When the test is carried out with polymer inside the environmental matrix, the system recognises the characteristic markers of the polymer, allowing its fast identification and quantification (Dümichen et al., 2017).

The Py-GC/MS follows the same concept of TED and TDS-GC/MS, with the identification of some specific degradation products of the polymers, to be found when

the environmental matrix is submitted to the test (Hermabessiere et al., 2018; Horton et al., 2017).

The thermal techniques have been carried out on a wide range of polymer: PP, PE, PS, PMMA, PET and PVC. For all the polymer at least one characteristic degradation product has been found to be a good marker for the identification in the environmental matrix. However, an issue of these techniques is related with the amount of the sample and the concentration of microplastics in it.

Even though in the TED-GC/MS it is usable an amount of sample up to 20 mg, almost 200 times higher than pyrolysis, the methods have been tested for a concentration of microplastics of 10% in a sample of matrix simulated in laboratory. This can be found to a be a limit of the thermal techniques when applied on environmental samples with a lower concentration of microplastics.

Spectroscopy

Infrared spectroscopy has a wide use for microplastics identification after recovery from the environmental matrix. This technique is applied with the double purpose to avoid misclassification of the polymer with organic matter or other materials present in the sample, and to characterize the polymer type (Lavers et al., 2016).

The most easy and cost-effective technique is the FTIR (Shim et al., 2017): in some cases studies, it is directly applied on residues from flotation or digestion (Quinn et al., 2017; Tagg et al., 2015), while in some others it is employed to provide a characterization of polymers already identified under fluorescence microscope (Shim et al., 2016; Maes et al., 2017).

Beside this, spectroscopy has found a new application coupled with automated tracking systems which scan slices of sample surface to detect the presence of microplastics. Atomic Force Microscopy – Infrared (AFM-IR) is a technique that couples an infrared laser source to an atomic force microscope: the concept of the technique is to excite molecular vibrations in the sample using a pulsed infrared laser. Infrared absorption results in rapid heating of the sample causing a quick thermal expansion of the sample, which gives rise to oscillations of the AFM cantilever. The oscillations are detected by the laser beam deflection system typical of AFMs, and the oscillation ringdown pattern is analysed by FTIR that allows extracting the frequencies and amplitudes of the oscillations. As the local absorption is proportional to the cantilever oscillation, an infrared spectrum can be acquired by collecting this amplitude versus wavelength (Dazzi et al., 2010). The method was carried out on PU nanoplastics which has been identified in the solid matrix thanks to the characteristic spectrum of the polymer (Dazzi et al., 2015).

Another methodology for microplastics detection and characterization is the Raman spectrometer, with a wavenumber range normally between 200 to 2000 cm^{-1}(S. Zhao et al., 2017; Shim et al., 2017). Raman can be also used in combination with the automated particle tracking for images analysis. The matrix volumetrically measured and nebulised by high air pressure as a single layer onto a glass slide is automatically scanned and

microplastics are tracked one by one. Simultaneously, microscopic image and Raman spectroscopic analyses are also automatically conducted (Shim et al., 2016).

Detection of microplastics without extraction can be done with Vis-NIR (near infrared) spectroscopy (Corradini et al., 2019). A portable spectroradiometer measures the amount of light reflected from a surface within wavelength range of 350 to 2500 nm, giving a reflected percentage for each wavelength. This technique allows to identify microplastics in the surface level of soil. Thus, five or more reading should be taken after having mixed the soil, to obtain an average reading of microplastics concentration in soil.

Visual analysis

Visual analysis to detect plastics in the environment was widely used especially in beaches and coastal environment before the introduction of the concept of microplastics not identifiable by a naked eye (Heo et al., 2013).

Visual identification however allows a quick screen of microplastics, enough in many cases to differentiate plastic particles in solid matrices in a range of 5 to 0.25 mm size (Ballent et al., 2016). This method provides also a fast classification of plastic particles in accordance with their physical features: type, shape, size and colour (Rocha-Santos and Duarte, 2017). However, when going below the visible range, variable from 2 to 0.25 mm in accordance with the subjective attitude of the operator, it becomes frequent a misclassification of the microplastics due to our inability to differentiate them inside the bulk sample (Lavers et al., 2016).

The use of optical microscope (Song et al., 2015) and SEM (Cauwenberghe et al., 2013) can help in the identification of pieces no more in the range of visibility for a naked eye. Generally, either in the visual or in the microscopical inspection, a treatment to remove the organic matter is previously required. However, it is important to take into account that some of these methods can cause changes in the morphological characteristics of plastics, such as colour or size, which contribute to misidentification of microplastics (Claessens et al., 2013).

Hot needle test

The hot needle test is an alternative for identification of microplastics directly within a bulk sample or among residues of a previous treatment (Devriese et al., 2015). A hot needle carefully handled with tweezers enters in contact with the suspected microplastics. The needle makes plastics sticky and leaves a mark on their surface, while not-plastic particles are not changed by the contact with the hot needle (Griet et al., 2015). However, on the best of our knowledge, this method has not yet been validated in matrices others than biota, and it is still questionable to which range of microplastics it could be applied for a reliable identification.

Conclusions

Various techniques are available for microplastics recovery and identification in solid heterogeneous matrices. Recovery methodologies discussed in the review are all applicable on samples of few grams, exploiting physical and chemical properties of plastics (e.g. density and size), which however may be similar to some materials present in the environmental matrix, due to its heterogenicity. Expecting however the removal of most of the matrix, different identification methods can be tested on the residues. Most of these techniques exploits specific features of plastics, such as capability to fluoresce, specific chemical bonds of the polymers or markers. These properties allow not only to identify the presence of microplastics among different kinds of residues, but also to characterize the polymer type.

Most of the discussed studies combines one or two recovery methods, providing a first removal of great part of the bulk sample, with an identification method, which improves the results of microplastics detection and reduces the risk to misestimate their amount. The review sums up many studies concerning the matrices of interest and depicts the need for a combination of different recovery and identification methodologies, in accordance with the solid matrix typology. As anticipated in the introduction, starting from the already tested methodologies it should be encouraged the development of standardized protocols to detect microplastics in solid heterogeneous matrices.

Moreover, the presence on the market of a wide range of plastic types results, at the end of life, in a wide range of plastics waste which are released together into the environment, thus making it necessary to set up a methodology for microplastics recovery that can be applied to several and different plastic types. Some of the described methodologies would require preliminary analyses due to the various effect that they could have on different plastic types. In particular, new for both film and rigid products are having a great concern on the market. However, their biodegradability is not allowed in all the environmental conditions, but it requires specific process conditions, such as the ones implemented during composting. Thus, if some residues are released in ground and water environments, they can be transformed in microplastics as it happens for conventional plastics. Considering their relatively new development on the market and the wide assortment of ever up-to-date bioplastic types, their physical and chemical properties should be deepened to assess the applicability of those techniques that exploit these properties (e.g. fluorescence, spectroscopy, flotation) and the effects of those that can cause damages and significative changes to the microplastics during recovery (e.g. acid digestion, oxidation).

6 Microplastic residues in sludge and compost

6.1 A highly efficient multi-step methodology for micro-(bio)plastics quantification in sludge

Abstract

The present study develops a multi-step methodology for the identification and quantification in sludge of microplastics and micro-bioplastics (together called in the current work micro-(bio)plastics). In previous studies, different methods for microplastics extraction were devised for conventional plastics, while in the current research the developed methodology was tested on starch-based micro-bioplastics 0.1-2 mm size. Compostable bioplastics are expected to enter the anaerobic or aerobic biological treatments that lead to end-products applicable in agriculture; some critical conditions of treatments (e.g. low temperature and humidity) can slowdown the degradation process and be responsible for microplastics presence in the end-product. The methodology consists of an initial oxidation step, with H_2O_2 35% concentrated to clear the sludge and remove the organic fraction, followed by combination of flotation with NaCl and observation of the residues under a fluorescence microscope using a green filter. The workflow revealed an efficacy of 94% to 100% and from 92% to 96% for plastic fragments, 0.5-2 mm and 0.1-0.5 mm size, respectively. The methodology was then applied to samples of food waste pulp harvested after a shredding pre-treatment in an anaerobic digestion plant in Italy, where PE, starch-based Mater-Bi® (MB) and cellophane microplastics were recovered in amounts of $9\pm1.3/10$ g <2 mm and $4.8\pm1.2/10$ g ≥2 mm. By improving the background knowledge about micro-(bio)plastics fate in biological treatments for the organic waste, the study highlights the need to lower the threshold size for plastics quantification in organic amendants, which is currently set by legislations at 2 mm.

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Introduction

As widely discussed in Chapter 3 and 4, the standards for compostability assessment provide specific conditions under which the degradation test is carried out. However, the process conditions (including time, temperature and humidity) of industrial biological treatments can largely differ from these standard testing conditions, especially regarding anaerobic digestion processes often conducted at mesophilic temperatures (37±2°C) and shorter time scale, both in sewage treatment plants and in organic waste biological treatments. Due to this issue and to the fact that some conventional plastic bags continue entering into treatment plants, in spite of the obligation to use bioplastic bags for the organic waste collection, usually the plants are provided with mechanical pre-treatments to remove larger plastic items (Vigneswaran et al., 2016). Even so, some microplastic items are found in compost (from aerobic composting) and stabilized sludge (from anaerobic digestion). Indeed, these microplastics are not only derived from conventional plastic bags, but also from incompletely biodegraded bioplastics (Weithmann et al., 2018).

The Italian legislation on compost quality (Legislative Decree 75/2010) imposes that no more than 0.5% dry weight of plastic is present in organic amendants. However, the threshold size set to define the presence of plastic materials is 2 mm, meaning that all the microplastics that eventually go through the meshes of a 2 mm sieve are considered analogous to compost. The Legislative Decree is an improvement on the previous threshold of 3.33 mm imposed by the Legislative Decree 217/2006, but a further decrease in this threshold is forecast with the introduction of a new legislation. The main issue here is related to the lack of a standardized methodology for the extraction from compost and sludge of microplastics below 2 mm. Indeed, recent research about microplastics mainly focused on detection and sampling in water environments (Prata et al., 2019). Soil, compost and sludge have been less investigated (Ruggero et al., 2020b). However, it is fair to say that especially the organic amendants for soils, typical end-products of the composting and waste sludge treatment, should be submitted to higher consideration as potential vehicle for the entry of microplastics in agroecosystem (Weithmann et al., 2018). The available studies refer to methodologies which exploit chemical and physical properties of microplastics. Generally, the bulk sample is sieved to study the fraction of interest (Qiu et al., 2016). Oxidizing agents or acids are applied for the removal of the organic matter from the investigated specimens (Li et al., 2018; Lares and Ncibi, 2018), which is highly present in compost and sludge hampering the identification, characterization and mass balance of microplastics, particularly the small size fraction (Jouraiphy et al., 2005). It is important to take into account that this method can cause changes in plastics colour or size, which contribute to misidentification of microplastics (Claessens et al., 2013). Therefore, a previous screening of the effect of the agents on the material should be provided. Visual analysis with naked eye or optical microscope has been also carried out to identify microplastics in various solid heterogeneous matrices (S. Zhang et al., 2018; Lares and Ncibi, 2018). This method allows a quick screen of microplastics of size around 5 mm (Ballent et al., 2016), but

unfortunately it can lead to high possibility of false positive and missing smaller transparent plastic particles (Shim et al., 2017). The small size and the poor contrast between the plastics and the matrix, make the particles not clearly visible (Weithmann et al. 2018; Prata et al., 2019). To this purpose, previous authors have improved the observations using SEM (Li et al., 2018; Cauwenberghe et al., 2013).

The present study intends to face the need of a repeatable and efficient methodology to be promoted as reference for micro-(bio)plastics extraction, imaging and quantification in samples from industrial plants. To this purpose, the aim is to implement and validate a novel methodology with samples of sludge and organic waste pulp, analogous to sludge, obtained from shredding pre-treatment. In the study, it is developed a procedure involving subsequent steps, where each step entails a higher technical complexity but also a higher detectability of smaller sizing microplastics.

The methodology, validated in a sludge matrix, is then applied to a pulp of shredded organic waste harvested from the field, containing endogenous micro-(bio)plastics, including Mater-Bi®.

Materials and methods

MB microplastics sample preparation

While in previous studies different methods for microplastics extraction were devised for conventional plastics, the current research tested the methodology on Mater-Bi®. Density, experimentally measured with a pycnometer, is 1.1-1.2 g/cm3.

Microplastics were generated from MB biobags for organic waste collection, available in Italian supermarkets. Bags were frozen with liquid nitrogen and manually shredded. The pieces obtained with this procedure were sieved to subdivide them into three granulometric fractions: 1-2 mm, 0.5-1 mm, 0.1-0.5 mm.

Preliminary assessment of the effect of H_2O_2 on MB microplastics

Digestion with oxidizing agents was applied in previous studies to remove the organic matter in sludge, biota and sediments (Claessens et al., 2013, Prata et al., 2019). Other groups have used acid and alkali digestion, respectively with HCl or HNO_3 and with NaOH or KOH, to digest the microplastics, however these melt, age or totally destroy the pieces of plastic, leading to an underestimation of their quantity (Karami et al., 2017; Naidoo et al., 2017). Digestion with 30-35% concentrated H_2O_2 has proven to efficiently remove organic matter without significantly alter the polymeric structure of a number of plastics, including PE, PP, PS, PVC and PET (Nuelle et al., 2014). Indeed, in the current study 34.5-36.5% concentrated H_2O_2 (purchased from Sigma Aldrich) was selected to be used in the procedure.

However, from the best of my knowledge, the effect of H_2O_2 oxidation on MB has not yet been proven in literature; therefore, it was preliminarily assessed if the material was

strongly or negligibly aged by the oxidizing agent. Indeed, five MB pieces of each granulometric fraction were weighed with analytical XS Balance mod. BL 224, repeatability 0,0001 g, and submitted to the oxidation process.

The microplastics were deposited at the bottom of a 60 ml beaker and 1 ml of 34.5-36.5% concentrated H_2O_2 was added in a proportion with MB 100:1. The solution was shaken, then deposited for 4 hours at room temperature in a fume hood. The solution was filtered through 0.45 µm pore size, with a Buchner funnel under vacuum; the beaker was rinsed with distilled water and poured into the filter to ensure the removal of all microplastics from the sides of the beaker. After the vacuum filtration, the paper filter was moved into a glass dish with a proper cover for drying into the oven at 60°C overnight.

MB microplastics recovered from the filter were characterized by mean of chemico-physical analyses: weight, FTIR, SEM.

Weight residual after treatment R (%) was measured with Equation 6.1; where W_i and W_f are the weight of MB microplastic samples before and after H_2O_2 treatment, respectively.

Equation 6.1

$$R(\%) = \frac{W_f}{W_i} * 100$$

FTIR analysis was carried out in total reflectance mode (ATR) using a Thermo Scientific™ Nicolet™ iS™10 FTIR Spectrometer with 2 cm^{-1} spectral resolution, coupled with OMNIC software. The sampling area was around 1 mm diameter, in the centre of the crystal. To perform the analysis on the paper filter, the square of interest was placed centrally on the crystal. The SEM was a JEOL JSM-6010PLUS/LA analytical SEM, coupled with JEOL software. The SEM was set with a working distance 18 mm and accelerating voltage 20 kV.

Both FTIR and SEM analyses were carried out on MB microplastic samples before and after H_2O_2 treatment.

Importantly, Mater-Bi® is recognizable from the main peaks assigned to starch and PBAT. Here it is reported a summary of them, while further details were discussed in Chapter 3.1. The wavenumbers for starch are: 2921 cm^{-1} (asymmetric C-H_2 stretch typical of *n*-Alkanes), 1274 cm^{-1} (C-O in ester linkage) and 1163 cm^{-1} (CH_2OH). The main peaks assigned to PBAT are: 1717 cm^{-1} (C=O stretch), 1018 cm^{-1} (ring vibration of benzene) and 726 cm^{-1} (symmetric skeletal stretch of butyl group) (Elfehri Borchani et al., 2015; Herrera et al., 2002; Weng et al., 2013).

Extraction of MB microplastics from sludge using a multi-step procedure

The multi-step procedure devised for microplastics extraction in sludge is schematized in Figure 6.1.

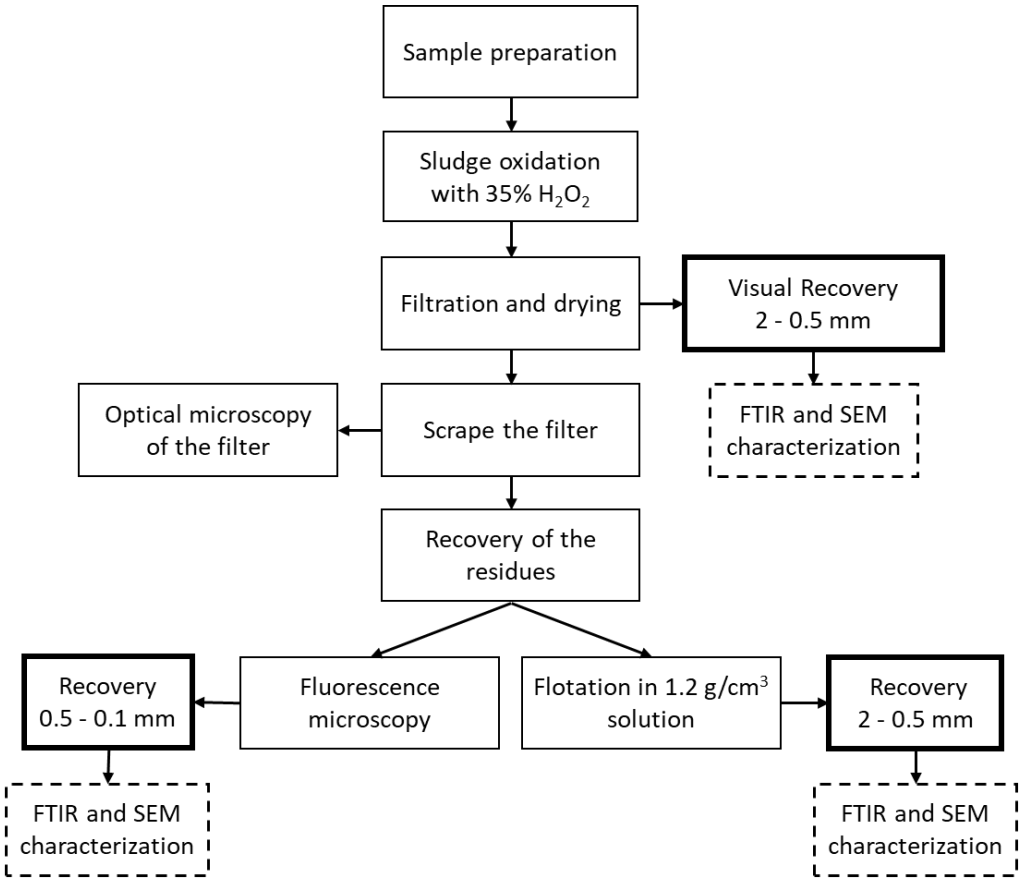

Figure 6.1 Methodology used to recover and quantify microplastics (0.1-2 mm) in sludge.

To test the procedure, primary sludge was collected from waste water treatment plant (WWTP) located in Eastern England; before entering the lab, sludge was disinfected and sieved with a 0.1 mm mesh, to remove the microplastics ≥ 0.1 mm. TS of the sludge were measured in accordance with ISO 11465, 1993 resulting in 6.5 %. Density of the sludge was 1.3 g/cm^3.

Synthetic samples were prepared in laboratory by adding manually generated MB microplastics to plastic-free primary sludge. Before preparing the samples, microplastics, handled with tweezers, were counted and weighed. Then, they were carefully transferred to the bottom of a 60 ml glass beaker. The beaker was filled with 10 ml of primary sludge (W_s). Table 6.1 reports the samples generated: five replicates of each MB microplastic granulometric fractions and three replicates containing mixed pieces of all the fractions. Three blanks, consisting on 10 ml primary sludge without addition of MB microplastics, were also submitted to the procedure as negative controls for handling contamination.

Table 6.1 Initial number of pieces (#i) and weight (Wi) of Mater-Bi® microplastics used to assess the methodology.

Sample	**1-2 mm**		**0.5-1 mm**		**0.1-0.5 mm**		**0.1-2 mm**	
	Pieces	W_i (g)	Pieces	W_i (g)	Pieces	W_i (g)	Pieces	W_i (g)
I	10	0.0017	10	0.0005	7	0.0006	16	0.0018
II	8	0.0012	8	0.0005	6	0.0004	15	0.0014
III	11	0.0019	11	0.0007	7	0.0005	16	0.0019
IV	10	0.0016	9	0.0005	8	0.0004		
V	10	0.0014	10	0.0004	8	0.0004		

Oxidation with 34.5-36.5% concentrated H_2O_2

For the oxidation step, 30 ml of 34.5-36.5% concentrated H_2O_2 were added to the 60 ml glass beaker already containing the mixture of sludge and microplastics. The proportion sludge:H_2O_2 was 1:10 by weight. To homogenize the solution, the beaker was shaken on IKA® KS 130 basic orbital shaker at 160 osc/min for 5 min. The use of the orbital shaker allowed a gentle mixing of the solution, preventing eventual loss of the microplastics. The oxidizing agent was left to act for 4 hours at room temperature under a fume hood. However, having observed that after 2 hours the undissolved organic matter accumulated on the top of the beaker, the solution was shaken again at 160 osc/min for 20 min, until observing a homogeneous solution. It was filtered under vacuum with a Buchner funnel that was 90 mm diameter. The contents of the beaker were transferred into filter paper 0.45 µm pore size, then the beaker was carefully washed out with distilled water to remove any residual microplastics. Filter paper was gently recovered with tweezers and deposited on a covered glass dish. The sample was dried in the oven at 60 °C overnight.

Validation of extraction

Dried filter was visually analysed to identify MB microplastics: pieces were extracted with tweezers. The efficacy of microplastics recovery $R_{MP}(\%)$ was evaluated on the basis of the recovered number of microplastics (Equation 6.2).

Equation 6.2

$$R_{MP}(\%) = \frac{\#_r}{\#_i} * 100$$

Where $\#_i$ and $\#_r$ represent the initial number of microplastics added and the number of microplastics recovered, respectively. Recovered items were also analysed by mean of FTIR and SEM to verify they were effectively Mater-Bi® microplastics.

Afterwards, the weight loss of sludge due to H_2O_2 treatment was measured with Equation 6.3, by mean of a simple procedure: paper filter was weighed (W_{r+pf}); residue was scratched off; paper filter was weighted again (W_{pf}).

Equation 6.3

$$Weight\ loss\ (\%) = \frac{W_s - (W_{r+pf} - W_{pf})}{W_s} * 100$$

Where W_s is the weight of sludge added at the beginning of the test. Paper filter was kept for subsequent observation under an optical microscope, to assess whether any residual microplastics have remained adhered to its surface after the residue was brushed off. The observations were conducted on an Olympus BX51 microscope.

Flotation in NaCl solution

After oxidation, the residue (and microplastics not recovered in the previous step) were subjected either to flotation or to fluorescence microscopy. Indeed, every step increased the risk of microplastics loss and sample contamination. Equation 6.2 was again applied to determine the efficacy of recovery $R_{MP}(\%)$.

For the flotation, a solution of NaCl 1.2 g/cm^3 was prepared; the difference in density between plastic and sludge allowed flotation of the microplastics and deposition of the sludge residue. NaCl solution was added to the residue in a 20 ml glass vial. After manual shaking for 1 min, another 10 min were needed to observe that the particles inside the vial had reached equilibrium inside the water column. The surfactant was then recovered from the vial.

Fluorescence microscopy with green filter

This step involved to image the residue by fluorescence microscopy. Samples were placed on a glass slide and a drop of distilled water was added to the corners of the slide and a cover glass was deposited over the sample. A Brunel SP105F with pE300 LED inverted fluorescence microscope, with transmitted illumination - 6v 30 watt (bright field and phase contrast available) was used for imaging. The available fluorescence channels in the microscope are: DAPI, FTIC and Texas Red, corresponding to violet (emission wavelengths 395 to 410 nm), blue (490 to 505 nm) and green (560 to 580 nm) filters respectively. Imaging was carried out using an objective lens strength of 4x. The microscope was fitted with Canon EOS 1200D camera to capture the images of the samples.

The three spectral ranges were tried first on Mater-Bi® and sludge residues separately to observe the tendency of each matrix to fluoresce. After this preliminary observation, full analysis was carried out.

Application to harvested samples

The methodology was applied on organic waste samples harvested from a treatment plant located in the Northern Italy. The plant consists of a dry mesophilic (37±2°C) anaerobic digester, followed by a composting treatment. However, the digestion condition may not be suitable for the degradation of bioplastic materials. Beside this issue, the plant has also to handle some bags made from conventional not compostable plastics (mainly PE), used for organic waste collection in spite of the ban by the Italian regulators. Therefore, the plant is provided with a pre-treatment that aims to remove both bioplastic and plastic bags and to shred the organic waste until a homogeneous pulp has been obtained. As the two processes are developed inside the same press machine, microplastics may be generated from the shredding of some plastic items remaining in the organic waste after separation. Samples of the organic waste pulp obtained after the pre-treatment were harvested and the presence of microplastics was investigated using the developed methodology. In order

to characterise the plastic types expected in the samples, FTIR analysis was randomly carried out on bags removed by the separation pre-treatment.

Extraction and quantification of microplastics from harvested samples

The samples consisted of 10 g of organic waste pulp, a matrix analogous to sludge but more heterogeneous. The waste pulp samples, with initial TS 10%, were diluted with tap water to obtain the same content of dry matter of the sludge used to validate the method (*i.e.* 6.5%). After 4 hours oxidation, the samples were recovered from the beaker, filtered under vacuum in a 90 mm paper filter and dried in the oven overnight at 60°C. Due to the heterogeneity of the residue on the filter, mainly pieces of cellulosic material and food peels, the methodology was applied until the visual recovery step: the filter was divided into a grid 1x1 cm squares and each square was visually analysed. The items identified as microplastics were recovered and analysed by FTIR to verify and define the plastic type. The squares of the filter in which microplastics could not be detected visually, were analysed with FTIR. To prepare the background, a paper filter was wet using tap water, 34.5-36.5% H_2O_2 and water filtered from the organic waste pulp. One point of each square visually "free" of plastic was analysed with FTIR to check the presence of eventual microplastics not visually identified.

In some cases, the identification of microplastics was masked due to degradation and organic matter attached to the surface of the material. Thus, the Cauchy-Schwarz inequality was used to calculate the numeric score which describes the differences between two spectra, and on which a library search was based. In accordance with the Equation 6.4, the score ranged from 0 to 1; a score ranging from 0.8 to 1 is generally considered a good basis for polymer assignment (Rocha-Santos and Duarte, 2017).

Equation 6.4

$$score = \frac{(sample, reference)}{||sample|| \cdot ||reference||}$$

Statistical analysis

Five samples of 10 g of diluted organic waste pulp were analysed. For each sample 71 squares were drawn in the grid filter, for a total of 355 squares analysed with FTIR.

The error of visual recovery of microplastics in each sample was estimated from the experimental data. Two errors were defined: an error of overestimation, called ε_{over}, that refers to the number of items visually recovered which are not plastics; and an error of underestimation, called ε_{under}, that means the number of squares in the filter which contain microplastics that could not be identified visually. From the experimental data is finally possible to derive the Equation 6.5 for microplastics estimation, starting from the number of visually recovered items:

Equation 6.5

$$MP = MP_{VR} - \varepsilon_{over} + \varepsilon_{under}$$

Where MP refers to the corrected estimation of microplastics present on the filter and MP_{VR} refers to the microplastics visually recovered from the filter after the oxidation process.

Results and discussion

Preliminary assessment of the effect of H_2O_2 on MB microplastics

The initial and final weights of the five samples of microplastics used to assess the effect of H_2O_2 on Mater-Bi® material are shown in Table 6.2. $R(\%)$ has been calculated using Equation 6.1, as an average of the five samples in each size range; results highlighted a negligible weight loss of the material during the oxidation.

Table 6.2 R (%) of microplastics after oxidation with H_2O_2.

Sample	**1-2 mm**			**0.5-1 mm**			**0.1-0.5 mm**		
	W_i (g)	W_f (g)	R (%)	W_i (g)	W_f (g)	R (%)	W_i (g)	W_f (g)	R (%)
I	0.011	0.011	100	0.010	0.009	90	0.005	0.005	100
II	0.009	0.009	100	0.010	0.010	100	0.006	0.006	100
III	0.010	0.010	100	0.010	0.009	90	0.005	0.005	100
IV	0.010	0.010	100	0.010	0.010	100	0.005	0.005	100
V	0.010	0.010	100	0.010	0.010	100	0.005	0.005	100

The recovered microplastics were characterised by SEM and FTIR; SEM micrographs, which compare MB microplastic surface before and after oxidation with 34.5-36.5% concentrated H_2O_2 for 4 hours, show that Mater-Bi® was heterogeneous containing some grains within a smooth film. After oxidizing treatment, some holes were visible on the surface of the Mater-Bi. Since Mater-Bi® is composed of starch grains and PBAT, these micrographs suggest that starch has been partially degraded by hydrogen peroxide (Deschamps et al., 2008; Szymońska et al., 2009).

FTIR showed representative spectra for 0.5-1 mm and 1-2 mm MB microplastics recovered after oxidation in comparison with a spectrum of the material taken before the treatment. The material maintained its characteristic absorption peaks after the oxidation process. The main peaks in the spectra matched the components of Mater-Bi® molecular structure, and are comparable with standards given in references. The peak 2921 cm^{-1} related to starch, was smoother after the oxidizing treatment, thus confirming the observations derived from SEM micrographs about a partial degradation of starch by hydrogen peroxide, which however does not compromise the recoverability of MB pieces.

The adopted methodologies seem to provide accurate information about materials change after oxidation with H_2O_2. Other authors tried to measure the oxidation effects on plastic

materials with optical analysis and calculation of surface area reduction. However, these methods were not found to be highly suitable for this purpose (Nuelle et al., 2014).

Extraction of MB microplastics from sludge using a multi-step procedure

The oxidation of sludge in H_2O_2 led to a reduction of the sludge weight of more than 99.96% for all the samples, in accordance with Equation 6.3. A removal rate of organic matter near 100% was also found in previous studies dealing with solid matrices, such as sand and biota (Karami et al., 2017; Shim et al., 2016).

Microplastics from sludge residues were recovered in accordance with the two branches of the described methodology. Samples containing microplastics 1-2 mm and 0.5-1 mm were recovered using the flotation protocol. The efficacy of recovery, calculated with Equation 6.2, of 1-2 mm microplastics was 93.7±2.9% from visual recovery after oxidation, rising to 100% after flotation. For samples containing microplastics 0.5-1 mm size, $R_{MP}(\%)$ was 83.9±5.8% after oxidation, rising to 93.7±2.8% after flotation. The time required for visual recovery after oxidation procedure did not exceed 5 min for 1-2 mm microplastics fraction, and 15 min for 0.5-1 mm fractions. The samples containing microplastics <0.5 mm were imaged in the fluorescence microscope. The recovery efficacy of microplastics of size 0.1-0.5 mm was 60.8±6% using visual recovery after oxidation, and 91.8±4.5% using fluorescence microscopy. The average recovery of the three samples containing microplastics in the size range of size 0.1-2 mm, was 80.8±3.2% after oxidation, and 95.8±2.1% with fluorescence. Figure 6.2 summarizes the four categories of samples: each column represents the mean value of the samples, with the standard deviation of the total efficacy of recovery $R_{MP}(\%)$.

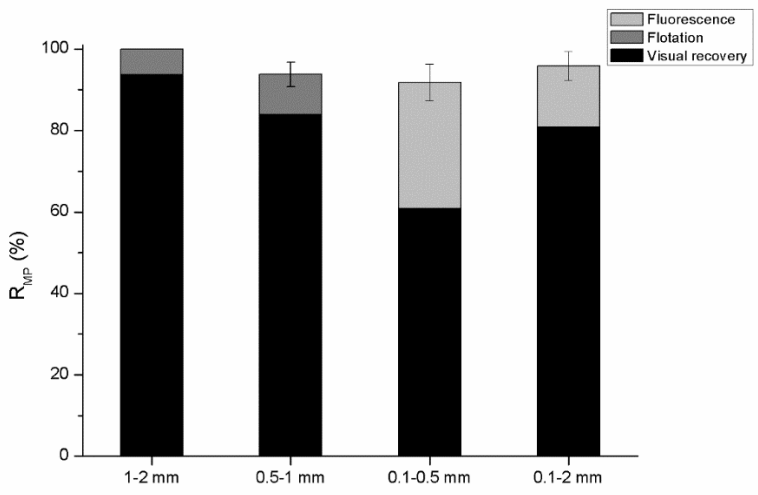

Figure 6.2 Recovery efficacy (R_{MP}%) of microplastics 1-2 and 0.5-1 mm, after oxidation and flotation. Microplastics shown in the third and fourth column (0.1-0.5 and 0.1-2 mm) are recovered after applying an oxidation treatment and fluorescence microscopy. The standard deviation of the final recovery is indicated for each column respectively.

In the three blanks no microplastic items were found neither with visual recovery nor with microscopic observation of the filter: indeed, it is fair to assume that contamination of the samples was prevented during the procedure with careful samples handling.

Almost all of the <0.5 mm microplastics missed with visual recovery were identified using fluorescence microscopy. Indeed, it was expected that fluorescent plastic particles >0.1 mm could be counted easily and had a recovery not lower than 95% (Maes et al., 2017). A total of 11 items were recovered from the five samples containing microplastics with a size of 0.1-0.5 mm and 8 microplastics with a diameter of 0.1 to 2 mm were found in the residual powder of the sludge. Figure 6.3 reports two examples of MB microplastics that fluoresce under a green filter, while the surrounding residues of sludge were much less sensitive to fluorescence. The FTIR spectra confirmed that the fluorescing pieces in Figure 6.3 are MB microplastics. Moreover, filters after scraping were analysed under microscope, to check if any residues of microplastics were still attached to the filter surface. Only two items were discovered.

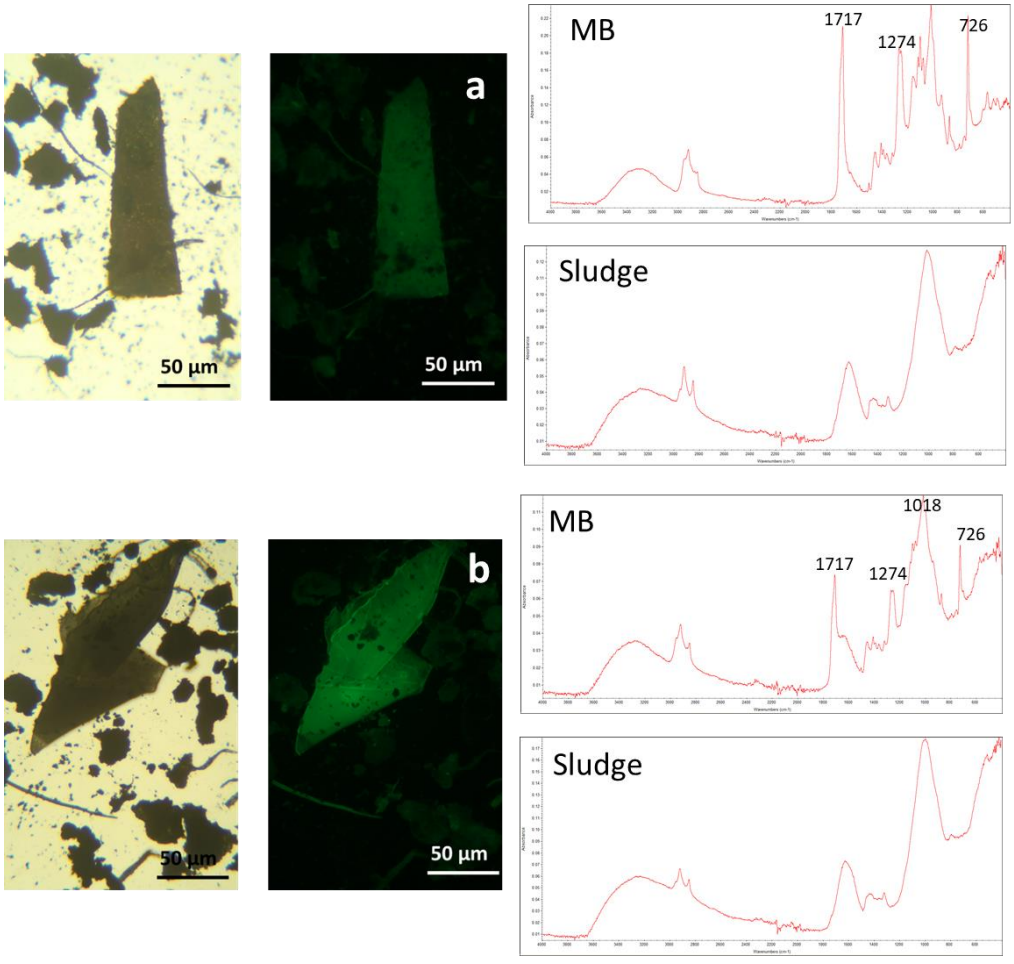

Figure 6.3 Optical micrographs (left) and green fluorescence images of microplastics of Mater-Bi®, compared to observations without using a green filter. The microplastics were recovered from samples containing items of 0.1-0.5 mm, IV (a) and V (b); the spectra confirm that the fluorescent items are MB microplastics, whilst the surrounding powder was sludge residue.

Application to harvested samples

The plastic bags collected after separation pre-treatment were identified with FTIR and the peaks were assigned to characteristic peaks from Mater-Bi® and polyethylene bags. Only one polypropylene bag was found. The FTIR analyses on the microplastics recovered from the filters identified also one polystyrene item and some microplastics of cellophane. Some reference peaks of each plastic type have been identified; they are reported in Table 6.3.

Table 6.3 Characteristic peaks (cm^{-1}) of plastic items found in the harvested organic waste samples.

Mater-Bi®	**Polyethylene**	**Polypropylene**	**Cellophane**	**Polystyrene**
1274 (C-O)	2914 (CH_2)	2950 (CH_2)	2923 (CH_2)	3100 (aromatic CH)
1717 (C=O)	2845 (CH_2)	2845 (CH_2)	2845 (CH_2)	1426 (ring stretch)
1018 (C_4H_9)	1463 (CH_3, CH_2)	1576 (C=C)	1065 (C=S)	765 (ring stretch)
726 (C_6H_6)	721 (CH)	1538 (C=C)	1030 (CH)	726 (C_6H_6)

The presence of some cellulosic and powder residues on the filter was also assessed from FTIR analysis. The same issue was observed by previous authors working on microplastics recovery from sludge. Therefore, proper characterization of the residual items present on the filter was required, something that is lacking in many studies (Ziajahromi et al., 2017).

Figure 6.4 shows an example of the analysis carried out on the paper. FTIR spectra of PE, MB and PS microplastics visually recovered from C8, E9 and D6 squares are displayed to the right of the paper filter.

After correction with FTIR analysis, the efficacy of the visual recovery from the filter is found to be in a range between 87.5% (filter III) and 100% (filters I and II). In filters IV and V the recovery efficacy was 93.3% and 92.3% respectively. These values are comparable with those previously assessed for the visual recovery method (Figure 6.2).

The average number of microplastics recovered in 10 g sample is 14±1.5, of which 8.4±0.5 are MB and 5.4±1.7 are conventional microplastics, including cellophane, PE and PS. The histograms in Figure 6.5 depict the number of microplastics recovered in the five paper filters analysed. Moreover, the average number of microplastics with diameter < 2 mm, meaning that at least one dimension is smaller than 2 mm, is 9±1.3 and ≥ 2 mm 4.8±1.2. The data point out that the current threshold of 2 mm devised for plastics quantification should be lowered, as the microplastics smaller than this size are almost the double that of items ≥ 2 mm.

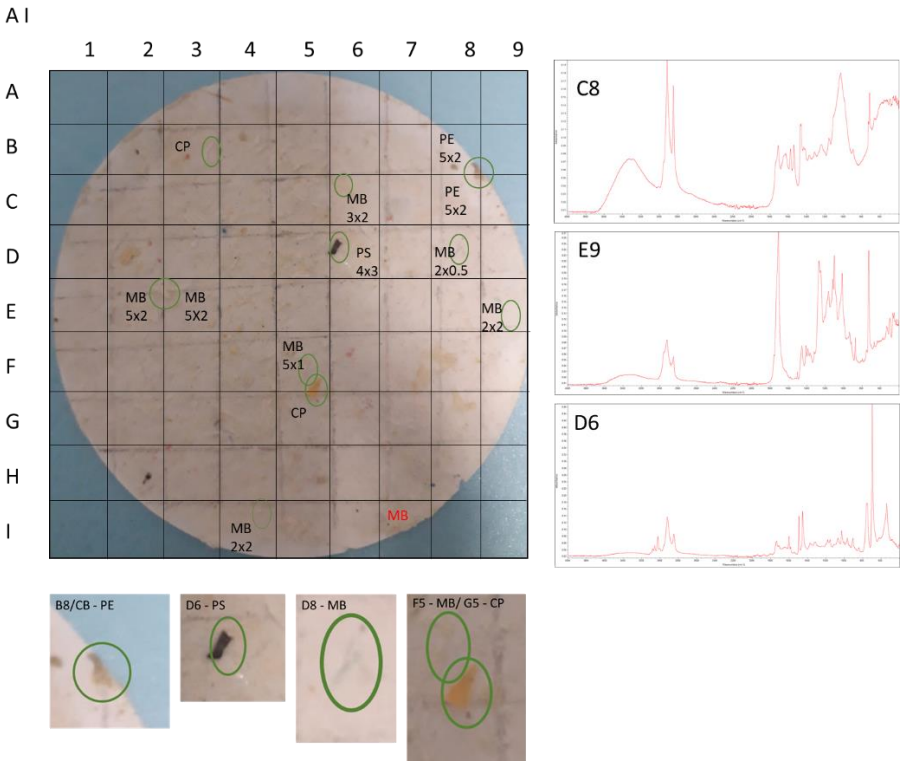

Figure 6.4 Filter paper with a grid of 1x1 cm squares. The microplastics visually identified on the filter surface are circled in green, and three spectra are shown to the right of the image. They correspond to squares C8, E9 and D6, which identify PE, MB and PS microplastics, respectively. Square I7 contains an MB item (written in red) on its surface which was not visually identified.

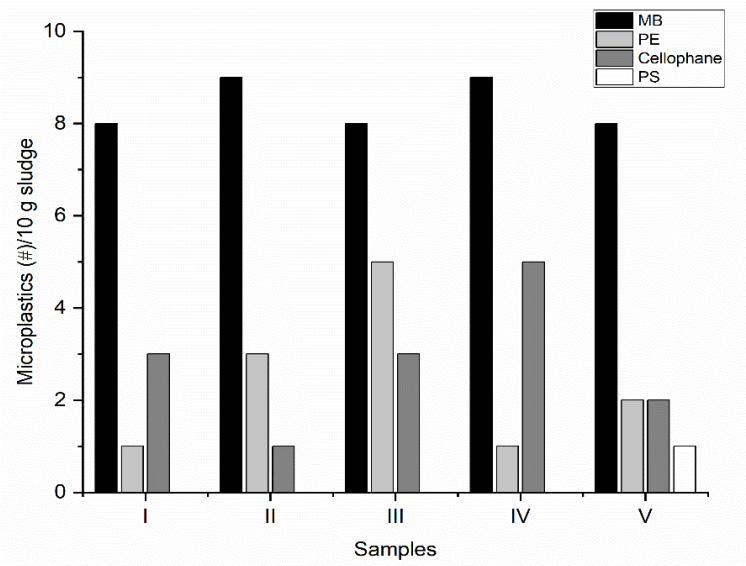

Figure 6.5 Quantification analysis made on five harvested samples of pre-treated food waste: the bar chart shows the number of microplastics recovered in 10 g of each sample (numbered from I to V).

From the experimental data, ε_{over} and ε_{under} in the Equation 6.5 have been determined to be 3±4% and 9±3%, respectively. The statistical elaboration of the 355 FTIR data, through which the experimental Equation 6.5 is built, appears to provide a simplification of the protocol for microplastics quantification. In a highly heterogeneous matrix where no further extraction techniques are applicable after visual inspection of the oxidized

sample, the experimentally defined equation makes it possible to implement a pretty good correction in microplastics quantification.

Conclusions

The current study devised a multi-step procedure to extract and measure the amount of microplastics film, both Mater-Bi® and conventional plastics, in sludge and pulp of food waste pre-treated before digestion. The protocol is based on a combined methodology of oxidation using H_2O_2, flotation in NaCl and fluorescence microscopy, with a preliminary assessment of the negligible effect of H_2O_2 on the bioplastic. The devised multi-step procedure allows to use either flotation or fluoresce microscopy on the basis of the size of microplastics, which have to be detected. Indeed, the faster and easier branch of the methodology (oxidation + flotation) is applicable on 0.5-2 mm sizing microplastics: the protocol leads to extract from 94% to 100%. Microplastics sizing 0.1-0.5 mm were instead recovered with an efficacy ranging from 92% to 96%, implementing oxidation and fluoresce microscopy. The investigation of samples of food waste pulp harvested from the field after shredding pre-treatment found in the matrix PE and MB microplastics, as well as cellophane. The recovered microparticles were furtherly confirmed with FTIR analysis to avoid misidentification with items such as food peels and cellulosic microparticles. Importantly, the average number of the microplastics extracted was $9 \pm 1.3/10$ g with size <2 mm and $4.8 \pm 1.2/10$ g with size ≥ 2 mm.

To conclude, the work is topical as the market and the use of compostable bags and single-use cutlery is increasing, and so these bioplastic products are expected to be delivered in increasing volumes with the organic waste fraction. Both bio and conventional plastics are destined to enter the digesters. However, the process conditions (of anaerobic digestion plants can largely differ from those of the standard tests and thus may not allow a complete degradation of MB bioplastics. Some anaerobic digestion plants are provided with a further composting treatment, which can improve the degradation of bioplastics. Conversely, conventional plastics are not supposed to be degraded during these treatments, thus microplastics are expected to be found in compost. Taken together, these findings highlight the need to lower the current threshold of 2 mm devised for micro-(bio)plastics quantification in the organic amendants. The study seems to be a step towards filling the gap between the need to improve the current legislation and the lack of a standardized protocol for microplastics <2 mm quantification.

6.2 Characterization of bioplastic micro-residues at the end of composting: are they assimilable to compost?

Abstract

The current legislations about plastics in compost (Legislative Decree 75/2010) and bioplastics compostability (EN 13432:2000) require monitoring plastic content only in the retained fraction of 2 mm sieve. The undersieve is considered assimilable to compost without further analysis. Starting from this statement, the present research aims to observe and characterize some bioplastic micro-residues found in the undersieve of a lab scale composting test, carried out on a matrix of organic food and green waste. FTIR and TGA analysis were performed to compare compost and recovered micro-residues. The results reported a high compatibility in terms of chemical composition between compost and bioplastic micro-residues. However, data elaboration from TGA revealed that a small percentage (5.6%) of micro-residues chemical composition strictly differed from compost and consisted on a residual part of PBAT. The presence of residual PBAT was confirmed by two peaks in the wavenumbers 1018 cm^{-1} and 726 cm^{-1}, corresponding to disubstituted benzene and $(CH_2)_{n=4}$ in butanediol terephthalate. Compost is directly accessible to living organisms due to the wide use in agriculture. Therefore, the present study outlines the need to strengthen the inspection requirements about plastic content in compost below the threshold size of 2 mm.

Introduction

The present work is focused on micro-residues potentially originated during composting and released into the environment within compost. Bioplastic micro-residues are generated by contributing causes: (i) macro-scale mechanical factors, such as stresses in manual operations during the process and abrasion of the material with other waste (Lucas et al., 2008). (ii) micro-scale phenomena: breaks and fractures along the superficial layers contribute to erode the material and disintegrate the surface (Adhikari et al., 2016), (Arrieta et al., 2014). (iii) since mechanical and physical factors reduce the size of bioplastics, the surface available for microbial and chemical activities increases. Therefore, oxidation processes and hydrolysis, as well as bacterial colonization, work both to disintegrate the pieces and to degrade them.

It is fair to remark that the method to monitor the disintegration of bioplastics during composting is the disintegration degree, which is based on weight loss experimentally measured. Bioplastics weight after degradation is compared with the weight of fresh material introduced in the test. Standard measurement procedure encloses three steps: sieving with 2 mm mesh and recovery of the oversieve; washing with distilled water; drying at 40±2°C and weighting. It is generally followed not only by industries for new compostable materials. Many scientific studies were carried out in the last decade referring to the disintegration degree methodology. The results obtained by different authors, for starch-based bioplastics, PLA and PHA, are not lower than 80% during composting of 6-12 weeks at 58°C (Sarasa et al., 2009; Javierre et al., 2015). Despite the high weight loss measured, pictures of the residues after the test reported small fragments, very similar to compost in the aspect (Balaguer et al., 2016). They are not accounted in the weighting for their size below the set threshold but also because, in accordance with visual observations as required by EN 14045 (EN 14045, 2003), they have colour, shape and consistency similar to compost.

With the effort to overcome the mentioned methods which account only physical aspects, this study aims to characterize the chemical features of bioplastic micro-residues at the end of composting process, before compost delivery for fertilization purposes. These chemical features are an important unknown parameter to determine the nature of bioplastic micro-residues, considering that they are meant to be released into the environment. The characterization of bioplastic micro-residues was assessed by combining FTIR and TGA results. Furthermore, to test micro-residues assimilability to compost, not only in terms of physical aspect but also of chemical composition and structure, the analyses were carried out both on compost and on micro-residues.

Materials and methods

Experimental setup

Bioplastic micro-residues were generated during a lab scale composting with Mater-Bi® carrier bags available in Italian supermarkets. The composting test consisted on a

thermophilic phase at 58±2°C for 20 d, followed by a maturation phase at 37±2°C for 25 d. It was carried out in a 12 l vessel with 5 kg of a homogeneous mixture of organic waste (food and green waste). MB bags were manually cut in 5x5 cm size and added in 1% wt to the waste matrix. The same lab scale test was carried out in Chapter 3.1, were all details are provided. However, unlike the previous test, bioplastic pieces were integrated into the waste matrix without nets. Furthermore, at the end of the 45 d test it was arranged the recovery of microscopic residues, on which it was focused the objective of the work. Micro-residues were recovered from compost after sieving it through a 2 mm sieve. To improve the identification of the smallest particles, down to the size of 1 mm, the recovery procedure was enhanced by a magnifying glass, diameter 50 mm and magnification 5x, and carried out for the same sample at least three times, to reduce the subjectivity of the naked eye.

Thermogravimetric analysis

TGA was performed using a TA Instruments Q-600 (DTA-TG) apparatus using open aluminum pans under nitrogen atmosphere. Measurements were performed in a dry nitrogen flow of 100.0±0.5 cm^3 min^{-1} by increasing the temperature from room temperature up to 500°C at 10°C min^{-1}. The analysis was carried out on fresh MB (before degradation), on five MB micro-residues (<2 mm) recovered from composting test and on sample of compost from the undersieve 2 mm. Before submitting compost to the analysis, it was manually grinded with a pestle in a mortar in order to obtain a better homogenization of the matrix. From this analysis we were interested in two main observations: (i) the degradation of MB during composting and (ii) the comparison between MB residues and compost obtained at the end of the test from the organic waste. The mentioned observations can be derived from both the semi-quantitative information about percentage weight loss of the material and the decrease of the peak temperature of thermal conversions, occurring during the analysis.

To this purpose, the raw data were divided in four sections. The sections were identified in the DTGA graph of the fresh MB thanks to the thermal conversion processes which take place within the polymer during the analysis. The first section corresponds to the temperature range (Tr): 125-250°C, with an almost flat curve in the graph. The second area is in the Tr: 250-340°C, associated to the conversion of the starch. PBAT component takes a higher temperature to start the thermal conversion, which is concluded within the Tr: 340-440°C. The last area corresponds to 440-500°C, relatable to residual ashes remaining at the end of the analysis. These four sections are used as reference also in the data elaboration of micro-residues and compost.

However, it is necessary to explicit that the conversion Tr for starch and PBAT is not directly identifiable in the DTGA graph of MB. In the graph of the polymer, the two thermal conversions are subsequent and the end of starch conversion, as well as the beginning of PBAT conversion, is not clear. Therefore, it was carried out a TGA analysis on pristine starch and on pristine PBAT Ecoflex® F Blend C1200, supplied by BASF.

From this analysis it was possible to clearly delimitate starch and PBAT conversion Tr (Figure 6.6).

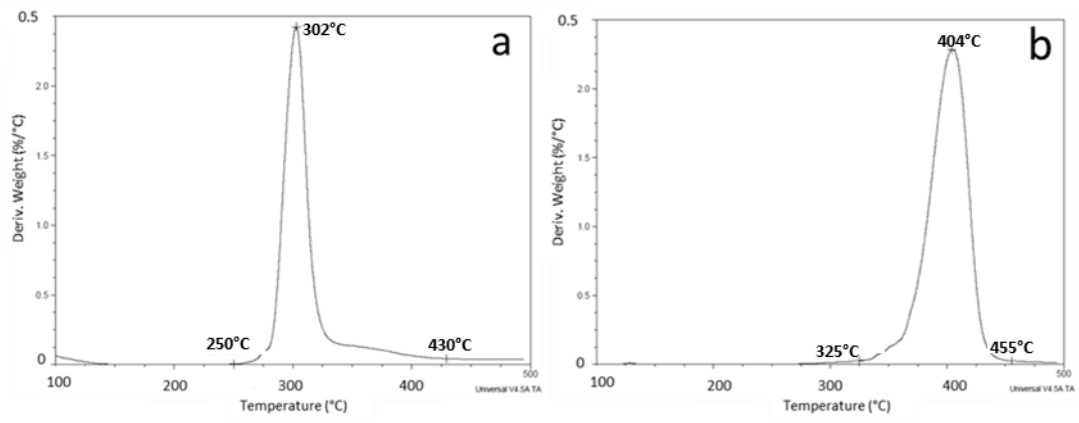

Figure 6.6 TGA and DTGA graphs of starch (a) and pristine PBAT (b).

A precise indication of the peak temperatures (T_{peak}) associated to the thermal conversions of starch and PBAT was directly provided by the software TA Universal Analysis.

On the contrary, the procedure to get information about the percentage weight corresponding to each section requires a mathematical elaboration. Exploiting the raw data of DTGA, the underlying area (or peak area PA) of each Tr is extrapolated from the integral in the Equation 6.6.

Equation 6.6

$$\int_{T_i}^{T_f} \frac{dw}{dT}\, dT = (PA)_{Tr}\ (\%)$$

Where T_i and T_f are the initial and final temperatures in each temperature range defining the four sections.

Then, with the Equation 6.7 each peak area needs to be normalized on water content, which may vary from one sample to the other. Water content corresponds to the peak area of Tr: 0-125 °C.

Equation 6.7

$$(PA)_{Tr}\frac{100-(PA)_{0-125°C}}{100} = (PA)^*_{Tr}\ (\%)$$

Fourier transform infrared

FTIR analysis was carried out in total relfectance mode (ATR) using a Thermo Scientific™ Nicolet™ iS™10 FTIR Spectrometer with 2 cm^{-1} spectral resolution, coupled with OMNIC software. The investigated wavenumber range is 4000-500 cm^{-1}.

The sampling area was around 1 mm diameter, in the centre of the crystal. Samples analysed were mostly bigger than this size, allowing to easily menage the intrument. However, for the pieces smaller than 1 mm size the analysis was feasible by placing the item centrally on the crystal. The variation of peaks intensity and wavenumbers provides qualitative information about the chemical change of the polymeric structure and about the specific degradation process of starch and PBAT.

Visual inspection

Micro-residues <2 mm recovered from the waste matrix were also observed in accordance with the criteria described by EN 14045 (EN 14045, 2003): distribution of particle size, consistency of the material, discolouring, erosion signs on the surface and lateral erosion signs.

Results and discussion

Thermogravimetric analysis

Figure 6.7a reports fresh MB and one replicate of MB micro-residue recovered after composting. The five replicates of bioplastic micro-residues do not present a high variability neither in the T_{peak} (315±1°C for starch and 385±1°C for PBAT) nor in the peak areas $(PA)^*$ of the different temperature ranges.

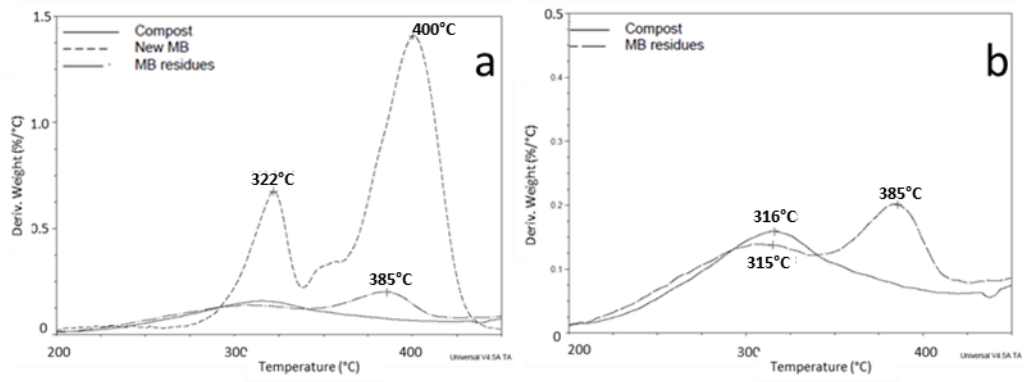

Figure 6.7 DTGA outputs with temperatures of the peaks of conversion. In Figure a) are depicted fresh MB, on replicate of MB residues and compost, while Figure b) is a focused on the sections of main interests (250-340°C for starch degradation and 340-440°C for PBAT).

Higher is the complexity of the material, higher is the T_{peak} of the thermal conversion (Dümichen et al., 2017). After degradation took place, it was observable that T_{peak} of starch and PBAT in MB residues were lower respectively of 10°C and 16°C than in fresh MB. A decrease from 10 to 20°C in a period exceeding 20 d of composting, is directly attributable to the chain scission and depolymerization to oligomers (Luzi et al., 2015), here occurring both in starch and in PBAT components. A further consideration should be done on the small peak of temperature 340-360°C, corresponding in the fresh MB to

additives for the extrusion of the polymer (Elfehri Borchani et al., 2015). From the figure it is clearly visible that the peak disappeared after the 45 d of the test. However, recent studies disclosed some relevant issues about the potential of additives to migrate from the plastic products to the medium in contact with the product. Generally this phenomenon can occur due to the comparatively low molecular weight of the additives (300-600 g/mol) (Hahladakis et al., 2018). In light of this finding, the disappearance of the peak related to additives in the degraded Mater-Bi® residues may be related either to effective degradation or to partial or complete migration into the compost matrix. This outbreaking topic is foreseen to find in the future research further helpful findings.

The $(PA)^*$ was calculated with Equation 6.7, in the sections correspondent to starch and PBAT, both on fresh MB and on degraded micro-residues. The weight loss of starch and PBAT after degradation corresponded to 44.5% and 87.2%, respectively.

Starch apparently had a lower weight loss than expected: to this purpose, it is fundamental to compare the DTGA results of MB micro-residues and compost. Figure 6.7b provides a first focus on this comparison: in the T range 250-340°C, it depicts a broad peak both in MB residues and in compost in the surrounding of 315°C. On the contrary, in T range 340-440 °C it is visible a flat curve in compost instead of the sharp peak of the polymer.

The results of peak areas calculation for the four sections, normalized in accordance with Equation 6.7 (water content of 5.26±0.44% in MB residues and of 13.66% in compost), provided a deeper understanding in the comparison between compost and micro-residues. In Figure 6.8 the peak areas, equivalent to the percentage weight of each section, are reported in form of histograms.

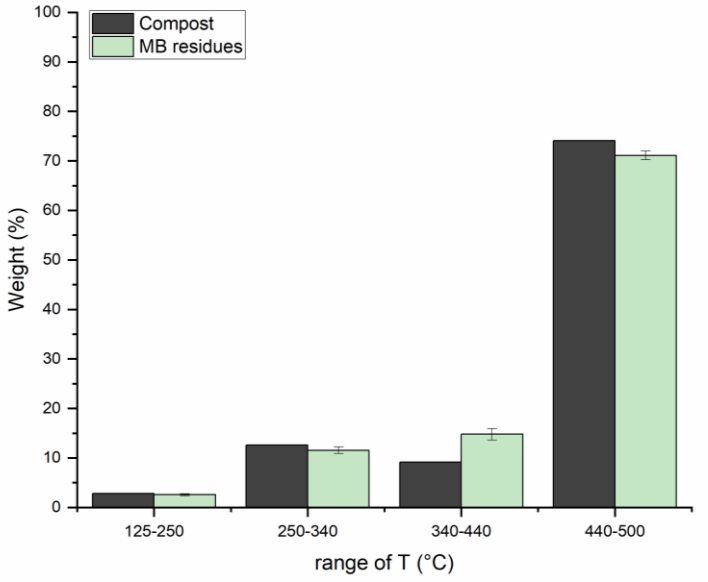

Figure 6.8 Weight (%), or peak areas, from TGA normalized on water content in accordance with Equation 6.7: the comparison between mature compost and MB residues was done for different ranges of temperature. In particular, 250-340°C corresponds to starch, while 340-440°C corresponds to PBAT in the MB polymer.

Each section was studied separately: the residual ashes (440-500°C) were the highest amount in both MB and compost, 71±0.9% and 74%, respectively. The range 250-340

°C presented just a difference of 0.5±0.5% between the compared samples. Thus, beside the samples T_{peak} in the considered temperature range, the weight result suggested a similarity between compost and MB residues. Moreover, the broad peak visible in the DTGA graph in the range 200-350°C could be easily related to the main step of conversation of the organic matter from soil, compost and sludges (Miyazawa et al., 2000). Instead, in the TGA analysis the peaks in the range 350-400°C are normally relatable to polymers conversion (Dümichen et al., 2017). In the current study, analysing the Tr 340-440°C, it was found a gap between compost and degraded MB. While MB micro-residues displayed a percentage weight of 14.8±1.2%, in compost it was 9.1%. The 5.6% weight difference could be related to PBAT backbone remaining in bioplastic micro-residues. More details are stressed in the next paragraph, exploiting the FTIR analysis.

Fourier transform infrared

Figure 6.9a shows both the spectrum of the pristine MB and of the residue. The graph highlights the disappearance of some peaks in the residue and the decrease of the absorbance of the remaining peaks.

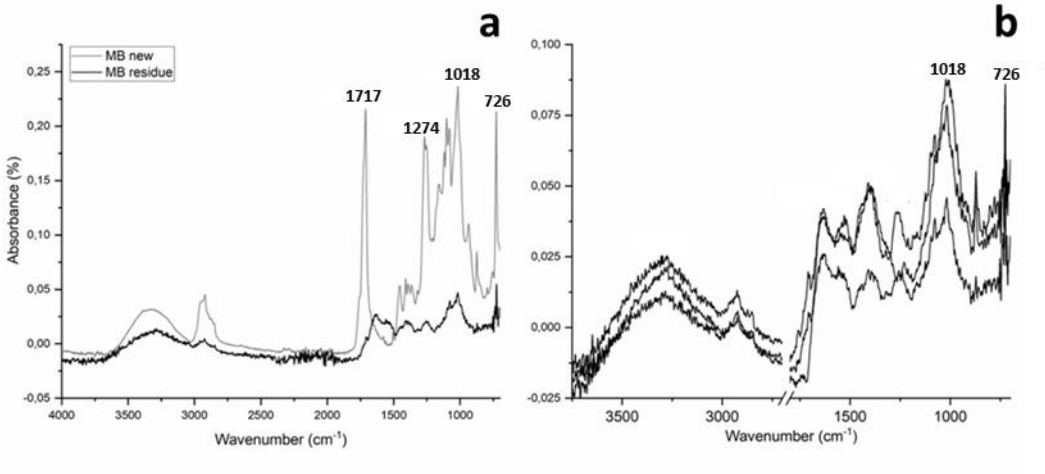

Figure 6.9 Spectra of new MB compared with residue (a) and variability of the spectrum of the residues reported with three replicates (b).

The absorbance, in accordance with the Lambert Beer law, is dependent both on the thickness of the material and on the concentration of the absorbent bonds. It is assumable a contribution of both factors: on the one hand, physical erosion and mechanical abrasion act on the material, thus weakening and thinning the residual plastic (Lucas et al., 2008). On the other hand, TGA outlined that micro-residues have a simpler composition than the fresh material, which is more similar to compost than to a polymer. In Figure 6.9b, the spectra of three analysed residues are reported. It is fair to observe a common trend in the three replicates: the disappearance of the peak 1163 cm^{-1} and of the smallest peaks in the group 1150-950 cm^{-1} associated to starch (Elfehri Borchani et al., 2015; Mihaela et al., 2018) and finally of the peak 1717 cm^{-1} related to C=O in PBAT. The peak 1274 cm^{-}

[1], related to ester linkage again in PBAT, is visible in one spectrum, but with a very low absorbance. The peaks 726 cm^{-1} and 1018 cm^{-1} are still identifiable in the spectra: the first corresponds to $(CH_2)_{n=4}$, present in both the monomers composing PBAT (1,4-butanediol terephthalate and 1,4-butanediol adipate). The second is associable to disubstituted benzene in 1,4-butanediol terephthalate.

In order to provide an easier understanding of the MB molecular structure and of the specific bonds identified with the FTIR, Figure 6.10 represents a scheme of the monomers composing the MB molecule.

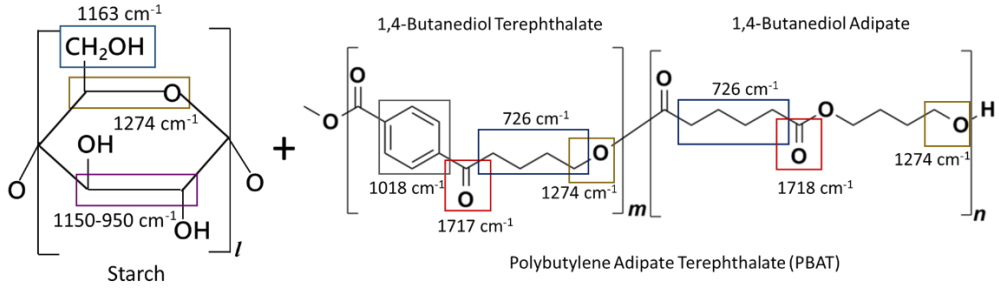

Figure 6.10 Molecular structure of the MB components, with the assignments of the main peaks identifiable in the FTIR spectrum.

The observations from the FTIR confirmed that part of PBAT chemical structure had not yet been degraded. From the peak 1018 cm^{-1} identified in the spectra, the 5.6% of residual polymeric weight was related to terephthalate (benzene linked to two carboxylic acids) in PBAT. Moreover, it was expected that the degradation of PBAT generated hydrolysed products rich in adipate and residues of the polymer mainly containing terephthalate (Zumstein et al., 2018). Terephthalate is better known for being part of PET, but its degradation pathways into the environment are mostly unknown. A previous study investigated a combination of photolytic and hydrolytic degradation pathways of terephthalate under natural aging conditions. However, the environmental factors which promote one pathway over another remained to be discovered and novel accelerated weathering techniques should be acquired in order to predict the lifetimes of this compound in different natural environments (Sang et al., 2020).

Visual inspection

Bioplastic micro-residues recovered in the undersieve <2 mm are reported in Figure 6.11. Fragments presented erosion signs, discolouring and high similarity with compost grains. Generally, when physical features of micro-size bioplastics make them assimilable to compost in accordance with EN 14045, no other analyses are performed on them (Weng et al., 2011; Balaguer et al., 2016). However, the experimental results obtained in the current study with TGA and FTIR on MB micro-residues suggested that they are not at all assimilable to compost. Considering the recent findings on potential impact of microplastics on the overall environment and on biota (Roex et al., 2013; De Souza

MacHado et al., 2018), more exhaustive analyses than visual inspection should be promoted in the requirements for compostability assessment.

Figure 6.11 Bioplastic micro-residues recovered from the undersieve of composting (<2 mm).

Conclusions

The current research confirmed that some micro-residues are expected to be generated from bioplastics degradation in composting. Despite the physical similarity between bioplastic residues and compost, deeper analyses revealed that the polymer was still partially intact. Indeed, bioplastic micro-residues and compost had a 5.6% difference in terms of chemical composition detected with TGA. FTIR analysis provided a deeper understanding of this difference, identifying it in the 1,4 butanediol terephthalate from PBAT degradation.

The current legislations about bioplastics compostability and about compost quality, which consider assimilable to compost all the residues in the undersieve <2 mm, date back to twenty years ago. But the recent developments in the research about bioplastics suggested the need to strengthen the inspection requirements for the undersieve, where microplastics are expected to be found. Indeed, compost may be a vehicle to spread bioplastic micro-residues into soil environment. Moreover, bioplastic waste conferred to composting are increasing in parallel with bioplastics market, promising micro-residues release into the environment.

Finally, some unknowns remained to be investigated about residual bioplastics once released into the environment. Issues to be accounted may be the lifetime and the environmental factors involved to completely degrade residual 1,4 butanediol terephthalate, the ecotoxicity in soil, where residues are first released as part of compost, as well as the bioaccumulation in the food chain.

Acknowledgments

Experimental methodology for microplastics recovery, as well as microplastics characterization, has been carried out at Imperial College London, Royal School of Mines. An EPSRC grant EP/S025456/1 to AEP is acknowledged for funding this part of my PhD research.

7 Conclusions and future perspectives

The present research disclosed important observations about the fate of bioplastics from the treatment with the OFMSW to the release into the environment, mostly in form of compost, but partially as residues not completely degraded.

In this final chapter, the most significant conclusions outlined by the research will be presented, together with some future perspectives about the topic.

Improving the monitoring methodologies

The synergic approach of methodologies developed during the research path allowed to improve the knowledge about the degradation level assured during composting.

Starting from the method of experimental weight loss, generally adopted in accordance with the standards, it was preliminarily observed that the method is technically simple but has some limitations. It does not address the complexity of the degradation process, neither include the fragments smaller than 2 mm.

Conversely, joining this method with more sophisticated ones, such as TGA, FTIR, SEM and GPC, complementary information can be provided to deeper understand the degradation process. TGA and GPC give quantitative indications about the degradation level, at macro and molecular level, respectively. Moreover, TGA follows the fate of each single compound in composite materials, such as starch-based and PLA-based polymers which include PBAT. This technique can be particularly useful if the weight loss methodology displays a partial degradation. Indeed, the TGA reveals the composition of the residual undegraded material. The use of FTIR can furtherly enhance the identification of the strongest chemical bonds within the residual polymer.

Generally, in the spectra of the degraded materials some specific peaks can be relatable to the hydrolytic and microbial activities, and are clear signs of the degradation process occurring. SEM micrographs show the activity of microorganisms, both on the surface of the material and inside cracks and pits. These are formed during degradation processes, such as grains abrasion or oxidation, and provide suitable places for microorganisms to adhere and growth degrading the polymers. SEM technique is also an improvement with respect to visual inspections, showing erosion signs and morphological changes not visible at macroscopic level.

Interestingly, this synergic approach of methodologies can be useful to overcome visual inspections in verifying the nature of items smaller than 2 mm. Indeed, these fragments can be considered compost if they are visually similar, based on standards for compost quality and bioplastics composability assessment (i.e. Legislative Decree 75/2010 and EN 13432:2000). However, the present research exploited TGA and FTIR to analyse some of these 2 mm residues. The analyses disclosed that they are partially composed of polymeric material.

In conclusion, a synergic approach of complementary techniques should be applied for research purposes or on novel bioplastics to be immitted on the market. In fact, it

strengthens the knowledge of the polymer fate during degradation and of the composition of eventual residues.

Influence of composting conditions on bioplastics degradation

The operative conditions of industrial composting can significantly vary on the basis of the composting technologies (e.g. covered windows, biocells, open-air storage), mechanical and manual operations during the process (e.g. turning, moistening, aeration) and climate conditions, especially in the case of completely or partially open-air treatments.

During composting, the initial thermophilic phase is followed by a longer maturation phase under lower temperature and humidity conditions. This natural process is generally not included in the standards for bioplastics compostability assessment, where temperature and humidity are kept constant at the suitable values of the thermophilic phase. From this observation, it rose the need to perform some tests under some suboptimal composting conditions, more similar to industrial plants than those of the standards.

From the experimental tests carried out in the present research, it was found that both temperature and humidity play a substantial role, influencing bioplastics degradation. A significant variation of the time required to fulfil the degradation requirements (i.e. 90% degradation, within 6 months (EN 13432, 2000)) was shown when changing the length of the thermophilic phase and the humidity. Moreover, the influence of the conditions was much higher on rigid materials than on film bags.

Concerning film Mater-Bi® bags, made of 20% starch plus few additives and 70% PBAT, the time required to degrade was 30 days, at 58°C and 60% humidity. With a thermophilic phase shorter than two weeks and humidity decrease down to 30-20% during the maturation phase, the degradation process took twice as long and led to the release of residues as part of compost. The results were more alarming in the case of rigid bioplastics. The experimental tests under different composting conditions were carried out on material made of 15% PBAT and 85% PLA. The degradation process clearly benefited from a thermophilic phase of 45-60 days, with an almost constant rate when the temperature did not decrease below 58°C. On the contrary, the change towards mesophilic conditions with temperature below 40°C and humidity of 20-30%, significantly slowed down the degradation rate. Thus, the degradation requirements were fulfilled within 6 months only in the case of a thermophilic phase of at least 60 days, while this time rose to 2 or 3 years in less suitable conditions of humidity and temperature.

As previously introduced, the most critical point is related to the difference between the standard conditions for compostability certification and the operative conditions of industrial plants. While the tests for compostability assessment can last for a maximum of 6 months at 58°C and constant humidity not lower than 50-60%, the industrial plants are generally equipped to maintain the thermophilic conditions for a maximum of 20-30 days. Moreover, during the maturation phase aeration and moistening are normally not provided, because the degradation of most of the organic waste has almost completed and the volume of compost needs to be reduced for a better selling of the product.

The observed results outlined that humidity and temperature particularly influenced the degradation of PBAT component in the composite bioplastics tested. In fact, this material has a more complex chemical structure, which requires longer time under suitable conditions to ensure the deterioration into oligomers and monomers readily degradable by most of bacteria.

It was finally observed an influence of suboptimal composting conditions on the final quality of compost, obtained from the fresh and synthetic waste matrices. In the present work, the main parameters considered were the final C/N, pH and humidity. The current Italian Legislative Decree 75/2010 is very strict in the requirements for a good compost quality. Indeed, it would be interesting for future developments of this project to promote beside the monitoring of bioplastics degradation, the monitoring of the compost quality under suboptimal composting conditions.

Matrices for compostability tests

A further not negligible consideration emerged from the experimental tests. It is briefly remined that the tests were carried out in three different ways. In Chapter 3.1, by using a homogeneous mixture of organic waste directly collected from the bins, as suggested in the EN 13432. In Chapter 3.2, by reproducing the synthetic organic matrix of the ISO 20200. In Chapter 4, by carrying out the test in mature compost harvested from a plant, in accordance with the EN 14855. It is fair to underline that all the standards are valid to test the treatability of bioplastics with the organic fraction of MSW.

Some significant differences have been observed between the results. In particular, the degradation in the organic waste collected from the bins was slower than in the synthetic matrix. Under the same temperature and humidity conditions (58°C and 50-55%), Mater-Bi® film bioplastics degraded within 30 days in the synthetic matrix, while in the organic waste it had not yet ended after 45 days and some micro-residues were still visible. Therefore, it emerged that the synthetic waste matrix, in which compostability tests are generally performed, slightly encouraged the degradation process, probably because organic matter and nutrients are well balanced.

In compost the degradation was even slower. In fact, the microbial community already developed in the environment must adapt to the introduction of bioplastic materials. Therefore, the lag phase is longer than in a fresh waste matrix. However, by testing the degradation directly in compost, it was possible to follow the evolution of the microbial community and its specialization towards bioplastics degradation.

To conclude, synthetic matrix has the advantage of ensuring homogeneity when analysing many replicates, as well as compost. Compost allows to study the microbial community evolution. Furthermore, a slightly higher concentration of bioplastics (3% instead of 1%) enhances the community to become more specialize in degrading polymers, even though it results in lowering the degradation. Finally, matrix of food and green waste better reflects waste conferred to industrial plants, but it is less homogeneous and less reproducible than the previous ones. Therefore, it should be known the effect of the matrix in the degradation process and accounted based on the objective of the test.

The role of the microbial community in the degradation of bioplastics

Many studies from literature have highlighted the fundamental role of some specialized bacteria in the degradation of polymers. The research in this filed mainly accounts water and soil, which are of great concern for the issue of microplastics accumulation in the environment and in the food chain. The present work started from the interesting findings of previous research about polymer degrading microorganisms, to investigate specifically bioplastics-associated bacterial communities present in composting environment. Both the thermophilic and the mesophilic composting conditions were considered, performing two separated analyses of the microbial community.

During lab-scale composting carried out in this study, the tested bioplastics showed deep signs of erosion, in particular Mater-Bi®. On the contrary, conventional LDPE was found not to be subjective to a significative degradation. However, the bacterial community profile, disclosed with a 16S rRNA amplicon sequencing, outlined that the communities on bioplastics and LDPE samples became more specialized in degrading polymers. Moreover, the amplicon analysis found distinct bacterial genera in mesophilic and thermophilic conditions. Among the most abundant genera associated in this study to bioplastics and LDPE degradation there are: *Streptomyces*, *Pseudomonas*, *Aeribacillus*, *Schlegellela*, *Limnobacter*, and *Cohnella*.

Interestingly, some bioplastics degrading bacteria were found to be dependent on the plastic nature, but a larger variety of specialists were present on two or more plastic types. This finding is important in the promotion of research studies on bioaugmentation. In fact, an increasing variety of bioplastic types is conferred to composting plants; therefore, wider is the variety of bioplastics degradable by bioaugmented bacteria, more efficient may be the bioaugmentation practice. Aiming to investigate the effective possibility to culture some specific bioplastics degrading species, an enrichment approach was also applied. After 2 months cultures of bioplastics and LDPE in a poor carbon source liquid medium, the phylum of *Firmicutes* completely predominated in the colonization of the tested materials, under both thermophilic and mesophilic conditions. With the cultivation approach, again most of the identified species were capable to be specialist of one or more tested bioplastics, such as *Aeribacillus pallidus* and *Brevibacillus borstelensis*.

Fate of bioplastic residues into the environment

From the composting tests, it was clearly observed that some residues were still present in the final compost. Thus, it was of great interest to follow their fate throughout soil and water environment, performing experimental lab scale tests.

As expected, the change of the environmental conditions significantly slowed down the degradation of the residues of all the monitored bioplastics: Mater-Bi®, PBAT and PLA. In particular, in soil and sand a much lower humidity than compost prevented the microbial activity. The only contribution to physical deterioration of the material was the mechanical abrasions with grains. In fresh and saltwater, the degradation continued thanks to both physical factors (i.e. wave motions and UV light) and microbial community,

which found in water a suitable environment to grow. Nevertheless, the slowdown of the process was not negligible, resulting in a long-expected time needed to be completed. This lab scale test was performed because very few studies are available about bioplastics fate in environments outside the biological treatments for the organic waste, and it was observed a lack of knowledge about the fate of bioplastic residues once transported as part of compost into soil and water. The results highlighted the need to raise the awareness on a double front: on the one hand, citizens should be better acknowledged about the difficulties of bioplastics to degrade in outside environments. Indeed, citizens seem to be comfortable in discarding bioplastics because of their biodegradability, but the term is strictly related to the environmental conditions. In fact, generally bioplastics are not made to be discarded in water or soil. On the other hand, the knowledge of the consequences of an incorrect management of bioplastics should promote the efforts to improve the industrial treatments efficiency.

Microplastics in the organic amendants

Microplastics in compost from aerobic composting, as well as in sludge from anaerobic digestion, should be considered an issue of great concern because amendants can be employed for soil improvement in agriculture. Microplastics can derive from incompletely degraded bioplastics, but also from conventional plastics which continue to be incorrectly conferred into the organic waste. Farmers feel uncomfortable in using amendants if microplastics are visible due to their shape and color. However, the most critical point is the gap of knowledge about the fate of microplastics, both bio and conventional, once released into the environment, i.e. interaction with flora and fauna, accumulation in the food chain, degradation timings.

The current legislation is not very strict. There is a lack of standardized methodologies to identify, recover and quantify microplastics in compost and sludge. Therefore, the main contribution in microplastics identification is visually provided. Consequently, most items of microscopic sizes are missed, as well as those which look like compost. To this purpose, the present work provided a contribution by developing a multi-step methodology for recovering and quantifying bio and conventional microplastics down to a size of 0.1 mm. The protocol included digestion of the organic matter with hydrogen peroxide, followed by filtration and desiccation. This step allowed to preliminarily remove the largest items, with size 1-2 mm. Then, both flotation in NaCl and fluorescence were employed to detect the smallest microplastics. Moreover, the protocol was tested on a heterogeneous matrix, including FTIR analysis to avoid misidentification of cellulosic and inorganic items with microplastics.

Future perspective in bioplastics research

The amount and types of bioplastic products on the market are continuously increasing in the last years. Both film biobags and rigid cutleries, dishes or coffee capsules are easily available in the supermarkets. Thus, some improvements should be promoted to enhance

the degradation in the industrial plants for organic waste treatment, as well as protocols for bioplastics and microplastics monitoring in the final products.

If necessary, a proper degradation time in the industrial plants may be achieved by recirculating bioplastic residues after refining treatments. Moreover, to promote a suitable humidity range, a moistening treatment may be considered before recirculating the dry bioplastics derived from the maturation phase. To this purpose, a further important issue emerged during the research work. The LDPE, used as a benchmark, showed an almost negligible weight loss and not significant chemico-physical changes after composting. Therefore, if some LDPE bags are conferred with the organic waste, their treatment cannot be achieved anyway. Thus, regulations should force the correct conferment of only bioplastics in the organic waste.

Finally, perspectives about the bioaugmentation seem to be disclosed, thanks to the adaptability of some bacteria to exploit bioplastics as substrate. Therefore, it is fair to say that interesting possibilities have been disclosed in this field. Enlarging the microbiological studies, bioaugmentation may be considered a further way to improve bioplastics degradation in the industrial plants.

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