The fate of biodegradable plastic during the anaerobic co-digestion of excess sludge and organic fraction of municipal solid waste

Assessing bioplastics biodegradability by standard and research methods: current trends and open issues

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Abstract

Bioplastics are currently and increasingly used as substitutes of conventional plastics; furthermore, they are mainly utilized in order to cope with problems related to plastic-based pollution. Certified international standard methods identify the criteria a bioplastic must comply with in order to be labelled as compostable and/or biodegradable. In addition, this is particularly the case when operating under the conditions that are expected in full-scale waste facilities. However, biodegradation in natural environments occurs under a manifold of different conditions, such that the aim of research studies is to estimate the extent to which a bioplastic can biodegrade under simulated natural conditions. For this reason, specific indexes are used to quantitatively estimate the degree of degradation. In the present paper, a description of the standard methods, research methods, and the indexes used to assess the biodegradability of bioplastics under different environmental conditions is provided. By summarising the results obtained by this study, it can be concluded that: (i) biopolymers claimed as biodegradable bioplastics may not degrade in full-scale plants due to the fact that the process conditions present in industrial waste treatment plants cannot completely reproduced at lab-scale; (ii) the static conditions set by the standard methods are not representative of the dynamic processes that occur in natural or industrial environments; and (iii) experimental tests are difficult to compare to one other due to the differences in the multitude of matrixes that can be used (i.e., inocula, soils, and biopolymers).

Keywords: bioplastics; standard methods; degradation indexes; plastic pollution.

1 Introduction

Plastics, since their early developments in the 1950's [1], have covered a crucial role in everybody daily life and represented a real "game-changer" in every industrial sector they have been used. This is due to their convenience, easy production, resistance to corrosion, and low cost [2,3]. Plastics annual production has been estimated to account for more than 367 million tonnes [4,5]. However, the improper management and disposal of wasted plastics have converted their usefulness into a serious issue [3] due to their persistence in the environment and to the release of possible toxic compounds (generally used for their production) during plastics degradation [6]. Moreover, plastics debris represents a significant economic and environmental damage to several activities, such as tourism, fishery production, and shipping [7]. In fact, up to 4% of yearly plastic production ends up in the oceans [8], constituting the main component of the marine litter (> 80%) [7]. Another issue related to the leakage of plastics into the environment is their disintegration in small pieces - below 5 mm - known as microplastics, which can be ingested by marine creatures and also enter into the food chain, even the one concerning humans [7,9]. A variety of human health problems, such as cancer, respiratory, and reproductive problems, may be attributed to plastics assimilation via ingestion (e.g., contaminated food) as well as by inhalation (e.g., dust or contaminated air) [6]. A study that was conducted analysing data collected from world's oceans expeditions in the period 2007 – 2013 [10], estimated over 5 trillion plastic particles weighing over 265,000 tons floating in the oceans. This was deemed to be the result of accumulation of plastics litter over the years - due to the increasing growth of 'single-use' plastics (such as disposable cups, lids, straws and cutlery) - which are rarely recycled and usually disposed of uncontrolledly, ending up in the environments and especially in oceans [7]. Around 13% (w/w) of the total weight estimated [10] was attributed to microplastics. Indeed, there are even smaller pieces of plastics than microplastics, within the size range of 1 to 1000 nm, which are known as nanoplastics [11]. Due to their size dimension, nanoplastics demonstrate a colloidal behaviour that prevents them from sedimentation [11]. Nanoplastics are more harmful of microplastics than microplastics due to the fact that they can cross biological barriers [12]. However, due to the lack of suitable methods for the detection and characterisation of nanoplastics, few studies have been conducted regarding their influence on the environment and living organisms [6]. Moreover, traditional plastics are commonly created from products of fossil-fuel origin, such that their production cannot be considered environmentally friendly.

In order to overcome, at least partially, the problems related to plastic goods production and end-of-life, bioplastics were developed in the last few decades as a valid substitute to conventional plastics. A multitude of materials belong to the family of bioplastics. Indeed, they largely differ from each other depending on the polymer they are composed of, as well as in respect to the structural characteristics that mainly affect their persistence in the environment when released. Hence, the term bioplastics refers to both bio-based plastics (i.e., plastics that composed of biogenic materials, such as crop-based feedstock [13] or organic waste [14,15]) and biodegradable plastics. In respect of issue, it must be noted that: (i) not all bioplastics are biodegradable; (ii) certain plastics of petrochemical origin can also be labelled as bioplastic due to their biodegradable properties. Therefore, a bioplastic is a material that is either bio-based, biodegradable, or both [16]. Moreover, they can be produced by biological fermentation or by chemical polymerisation [17,18]. In the first case, only renewable feedstocks (such as corn, sugar cane, soybean, etc.) can be used as the base material, while chemical polymerisation can occur independently from the raw material used [16]. The use of microalgae for the production of bioplastics (e.g., the extraction of lipids and cellulose from microalgae biomass) has been receiving much attention in recent times. This is likely due to the fact that bioplastics derived from microalgae can be considered as both bio-based and biodegradable [19]. Examples of bio-based bioplastics are: poly-hydroxyalkanoates (PHA); polyhydroxybutirate (PHB); polylactic acid (PLA); bio-polyethylene (Bio-PE); bio-polyethylene terephthalate (Bio-PET); bio-polyvinyl-chloride (Bio-PVC); and bio-polyurethane (Bio-PU) [20–23]. Meanwhile, examples of fossil-based bioplastics are: poly (butylene succinate) (PBS); poly(e-caprolactone) (PCL); poly (butylene adipate-co-terephthalate) (PBAT); and poly(butylene succinate-co-butylene adipate) (PBSA), [20,24,25].

The global world bioplastics production in 2021 was around 2.4 million tonnes and is expected to increase (i.e., an over 200% growth rate) to 7.5 million tonnes in 2026, thereby accounting for the 2% of the expected global production of plastics [26]. The continuous increase in the global bioplastics production can be attributed to their versatility in several applications (such as in respect to packaging and consumer products, as well as in electronics and automotive industries [4]). Packaging, for instance, representing 48% of the total bioplastics market in 2021 [4] is one of the most promising and important uses. One of the main advantages in the use of many bioplastics consists in the absence of toxic compounds released in the environment after degradation [27]. Furthermore, bioplastics production does not necessary entail competition with feedstock for food and feed, due to the fact that the land used for the renewable feedstock growth for the purposes of bioplastics production accounts for only 0.01% of the global available agricultural area [26]. In addition, the land use share in 2026 will not exceed 0.06% [26]. Moreover, bioplastics can be produced from organic waste [14,15], thus positively contributing to the management of the organic waste through the perspective of the circular economy. On the other hand, the absence of clear labelling and/or inadequate collection, and/or the processing of wasted bioplastics does not prevent the risks that are related to plastics leakage [7], nor in respect to microplastics and nanoplastics pollution [6], however the lower persistence in the environment od biodegradable plastics could reduce the problems related to plastic pollution. As such, the management of bioplastics is extremely important - specifically in terms of the circular economy, especially when referring to their end-of-life options, i.e., recycling, incineration, landfilling, and biodegradation. Due to the variety and heterogeneity of bioplastics, the sorting of and the processing, thus, recycling of bioplastics appears to not to be the most suitable option for their recovery. This may be due to the fact that recycled bioplastics generally show a quality reduction [28] as they may be too degraded to be utilized effectively [29]. In addition, the processes for their recycling are often not mature to be used at industrial level. The use of landfills is not considered to be suitable as end-of-life option, due to the fact that bioplastics can produced methane once landfilled [28,30]. Finally, incineration can be considered as a valid option for wasted bioplastics management if the bioplastics are produced from renewable feedstock; in fact, in this case, the CO_2 produced during combustion, being of renewable origin is not relevant for global warming [31]. Moreover, energy is also produced during this process thus increasing the environmental benefit of the process [29].

Biodegradation should convert the biopolymers into non-toxic compounds, such as into monomers, CO_2 and H_2O. Moreover, value-added products, such as compost and methane obtained by biological treatment processes, benefit the environment when compared to petroleum-based plastics [29]. Indeed, when compared to anaerobic digestion (AD) - in which the methane produced can be utilized for the purposes of energy production - industrial composting, in regard to it as end-of-life process for bioplastics, results in a high global warming potential [30]. This is due to the fact that composting-related operations are high-energy-consuming processes [32], and because energy recovery is not possible through this process either [32]. However, the real applicability of biological processes for the treatment of used bioplastics, grandly relies on their biodegradability that depends on the complexity of the bioplastics structure and on the type of raw materials used, such that potentially different waste streams should be adopted according to the bioplastics' characteristics. At the moment scientific literature does not report examples of full-scale plants destined specifically to bioplastics treatment.

Specific prevention policies in respect to the problem of plastic pollution have been adopted by several countries, such as: the ban of certain disposable plastic items (e.g., straws and plastic cutlery) or the replacement of lightweight plastic carrier bags with biodegradable ones. For instance, the use of compostable and biodegradable bags is now compulsory for the collection of food waste; further, this is addressed in to biological treatment plants in several European countries (such as Italy and Sweden) [33,34]. The extent to which a bioplastic can be labelled as compostable and/or biodegradable in a certain environment (such as aerobic or anaerobic) and under defined conditions (such as mesophilic or thermophilic temperature) is defined by certified international standard methods. These methods, required by national regulations and/or developed for marketing purposes, were established through considering realistic environmental conditions that occur in full scale utilities in respect to organic waste management especially when referring to composting and AD plants. The EU Directive 2015/720 firstly placed the attention on the necessity of proper labelling for biodegradable and/or compostable products. This was conducted due to the fact that non-biodegradable and biodegradable plastic items are generally not distinguishable to the public eye, due to their similar physical appearance [33,35]. Furthermore, as a consequence, they may be subjected to unproper treatment. Indeed, according to certain Italian legislation, biological treatment plants can only accept bioplastics that fulfil the requirements of the UNI EN 13432 and UNI EN 14995 directives in respect to packaging and other materials [36] respectively and therefore bioplastics disposed together with food waste must be labelled as compostable and clearly distinguishable from conventional plastics. In other countries (e.g., China), food waste is still collected by non-biodegradable plastic bags and treated in biological (generally anaerobic) treatment plants [37] possibly leading to negative effects on the mechanical equipment (i.e. feeding and mixing devices) and on the biological process [38] and on digestate suitability for agricultural use. Moreover, when it comes to the indiscriminate disposal of wasted bioplastics into the environment, the standard methods for the evaluation of their degradability cannot be applied as degradation/biodegradation processes occur in different conditions. For this reason, research studies were mainly focused on to the investigation of bioplastics' degradation in different, i.e., non-standardised environments.

For the reasons explained above, the biodegradable plastics industry, although still not fully mature, has already gained a prominent place in plastics global market. However, many issues, related to biodegradable plastics end-of-life and, more specifically, to their biodegradability in natural and industrial environments are still open. As such, in this paper following the description of the main standardised protocols that were adopted for the labelling of biodegradable bioplastics, the methods that were utilized in research studies in order to assess the degradability/biodegradability of bioplastics in different environments are discussed. This paper, summarizing the available information related to assessment of bioplastics biodegradability, aims at helping to re-shape future testing standards and research activities to cover the actual evident lack of knowledge in this field.

2 Bioplastics' (bio)degradability

The ability of a bioplastic to degrade or biodegrade in a specific environment does not depend on the type of materials that were used to synthesise it [39], but on the physico-chemical properties of the bioplastic itself [40], such as its thickness [20], hydrophobicity, molecular weight, and crystallinity [40] or the melting point of the biopolymer [20,34].

In addition to the biomaterial properties, the rate of decomposition of a bioplastic is affected by the specific environmental conditions [41] which must consider the possible presence of microorganisms (such as bacteria or fungi) [23]. The last condition is extremely important to define whether the decomposition of the material occurs only by abiotic processes (i.e., driven by heat, sunlight, moisture, etc.) and/or by the microbial activity (biotic processes) [35,42]. In the last case, it can be said that the decomposition of the bioplastic occurred via *biodegradation*, so that the material is mineralised to CO_2, H_2O, NH_4^+, N_2, H_2 and biomass through the biological action [23,27,41]. Both prokaryotic and eukaryotic microorganisms are responsible for the biodegradation of bioplastics [17,20,43], while endo- and exo-enzymes are functional in respect to the depolymerisation of biopolymers [44,45]. If this is not the case, then it is referred to *degradation* as a fragmentation of polymers' chains that occurs via abiotic processes [29] leading to the formation of persistent particles [20,46–48]. In any case, as one of the main advantages of bioplastics, the remaining residues of degradation/biodegradation should not be toxic for living organisms [23,27].

3 Biodegradability indexes

The biodegradability of a bioplastic is evaluated by the estimation of so called *biodegradability indexes* as defined in the international standard protocols. These indexes are related to both the structural properties (such as molecular weight and surface morphology) of the bioplastics and the microorganisms' activity, as estimated by the evolution of CO_2, O_2 and/or CH_4, which represents the main indexes for aerobic and anaerobic biological processes respectively. Weight reduction is often used as an indicator of biodegradation despite the fact that mass loss can also occur due to abiotic processes without the involvement of microorganisms [16].

Apart from, or in addition to, standard indexes, other biodegradability quantifiers are monitored during research activities, such as the decrease in the total carbon (TC) of the bioplastic [49], visual analyses as discoloration or surface erosion [3,50], ATP measurements for the assessment of oxo-degradable products [51] and spectroscopic spectrums [52].

A particular method, known as clear zone formation or the zone of clearance method, is also often used: (i) as a qualitative indicator of the presence of microorganisms-degrading bioplastics or, when microorganisms (e.g., bacterial strains) are isolated from a specific environmental matrix; as well as (ii) to define the best species able to degrade the biopolymer [27,53]. In other words, the clear zone is a method in which to test the microbial ability to hydrolyse a specific polymer [54] and/or for the assessment of the degradation potential of different microorganisms towards a polymer [55]. In the first case, the emulsified bioplastic contained in the basal medium agar plate represents the source of carbon for microorganisms' growth [25], such that after incubation of the inoculated microbial culture, the presence of a clear halo around the microbial colony represents the synthesis and the excretion of enzymes degrading the biopolymer [54–56]. The biopolymer degradation index (BDI) is then estimated as the ratio between the clear zone diameter and the colony diameter [53]. In the second case, the clear zone test in wells is used to identify the bacterial strains with the best biodegradation ability as higher halo zones formation indicates higher biodegradation activities of the tested microorganisms with respect to the bioplastic used as the substrate [25,27].

In general, the conversion of the carbon present in the bioplastic into CO_2 and/or CH_4 is used for the evaluation of the biodegradability of the test material under anaerobic conditions [52]. The biochemical methane potential (BMP) test is a method widely used to simulate anaerobic conditions at lab-scale [57]. The CO_2 production or the O_2 consumption are also used as indexes of biodegradability in aerobic environments [58].

As already mentioned, the extent at which a bioplastic can be biodegraded also depends on the environmental conditions the material is subjected for a certain period, such a temperature, humidity or UV light. The effects of the different combinations of biotic and abiotic processes on bioplastics degradability have been of increasing interest in the last few years in order to understand the mechanisms, and thus the impact, of bioplastic biodegradation in industrial and natural environments [16,59].

4 Standard and research methods for the assessment of bioplastics' degradability

When considering the multitude of existing bioproducts with their different properties and composition, standardisation and certification systems are of extreme importance in order to ensure compliance with national regulation, quality, and the appropriate labelling of the bioplastics [33,35,60]. On the other hand, the test methods described in the standard procedures do not cover all the variety of possible environmental conditions at which the bioplastics can be exposed. In this sense, research that has been conducted for the last few years in regard to better understanding the mechanisms of biodegradation of the different biopolymers has focused not only on the assessment of bioplastics' biodegradation within the common full-scale facilities for municipal waste management, but also on the extreme variability of conditions found in natural environments that can affect – under different aspects - the biodegradation process of a certain material. In other words, as is better explained in Section4.2, recent research has been mostly focused on the understanding of biodegradation mechanisms under non standardised conditions, due to the fact that wasted bioplastics may enter into the environment without being treated or recovered in the proper plants.

4.1 Standardised Methods

Certain important normalization institutes are active in the field of biodegradable materials, especially in respect of setting standards for biodegradable and compostable plastics. The main institutes, classified according to their geographical location, are reported as below:

- USA:
    - ASTM (American Society for Testing and Materials) operating in USA-Canada [61];

- EU:
  - CEN (Comitè Europèen de Normalization - European Committee of Standardisation) operating in EU and EFTA countries (Iceland, Norway, Switzerland, etc.) [62];
  - UNI (Ente italiano di normazione – Italian Institute of Standardisation) operating in Italy [63];
  - DIN (Deutsches Institut fur Normung - German Institute for Standardisation) operating in Germany[64];
- Asian countries:
  - JAS (Japanese Standard for Association) operating in Japan[65];
- Australia:
  - AS (Australian Standard) operating in Australia and New Zealand. [66];
- Worldwide:
  - OECD (Organisation for Economic Cooperation and Development) operating in OECD Countries [67];
  - ISO (International Organisation for Standardization) operating worldwide[68].

The standards from these organizations played an important role in respect for helping the industry to create biodegradable and compostable products that meet the increasing worldwide demand for more environmentally friendly plastics.

Various norms that describe biodegradation test methods are available; further, they all possess a few basic aspects in common. First of all, they list test procedures and set the testing conditions, e.g., pH, nutrients, temperature, concentration and source of inoculum, etc. The test conditions are set depending on the specific disposal environments, such as those found in: industrial composting, marine environment, anaerobic digestion, landfill and home composting. However, these tests have a common important limiting factor which is the carbon source restricted to the bioplastic sample only. In fact, usually, in all the environments, additional carbon sources are present. Moreover, the tests are conducted under optimum conditions for the purposes of biodegradation with regard to temperature, moisture, presence of nutrients and micronutrients etc. In respect to inoculum, the biological quality should be assured by the number and the biodiversity of the species present [69].

Biodegradation standards are described in the following sections. In particular, a distinction between standard specifications and standard test methods is explained.

The various standards are indeed divided into these two groups: (i) standard specifications that describe product requirements and set a test scheme combining different tests, criteria, and pass levels, and (ii) testing standards that describe detailed procedures for the execution of the test methods as well as the evaluation of tests and the permissible limiting values.

Standardised methods are summarized in Figure 1.

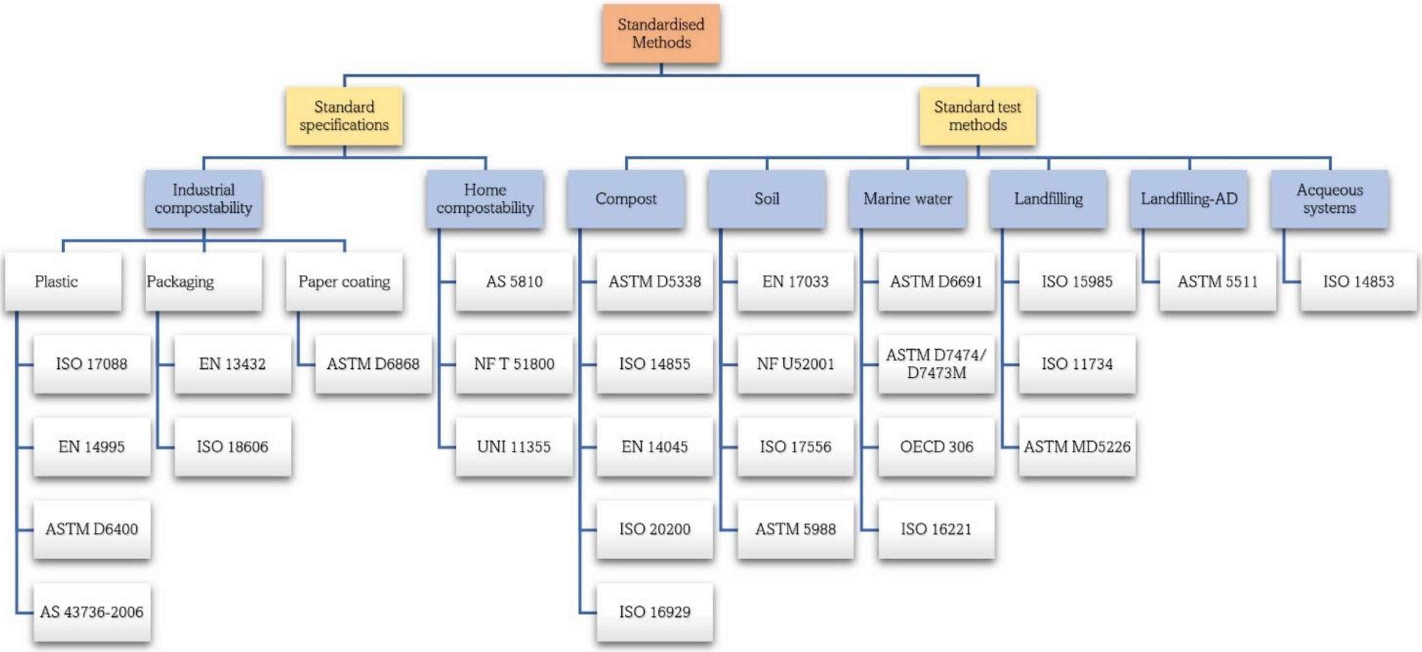

Figure 1 - Standardised methods

4.1.1 Industrial compostability

The specification standards defining the requirements for the industrial compostability of bioplastics are listed in Table 1 [69]. There is a large similarity between these standards with only minor differences related to details.

Geographical Validity	Identifier	Materials covered
	Plastics	
Worldwide	ISO 17088	Plastics — Organic recycling — Specifications for compostable plastics
European Union	EN 14995	Plastics - Evaluation of compostability - Test scheme and specifications
USA	ASTM D6400	Compostable Products Testing – Composting
Australia	AS 43736 -2006	Biodegradable Plastic - Biodegradable Plastics Suitable for Composting and other Microbial Treatment
	Packaging	

European Union	EN 13432	Packaging - Requirements for packaging recoverable through composting and biodegradation - Test scheme and evaluation criteria for the final acceptance of packaging
Worldwide	ISO 18606	Packaging - Procedures and requirements for packaging suitable for organic recycling.
Paper coating		
USA	ASTM D6868	Standard Specification for Labelling of End Items that Incorporate Plastics and Polymers as Coatings or Additives with Paper and Other Substrates Designed to be Aerobically Composted in Municipal or Industrial Facilities

Table 1 - Overview of industrial compostability standards related to material and geographical validity

As already mentioned, these standards are specifications and define two requirements [70]:

- a set of scientific tests that can be used to measure the properties of a biopolymer;
- a set of criteria (i.e., threshold values) that these measurements must meet for the biopolymer to be considered "compostable".

The standards EN 13432:2002, EN 14995:2007, ISO 17088:2021, and ASTM D6400-21 define the same test scheme for the characterization of a product as compostable.

According to these four standards, in order to be compostable, a product must strictly adhere to the following criteria:

1. Characterization of material composition: identification of the different constituents (e.g., by IR), organic matter content (represented as volatile solids that must be at least 50% on dry weight), and heavy metals concentration level. Several metals, each with a specific limit, are considered in these standards. They refer to heavy metals limits that are required in order to check compost quality. Polymers or basic packaging materials, usually, pose little problems. However, heavy metals requirements differ among norms both in reference to the type of metal and limit value. In both the EN 14995 and EN 13432 standards, the concentration of any substance (e.g. Zn, Cu, Ni, Cd, Pb, etc.) shall not exceed the tabulated values (e.g., the limit value for Zn is 150 mg/kg substance) [71]. In these cases, it is assumed that 50 % of the original mass of the plastic material will remain in compost following biological treatment together with the complete amount of hazardous substances [71,72]. In addition, ASTM D6400 standard permits higher values for heavy metals within the material than the EN standards allow. For instance, the limit value for Zn is 2800 mg/kg; another example is As, whose limit in ASTM is 41 against the 5 mg/kg established in aforementioned EN standards [73].

2. Disintegration: disintegration requirements are incredibly similar in all four standards. At least 90% of the original dry weight disintegrates into particles having a size of less than 2 mm (maximum of 10% of original dry weight may remain after sieving on a 2.0 mm sieve) after a specified time. Moreover, EN standards require a maximum of 12 weeks of aerobic composting,

5 weeks of anaerobic biogasification, (which is optional and which possesses the option of extension), and the test duration may be modified as necessary as a result of the testing currently being carried out. In the ASTM D6400 standard, test duration is 12 weeks. In respect to the ISO 17088 standard, the time is 45 days (with the option of an extension of up to 6 months). Furthermore, the ASTM D6400 standard allows the use of other test methods, such as those found in ASTM 5338 and ISO 16929, in order to determine the details of the disintegration. As alternative test methods for disintegration - other than those found in ISO 16929 - the ISO 17088 standard includes mentions of the methods detailed in ISO 14855 and ISO 20200. The issue of test duration and fragmentation are two of the most serious within the field and will be discussed further in this paper.

3. Biodegradation: conversion of the material to carbon dioxide, water, and biomass within a period of 6 months to the extent of 90% for the EN 13432, EN 14995, and ISO 17088 standards. The pass level of 90% is given in respect to biodegradation in absolute terms, or in relative terms when compared with the positive reference (e.g., cellulose). That is to say that 90% of the organic carbon in the whole item or for each organic constituent, which is present in the material at a concentration of more than 1% (i.e., by dry mass), shall be converted to carbon dioxide by the end of the test period when compared to the positive control or in the absolute. The standard ASTM 6400 sets a less stringent threshold of 60% biodegradation within six months for homopolymers or random copolymers, and 90% for copolymers and polymer blends.

4. Compost quality: the performance of ecotoxicity tests in respect to the finished compost. Final compost quality should not be negatively influenced by the addition of a biodegradable plastic into the original substrate that is to be composted. This is evaluated by comparing a blank compost to a test compost that contains composted bioplastic. As such, the pilot-scale composting test for the measurement of biodegradation and ecotoxicity test can be combined. In addition, the physico-chemical parameters such as pH, salt content, density, are analysed.

The ecotoxicity tests are generally carried out via pot tests in which a comparison between a blank compost and test compost is conducted with regard to their respective seeds germination and plant growth. In all four standards the ecotoxicity tests are performed in accordance with OECD 208, which is a terrestrial plant test that is used to determine if composted material is toxic to plants. The ASTM, ISO, and EN norms have the same two requirements as concerning ecotoxicity: (i) the plastic should have concentrations of regulated metals that are lower than 50% of those prescribed for sludges or composts in the country where the product is sold (these values are tabulated for each country); and (ii) the germination rate and plant biomass of the sample composts shall be no less than 90% than that of the corresponding blank compost for two different plant species (when following the OECD Guideline 208 with the modifications found in the Annex E of the EN 13432 standard). By fulfilling requirements (i) and (ii), a plastic product can demonstrate satisfactory territorial safety and the ecotoxicity test is, thus, passed. Furthermore, only AS 4736-2006 guideline deviates from other standards, thereby requiring an earthworm toxicity test as well as two plant toxicity tests.

Another interesting standard that is present in the USA only is related to the use of bioplastics in composite materials (e.g., in packaging).

ASTM D6868-21: Standard Specification for the Labelling of End Items that Incorporate Plastics and Polymers as Coatings or Additives with Paper and Other Substrates Designed to be Aerobically Composted in Municipal or Industrial Facilities

This is a standard specification for the labelling of end items that incorporate plastics and polymer as coatings or additives with paper, as well as and other substrates that are designed to be aerobically composted in municipal or industrial facilities. The scope is to provide requirements for the purposes of labelling of materials and products (including packaging). Further, this is applicable wherein a biodegradable plastic film or coating is attached to compostable substrates and the entire product or package is designed to be composted in municipal and industrial aerobic composting facilities. Having said this, there is no known ISO equivalent for this standard.

In order to be composted satisfactorily, the product must demonstrate each of the following three characteristics as follows:

1. Proper disintegration during composting; after twelve weeks in a controlled composting test, no more than 10% of its original dry weight remains after sieving the material through a 2.0 mm sieve. Please note, sieving is further discussed below and is a critical part of the test.

2. Adequate level of inherent biodegradation: an end item, possessing a plastic coating(s) or additives, is considered to have achieved a satisfactory level of biodegradation if the plastic coating or polymeric additives meet the requirements of ASTM 6400 (as previously reported). Moreover, the substrates of the end item are to individually demonstrate that 90% of the organic carbon is converted to carbon dioxide using Test Method D5338 within 180 days at 58°C (to a maximum of 62°C), when compared to the positive control. End items composed of ligno-cellulosic substrates are permitted to fulfil previous requirements by demonstrating that they are materials of natural origin and therefore they are biodegradable by showing that over 95% of their carbon derives from biobased resources. A problematic issue in respect of this test is that usually the amount of carbon dioxide produced by bioplastic biodegradation is quite limited, thereby affecting the precision of the measurement and the replicability of the experiment (in regard to the comparison with the background CO_2 or with a positive control).

3. No adverse impacts on the ability of compost to support plant growth: an end item that incorporates a plastic or polymer, after composting, is demonstrated to fulfils two requirements. These two requirements are: the concentrations of heavy metals that are less than 50% of those prescribed in 40 CFR Part 503.13; as well as that the germination rate and the plant biomass resulting from the testing of the sample composts shall be no less than 90% than that of the corresponding blank composts in respect of the two different plant species that follow the requirements detailed in the OECD Guideline 208 (which is in conjunction with the modifications found in Annex E of the EN 13432 standard).

ISO 18606:2013 - Packaging and the environment — Organic recycling

The ISO 18606:2013 standard specifies procedures and requirements for packaging that are suitable for the purposes of organic recycling. As is the case with EN 13432, packaging is considered recoverable via organic recycling only if all the individual components meet the requirements.

In respect to each of the packaging components the following four aspects are addressed: biodegradation; disintegration during biological waste treatment processes; negative effects on the biological process; and the negative effects on the quality of the resulting compost, including the presence of high levels of regulated metals and other substances that are hazardous to the environment.

In addition, the ISO 18606 standard does not provide information on the requirements for the biodegradability of used packaging which ends up in the soil environment as litter, due to the fact that littering is not considered as a recovery option. It is also not applicable to biological treatment undertaken in small installations by householders.

4.1.2 Home compostability

Home composting is an important waste management option in various countries. Furthermore, although there are still opposing views concerning hygienic aspects, this does represent a sustainable and valuable option for the purposes of waste reduction. Moreover, temperature trends during the process represents the major difference with industrial composting, in which it is possible to control the environmental conditions [29]. Moreover, while the heat generation is the same in respect to industrial composting, there are greater heat losses and a lower reaction velocity. Therefore, usually, temperatures are slightly higher than those found in the environment. Indeed, certain biodegradable polymers require a thermal trigger in order to commence hydrolysing. As such, this can make quite a difference.

The Belgian certifier TÜV Austria Belgium had developed the first "OK compost" home certification scheme, whereby it was required that there be at least a 90% degradation in 12 months at ambient temperature. The requirements of the OK compost HOME programme, as defined in 2003, have served as the basis for the drafting of several standards, such as:

- Australia: AS 5810 (2010) – Biodegradable plastics: Biodegradable plastics suitable for home composting. This standard specifies the requirements and procedures in which to determine whether a plastic material is biodegradable in home-composting conditions. In addition, it provides the basis to allow one to label materials and products constituted of plastics as “home compostable” for use in home composting systems. Moreover, this standard stipulates pass/fail criteria that specifically address biodegradability, disintegration during biological treatment, the effect on the biological treatment process, and the effect on the quality of the resulting home compost. Therefore, these requirements are mainly similar in respect to the industrial composting requirements, but it this case is required to determine the degree of biodegradation and disintegration at an ambient temperature.

- France: NF T 51800 (2015) – Plastics : Specifications for plastics suitable for home composting. This norm strictly follow “OK compost” scheme described above.

- Italy: UNI 11355:2010 - Plastic items biodegradable in home composting: Requirements, test methods, and the UNI 11183:2006 standard. In addition, this also concern plastic materials that are biodegradable in terms of home composting, i.e., in respect to requirements and test methods. As it will be discussed in a following section, the twelve months requirements for composting time required in this method can be considered, in most cases, unrealistic.

4.1.3 Biodegradation testing standards

A testing standard or test method is a kind of standard that defines: (i) an exact scientific experimental procedure that can be applied to a material in order to produce a test result; as well as (ii) an exact way in which to measure and calculate the results of the test.

The testing standards contain detailed descriptions of the test methods that must be performed according to the stipulations of the aforementioned standard specifications. In addition, the biodegradation testing standards are subdivided into various categories depending on the environmental conditions during the biodegradation tests, as reported in Table 2 [70].

Environment/Treatment					
Compost	**Soil**	**Marine water**	**Landfilling -AD**	**Landfilling**	**Aqueous System - Anaerobic**
ASTM D5338 (BIO)	EN 17033 (BIO)	ASTM D6691 (BIO)	ASTM 5511 (BIO)	ISO 15985 (BIO)	ISO 14853 (BIO)
ISO 14855 (BIO)	NF U52001 (BIO)	ASTM D7474 /D7473M (BIO)		ISO 11734 (BIO)	
EN 14045 (DIS)	ISO 17556 (BIO)	OECD 306 (BIO)		ASTMD 5526 (BIO)	

ISO 20200 (DIS)	ASTM 5988 (BIO)	ISO 16221 (BIO)			
ISO 16929 (DIS)					

Table 2 - Biodegradation (BIO) and disintegration (DIS) testing standards

4.1.4 Composting biodegradation and disintegration standards

Biodegradation during composting is evaluated using the following ISO 14855 and ASTM D5338 testing standards while the evaluation of disintegration during composting follows three main test standards: EN 14045, ISO 20200 and ISO 16929.

ISO 14855-1:2012 "Determination of the ultimate aerobic biodegradability of plastic materials under controlled composting conditions — Method by analysis of evolved carbon dioxide — Part 1: General method"

The standards of ISO 14855-1:2012 specify a method for the determination of the ultimate aerobic biodegradability of bioplastics. This is performed under controlled composting conditions, based on organic compounds, via the measurement of the amount of carbon dioxide that has evolved and the degree of disintegration of the plastic at the end of the test.

The composting takes place in an environment wherein temperature, aeration and humidity are closely monitored and controlled. The test method is designed to yield the percentage conversion of the carbon in the test material that has evolved to carbon dioxide, as well progressed in respect of the rate of conversion.

The principle of the test is found in respect to the item that is mixed with mature compost and incubated under batch conditions at 58°C under optimum O_2 and moisture conditions. The mature compost acts at the same time as the carrier matrix, the source of the microorganisms and the source of nutrients. The mixture is continuously aerated and the exhaust air is analysed in terms of produced CO_2 [69].

The maximum test duration is 6 months, while a typical minimum duration is 45 days. Further, CO_2 production is continuously measured. After subtracting the background CO_2 production from the blank compost inoculum, the percentage of biodegradation is determined by the net amount of carbon in respect of the test item that is converted to CO_2. A positive reference control, cellulose, is tested in parallel to check the activity of the inoculum. Furthermore, strict requirements are imposed on the results for cellulose in order to validate the test. The test item is preferably added in the form of a fine powder. Again, here the test conditions (e.g., temperature and duration) are the most severe issues. Furthermore, the addition of the material as a fine powder is also quite unrealistic. Moreover, the measurement and comparison of the produced CO_2 with a background production are complicated in terms of precision and reproducibility, especially in respect of the compost heterogeneity.

ISO 14855-2:2018 "Determination of the ultimate aerobic biodegradability of plastic materials under controlled composting conditions — Method by analysis of evolved carbon dioxide — Part 2: Gravimetric measurement of carbon dioxide evolved in a laboratory-scale test"

The standard ISO 14855-2:2018 specify a method for determining the ultimate aerobic biodegradability of plastic materials under controlled composting conditions via the gravimetric measurement of the amount of carbon dioxide that has evolved. The method is designed to yield an optimum rate of biodegradation by adjusting the humidity, aeration and temperature of the composting vessel. The degradation rate is periodically measured by determining the mass of the evolved carbon dioxide using an absorption column filled with soda lime and soda talc on an electronic balance.

The test material is mixed with an inoculum that is derived from mature compost in conjunction with inert material, such as sea sand. The sea sand plays an active part by acting as a holding body for humidity and microorganisms. When compared with the ISO 14855-1 standard, the amounts of compost inoculum and test samples that are detailed in this document are of a one-tenth size. In order to ensure the activity of the compost inoculum, inert material that provides the mixture with the same texture as soil is mixed into the inoculum. The carbon dioxide that evolves from the test vessel is determined by absorbing it in a carbon dioxide trap, as well as by carrying out gravimetric analyses of the absorbent components. In this method, the degree of biodegradation - expressed as a percentage- is calculated by comparing the amount of carbon dioxide that has evolved with the theoretical amount.

Composting vessels are incubated at a constant temperature of 58°C. In addition, the test is terminated when the plateau phase is reached. The standard time for termination is 45 days, but the test could be continued for up to six months. As such, the same issues raised for previous tests are present in this one too.

ASTM D5338-15 - Biodegradation Test – Composting

The ASTM D5338 -15 standard also details a test method that determines the degree and rate of the aerobic biodegradation of plastic materials in respect to their exposure to a controlled-composting environment under laboratory conditions, at thermophilic temperatures. In addition, the ASTM-D5338 standard is not a pass/fail test. The reports indicate what percentage biodegraded over the tested time period, which can be selected by the test requestor. The principle used is the same as that found in ISO 14855. Moreover, this test does not include any testing for the purposes of measuring disintegration.

The evaluation of disintegration during composting has been evaluated in various test procedures standardised as *ISO 16929 - Determination of the degree of disintegration of plastic materials under defining composting conditions in a pilot-scale test.*

The same procedure was also published in another testing standard *EN 14045 - Packaging Evaluation of the disintegration of packaging materials in practical oriented tests under defined composting conditions.*

The principle of the test, however, is that the test material is mixed in with a precise concentration of fresh biowaste and introduced into a pilot-scale composting bin (which possesses a volume of a minimum of 140 l), after which the biological composting process spontaneously starts. A natural ubiquitous microbial population will start the composting process and temperature increase will happen spontaneously. During this process, the composting mass is regularly mixed. Furthermore, the temperature, pH, moisture content and gas composition within the composting material are regularly monitored and are required to fulfil certain requirements in order to ensure sufficient and appropriate microbial activity. After 12 weeks of composting, the test is terminated. Disintegration is evaluated in a quantitative way by sieving over 2 mm, 10 mm and through a mass balance. The compost obtained at the end of the process can be used for further measurements such as chemical analyses and ecotoxicity tests.

A composting environment may be either a pilot-scale composting bin or nets that are buried in a pilot-scale composting bin. The volume of each bin shall be high enough for natural self-heating to occur. In addition, sufficient aeration shall be provided by an appropriate air supply system. In order to standardise conditions for the test, the composting trials can be run in bins which are placed in a climatic chamber with a constant chamber temperature. If, during the spontaneous thermophilic phase, the compost reaches temperatures higher than 65°C, then the diversity of the microbial species can be reduced, and the compost can be re-inoculated with mature compost.

The EN 14045 and ISO 16929 standards share the same procedure, but they differ with respect to bin volume which is smaller in the ISO standard (i.e., a minimum volume of 35 l).

ISO 20200-Plastics - Determination of the degree of disintegration of plastic materials under simulated composting conditions in a laboratory-scale test

The ISO 20200 method is easier to perform when compared to ISO 16929*.* There are certain differences when compared to this test, such as the use of smaller reactors (i.e., a volume between 5 l and 20 l), whereas disintegration is determined in a similar manner.

The method determines the degree of disintegration in respect of test materials on a laboratory scale under conditions simulating an intensive aerobic composting process. The solid matrix used consists of synthetic solid waste that is inoculated with mature compost, which is taken from municipal or industrial compost plants. Pieces of the plastic test material are composted with this prepared solid matrix. Furthermore, the degree of disintegration is determined after a composting cycle, by sieving the final matrix through a 2 mm sieve in order to recover the non-disintegrated residues. The reduction in mass of the test samples is considered as disintegrated material and used to calculate the degree of disintegration. In this test there is a minimum period of 45 days and a maximum of 90 days in which reactors are maintained at a constant thermophilic temperature (58°C). It is then followed by a mesophilic incubation period at room temperature for a maximum period of additional 90 days.

The common issue for all disintegration tests is the feasibility of sieving. The recovery and identification of small pieces of bioplastics is complicated and results can often be unreliable.

4.1.5 Soil biodegradability

The main standard test methods for the purposes of measuring the biodegradation of plastics in soil (i.e., the ISO 17556, ASTM D5988, NF U52-001, UNI 11462 and EN 17033 methods) determine the rate of biodegradation under normalised conditions. The standard testing procedures are designed to determine the inherent biodegradability of plastics in soil under optimal controlled conditions. Criteria for the biodegradation of materials used in agriculture and horticulture are defined in standard specifications NF U52-001 and UNI 11462, together with the criteria for environmental safety. In the French specification, the evaluation of the biodegradation in soil is not obligatory. The main requirements for mulching films are that: (i) biodegradation achieves at least 90% within 24 months; as well as (ii) material shall not contain heavy metals and no ecotoxicological effects should occur due to the films' biodegradation. A first issue is that it would be difficult to carry out biodegradability tests for such a long period; moreover, standards refer to a reference biomass (e.g., cellulose) in order to compare the extent of biodegradation, but no reference soil is indicated, as neither microorganisms nor communities are required to be identified.

EN 17033-Plastics - Biodegradable mulch films for use in agriculture and horticulture - Requirements and test methods.

The EN 17033 document specifies the requirements for biodegradable plastic mulch films (BDMs), which are manufactured from thermoplastic materials, and are to be used for mulching applications in agriculture and horticulture. In so doing their composition is taken into account, as well as their biodegradability in soil, the effect on the soil environment (ecotoxicity), their mechanical and optical properties (e.g., thickness, tensile stress, light transmission), and the test procedures for each of the listed categories. Furthermore, a unique aspect of EN 17033 is its focus upon BDMs rather than conventional plastics.

The biodegradability index is represented by the conversion of the carbon source, which is present in the biomaterial into CO_2. In respect of this, it is required to demonstrate a ≥ 90% conversion of film carbon into CO_2 within 2 years under ambient soil conditions. The test method used is the one described in the ISO 17556 standard.

ISO 17556:Plastics — Determination of the ultimate aerobic biodegradability of plastic materials in soil by measuring the oxygen demand in a respirometer or the amount of carbon dioxide evolved.

The ISO 17556 document specifies a method for determining the ultimate aerobic biodegradability of plastic materials in soil by the measuring of the oxygen demand in a closed respirometer, or in regard to the amount of carbon dioxide that has evolved. The method is designed to yield an optimum degree of biodegradability by adjusting the humidity of the test soil. Further, the plastic material is mixed with soil, then the mixture is allowed to stand in a flask over a period of time during which the amount of oxygen consumed (BOD) - or the amount of carbon dioxide evolved - is determined. Provided the CO_2 that has evolved is absorbed, the BOD can be determined by, for example, measuring the amount of oxygen that is required to maintain a constant gas volume in a respirometer flask. The respirometer is set up in a temperature-controlled environment and contains test vessels, each fitted with a CO_2 absorber in the headspace, a coulometric oxygen production unit, a manometer, as well as an external monitoring device and recorder. The test vessels are filled to about one third of their volume with the test mixture. If biodegradation takes place, the microorganisms consume oxygen and produce carbon dioxide - which, in turn, is completely absorbed. The pressure drop is detected by a manometer and used to initiate the electrolytic generation of oxygen.

4.1.6 High-solids anaerobic/landfill simulation biodegradation

An anaerobic biodegradation test can be divided into two main categories according to moisture content: aquatic tests and high solids tests. These test procedures are intended to apply to any plastic substance that is not toxic to the microorganisms found in anaerobic digesters that process household waste.

The biodegradation of bioplastic within a high-solids anaerobic digestion unit is an important phenomenon. This is due to the fact that their presence can affect both the decomposition of other waste materials, which are enclosed by and/or surround the plastic and the resulting quality and appearance of the digestate/compost after the anaerobic digestion process.

This procedure was developed in order to permit the determination of the rate and degree of anaerobic biodegradability in respect of plastic products when placed in a high-solids anaerobic digester.

One of the earlier high-solids anaerobic biodegradation test methods for bioplastics was developed by ASTM in the form of the ASTM D5511 standard.

ASTM D5511: Anaerobic Biodegradation.

The ASTM D5511 test method covers the determination of the degree and rate of anaerobic biodegradation of plastic materials in high-solids environments (more than 30% total solids) under anaerobic conditions and static (non-mixed) conditions. Thereafter, the same method was published by

ISO as: *ISO 15985-Plastics - Evaluation of the ultimate anaerobic biodegradability and disintegration under high solids anaerobic digestion conditions - Method by analysis of released biogas.*

Both standards describe tests that utilize a TS concentration higher than 20% (i.e., a high-solid condition) at a thermophilic temperature (about 52 °C in the ISO 15985 standard) or mesophilic temperature (about 35 °C in the ASTM D5511 standard). This is performed in conjunction with mixed inocula that are derived from anaerobic digesters operating only on pre-treated household waste. The volume of biogas produced is measured and used in order to calculate the percentage of biodegradation, which itself is based on carbon conversion.

Even in this standard, the issue regarding the correct evaluation of the difference between biogas production in the reactor that contains the bioplastic and the same production in the blank is a key factor for the reliability of the test.

Landfill simulation tests represented another category of dry, anaerobic biodegradation testing.

The decomposition of a bioplastic within a landfill environment involves biological processes that will affect the decomposition of other materials that are enclosed by or are in close proximity to the plastic.

The rapid degradation of the bioplastic materials would have the ability to increase the economic feasibility of landfill gas recovery, to minimize the duration of after-care of the landfill, and render possible the recovery of the volume generated thanks to the biodegradation of the bioplastics during the active life of the landfill. This procedure was developed in order to permit a better determination of the anaerobic biodegradability of plastic products when placed in biologically active environments simulating landfill conditions.

In this simulation tests, there is a lower concentration of microorganisms, which thus determines a slower biological activity if compared to high solids anaerobic digestion tests. Biodegradation is evaluated through a measurement of biogas as in the ASTM D5526 standard. Furthermore, it provides the percentage of conversion in respect of carbon in the test sample to carbon in the gaseous form (CH_4 and CO_2) under conditions that mimic landfill conditions. This test method covers the determination of the degree and rate of anaerobic biodegradation of plastic materials in an accelerated-landfill test environment. Furthermore, this test is carried out at a constant temperature; moreover, it can be run for as long as required in order to establish the time it takes for the bioplastic sample to degrade.

4.1.7 Aquatic, anaerobic biodegradation

Fresh and marine waters became the most vulnerable environments in respect to plastic pollution. Plastic contamination - especially plastic debris such as microplastics and nanoplastics - is currently one of the most serious problems in both marine and freshwater aquatic ecosystems.

In the field of bioplastic production and in relation to aquatic environment, the main test standard that applies is the ISO 14853 standard.

ISO 14853-Plastics - Determination of the ultimate anaerobic degradability in an aqueous system - Method by measurement of biogas production.
The ISO 14853 standard specifies a method for the determination of the ultimate anaerobic biodegradability of plastics by anaerobic microorganisms in an aqueous environment. The principle is placing the test item in an aqueous inoculated (anaerobic sludge) medium and is conducted under batch conditions at a mesophilic temperature.

In detail, incubation should take place in sealed vessels at a constant temperature of 35 (± 2) °C, which is a normal temperature for an anaerobic digester. Further, it must be noted that the normal test duration is 60 days. Furthermore, the test may be termined earlier if the biodegradation curve obtained from the pressure or volume measurements has reached a plateau phase. On the contrary, it can be extended until the plateau phase is reached; in addition, in respect of this, the maximum test duration is nevertheless limited to 90 days. The period of exposure regarding the test material in this test is longer than the normal sludge retention time (i.e., 25 - 30 days) in an anaerobic digester while temperature is significantly higher of that of aqueous natural environments.

The amount of microbiologically produced biogas carbon is calculated from the net biogas production in respect to a blank.

4.1.8 Marine biodegradation

Marine environments cover two-thirds of the Earth's surface area and include a great variety of habitats, from open-ocean and coastal ecosystems to deep-sea environments.

The first specific standards for marine biodegradation of plastic were published in the OECD 306 standard.

OECD 306: Biodegradation Test – Seawater
The OECD 306 norm provides a first evaluation of biodegradability in seawater by describing two methods: the shake flask method and the closed bottle method.

1. The shake flask method consists of a dissolution of a pre-determined amount of the test substance in the test medium in order to yield a concentration of 5 - 40 mg L^{-1} dissolved organic carbon (DOC). Five flasks, at least, should be used: two for the test suspension, two for the blank and one for procedure control. The solution of the test substance in the test medium is incubated, under agitation in the dark or in diffuse light under aerobic conditions, at a fixed temperature which normally is within the range of 15 – 20°C. The recommended maximum test duration is around 60 days. Furthermore, degradation is followed by DOC measurements (i.e., in the form

of ultimate degradation) and, in some cases, by specific analysis (primary degradation). However, it must be noted that this method is rarely used for biodegradable plastic.

2. The closed bottle method consists of a dissolution of a pre-determined amount of the test substance in the test medium in a concentration of usually 2 - 10 mg L^{-1} (one or more concentrations may be used). The solution is kept in a full and closed bottle in the dark; further, it is kept in a constant temperature bath or enclosure that is controlled within a range of 15 – 20 °C. The degradation is then followed by oxygen analyses over a 28-day period. However, if the blank biological oxygen demand value remains within the 30 % limit, the test could be prolonged. Twenty-four bottles are at least used (eight for the test substance, eight for reference compound and eight for seawater plus nutrient). All the analyses are performed on duplicate bottles. Moreover, four determinations of dissolved oxygen, at least, are performed (i.e., days 0, 5, 15 and 28) using a chemical or electrochemical method.

This test provides a first impression of biodegradability within seawater. The degradation of organic chemicals in seawater has generally been found to be slower than that experienced in freshwater, activated sludge, and sewage effluent. Therefore, a positive result obtained during 28 days in a biodegradability seawater test (> 60% ThOD – theoretical oxygen demand - and > 70% DOC) can normally be regarded as an indication of ready biodegradability. Both the methods described in the OECD 306 standard are not, in actuality, suitable for bioplastics even if these were the first standards used in order to test biodegradability of plastic in general.

As an aside, a standard for measurement of marine biodegradation for bioplastics was published also by ASTM.

ASTM D6691 - Standard test method for determining aerobic biodegradation of plastic materials in the marine environments by a defined microbial consortium or natural seawater inoculum.

The ASTM D6691 test method establishes the procedures, equipment, materials, and conditions that are required in order to measure the degree and rate of biodegradation of plastic materials under aerobic mesophilic marine water conditions.

Furthermore, this method is designed to index polymer materials that are possibly biodegradable in an aerobic marine environment. The test method consists of preparing a uniform inoculum of marine water, exposing the plastic samples to marine water, measuring biodegradation with a carbon dioxide respirometer or equivalent measurement method, and assessing the percentage of carbon conversion in the plastic carbon dioxide.

ASTM D7473/D7473M: Standard Test Method for Weight Attrition of Non-floating Plastic Materials by Open System Aquarium Incubations

The ASTM D7473/D7473M standard is another standard that concerns the measurement of biodegradation in a marine environment. This test method is used to determine the weight loss as a function of time in respect of non-floating plastic materials. The method entails the materials being incubated under changing marine aquarium conditions. These conditions are representative of aquatic environments near the coastal regions and near the bottom of a body of water, particularly in respect to an absence of UV light and visible portions of the electromagnetic spectrum.

The aquarium-incubated plastic materials are examined in respect of determining the extent of visual degradation and dry weight loss over time. This test does not provide information on ultimate biodegradation (that is, it is not a replacement for Test Method D6691), but it is an ASTM method that can be utilized for purposes of assessing weight attrition. The standard addresses only weight loss as a function of time of the plastics materials in a marine environment and cannot be used for the purposes of demonstrating ultimate biodegradation. In addition, it is considered insufficient for establishing biodegradability on its own and is only completed for materials achieving at least 30% biodegradability in the ASTM D6691 standard.

Furthermore, the aquarium incubation test method allows for the assessment of representative indigenous microorganisms that are present in seawater and marine sediment in terms of how they can be enriched for and can carry out the biodegradation. It is recommended that the test be carried out in the geographical vicinity (latitudinal area) where the test materials are likely to be used. These aquarium studies are conducted in indoor environments, hence any sunlight-induced effects on degradation, or biodegradation, or both, are not taken into account.

In addition, this test method also consists of exposing film pieces in the absence of light to natural flowing seawater or sediment surfaces under natural flowing seawater in open tray incubators. Further, this should be conducted in a marine aquarium at seasonally varying water temperatures; however, this can vary depending on in situ conditions.

Film pieces are harvested at varied time intervals in order to assess visual impacts of exposure and degradation , as well as in respect of determining the percentage loss in terms of dry weight and weight loss per unit area. It is required the prior determination of its organic carbon biodegradability to CO_2, which is based on the outcome of Test Method D6691. It must also be noted that the test entails a maximum duration of 180 days.

The goal of this test is to obtain data that can be used to assess the potential for physical degradation of the test material.

As already demonstrated, the standard test in the marine environment has aided researchers in foreseeing that a minimum duration of 28 days and maximum duration of 6 months is sufficient. However, in that timeframe the item can still cause harm to marine life via ingestion, entanglement, etc. This is one of the most limiting aspects related to the marine environment standard. Moreover, due to the high variability in marine conditions (i.e., temperature, salinity, exposure to light, etc.) the standard tests that are based on laboratory procedures cannot mimic completely the full spectrum of marine conditions that can be encountered (such as, the cool water in the northern and southern hemisphere [29]). Another important, and undervalued, aspect is in the fact that it is almost impossible for this test to replicate the abiotic degradation that is caused by exposure to light, waves agitation, etc.

4.2 Methods used in research activities

The test methods that are used in research activities generally refer to the standard methods. As previously reported, these standards are utilized in order to focus on assigning rules that a product must comply with before it could be labelled as a bioproduct and/or as biodegradable under certain environmental conditions. However, standards cannot cover all the possible existing environmental conditions in the treatment plants and in natural environments. For this reason, research experiments aim at simulating a great variety of different environments in order to assess the degradability, or rather the biodegradability, of a certain product in a specific condition by studying the kinetic variations of selected parameters (such as mass weight, molecular structure of the biopolymer, as well as the chemical and microbiologic composition of the soil or other biological mediums [39]).

Moreover, in considering the variety of base materials that can be used for the production of bioplastic products, research activities are often conducted on novel "lab-produced" bioplastics rather than on the ones that are already labelled and marketed as bioplastic material. Indeed, the focus of many studies is to develop bioplastics (for specific issues, such as food packaging [74] or the replacement of disposable plastics [75]) that can be completely degraded as much and as easily as possible after their use. Therefore, the tested materials refer to both certified bioplastics products (such as starch-based shopping bags and PLA goods [36,57,76,77], and bottles used for the packaging of water [78]) and novel lab-made bioplastic blends (such as silk fibre + glycerol + wheat gluten [75], corn starch + PCL + biochar [79], and PLA + PHA [80]).

In the following subsections, the main test methods and the parameters used at research level to evaluate the degree of degradation of bioplastics in different environments (specifically in soil, composting/anaerobic digestion plants and aquatic environments) are summarised.

4.2.1 Soil

Tests carried out in soils are mainly addressed within the definition of the biodegradability of bioplastics when improperly disposed of in the environment, such that they are accidentally buried in soils. Biodegradability experiments in soils are carried out both in natural field or at lab-scale, generally by the use of small pots or larger containers. The biodegradability of the tested bioplastic is mostly affected by the type of selected soil in which specific microorganisms are naturally present [39]. This leads to a difficulty in comparing the biodegradability of the same material within different soils, due to the fact that the biodegradation mechanisms change not only over the season but also from place to place [81]. For instance, sandy soils do not generally represents a favourable environment for the purposes of biopolymer degradation. This is due to the fact that they are characterised by low water content (which is the medium for most microorganisms is soil)[16].

The natural environment at lab-scale is simulated by varying temperature, humidity, depth, and the size of the buried samples, as reported in Table 3. The test is generally stopped when no variation in selected parameters (e.g., weight loss) is observed, such that - depending on the tested materials and the environmental conditions - test duration varies from a few weeks up to one year. In addition, biodegradability can also vary from less than 5% up to complete (almost 100%) degradation (Table 3).

Mass loss (which is periodically measured) is the main index that is used to assess the biodegradability of bioplastics in soil. This is because it is assumed that (i) microorganisms are present in the soil and that (ii) they would be able to degrade the material. For the same reason, disintegration is also considered an index of biodegradability. Furthermore, the analysis is usually conducted by sieving the final matrix through a 2 mm sieve in order to recover the non-disintegrated residues [76]. Less frequently, microstructure characteristics that are determined via FTIR spectroscopy or X-ray diffraction (XRD) are analysed [82]. In some cases, analyses on quantification and biomass diversity are carried out in order to define a relationship between the degradation of bioplastics and the bacterial biomass in the soil [49]. Conversely, specific microbial culture from soil are isolated, by means of certain methods - such as the already mentioned clear zone formation [25,83]- in order to investigate the relationship between bioplastic biodegradation and microbial colonisation [84]. For instance, bacteria (*Pseudomonas* and *Bacillus* strains), fungi (*Geomyces*, *Sclerotinia*, *Fusarium* and *Mortierella* strains) and yeast (*Hansenula anomala*) that are all isolated from Antarctic soil samples were found to be good candidates for effective PCL, PBS and PBSA degradation at low temperatures (< 20°C) [25]. In addition, fungal strains (*Apiotrichum porosum*, *Penicillium samsonianum*, *Talaromyces pinophilus*, *Purpureocillium lilacinum*, and *Fusicolla acetilerea*) that were isolated from terrestrial environments in various region of Korea were able to degrade PLA and PCL polymers [83]. Moreover. bacteria from the genus *Amycolatopsis sp*., which were isolated from agricultural soils collected in northern Thailand, showed enzymatic activity for both PLA and PCL [53]. When no microbial analysis is conducted, the presence of microorganisms is confirmed via the monitoring of the production of CO_2 [23,85] in relation to a blank.

Environmental conditions in soil biodegradability tests		
Test parameter	**Range**	**References**
Temperature	20 – 60 °C	[23,82,86]
Humidity	30 - 80%	[86–88]
Soil Depth	0.05 – 0.15 m	[77,89]
Size of the sample	from 0.015 m x 0.015 m to 0.4 m x 0.2 m	[14,75]
Test duration	few weeks to one year	[42,90–92]
Biodegradability indexes		
Mass loss		[23],[49]
Disintegration		[76]
FTIR spectroscopy - X-Ray Diffraction (XRD)		[82]
Biomass diversity		[49]
Isolation of microbial culture		[25,83]
CO_2 production		[23,85]
Biodegradability	< 5% - 100%	[14,41,42,49,75–77,88,93]

Table 3 – Summary of the environmental conditions, biodegradability indicators and biodegradability achieved in soil environment

When compared to tests carried out according to a standard method (such as for marketing purposes), research studies mainly focus on the evaluation of the degree biodegradation, thereby often omitting the importance of carrying out ecotoxicity tests (e.g., by evaluating the seed germination indexes [85]). Even if the degradation of the bioplastic material does not imply a release of toxic compounds, certain disturbances to the soil microorganisms may occur due to the possible accumulation of metabolic intermediates, oxygen depletion in soil (due to the fact that, it would be consumed during the process of bioplastics biodegradation), as well as in regard to the variation in the soil's physico-chemical characteristics. Although soil quality could be deeply affected by the degradation of the buried bioplastics, a few studies have specifically investigated on its effects in respect to soils. Abe et al. [42] found that the degradation of the biopolymer (which was specifically a starch-xylan blend) in soil did not inhibit the growth of *S. cerevisiae*; similarly, Bhowmik et al. [75] found that soil quality was not significantly affected by the degradation of a bioplastic blend (i.e., waste Kibisu silk fibre + wheat gluten). It is important to highlight that these results are for single lab-scale tests and, consequently, cannot be representative of the degradation's effects that may occur in natural real conditions, whereby the high amount of heterogeneous biodegradable materials can accidentally or purposely (such as in respect to mulch films) enter into the soil.

4.2.2 Aquatic or marine environments

As for the soils, the bioplastics degradation when discharged in an aquatic environment is a major issue for research investigations. However, the majority of the studies that were conducted on this topic, have investigated bioplastics degradation in terrestrial systems rather than in marine environments [3,17]. Bioplastics' degradation in aquatic environments refers to freshwater, seawater, and river water environments; furthermore, it implies both aerobic and anaerobic biodegradation. Almost all the research activities present in scientific literature are carried out at laboratory scale, most likely due to the difficulty in managing the degradability test in a real environment. In a few cases- e.g. in [77], [94], [95] and [94]- via in-situ tests and by the recreation of an eutrophic reservoir, the experiments were conducted under uncontrolled conditions; this, therefore, means that they were conducted within a real existing environment. In all the other cases, environmental biotic (such as the type of microorganisms involved and the nature of incubation) and abiotic (such as heat, light, water pH or salinity) parameters were set and applied for a certain period.

In general, the samples are prepared by cutting the biomaterial into small pieces; then, they are immersed in water at the set testing conditions such as: temperature, pH, static (flasks) or dynamic (i.e., an aquarium with samples subjected to continuous flow of water) [96], natural or inoculated water [94,96], with or without contact on a sediment surface [3,96] or buried in wet sediments [3], an alternation of light and dark periods, as well as in aerobic or anaerobic conditions (Table 4). Depending on the type of bioplastic and the set environmental conditions, the testing time ranged from few days up to one year, while the degree of biodegradability varied from less than 2% to almost complete (> 90%) biodegradation (Table 4).

Weight loss and visual inspection are the main parameters used as the degradability indexes. In addition, other physico-chemical analyses (e.g., Raman measurements [97]) were conducted in order to understand the extent of the polymers' degradation. A solubility test was also seldom used for the estimation of the soluble fraction of the bioplastic [74] and chemical parameters (e.g., the chemical oxygen demand - COD) were determined on the test water in order to evaluate the release from the various bioplastics [36]. The degree of biodegradation and the microorganisms' activity are specifically determined by indicators, such as CO_2 production [98], the evolution of the BOD by respirometry tests [3,40,99], the production of biogas [100], the formation of the clear zone [101], or by the selecting of specific mixed culture, such as bioplastic degrading bacteria [91,101]. For instance, thermotolerant and halotolerant *Bacillus sp.* JY14 bacteria, when isolated from marine soil, was found to be capable of degrading PHB and various PHAs [101]. The *Microbulbifer* genus strains, which reside in high-salt environments, also showed a great ability to degrade PHB [102,103]. The bacterial species *Pseudomonas pachastrellae* was found to be involved in the degradation of PCL in coastal environment [104]. *Shewanella*, *Moritella*, *Psychrobacter* and *Pseudomonas* genera were isolated from deep-sea environments at depth of over 5,000 m from the Kurile and Japan Trenches for testing their ability in the PCL degradation [105]. *Enterobacter sp.*, *Bacillus sp.* and *Gracilibacillus sp.* strains were isolated from seawater environments and used for the purposes of PHA biodegradation [95], while phylogenetic groups of *Cytophaga-Flavobacterium-Bacteroides*, *g-Proteo-* bacteria and *b-Proteo*-bacteria were identified in a reservoir-within the Bugach river (Russia) and they were found to be able to utilise PHA [94].

Environmental conditions in aquatic or marine tests		
Test parameter	Range	Ref.
Temperature	20 – 32 °C	[101,106]
pH	7.0 – 8.1	[40,95,96,106]
Solar radiation exposure	Alternance light/dark	[59]
Size of the sample	0.02 – 0.04 m dishes/square samples or larger (> 0.1 m)	[74,94,95]
Conditions	Aerobic or anaerobic	[36,40,91,100]
Test duration	< 10 days – 1 year	[59,91,101,107]
Biodegradability indexes		
Weight loss and visual inspection		[3]
Raman measurements		[97]
COD (on test water)		[36]
CO_2 production		[98]
BOD		[3,40,99]
Biogas production		[100]
Clear zone formation		[101]
Biodegradability	< 2% - 90%	[59,77,96,106]

Table 4 - Summary of the environmental conditions, biodegradability indicators and biodegradability achieved in aquatic and marine environment

As for the soils, the interaction between the different types of aquatic environment and the microbial communities could not render possible the comparison among the tests that were conducted, even in respect to the same type of bioplastics. Therefore, a wide range in respect of the degree of degradability can be found in the literature (Table 4). In addition to the environmental conditions, the size and dimension of the samples tested were found to affect the rate and degradability of PHB more than chemical composition [95]. Indeed, this could be due to the higher surface that is available for microorganisms in smaller fragments.

4.2.3 Composting environment

Bioplastic's biodegradation during a composting process has been deeply investigated. This has been performed due to the fact that bioplastics are commonly used for the purposes of household organics collection. Most of the purchased bioplastics, indeed, are compostable [16] and biodegradable [24], as composting represents the main organic waste management practice in several countries. Most likely in respect to the wide presence of composting facilities, certain research activities were conducted at industrial scale [108] and in field conditions [78,109]. The simulation of composting at the lab-scale was obtained by setting the temperature, water content, pH, carbon to nitrogen ratio (commonly adjusted to 30:1 [109]), sample dimensions, type of compost (purchased from [77], obtained from composting facilities [109] or synthetically reproduced in the experiment [22]) and feedstock composition (mixed food and green waste [110], i.e., the digested mixture of bioplastics and the organic fraction of municipal solid waste, OFMSW [76]) (Table 5). The composting tests were conducted for periods ranging from less than 2 weeks to over 150 days; moreover, bioplastics degradability varied from about 10% to over 90% (Table 5). Due to the fact that the compost itself (in which microbial communities are spontaneously developed) was used as a natural environment for the test other types of inoculum were not used. Moreover, both compost and soil are characterised by higher microbial diversity when compared to other environments that facilitate the presence of bioplastics degrading microorganisms [24]. As reviewed by Emadian et al. [24], indeed, bacteria (such as *Stenotrophomonas*), fungi (such as *Penicillium*, *Aspergillus*, *Thermomyces*, *Fusarium*, *Clonostachys*, *Verticillium*, *Lecanicillium*, *Cladosporium*, *Mortierella* and *Doratomyces*) and actinobacteria species (such as *Streptomyces*) are all able to biodegrade different biopolymers when they were all isolated from compost environments. The main gene sequences involved in the biodegradation of PLA were found to be *Paecilomyces*, *Thermomonospora*, and *Thermopolyspora* [111]. Moreover, the thermophilic actinomycete (*Streptomyces thermonitrificans* PDS-1) when supplemented with other microorganisms (*Bacillus licheniformis* HA1), showed a synergistic effect in respect to the degradation of PCL under composting conditions [112].

Following a visual inspection of the residues, the disintegration and mass loss were the most usual biodegradation indicators. Indeed, changes in the polymeric structure were observed in other investigations, such as those found in the application of the FTIR analysis [110]. The CO_2 production was used, more correctly, to evaluate the extent to which the biomaterial was degraded by the action of microorganisms [78,113], as composting is an aerobic process. However, field-scale testing may render difficult, or perhaps even not possible, the tracing of the CO_2 production [109]. The observation of microbial growth in compost, generally in proximity of the bioplastic, is also a qualitative indication of disintegration and biodegradation [42].

Environmental conditions during composting process		
Test parameter	Range	Ref.
Temperature	25 – 60 °C	[42,77,114,115]
Water content	55 – 80%	[88,116]
pH	7.0 - 8.5	[78,112,114]
Size of the sample	0.15 – 0.7 m	[77,110]
Test duration	< 14 – 150 days	[112,114,117]
Biodegradability indexes		
Visual inspection of the residues, disintegration and mass loss		[22,42,115]
FTIR analysis		[110]
CO_2 production		[78,113]
Biodegradability	10 - 90%	[22,42,88,113,116]

Table 5 - Summary of the environmental conditions, biodegradability indicators and biodegradability achieved in compost environment

When compared to industrial composting, home composting temperatures are usually lower; as such, longer periods of time for the purposes of biodegradation may be required. Most of the analysed studies were conducted according to the standard methods- such as the ASTM D6400, ISO 20200, and ISO 14855-1 standards. As defined in these standards, at least 90% of weight loss (as well as the disintegration of the mass into fragments that are less than 2 mm) should occur, within six months in order to label a bioproduct as compostable. However, the existing composting plants were not designed to treat bioplastics; as such, their processing may be problematic for this reason [33]. It must be noted that although residual fragments can affect the compost quality, ecotoxicity tests in research studies are barely applied to the final compost.

4.2.4 Anaerobic environment

The aim of anaerobic tests that are carried out using bioplastics as a substrate is to simulate the environmental conditions that take place in common waste facilities, specifically anaerobic digestion plants [36], the anaerobic phases of wastewater treatment plants [109] and landfills [118]. Compostable bags for the purposes of food collection can also enter into AD plants. Indeed, this is even the case when a mechanical sorting in order to remove the bags is applied. For this reason, it is important to evaluate the biodegradability of bioplastics under anaerobic environments, due to the fact that they are not supposed to be processed by in this manner and therefore the design of the plants do not consider their presence. Incomplete degradation in respect of the bioplastics within AD plants results in the presence of fragments in the digestate [32].This is due to the fact that only disintegration may occur during the anaerobic process. Furthermore, complete biodegradation of the bioplastics may occur within the aerobic phase usually applied for the final stabilization of the digestate.

The environmental conditions are simulated by setting the main process parameters (Table 6), which are: temperature (mesophilic and/or thermophilic), type of digestion (wet or dry), type of test (discontinuous batch or semi-continuous), inoculum used (commonly collected from full-scale AD plants treating OFMSW [37], substrate used (green waste and/or food waste [37], as well as cow manure and vegetable waste [119]), the possible presence of a co-substrate, or of single type [57,119] or mixed bioplastics [80], the dimension of the bioplastics samples, organic loading rate (bioplastics OLR of 0.75 $g_{ThOD}·L^{-1}·day^{-1}$ [120], 0.25 $kg_{CODbioplastics}·m^{-3}·day^{-1}$ [36] and 0.04 $kg_{VSbioplastics}·m^{-3}·day^{-1}$ [121]); the hydraulic retention time (HRT), and the food-to-microorganisms ratio. Although the long test duration, which generally exceeds 30 days up to over 250 days [122], bioplastics show low biodegradability under anaerobic conditions. Only powdered PHB was found to biodegrade (> 90%) within 10 days in the mesophilic AD process [122]. Indeed, even when co-digested with other substrates (such as food waste or sludge), bioplastics degradability was lower than 30% [36,76,109]. The procedure for the evaluation of bioplastics' biodegradability under anaerobic conditions consists in the application of the biochemical methane potential (BMP) test, such that the degree of biodegradability of the biopolymer is generally estimated by the means of the biogas that is produced during the process. In addition to the biogas and/or CH_4 and/or CO_2 production, the weight loss and visual inspection of the residues after sieving (with a 2 mm mesh) are traditionally, and commonly estimated. Other laboratory analyses - such as the differential scanning calorimetry for the evaluation of the thermal properties both before and after the process [36], spectroscopic analysis [37], thermogravimetric analysis [37] and discoloration [123]- were also used in these tests as indicator of degradability of the tested material.

PLA-based biopolymers were decomposed by the microbial communities at the phylum level of *Firmicutes*, *Bacteroidota* and *Proteobacteria*, while *Methanosarcina*, *Methanoculleus* and *Methonothermobacter* at the genus level were involved in their degradation within mesophilic conditions [37]. Organisms that are identical (i.e., over 97%) to *Peptococcaceae bacterium* Ri50, *Bacteroides plebeius*, and *Catenibacterium mitsuokai* were involved in the biodegradation of PHB, while *Ureibacillus sp.*. *Bacillus infernus*, and *Propionibacterium sp.* were implicated in the anaerobic biodegradation of PLA [100].

Environmental conditions during AD process		
Test parameter	Range	Ref.
Temperature	30 – 55°C	[37,57,124,125] [32]
TS content	< 10% – 30%	[37,76,125]
Type of test	BMP (Batch)/Continuous	[36,57,121]
Type of co-substrate	Food waste	[36,37,76,80]
	Pig slurry	[124]
	Synthetic wastewater treatment plant (WWTP) primary sludge	[120]

	Sewage sludge	[32]
	Mixed primary and secondary WWTP sludge	[109]
Shape/Size of the sample	Square/ 0.01 – 0.1 m	[57,109,121]
	Powdered /125 - 250 μm	[100,119,120,125]
HRT	15 - 40 days	[36,76,120]
Food to Microorganisms ratio	0.25 – 2	[36,57,80,109,123]
Test duration	up to 250 days	[122]
Biodegradability indexes		
Biogas and/or CO_2 production		[56,118,122]
CH_4 production, weight loss and visual inspection		[57]
Differential scanning calorimetry		[36]
Spectroscopic and thermogravimetric analyses		[37]
Discoloration		[123]
Biodegradability	< 10 - 70%	[23,36,57,109,118,120,122]

Table 6 - Summary of the environmental conditions, biodegradability indicators and biodegradability achieved in anaerobic environments

The main issue concerning the methods for testing the biodegradability of bioplastics under anaerobic conditions is the low comparability among the tests. This is mainly due to the variability in the inoculum sources used. Even if the same environmental conditions (such as temperature, and the pH of C/N ratio) are reproduced, the type of inoculum used cannot be standardised, due to the fact that it widely varies according to its origin. Moreover, better performances were obtained under thermophilic conditions. However, most real plants work with mesophilic temperatures. In addition biopolymers, such as compostable bags constituted of starch-derived bioplastics, are not completely degraded under normal HRT [57]. Moreover, there is a lack of studies that have investigated AD plants at full-scale [32] and this is a strong limitation since conditions and equipment commonly used in biodegradability assessments at lab-scale do not fully mimic full-scale AD processes [32].

It must be noted that bioplastics' biodegradation in landfills has not been sufficiently studied. As such, it can be assumed that biodegradation of bioplastics in landfills could occur slowly due to the lack of water and phosphorus or to the presence of inhibiting substances such as heavy metals [29].

5 Drawbacks, future prospects and challenges

The increasing use of bioplastics worldwide is an important component in the drive to lower the global carbon footprint, to reduce the degree of climate change, and decrease plastic-based pollution [29]. Although the production of bioplastics and its related market have been well established, certain issues related to the proper labelling of these materials as biodegradable still remains. Firstly, the environmental conditions that are suggested in the standard methods as optimal for biodegradation to take place cannot be reproduced in common full-scale treatment plants. In particular, most of the compostability standard tests set duration and process temperatures that are unrealistic. This is due to the fact that the standards advise much longer durations and higher process temperature when compared to those of real full-scale plants, where bioplastics are supposed to be treated in reality [16,34]. Consequently, there is a discrepancy between the time required for working operations that are applied in full scale applications, as well as in respect to the maximum period of degradation set in the supposed norm. Similarly, the recommended temperature used in the various standards are unrealistic when compared to the ones found in actual environmental conditions. Indeed, advisable range is, in actual fact, between 15 - 28 °C and reaching 58 °C in the industrial composting field. However, the average environmental temperature in the EU is 9 °C in respect to marine environment, 12 °C in freshwater environments and soil environments and can reach about 55 °C - but only for a few days - in industrial composting. As a consequence, materials may degrade in laboratory conditions, according to the requirements detailed in the standard methods, but not in the waste treatment facilities [126]. Moreover, the requirements within standards do not cover all the natural environment that the bioplastics are accidentally disposed within. This is the other issue related to the assessment of biodegradability at lab-scale: the laboratory testing cannot completely and accurately enough reproduce the complexity of the dynamics that take place within those systems. On the other hand, it is important to state that the main purpose of research studies is to evaluate the biodegradability of the bioplastics outsides the treatment facilities that they should be addressed to. Having said this, there is the increasing attention of the public in respect to the proper disposal of waste items and, plastics to contend with, as well as the fact of bioplastics leakage into the environment, which represents a serious problem. For this reason, one of the main questions that the ongoing research is required to solve is whether a material labelled as a bioplastics is able to biodegrade under different natural environmental conditions. In order to perform this, the conditions imposed by the standard methods cannot always be applied within experimental tests, due to the fact that the natural environment may significantly differ from the standardised one in respect of waste treatment. Additionally, the indicators used for the assessment of bioplastics' biodegradability may differ from those reported in the standard methods.

The main indicators used for the evaluation of bioplastics' biodegradation consist in: the definition of the mass loss, the visual inspection of the tested material, the degree of disintegration, the discoloration, the changes within the morphology and structure of the biopolymer and the evaluation of the soluble components released by solubility tests. However, it is important to highlight that the correct evaluation of the biodegradability of a material should be assessed, even in presence of severe problems related to the implementation of the needed measurements, by monitoring the evolution of parameters, such as BOD, CO_2, O_2, CH_4 or biogas, as these components are directly correlated to the presence of microbial activities. Among the experiments observed in this study, only the biodegradability of bioplastics under anaerobic conditions was always evaluated by methane and/or biogas production, compared to the tests carried out in the other environments (i.e., aquatic, soil, and composting).

Under a strictly technical point of view, certain problems remain open. The first that requires mentioning is the difficulty to reproduce and analyse a biological system that treats biodegradable waste and bioplastics at the same time. This is a problem due to the inherent heterogeneity and high biodegradability of the substrate (biowaste) in relation to the low biodegradability of certain bioplastics. For these reasons, it is nearly impossible to evaluate the degree of the biodegradation of bioplastics assessing the difference between a system containing them and a blank (i.e., the same system fed only with biowaste). On the other hand, simulating the bioplastic biodegradation inside mature compost leads to the creation of a system where the rate of biological activity is completely different from that of a pile during active composting or from that of an AD plant.

Another key issue is related to bioplastic disintegration. Indeed, for practical reasons, during all the tests (both the standard methods and most of the research ones) particles with a size of < 2 mm (i.e., those belonging to the group of microplastics) were considered to be "disintegrated" included in the "mass loss" and thus considered degraded. As such, they can represent a noticeable fraction in respect of compost, thus leading to a possible non-compliant one [33,76]; moreover, bioplastics - such as PBAT, PBS, PCL and PLA - are generally not biodegradable under AD conditions, such that disintegrated fragments are present in the digestate [2,37]. The idea behind the set threshold of 2 mm is that the sieving operation is performed manually; further the identification of the bioplastics fragments is carried out visually. Therefore, for particles that are too small (i.e., < 2 mm) it is nearly impossible to detect and collect them; this leads to profoundly serious practical problems. At the moment, the behaviour - in terms of both fate and the effects - of micro-bioplastics that are released in natural environments is essentially unknown and thus it is not completely safe to release them within compost or digestate at this time. For these reasons, many plant managers must adopt specific strategies in order to reduce the problem related to biopolymer fragments. At full scale, the solution that is mainly applied consists in the removal of the bioplastic bags before the treatment. This is, while research activities pose the attention on three alternatives [2,76,127], which are: (i) the assessment of physico-chemical pre-treatments on bioplastics in order to facilitate the polymer degradation during the subsequent processes; (ii) the implementation of post-treatment methods in order to allow complete bioplastics decomposition and/or the removal of residual fragments from compost and (iii) the assessment of innovative blends of bioplastics that should be able to biodegrade in the working time of conventional biological treatment plants. Indeed, thermophilic conditions have been suggested for the purposes of degradation of bioplastics requiring long HRT.

Finally, bioplastics' degradation should be characterised by the release of non-toxic compounds. However, the effects of the released compounds in regard to the environment have not been fully investigated. In addition to biodegradation tests, physico-chemical modification of the environment, for instance by phytotoxicity tests, should be carried out in order to evaluate the possible negative or positive impact of the bioplastics' biodegradation process in respect to the environment. In fact, natural ecosystems - such as soils and marine environments - demonstrate a complex range of physical and chemical conditions as well a variety of bioplastics (especially when fragmentated). Therefore, such facts are notable in respect to inducing a high variability and complexity to the conditions in which to assess biodegradation, thereby rendering it difficult to develop environmentally sound criteria for biodegradation in all the affected environmental compartments.

In summary, there is a discrepancy between the results, in terms of the degree of biodegradability. This discrepancy is obtained by following the standard methods, in full-scale treatment systems and laboratory tests. These differences can be attributed to the unrealistic conditions set in the standard methods that cannot be replicated in full-scale treatment processes. Therefore, certain labelled biodegradable bioplastic materials that fulfils the requirements under the standard method testing conditions may eventually not biodegrade under the expected treatment conditions nor under uncontrolled natural conditions, when improperly disposed of. In the attempt to assess the bioplastics biodegradability in natural environments, the standards are set too far apart, as they do not consider the dynamic mechanisms involved in natural environments. Moreover, a comparison between the experimental studies is almost impossible. This is due to the fact that there is no particular indication regarding, for instance, the soil to be used as a "reference soil" when testing the biodegradability of bioplastics within various soils. The same considerations can also be applied for the other tests. Indeed, there is a multitude of composts or inocula that can be used as sources of microorganisms as well as manifold natural water conditions (e.g., river water, seawater, etc.). This is such that every test differs from one another and the results that are obtained cannot be thus related to any "standard" condition. Under this perspective, the use of the standard methods loses its original meaning, especially considering the fact that the major issue in the management of bioplastics is the prevention of microplastics leakage into the environment or in other words, the complete biodegradation of bioplastics that are improperly disposed of. The further revision and the harmonisation of the standards are required; in addition, more stringent conditions should be adopted in order to label a product as a biodegradable bioplastics. For instance, complete biodegradation should occur at less than favourable environmental conditions than that of the common waste treatment plants. This could facilitate the biodegradation of items that are discharged outside the proper treatment systems. Moreover, the standards should better represent the dynamic processes that occur in both industrial and natural environments; that is to say the parameters, such as temperature or pH, may vary continuously over time as well as the microbial community that are susceptible to change within changing environmental conditions. In addition to a revision of the standards, the other strategy to render bioplastics as easier to biodegrade could be the implementation of new bioproducts by means of the modulation of the chemical structure of the biopolymer. Indeed, it is known-that chemical composition can strongly affect the degradation kinetic of the biopolymer. However, a countereffect could be a reduction in the characteristics that render bioplastics as easily marketable (e.g., their mechanical properties). The exploitation of new easily biodegradable bioplastics blends could also improve the bio-recycling of bioplastics. In this sense, financial incentives can help in achieving a large-scale bioplastics market with a sustainable impact [28].

Finally, the harmful effects of microplastics as well as the influence of biodegradation products on the environment need to be further investigated. Ecotoxicity tests should be part of every biodegradation experiment and the effects of the biodegradable plastics on human heath requires further investigation also.

6 Conclusions

The assessment of bioplastic~~s~~’ biodegradability is extremely influenced by the conditions of the standard experiments. Standard tests are often inadequate due to the fact that the experimental environmental conditions (such as temperature, mixing and test duration) may not reflect the real conditions in waste treatment plants, thus not resulting in a correct estimation of bioplastic~~s~~ fragmentation and biodegradation.

In respect of this issue, it appears that biodegradation standards were addressed more in order to demonstrate that bioplastics are the panacea for solving the problems related to plastic pollution rather than providing an environmentally sound tool for the purposes of evaluating the properties of a given material. In fact, the available literature often demonstrates that biodegradation in real environmental or plant conditions is lower than expected and sometimes negligible.

Laboratory methods possess the advantage of being able to set and keep control of the experimental conditions (temperature, humidity, pH, oxygen supply, and test duration) [39]. On the other hand, lab-scale experiments aim at simulating specific process conditions (i.e., in natural environments or in waste treatment plants) but cannot exactly reproduce the conditions present in the multitude of natural and industrial environments.

In respect to small scale laboratory tests, more reliable data can be obtained by the application of full- or field-scale tests in which the kinetics and mechanisms of bioplastics’ degradation occur in real conditions. However, as expected, the results obtained can be subjected to different interpretations due to the continuous changes in the environmental conditions and due to microbiological composition [39]. For this reason, research activities are rarely conducted at full-scale and the procedures applied for the assessment of the biodegradability sensibly differ from the standardised protocols, as well as also in how they differ from one study to another. This specifically happens in regard to anaerobic biodegradation, as standardisation is not fully developed and is still in an early stage [16].

The main outcome of this study is that the comparisons between experimental (at either lab- or full-scale) and standard tests are generally not possible. This is due to several factors, specifically, the differences in the microbial sources, the varieties of the environments tested, the heterogeneity of the biopolymers, the difficulty in reproducing at lab-scale the complexity of natural spontaneous processes, and the different indexes used for the assessment of biodegradability. An improvement on the current standards tests and analytical methods (especially in terms of methods for assessing biodegradation and the presence of fragments) is necessary and should include the field-testing of the biodegradable polymer as well as of the finished product in order to ensure all criteria are met in real-life conditions. Environmental conditions set in the future standard methods should be far from that indicated as "optimal" for biodegradation, as bioplastics eventually end up in environments where conditions can vary significantly vary from that which is reported in the standards. Although research testing methods can differ from standard protocols since they aim at testing bioplastic biodegradability in very diverse environmental conditions, future research activities should be oriented at an harmonization of the applied procedures in order to increase the comparability of the results obtained in different studies.

Moreover, a future challenge in the bioplastics market could be the production of new blends of biopolymer that are more easily biodegradable without losing the characteristics (such as mechanical strength or flexibility) that make the bioplastics attractive in the first place.

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