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1 Assessing bioplastics biodegradability by standard 2 and research methods: current trends and open 3 issues

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8

9 **Abstract**

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

26

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

28

29 **1 Introduction**

30 Plastics, since their early developments in the 1950's [1], have covered a crucial role in everybody daily
31 life and represented a real "game-changer" in every industrial sector they have been used. This is due to
32 their convenience, easy production, resistance to corrosion, and low cost [2,3]. Plastics annual
33 production has been estimated to account for more than 367 million tonnes [4,5]. However, the
34 improper management and disposal of wasted plastics have converted their usefulness into a serious
35 issue [3] due to their persistence in the environment and to the release of possible toxic compounds
36 (generally used for their production) during plastics degradation [6]. Moreover, plastics debris
37 represents a significant economic and environmental damage to several activities, such as tourism,
38 fishery production, and shipping [7]. In fact, up to 4% of yearly plastic production ends up in the oceans
39 [8], constituting the main component of the marine litter (> 80%) [7]. Another issue related to the
40 leakage of plastics into the environment is their disintegration in small pieces - below 5 mm - known as
41 microplastics, which can be ingested by marine creatures and also enter into the food chain, even the
42 one concerning humans [7,9]. A variety of human health problems, such as cancer, respiratory, and
43 reproductive problems, may be attributed to plastics assimilation via ingestion (e.g., contaminated food)
44 as well as by inhalation (e.g., dust or contaminated air) [6]. A study that was conducted analysing data
45 collected from world's oceans expeditions in the period 2007 - 2013 [10], estimated over 5 trillion
46 plastic particles weighing over 265,000 tons floating in the oceans. This was deemed to be the result of
47 accumulation of plastics litter over the years - due to the increasing growth of 'single-use' plastics (such
48 as disposable cups, lids, straws and cutlery) - which are rarely recycled and usually disposed of
49 uncontrolledly, ending up in the environments and especially in oceans [7]. Around 13% (w/w) of the
50 total weight estimated [10] was attributed to microplastics. Indeed, there are even smaller pieces of
51 plastics than microplastics, within the size range of 1 to 1000 nm, which are known as nanoplastics
52 [11]. Due to their size dimension, nanoplastics demonstrate a colloidal behaviour that prevents them
53 from sedimentation [11]. Nanoplastics are more harmful of microplastics than microplastics due to the
54 fact that they can cross biological barriers [12]. However, due to the lack of suitable methods for the
55 detection and characterisation of nanoplastics, few studies have been conducted regarding their
56 influence on the environment and living organisms [6]. Moreover, traditional plastics are commonly
57 created from products of fossil-fuel origin, such that their production cannot be considered
58 environmentally friendly.

59 In order to overcome, at least partially, the problems related to plastic goods production and end-of-life,
60 bioplastics were developed in the last few decades as a valid substitute to conventional plastics. A
61 multitude of materials belong to the family of bioplastics. Indeed, they largely differ from each other
62 depending on the polymer they are composed of, as well as in respect to the structural characteristics
63 that mainly affect their persistence in the environment when released. Hence, the term bioplastics refers

64 to both bio-based plastics (i.e., plastics that composed of biogenic materials, such as crop-based
65 feedstock [13] or organic waste [14,15]) and biodegradable plastics. In respect of issue, it must be noted
66 that: (i) not all bioplastics are biodegradable; (ii) certain plastics of petrochemical origin can also be
67 labelled as bioplastic due to their biodegradable properties. Therefore, a bioplastic is a material that is
68 either bio-based, biodegradable, or both [16]. Moreover, they can be produced by biological
69 fermentation or by chemical polymerisation [17,18]. In the first case, only renewable feedstocks (such
70 as corn, sugar cane, soybean, etc.) can be used as the base material, while chemical polymerisation can
71 occur independently from the raw material used [16]. The use of microalgae for the production of
72 bioplastics (e.g., the extraction of lipids and cellulose from microalgae biomass) has been receiving much
73 attention in recent times. This is likely due to the fact that bioplastics derived from microalgae can be
74 considered as both bio-based and biodegradable [19]. Examples of bio-based bioplastics are: poly-
75 hydroxyalkanoates (PHA); polyhydroxybutirate (PHB); polylactic acid (PLA); bio-polyethylene (Bio-
76 PE); bio-polyethylene terephthalate (Bio-PET); bio-polyvinyl-chloride (Bio-PVC); and bio-polyurethane
77 (Bio-PU) [20–23]. Meanwhile, examples of fossil-based bioplastics are: poly (butylene succinate) (PBS);
78 poly(e-caprolactone) (PCL); poly (butylene adipate-co-terephthalate) (PBAT); and poly(butylene
79 succinate-co-butylene adipate) (PBSA), [20,24,25].

80 The global world bioplastics production in 2021 was around 2.4 million tonnes and is expected to
81 increase (i.e., an over 200% growth rate) to 7.5 million tonnes in 2026, thereby accounting for the 2%
82 of the expected global production of plastics [26]. The continuous increase in the global bioplastics
83 production can be attributed to their versatility in several applications (such as in respect to packaging
84 and consumer products, as well as in electronics and automotive industries [4]). Packaging, for instance,
85 representing 48% of the total bioplastics market in 2021 [4] is one of the most promising and important
86 uses. One of the main advantages in the use of many bioplastics consists in the absence of toxic
87 compounds released in the environment after degradation [27]. Furthermore, bioplastics production
88 does not necessary entail competition with feedstock for food and feed, due to the fact that the land used
89 for the renewable feedstock growth for the purposes of bioplastics production accounts for only 0.01%
90 of the global available agricultural area [26]. In addition, the land use share in 2026 will not exceed
91 0.06% [26]. Moreover, bioplastics can be produced from organic waste [14,15], thus positively
92 contributing to the management of the organic waste through the perspective of the circular economy.
93 On the other hand, the absence of clear labelling and/or inadequate collection, and/or the processing
94 of wasted bioplastics does not prevent the risks that are related to plastics leakage [7], nor in respect to
95 microplastics and nanoplastics pollution [6], however the lower persistence in the environment of
96 biodegradable plastics could reduce the problems related to plastic pollution. As such, the management
97 of bioplastics is extremely important - specifically in terms of the circular economy, especially when
98 referring to their end-of-life options, i.e., recycling, incineration, landfilling, and biodegradation. Due to
99 the variety and heterogeneity of bioplastics, the sorting of and the processing, thus, recycling of

100 bioplastics appears to not to be the most suitable option for their recovery. This may be due to the fact
101 that recycled bioplastics generally show a quality reduction [28] as they may be too degraded to be
102 utilized effectively [29]. In addition, the processes for their recycling are often not mature to be used at
103 industrial level. The use of landfills is not considered to be suitable as end-of-life option, due to the fact
104 that bioplastics can produced methane once landfilled [28,30]. Finally, incineration can be considered
105 as a valid option for wasted bioplastics management if the bioplastics are produced from renewable
106 feedstock; in fact, in this case, the CO₂ produced during combustion, being of renewable origin is not
107 relevant for global warming [31]. Moreover, energy is also produced during this process thus increasing
108 the environmental benefit of the process [29].

109 Biodegradation should convert the biopolymers into non-toxic compounds, such as into monomers, CO₂
110 and H₂O. Moreover, value-added products, such as compost and methane obtained by biological
111 treatment processes, benefit the environment when compared to petroleum-based plastics [29]. Indeed,
112 when compared to anaerobic digestion (AD) - in which the methane produced can be utilized for the
113 purposes of energy production - industrial composting, in regard to it as end-of-life process for
114 bioplastics, results in a high global warming potential [30]. This is due to the fact that composting-
115 related operations are high-energy-consuming processes [32], and because energy recovery is not
116 possible through this process either [32]. However, the real applicability of biological processes for the
117 treatment of used bioplastics, grandly relies on their biodegradability that depends on the complexity
118 of the bioplastics structure and on the type of raw materials used, such that potentially different waste
119 streams should be adopted according to the bioplastics' characteristics. At the moment scientific
120 literature does not report examples of full-scale plants destined specifically to bioplastics treatment.

121 Specific prevention policies in respect to the problem of plastic pollution have been adopted by several
122 countries, such as: the ban of certain disposable plastic items (e.g., straws and plastic cutlery) or the
123 replacement of lightweight plastic carrier bags with biodegradable ones. For instance, the use of
124 compostable and biodegradable bags is now compulsory for the collection of food waste; further, this is
125 addressed in to biological treatment plants in several European countries (such as Italy and Sweden)
126 [33,34]. The extent to which a bioplastic can be labelled as compostable and/or biodegradable in a
127 certain environment (such as aerobic or anaerobic) and under defined conditions (such as mesophilic
128 or thermophilic temperature) is defined by certified international standard methods. These methods,
129 required by national regulations and/or developed for marketing purposes, were established through
130 considering realistic environmental conditions that occur in full scale utilities in respect to organic
131 waste management especially when referring to composting and AD plants. The EU Directive 2015/720
132 firstly placed the attention on the necessity of proper labelling for biodegradable and/or compostable
133 products. This was conducted due to the fact that non-biodegradable and biodegradable plastic items
134 are generally not distinguishable to the public eye, due to their similar physical appearance [33,35].
135 Furthermore, as a consequence, they may be subjected to improper treatment. Indeed, according to

136 certain Italian legislation, biological treatment plants can only accept bioplastics that fulfil the
137 requirements of the UNI EN 13432 and UNI EN 14995 directives in respect to packaging and other
138 materials [36] respectively and therefore bioplastics disposed together with food waste must be labelled
139 as compostable and clearly distinguishable from conventional plastics. In other countries (e.g., China),
140 food waste is still collected by non-biodegradable plastic bags and treated in biological (generally
141 anaerobic) treatment plants [37] possibly leading to negative effects on the mechanical equipment (i.e.
142 feeding and mixing devices) and on the biological process [38] and on digestate suitability for
143 agricultural use. Moreover, when it comes to the indiscriminate disposal of wasted bioplastics into the
144 environment, the standard methods for the evaluation of their degradability cannot be applied as
145 degradation/biodegradation processes occur in different conditions. For this reason, research studies
146 were mainly focused on to the investigation of bioplastics' degradation in different, i.e., non-
147 standardised environments.

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

157

158 **2 Bioplastics' (bio)degradability**

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

163 In addition to the biomaterial properties, the rate of decomposition of a bioplastic is affected by the
164 specific environmental conditions [41] which must consider the possible presence of microorganisms
165 (such as bacteria or fungi) [23]. The last condition is extremely important to define whether the
166 decomposition of the material occurs only by abiotic processes (i.e., driven by heat, sunlight, moisture,
167 etc.) and/or by the microbial activity (biotic processes) [35,42]. In the last case, it can be said that the
168 decomposition of the bioplastic occurred via *biodegradation*, so that the material is mineralised to CO₂,
169 H₂O, NH₄⁺, N₂, H₂ and biomass through the biological action [23,27,41]. Both prokaryotic and eukaryotic

170 microorganisms are responsible for the biodegradation of bioplastics [17,20,43], while endo- and exo-
171 enzymes are functional in respect to the depolymerisation of biopolymers [44,45]. If this is not the case,
172 then it is referred to *degradation* as a fragmentation of polymers' chains that occurs via abiotic processes
173 [29] leading to the formation of persistent particles [20,46–48]. In any case, as one of the main
174 advantages of bioplastics, the remaining residues of degradation/biodegradation should not be toxic for
175 living organisms [23,27].

176

177 **3 Biodegradability indexes**

178 The biodegradability of a bioplastic is evaluated by the estimation of so called *biodegradability indexes*
179 as defined in the international standard protocols. These indexes are related to both the structural
180 properties (such as molecular weight and surface morphology) of the bioplastics and the
181 microorganisms' activity, as estimated by the evolution of CO₂, O₂ and/or CH₄, which represents the
182 main indexes for aerobic and anaerobic biological processes respectively. Weight reduction is often used
183 as an indicator of biodegradation despite the fact that mass loss can also occur due to abiotic processes
184 without the involvement of microorganisms [16].

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

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

203 In general, the conversion of the carbon present in the bioplastic into CO₂ and/or CH₄ is used for the
204 evaluation of the biodegradability of the test material under anaerobic conditions [52]. The biochemical
205 methane potential (BMP) test is a method widely used to simulate anaerobic conditions at lab-scale [57].
206 The CO₂ production or the O₂ consumption are also used as indexes of biodegradability in aerobic
207 environments [58].

208 As already mentioned, the extent at which a bioplastic can be biodegraded also depends on the
209 environmental conditions the material is subjected for a certain period, such a temperature, humidity
210 or UV light. The effects of the different combinations of biotic and abiotic processes on bioplastics
211 degradability have been of increasing interest in the last few years in order to understand the
212 mechanisms, and thus the impact, of bioplastic biodegradation in industrial and natural environments
213 [16,59].

214

215 **4 Standard and research methods for the assessment of bioplastics' degradability**

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

229

230 **4.1 Standardised Methods**

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

234 - USA:

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

- 236 - EU:
 - 237 ○ CEN (Comitè Européen de Normalization - European Committee of Standardisation)
 - 238 operating in EU and EFTA countries (Iceland, Norway, Switzerland, etc.) [62];
 - 239 ○ UNI (Ente italiano di normazione – Italian Institute of Standardisation) operating in Italy
 - 240 [63];
 - 241 ○ DIN (Deutsches Institut fur Normung - German Institute for Standardisation) operating
 - 242 in Germany[64];
- 243 - Asian countries:
 - 244 ○ JAS (Japanese Standard for Association) operating in Japan[65];
- 245 - Australia:
 - 246 ○ AS (Australian Standard) operating in Australia and New Zealand. [66];
- 247 - Worldwide:
 - 248 ○ OECD (Organisation for Economic Cooperation and Development) operating in OECD
 - 249 Countries [67];
 - 250 ○ ISO (International Organisation for Standardization) operating worldwide[68].

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

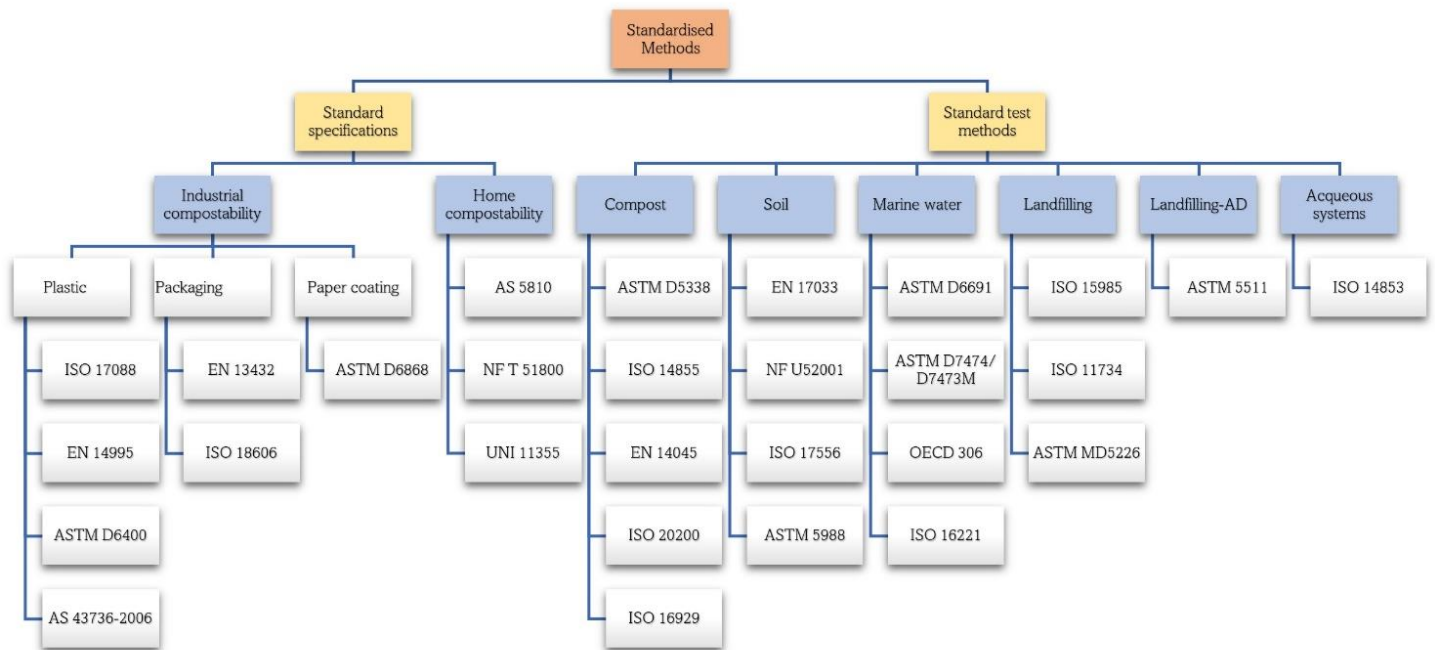
254 Various norms that describe biodegradation test methods are available; further, they all possess a few
255 basic aspects in common. First of all, they list test procedures and set the testing conditions, e.g., pH,
256 nutrients, temperature, concentration and source of inoculum, etc. The test conditions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

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

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

270 Standardised methods are summarized in Figure 1.

271



272

273 *Figure 1 - Standardised methods*

274

275 **4.1.1 Industrial compostability**

276 The specification standards defining the requirements for the industrial compostability of bioplastics
 277 are listed in Table 1 [69]. There is a large similarity between these standards with only minor differences
 278 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

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

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

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

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

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

- 288 1. Characterization of material composition: identification of the different constituents (e.g., by IR),
289 organic matter content (represented as volatile solids that must be at least 50% on dry weight),
290 and heavy metals concentration level. Several metals, each with a specific limit, are considered
291 in these standards. They refer to heavy metals limits that are required in order to check compost
292 quality. Polymers or basic packaging materials, usually, pose little problems. However, heavy
293 metals requirements differ among norms both in reference to the type of metal and limit value.
294 In both the EN 14995 and EN 13432 standards, the concentration of any substance (e.g. Zn, Cu,
295 Ni, Cd, Pb, etc.) shall not exceed the tabulated values (e.g., the limit value for Zn is 150 mg/kg
296 substance) [71]. In these cases, it is assumed that 50 % of the original mass of the plastic material
297 will remain in compost following biological treatment together with the complete amount of
298 hazardous substances [71,72]. In addition, ASTM D6400 standard permits higher values for
299 heavy metals within the material than the EN standards allow. For instance, the limit value for
300 Zn is 2800 mg/kg; another example is As, whose limit in ASTM is 41 against the 5 mg/kg
301 established in aforementioned EN standards [73].
- 302 2. Disintegration: disintegration requirements are incredibly similar in all four standards. At least
303 90% of the original dry weight disintegrates into particles having a size of less than 2 mm
304 (maximum of 10% of original dry weight may remain after sieving on a 2.0 mm sieve) after a
305 specified time. Moreover, EN standards require a maximum of 12 weeks of aerobic composting,

306 5 weeks of anaerobic biogasification, (which is optional and which possesses the option of
307 extension), and the test duration may be modified as necessary as a result of the testing currently
308 being carried out. In the ASTM D6400 standard, test duration is 12 weeks. In respect to the ISO
309 17088 standard, the time is 45 days (with the option of an extension of up to 6 months).
310 Furthermore, the ASTM D6400 standard allows the use of other test methods, such as those
311 found in ASTM 5338 and ISO 16929, in order to determine the details of the disintegration. As
312 alternative test methods for disintegration - other than those found in ISO 16929 - the ISO 17088
313 standard includes mentions of the methods detailed in ISO 14855 and ISO 20200. The issue of
314 test duration and fragmentation are two of the most serious within the field and will be
315 discussed further in this paper.

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

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

331 The ecotoxicity tests are generally carried out via pot tests in which a comparison between a
332 blank compost and test compost is conducted with regard to their respective seeds germination
333 and plant growth. In all four standards the ecotoxicity tests are performed in accordance with
334 OECD 208, which is a terrestrial plant test that is used to determine if composted material is
335 toxic to plants. The ASTM, ISO, and EN norms have the same two requirements as concerning
336 ecotoxicity: (i) the plastic-should have concentrations of regulated metals that are lower than
337 50% of those prescribed for sludges or composts in the country where the product is sold (these
338 values are tabulated for each country); and (ii) the germination rate and plant biomass of the
339 sample composts shall be no less than 90% than that of the corresponding blank compost for
340 two different plant species (when following the OECD Guideline 208 with the modifications

341 found in the Annex E of the EN 13432 standard). By fulfilling requirements (i) and (ii), a plastic
342 product can demonstrate satisfactory territorial safety and the ecotoxicity test is, thus, passed.
343 Furthermore, only AS 4736-2006 guideline deviates from other standards, thereby requiring an
344 earthworm toxicity test as well as two plant toxicity tests.

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

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

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

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

- 359 1. Proper disintegration during composting; after twelve weeks in a controlled composting test, no
360 more than 10% of its original dry weight remains after sieving the material through a 2.0 mm
361 sieve. Please note, sieving is further discussed below and is a critical part of the test.
- 362 2. Adequate level of inherent biodegradation: an end item, possessing a plastic coating(s) or
363 additives, is considered to have achieved a satisfactory level of biodegradation if the plastic
364 coating or polymeric additives meet the requirements of ASTM 6400 (as previously reported).
365 Moreover, the substrates of the end item are to individually demonstrate that 90% of the organic
366 carbon is converted to carbon dioxide using Test Method D5338 within 180 days at 58°C (to a
367 maximum of 62°C), when compared to the positive control. End items composed of ligno-
368 cellulosic substrates are permitted to fulfil previous requirements by demonstrating that they
369 are materials of natural origin and therefore they are biodegradable by showing that over 95%
370 of their carbon derives from biobased resources. A problematic issue in respect of this test is
371 that usually the amount of carbon dioxide produced by bioplastic biodegradation is quite
372 limited, thereby affecting the precision of the measurement and the replicability of the
373 experiment (in regard to the comparison with the background CO₂ or with a positive control).

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

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

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

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

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

394 *4.1.2 Home compostability*

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

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

- 408 • Australia: AS 5810 (2010) – Biodegradable plastics: Biodegradable plastics suitable for home
 409 composting. This standard specifies the requirements and procedures in which to determine
 410 whether a plastic material is biodegradable in home-composting conditions. In addition, it
 411 provides the basis to allow one to label materials and products constituted of plastics as “home
 412 compostable” for use in home composting systems. Moreover, this standard stipulates pass/fail
 413 criteria that specifically address biodegradability, disintegration during biological treatment,
 414 the effect on the biological treatment process, and the effect on the quality of the resulting home
 415 compost. Therefore, these requirements are mainly similar in respect to the industrial
 416 composting requirements, but in this case is required to determine the degree of biodegradation
 417 and disintegration at an ambient temperature.
- 418 • France: NF T 51800 (2015) – Plastics : Specifications for plastics suitable for home composting.
 419 This norm strictly follow “OK compost” scheme described above.
- 420 • Italy: UNI 11355:2010 - Plastic items biodegradable in home composting: Requirements, test
 421 methods, and the UNI 11183:2006 standard. In addition, this also concern plastic materials that
 422 are biodegradable in terms of home composting, i.e., in respect to requirements and test
 423 methods. As it will be discussed in a following section, the twelve months requirements for
 424 composting time required in this method can be considered, in most cases, unrealistic.

425 *4.1.3 Biodegradation testing standards*

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

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

433

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)					

434

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

436

437 *4.1.4 Composting biodegradation and disintegration standards*

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

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

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

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

452 The principle of the test is found in respect to the item that is mixed with mature compost and incubated
 453 under batch conditions at 58°C under optimum O₂ and moisture conditions. The mature compost acts
 454 at the same time as the carrier matrix, the source of the microorganisms and the source of nutrients.
 455 The mixture is continuously aerated and the exhaust air is analysed in terms of produced CO₂ [69].

456 The maximum test duration is 6 months, while a typical minimum duration is 45 days. Further, CO₂
 457 production is continuously measured. After subtracting the background CO₂ production from the blank
 458 compost inoculum, the percentage of biodegradation is determined by the net amount of carbon in
 459 respect of the test item that is converted to CO₂. A positive reference control, cellulose, is tested in
 460 parallel to check the activity of the inoculum. Furthermore, strict requirements are imposed on the
 461 results for cellulose in order to validate the test. The test item is preferably added in the form of a fine
 462 powder. Again, here the test conditions (e.g., temperature and duration) are the most severe issues.
 463 Furthermore, the addition of the material as a fine powder is also quite unrealistic. Moreover, the

464 measurement and comparison of the produced CO₂ with a background production are complicated in
465 terms of precision and reproducibility, especially in respect of the compost heterogeneity.

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

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

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

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

488 *ASTM D5338-15 - Biodegradation Test – Composting*

489 The ASTM D5338 -15 standard also details a test method that determines the degree and rate of the
490 aerobic biodegradation of plastic materials in respect to their exposure to a controlled-composting
491 environment under laboratory conditions, at thermophilic temperatures. In addition, the ASTM-D5338
492 standard is not a pass/fail test. The reports indicate what percentage biodegraded over the tested time
493 period, which can be selected by the test requestor. The principle used is the same as that found in ISO
494 14855. Moreover, this test does not include any testing for the purposes of measuring disintegration.

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

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

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

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

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

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

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

525 The method determines the degree of disintegration in respect of test materials on a laboratory scale
526 under conditions simulating an intensive aerobic composting process. The solid matrix used consists of
527 synthetic solid waste that is inoculated with mature compost, which is taken from municipal or
528 industrial compost plants. Pieces of the plastic test material are composted with this prepared solid
529 matrix. Furthermore, the degree of disintegration is determined after a composting cycle, by sieving the
530 final matrix through a 2 mm sieve in order to recover the non-disintegrated residues. The reduction in
531 mass of the test samples is considered as disintegrated material and used to calculate the degree of

532 disintegration. In this test there is a minimum period of 45 days and a maximum of 90 days in which
533 reactors are maintained at a constant thermophilic temperature (58°C). It is then followed by a
534 mesophilic incubation period at room temperature for a maximum period of additional 90 days.

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

537

538 *4.1.5 Soil biodegradability*

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

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

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

561 The biodegradability index is represented by the conversion of the carbon source, which is present in
562 the biomaterial into CO₂. In respect of this, it is required to demonstrate a ≥ 90% conversion of film
563 carbon into CO₂ within 2 years under ambient soil conditions. The test method used is the one described
564 in the ISO 17556 standard.

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

567 The ISO 17556 document specifies a method for determining the ultimate aerobic biodegradability of
568 plastic materials in soil by the measuring of the oxygen demand in a closed respirometer, or in regard
569 to the amount of carbon dioxide that has evolved. The method is designed to yield an optimum degree
570 of biodegradability by adjusting the humidity of the test soil. Further, the plastic material is mixed with
571 soil, then the mixture is allowed to stand in a flask over a period of time during which the amount of
572 oxygen consumed (BOD) - or the amount of carbon dioxide evolved - is determined. Provided the CO₂
573 that has evolved is absorbed, the BOD can be determined by, for example, measuring the amount of
574 oxygen that is required to maintain a constant gas volume in a respirometer flask. The respirometer is
575 set up in a temperature-controlled environment and contains test vessels, each fitted with a CO₂
576 absorber in the headspace, a coulometric oxygen production unit, a manometer, as well as an external
577 monitoring device and recorder. The test vessels are filled to about one third of their volume with the
578 test mixture. If biodegradation takes place, the microorganisms consume oxygen and produce carbon
579 dioxide - which, in turn, is completely absorbed. The pressure drop is detected by a manometer and used
580 to initiate the electrolytic generation of oxygen.

581 *4.1.6 High-solids anaerobic/landfill simulation biodegradation*

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

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

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

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

594 *ASTM D5511: Anaerobic Biodegradation.*

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

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

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

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

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

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

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

618 In this simulation tests, there is a lower concentration of microorganisms, which thus determines a
619 slower biological activity if compared to high solids anaerobic digestion tests. Biodegradation is
620 evaluated through a measurement of biogas as in the ASTM D5526 standard. Furthermore, it provides
621 the percentage of conversion in respect of carbon in the test sample to carbon in the gaseous form (CH₄
622 and CO₂) under conditions that mimic landfill conditions. This test method covers the determination of
623 the degree and rate of anaerobic biodegradation of plastic materials in an accelerated-landfill test
624 environment. Furthermore, this test is carried out at a constant temperature; moreover, it can be run
625 for as long as required in order to establish the time it takes for the bioplastic sample to degrade.

626 *4.1.7 Aquatic, anaerobic biodegradation*

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

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

632 *ISO 14853-Plastics - Determination of the ultimate anaerobic degradability in an aqueous system - Method*
633 *by measurement of biogas production.*

634 The ISO 14853 standard specifies a method for the determination of the ultimate anaerobic
635 biodegradability of plastics by anaerobic microorganisms in an aqueous environment. The principle is
636 placing the test item in an aqueous inoculated (anaerobic sludge) medium and is conducted under batch
637 conditions at a mesophilic temperature.

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

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

648 *4.1.8 Marine biodegradation*

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

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

653 *OECD 306: Biodegradation Test – Seawater*

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

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

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

665 2. The closed bottle method consists of a dissolution of a pre-determined amount of the test
666 substance in the test medium in a concentration of usually 2 - 10 mg L⁻¹ (one or more
667 concentrations may be used). The solution is kept in a full and closed bottle in the dark; further,
668 it is kept in a constant temperature bath or enclosure that is controlled within a range of 15 - 20
669 °C. The degradation is then followed by oxygen analyses over a 28-day period. However, if the
670 blank biological oxygen demand value remains within the 30 % limit, the test could be
671 prolonged. Twenty-four bottles are at least used (eight for the test substance, eight for reference
672 compound and eight for seawater plus nutrient). All the analyses are performed on duplicate
673 bottles. Moreover, four determinations of dissolved oxygen, at least, are performed (i.e., days 0,
674 5, 15 and 28) using a chemical or electrochemical method.

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

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

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

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

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

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

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

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

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

716

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

721 Film pieces are harvested at varied time intervals in order to assess visual impacts of exposure and
722 degradation, as well as in respect of determining the percentage loss in terms of dry weight and weight
723 loss per unit area. It is required the prior determination of its organic carbon biodegradability to CO₂,
724 which is based on the outcome of Test Method D6691. It must also be noted that the test entails a
725 maximum duration of 180 days.

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

728 As already demonstrated, the standard test in the marine environment has aided researchers in
729 foreseeing that a minimum duration of 28 days and maximum duration of 6 months is sufficient.
730 However, in that timeframe the item can still cause harm to marine life via ingestion, entanglement, etc.
731 This is one of the most limiting aspects related to the marine environment standard. Moreover, due to
732 the high variability in marine conditions (i.e., temperature, salinity, exposure to light, etc.) the standard
733 tests that are based on laboratory procedures cannot mimic completely the full spectrum of marine
734 conditions that can be encountered (such as, the cool water in the northern and southern hemisphere
735 [29]). Another important, and undervalued, aspect is in the fact that it is almost impossible for this test
736 to replicate the abiotic degradation that is caused by exposure to light, waves agitation, etc.

737

738 **4.2 Methods used in research activities**

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

748 Moreover, in considering the variety of base materials that can be used for the production of bioplastic
749 products, research activities are often conducted on novel “lab-produced” bioplastics rather than on the
750 ones that are already labelled and marketed as bioplastic material. Indeed, the focus of many studies is
751 to develop bioplastics (for specific issues, such as food packaging [74] or the replacement of disposable
752 plastics [75]) that can be completely degraded as much and as easily as possible after their use.
753 Therefore, the tested materials refer to both certified bioplastics products (such as starch-based
754 shopping bags and PLA goods [36,57,76,77], and bottles used for the packaging of water [78]) and novel
755 lab-made bioplastic blends (such as silk fibre + glycerol + wheat gluten [75], corn starch + PCL + biochar
756 [79], and PLA + PHA [80]).

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

760 4.2.1 Soil

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

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

776 Mass loss (which is periodically measured) is the main index that is used to assess the biodegradability
777 of bioplastics in soil. This is because it is assumed that (i) microorganisms are present in the soil and
778 that (ii) they would be able to degrade the material. For the same reason, disintegration is also
779 considered an index of biodegradability. Furthermore, the analysis is usually conducted by sieving the
780 final matrix through a 2 mm sieve in order to recover the non-disintegrated residues [76]. Less
781 frequently, microstructure characteristics that are determined via FTIR spectroscopy or X-ray
782 diffraction (XRD) are analysed [82]. In some cases, analyses on quantification and biomass diversity are
783 carried out in order to define a relationship between the degradation of bioplastics and the bacterial
784 biomass in the soil [49]. Conversely, specific microbial culture from soil are isolated, by means of certain
785 methods - such as the already mentioned clear zone formation [25,83]- in order to investigate the
786 relationship between bioplastic biodegradation and microbial colonisation [84]. For instance, bacteria
787 (*Pseudomonas* and *Bacillus* strains), fungi (*Geomyces*, *Sclerotinia*, *Fusarium* and *Mortierella* strains) and
788 yeast (*Hansenula anomala*) that are all isolated from Antarctic soil samples were found to be good
789 candidates for effective PCL, PBS and PBSA degradation at low temperatures (< 20°C) [25]. In addition,
790 fungal strains (*Apiotrichum porosum*, *Penicillium samsonianum*, *Talaromyces pinophilus*, *Purpureocillium*
791 *lilacinum*, and *Fusicolla acetilerea*) that were isolated from terrestrial environments in various region of
792 Korea were able to degrade PLA and PCL polymers [83]. Moreover. bacteria from the genus
793 *Amycolatopsis sp.*, which were isolated from agricultural soils collected in northern Thailand, showed

794 enzymatic activity for both PLA and PCL [53]. When no microbial analysis is conducted, the presence of
 795 microorganisms is confirmed via the monitoring of the production of CO₂ [23,85] in relation to a blank.
 796

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]

797

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

800

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

817

818 4.2.2 Aquatic or marine environments

819 As for the soils, the bioplastics degradation when discharged in an aquatic environment is a major issue
820 for research investigations. However, the majority of the studies that were conducted on this topic, have
821 investigated bioplastics degradation in terrestrial systems rather than in marine environments [3,17].
822 Bioplastics' degradation in aquatic environments refers to freshwater, seawater, and river water
823 environments; furthermore, it implies both aerobic and anaerobic biodegradation. Almost all the
824 research activities present in scientific literature are carried out at laboratory scale, most likely due to
825 the difficulty in managing the degradability test in a real environment. In a few cases- e.g. in [77], [94],
826 [95] and [94]- via in-situ tests and by the recreation of an eutrophic reservoir, the experiments were
827 conducted under uncontrolled conditions; this, therefore, means that they were conducted within a real
828 existing environment. In all the other cases, environmental biotic (such as the type of microorganisms
829 involved and the nature of incubation) and abiotic (such as heat, light, water pH or salinity) parameters
830 were set and applied for a certain period.

831 In general, the samples are prepared by cutting the biomaterial into small pieces; then, they are
832 immersed in water at the set testing conditions such as: temperature, pH, static (flasks) or dynamic (i.e.,
833 an aquarium with samples subjected to continuous flow of water) [96], natural or inoculated water
834 [94,96], with or without contact on a sediment surface [3,96] or buried in wet sediments [3], an
835 alternation of light and dark periods, as well as in aerobic or anaerobic conditions (Table 4). Depending
836 on the type of bioplastic and the set environmental conditions, the testing time ranged from few days
837 up to one year, while the degree of biodegradability varied from less than 2% to almost complete (>
838 90%) biodegradation (Table 4).

839 Weight loss and visual inspection are the main parameters used as the degradability indexes. In
840 addition, other physico-chemical analyses (e.g., Raman measurements [97]) were conducted in order to
841 understand the extent of the polymers' degradation. A solubility test was also seldom used for the
842 estimation of the soluble fraction of the bioplastic [74] and chemical parameters (e.g., the chemical
843 oxygen demand - COD) were determined on the test water in order to evaluate the release from the
844 various bioplastics [36]. The degree of biodegradation and the microorganisms' activity are specifically
845 determined by indicators, such as CO₂ production [98], the evolution of the BOD by respirometry tests
846 [3,40,99], the production of biogas [100], the formation of the clear zone [101], or by the selecting of
847 specific mixed culture, such as bioplastic degrading bacteria [91,101]. For instance, thermotolerant and
848 halotolerant *Bacillus sp.* JY14 bacteria, when isolated from marine soil, was found to be capable of
849 degrading PHB and various PHAs [101]. The *Microbulbifer* genus strains, which reside in high-salt
850 environments, also showed a great ability to degrade PHB [102,103]. The bacterial species *Pseudomonas*
851 *pachastrellae* was found to be involved in the degradation of PCL in coastal environment [104].
852 *Shewanella*, *Moritella*, *Psychrobacter* and *Pseudomonas* genera were isolated from deep-sea

853 environments at depth of over 5,000 m from the Kurile and Japan Trenches for testing their ability in
 854 the PCL degradation [105]. *Enterobacter sp.*, *Bacillus sp.* and *Gracilibacillus sp.* strains were isolated from
 855 seawater environments and used for the purposes of PHA biodegradation [95], while phylogenetic
 856 groups of *Cytophaga-Flavobacterium-Bacteroides*, *g-Proteo-* bacteria and *b-Proteo-*bacteria were
 857 identified in a reservoir-within the Bugach river (Russia) and they were found to be able to utilise PHA
 858 [94].

859

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]

860

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

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

870

871 4.2.3 Composting environment

872 Bioplastic's biodegradation during a composting process has been deeply investigated. This has been
 873 performed due to the fact that bioplastics are commonly used for the purposes of household organics

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

897 Following a visual inspection of the residues, the disintegration and mass loss were the most usual
898 biodegradation indicators. Indeed, changes in the polymeric structure were observed in other
899 investigations, such as those found in the application of the FTIR analysis [110]. The CO₂ production
900 was used, more correctly, to evaluate the extent to which the biomaterial was degraded by the action of
901 microorganisms [78,113], as composting is an aerobic process. However, field-scale testing may render
902 difficult, or perhaps even not possible, the tracing of the CO₂ production [109]. The observation of
903 microbial growth in compost, generally in proximity of the bioplastic, is also a qualitative indication of
904 disintegration and biodegradation [42].

905

906

907

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]

908

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

911

912 When compared to industrial composting, home composting temperatures are usually lower; as such,
913 longer periods of time for the purposes of biodegradation may be required. Most of the analysed studies
914 were conducted according to the standard methods- such as the ASTM D6400, ISO 20200, and ISO
915 14855-1 standards. As defined in these standards, at least 90% of weight loss (as well as the
916 disintegration of the mass into fragments that are less than 2 mm) should occur, within six months in
917 order to label a bioproduct as compostable. However, the existing composting plants were not designed
918 to treat bioplastics; as such, their processing may be problematic for this reason [33]. It must be noted
919 that although residual fragments can affect the compost quality, ecotoxicity tests in research studies are
920 barely applied to the final compost.

921

922 4.2.4 Anaerobic environment

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

932 anaerobic process. Furthermore, complete biodegradation of the bioplastics may occur within the
 933 aerobic phase usually applied for the final stabilization of the digestate.

934 The environmental conditions are simulated by setting the main process parameters (Table 6), which
 935 are: temperature (mesophilic and/or thermophilic), type of digestion (wet or dry), type of test
 936 (discontinuous batch or semi-continuous), inoculum used (commonly collected from full-scale AD
 937 plants treating OFMSW [37], substrate used (green waste and/or food waste [37], as well as cow manure
 938 and vegetable waste [119]), the possible presence of a co-substrate, or of single type [57,119] or mixed
 939 bioplastics [80], the dimension of the bioplastics samples, organic loading rate (bioplastics OLR of 0.75
 940 $\text{g}_{\text{ThOD}} \cdot \text{L}^{-1} \cdot \text{day}^{-1}$ [120], $0.25 \text{ kg}_{\text{CODbioplastics}} \cdot \text{m}^{-3} \cdot \text{day}^{-1}$ [36] and $0.04 \text{ kg}_{\text{VSbioplastics}} \cdot \text{m}^{-3} \cdot \text{day}^{-1}$ [121]); the hydraulic
 941 retention time (HRT), and the food-to-microorganisms ratio. Although the long test duration, which
 942 generally exceeds 30 days up to over 250 days [122], bioplastics show low biodegradability under
 943 anaerobic conditions. Only powdered PHB was found to biodegrade (> 90%) within 10 days in the
 944 mesophilic AD process [122]. Indeed, even when co-digested with other substrates (such as food waste
 945 or sludge), bioplastics degradability was lower than 30% [36,76,109]. The procedure for the evaluation
 946 of bioplastics' biodegradability under anaerobic conditions consists in the application of the biochemical
 947 methane potential (BMP) test, such that the degree of biodegradability of the biopolymer is generally
 948 estimated by the means of the biogas that is produced during the process. In addition to the biogas
 949 and/or CH_4 and/or CO_2 production, the weight loss and visual inspection of the residues after sieving
 950 (with a 2 mm mesh) are traditionally, and commonly estimated. Other laboratory analyses - such as the
 951 differential scanning calorimetry for the evaluation of the thermal properties both before and after the
 952 process [36], spectroscopic analysis [37], thermogravimetric analysis [37] and discoloration [123]-
 953 were also used in these tests as indicator of degradability of the tested material.

954 PLA-based biopolymers were decomposed by the microbial communities at the phylum level of
 955 *Firmicutes*, *Bacteroidota* and *Proteobacteria*, while *Methanosarcina*, *Methanoculleus* and
 956 *Methanothermobacter* at the genus level were involved in their degradation within mesophilic
 957 conditions [37]. Organisms that are identical (i.e., over 97%) to *Peptococcaceae bacterium* Ri50,
 958 *Bacteroides plebeius*, and *Catenibacterium mitsuokai* were involved in the biodegradation of PHB, while
 959 *Ureibacillus sp.*, *Bacillus infernus*, and *Propionibacterium sp.* were implicated in the anaerobic
 960 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]

961

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

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

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

977

978 **5 Drawbacks, future prospects and challenges**

979 The increasing use of bioplastics worldwide is an important component in the drive to lower the global
980 carbon footprint, to reduce the degree of climate change, and decrease plastic-based pollution [29].
981 Although the production of bioplastics and its related market have been well established, certain issues
982 related to the proper labelling of these materials as biodegradable still remains. Firstly, the
983 environmental conditions that are suggested in the standard methods as optimal for biodegradation to

984 take place cannot be reproduced in common full-scale treatment plants. In particular, most of the
985 compostability standard tests set duration and process temperatures that are unrealistic. This is due to
986 the fact that the standards advise much longer durations and higher process temperature when
987 compared to those of real full-scale plants, where bioplastics are supposed to be treated in reality
988 [16,34]. Consequently, there is a discrepancy between the time required for working operations that are
989 applied in full scale applications, as well as in respect to the maximum period of degradation set in the
990 supposed norm. Similarly, the recommended temperature used in the various standards are unrealistic
991 when compared to the ones found in actual environmental conditions. Indeed, advisable range is, in
992 actual fact, between 15 - 28 °C and reaching 58 °C in the industrial composting field. However, the
993 average environmental temperature in the EU is 9 °C in respect to marine environment, 12 °C in
994 freshwater environments and soil environments and can reach about 55 °C - but only for a few days - in
995 industrial composting. As a consequence, materials may degrade in laboratory conditions, according to
996 the requirements detailed in the standard methods, but not in the waste treatment facilities [126].
997 Moreover, the requirements within standards do not cover all the natural environment that the
998 bioplastics are accidentally disposed within. This is the other issue related to the assessment of
999 biodegradability at lab-scale: the laboratory testing cannot completely and accurately enough
1000 reproduce the complexity of the dynamics that take place within those systems. On the other hand, it is
1001 important to state that the main purpose of research studies is to evaluate the biodegradability of the
1002 bioplastics outside the treatment facilities that they should be addressed to. Having said this, there is
1003 the increasing attention of the public in respect to the proper disposal of waste items and, plastics to
1004 contend with, as well as the fact of bioplastics leakage into the environment, which represents a serious
1005 problem. For this reason, one of the main questions that the ongoing research is required to solve is
1006 whether a material labelled as a bioplastics is able to biodegrade under different natural environmental
1007 conditions. In order to perform this, the conditions imposed by the standard methods cannot always be
1008 applied within experimental tests, due to the fact that the natural environment may significantly differ
1009 from the standardised one in respect of waste treatment. Additionally, the indicators used for the
1010 assessment of bioplastics' biodegradability may differ from those reported in the standard methods.

1011 The main indicators used for the evaluation of bioplastics' biodegradation consist in: the definition of
1012 the mass loss, the visual inspection of the tested material, the degree of disintegration, the discoloration,
1013 the changes within the morphology and structure of the biopolymer and the evaluation of the soluble
1014 components released by solubility tests. However, it is important to highlight that the correct evaluation
1015 of the biodegradability of a material should be assessed, even in presence of severe problems related to
1016 the implementation of the needed measurements, by monitoring the evolution of parameters, such as
1017 BOD, CO₂, O₂, CH₄ or biogas, as these components are directly correlated to the presence of microbial
1018 activities. Among the experiments observed in this study, only the biodegradability of bioplastics under

1019 anaerobic conditions was always evaluated by methane and/or biogas production, compared to the
1020 tests carried out in the other environments (i.e., aquatic, soil, and composting).

1021 Under a strictly technical point of view, certain problems remain open. The first that requires
1022 mentioning is the difficulty to reproduce and analyse a biological system that treats biodegradable waste
1023 and bioplastics at the same time. This is a problem due to the inherent heterogeneity and high
1024 biodegradability of the substrate (biowaste) in relation to the low biodegradability of certain bioplastics.
1025 For these reasons, it is nearly impossible to evaluate the degree of the biodegradation of bioplastics
1026 assessing the difference between a system containing them and a blank (i.e., the same system fed only
1027 with biowaste). On the other hand, simulating the bioplastic biodegradation inside mature compost
1028 leads to the creation of a system where the rate of biological activity is completely different from that of
1029 a pile during active composting or from that of an AD plant.

1030 Another key issue is related to bioplastic disintegration. Indeed, for practical reasons, during all the tests
1031 (both the standard methods and most of the research ones) particles with a size of < 2 mm (i.e., those
1032 belonging to the group of microplastics) were considered to be “disintegrated” included in the “mass
1033 loss” and thus considered degraded. As such, they can represent a noticeable fraction in respect of
1034 compost, thus leading to a possible non-compliant one [33,76]; moreover, bioplastics - such as PBAT,
1035 PBS, PCL and PLA - are generally not biodegradable under AD conditions, such that disintegrated
1036 fragments are present in the digestate [2,37]. The idea behind the set threshold of 2 mm is that the
1037 sieving operation is performed manually; further the identification of the bioplastics fragments is
1038 carried out visually. Therefore, for particles that are too small (i.e., < 2 mm) it is nearly impossible to
1039 detect and collect them; this leads to profoundly serious practical problems. At the moment, the
1040 behaviour - in terms of both fate and the effects - of micro-bioplastics that are released in natural
1041 environments is essentially unknown and thus it is not completely safe to release them within compost
1042 or digestate at this time. For these reasons, many plant managers must adopt specific strategies in order
1043 to reduce the problem related to biopolymer fragments. At full scale, the solution that is mainly applied
1044 consists in the removal of the bioplastic bags before the treatment. This is, while research activities pose
1045 the attention on three alternatives [2,76,127], which are: (i) the assessment of physico-chemical pre-
1046 treatments on bioplastics in order to facilitate the polymer degradation during the subsequent
1047 processes; (ii) the implementation of post-treatment methods in order to allow complete bioplastics
1048 decomposition and/or the removal of residual fragments from compost and (iii) the assessment of
1049 innovative blends of bioplastics that should be able to biodegrade in the working time of conventional
1050 biological treatment plants. Indeed, thermophilic conditions have been suggested for the purposes of
1051 degradation of bioplastics requiring long HRT.

1052 Finally, bioplastics' degradation should be characterised by the release of non-toxic compounds.
1053 However, the effects of the released compounds in regard to the environment have not been fully

1054 investigated. In addition to biodegradation tests, physico-chemical modification of the environment, for
1055 instance by phytotoxicity tests, should be carried out in order to evaluate the possible negative or
1056 positive impact of the bioplastics' biodegradation process in respect to the environment. In fact, natural
1057 ecosystems - such as soils and marine environments - demonstrate a complex range of physical and
1058 chemical conditions as well a variety of bioplastics (especially when fragmented). Therefore, such
1059 facts are notable in respect to inducing a high variability and complexity to the conditions in which to
1060 assess biodegradation, thereby rendering it difficult to develop environmentally sound criteria for
1061 biodegradation in all the affected environmental compartments.

1062 In summary, there is a discrepancy between the results, in terms of the degree of biodegradability. This
1063 discrepancy is obtained by following the standard methods, in full-scale treatment systems and
1064 laboratory tests. These differences can be attributed to the unrealistic conditions set in the standard
1065 methods that cannot be replicated in full-scale treatment processes. Therefore, certain labelled
1066 biodegradable bioplastic materials that fulfils the requirements under the standard method testing
1067 conditions may eventually not biodegrade under the expected treatment conditions nor under
1068 uncontrolled natural conditions, when improperly disposed of. In the attempt to assess the bioplastics
1069 biodegradability in natural environments, the standards are set too far apart, as they do not consider
1070 the dynamic mechanisms involved in natural environments. Moreover, a comparison between the
1071 experimental studies is almost impossible. This is due to the fact that there is no particular indication
1072 regarding, for instance, the soil to be used as a "reference soil" when testing the biodegradability of
1073 bioplastics within various soils. The same considerations can also be applied for the other tests. Indeed,
1074 there is a multitude of composts or inocula that can be used as sources of microorganisms as well as
1075 manifold natural water conditions (e.g., river water, seawater, etc.). This is such that every test differs
1076 from one another and the results that are obtained cannot be thus related to any "standard" condition.
1077 Under this perspective, the use of the standard methods loses its original meaning, especially
1078 considering the fact that the major issue in the management of bioplastics is the prevention of
1079 microplastics leakage into the environment or in other words, the complete biodegradation of
1080 bioplastics that are improperly disposed of. The further revision and the harmonisation of the standards
1081 are required; in addition, more stringent conditions should be adopted in order to label a product as a
1082 biodegradable bioplastics. For instance, complete biodegradation should occur at less than favourable
1083 environmental conditions than that of the common waste treatment plants. This could facilitate the
1084 biodegradation of items that are discharged outside the proper treatment systems. Moreover, the
1085 standards should better represent the dynamic processes that occur in both industrial and natural
1086 environments; that is to say the parameters, such as temperature or pH, may vary continuously over
1087 time as well as the microbial community that are susceptible to change within changing environmental
1088 conditions. In addition to a revision of the standards, the other strategy to render bioplastics as easier
1089 to biodegrade could be the implementation of new bioproducts by means of the modulation of the

1090 chemical structure of the biopolymer. Indeed, it is known that chemical composition can strongly affect
1091 the degradation kinetic of the biopolymer. However, a countereffect could be a reduction in the
1092 characteristics that render bioplastics as easily marketable (e.g., their mechanical properties). The
1093 exploitation of new easily biodegradable bioplastics blends could also improve the bio-recycling of
1094 bioplastics. In this sense, financial incentives can help in achieving a large-scale bioplastics market with
1095 a sustainable impact [28].

1096 Finally, the harmful effects of microplastics as well as the influence of biodegradation products on the
1097 environment need to be further investigated. Ecotoxicity tests should be part of every biodegradation
1098 experiment and the effects of the biodegradable plastics on human health requires further investigation
1099 also.

1100

1101 **6 Conclusions**

1102 The assessment of bioplastics' biodegradability is extremely influenced by the conditions of the
1103 standard experiments. Standard tests are often inadequate due to the fact that the experimental
1104 environmental conditions (such as temperature, mixing and test duration) may not reflect the real
1105 conditions in waste treatment plants, thus not resulting in a correct estimation of bioplastics
1106 fragmentation and biodegradation.

1107 In respect of this issue, it appears that biodegradation standards were addressed more in order to
1108 demonstrate that bioplastics are the panacea for solving the problems related to plastic pollution rather
1109 than providing an environmentally sound tool for the purposes of evaluating the properties of a given
1110 material. In fact, the available literature often demonstrates that biodegradation in real environmental
1111 or plant conditions is lower than expected and sometimes negligible.

1112 Laboratory methods possess the advantage of being able to set and keep control of the experimental
1113 conditions (temperature, humidity, pH, oxygen supply, and test duration) [39]. On the other hand, lab-
1114 scale experiments aim at simulating specific process conditions (i.e., in natural environments or in waste
1115 treatment plants) but cannot exactly reproduce the conditions present in the multitude of natural and
1116 industrial environments.

1117 In respect to small scale laboratory tests, more reliable data can be obtained by the application of full-
1118 or field-scale tests in which the kinetics and mechanisms of bioplastics' degradation occur in real
1119 conditions. However, as expected, the results obtained can be subjected to different interpretations due
1120 to the continuous changes in the environmental conditions and due to microbiological composition [39].
1121 For this reason, research activities are rarely conducted at full-scale and the procedures applied for the
1122 assessment of the biodegradability sensibly differ from the standardised protocols, as well as also in

1123 how they differ from one study to another. This specifically happens in regard to anaerobic
1124 biodegradation, as standardisation is not fully developed and is still in an early stage [16].

1125 The main outcome of this study is that the comparisons between experimental (at either lab- or full-
1126 scale) and standard tests are generally not possible. This is due to several factors, specifically, the
1127 differences in the microbial sources, the varieties of the environments tested, the heterogeneity of the
1128 biopolymers, the difficulty in reproducing at lab-scale the complexity of natural spontaneous processes,
1129 and the different indexes used for the assessment of biodegradability. An improvement on the current
1130 standards tests and analytical methods (especially in terms of methods for assessing biodegradation
1131 and the presence of fragments) is necessary and should include the field-testing of the biodegradable
1132 polymer as well as of the finished product in order to ensure all criteria are met in real-life conditions.
1133 Environmental conditions set in the future standard methods should be far from that indicated as
1134 “optimal” for biodegradation, as bioplastics eventually end up in environments where conditions can
1135 vary significantly vary from that which is reported in the standards. Although research testing methods
1136 can differ from standard protocols since they aim at testing bioplastic biodegradability in very diverse
1137 environmental conditions, future research activities should be oriented at an harmonization of the
1138 applied procedures in order to increase the comparability of the results obtained in different studies.

1139 Moreover, a future challenge in the bioplastics market could be the production of new blends of
1140 biopolymer that are more easily biodegradable without losing the characteristics (such as mechanical
1141 strength or flexibility) that make the bioplastics attractive in the first place.

1142

1143 **References**

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