KBBPPS

Knowledge Based Bio-based Products' Pre-Standardization

Work package 6

Biodegradability

Deliverable N° 6.1:

Report on current relevant biodegradation and ecotoxicity standards

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5 Anaerobic environment

The review of the anaerobic environment has been executed by OWS.

5.1 Biodegradation

Anaerobic biodegradation tests in an aquatic environment are especially suitable for substances, which adsorb onto activated sludge, and which enter in this way in anaerobic digesters in wastewater treatment plants. No methods were developed especially towards lubricants and solvents.

Besides anaerobic biodegradation tests in the aquatic environment, also methods were developed in order to determine the anaerobic biodegradation of materials (especially biopolymers) under high-solids anaerobic-digester conditions or under landfill conditions.

5.1.1 OECD guidelines

There exists one OECD guideline with regard to the evaluation of the biodegradation of organic compounds in digested sludge: OECD 311 “Anaerobic Biodegradability of Organic Compounds in Digested Sludge: By Measurement of Gas Production” (Adopted 23 March 2006). This guideline is based on ISO 11734 (1995) “Water quality – Evaluation of the “ultimate” anaerobic biodegradability of organic compounds in digested sludge – Method by measurement of the biogas production”. This international standard is described in chapter 5.1.3. An overview of the main parameters of OECD 311 is given in Table 57.

Table 57. Overview of the main parameters as described in OECD 311.

Parameter	OECD 311
Inoculum	Washed digested sludge (preferably anaerobically digested for 5 days) from a sewage treatment plant treating predominantly domestic sewage is added to a mineral medium in order to obtain a total solids concentration of 1 g/l to 3 g/l in the vessels (IC test solution < 10 mg/l). Medium contains also resazurin.
Temperature	35°C ± 2 °C
Reference material	Sodium benzoate, phenol or polyethyleneglycol 400 (Figure 21)
Measurement technique	Measurement of gas production (CH_4 & CO_2) with a pressure meter connected to a syringe needle during the test and measurement of inorganic carbon (= evolved CO_2 which is transformed to hydrogen carbonate or carbonate) at the end of the test.
Bottle volume	0.1 l – 1 l
Amount of test item	20-100 mg organic C/l
Replicates	Triplicate for specimen bottles, blanks, reference compound and inhibition control
Duration	60 days (can be extended)
Validity	Anaerobic biodegradation reference > 60 % of the theoretical maximum. Bottles containing oxidized (pink) resazurin should be discarded. Gas production in inhibition vessels shall be at least equal to that in the vessel containing only reference substrate.

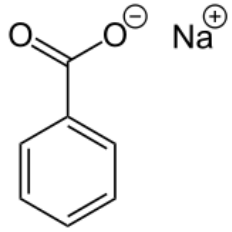
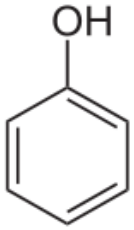
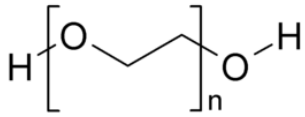
Sodium benzoate	Phenol	Polyethyleneglycol 400
		

Figure 21. Structural formula of the reference compounds as described by OECD 311.

5.1.2 European standards

The European committee for standardisation refers to the standard developed on international level (see chapter 5.1.3). An overview is given in Table 58.

Table 58. Overview of the European test method for the determination of anaerobic biodegradation of materials.

Standard	Description
EN ISO 11734 (1998)	Water quality – Evaluation of the "ultimate" anaerobic biodegradability of organic compounds in digested sludge – Method by measurement of the biogas production (ISO 11734:1995)

5.1.3 International standards

ISO has developed 2 standards with regard to the anaerobic biodegradation in digested sludge (Table 59). ISO 11734 is developed in order to evaluate the anaerobic biodegradation of organic compounds, while ISO 14853 is developed for more complex products (plastic materials). An overview of the main parameters of these two methods is given in Table 60.

ISO has also developed a standard referring to anaerobic conditions under high-solids anaerobic-digestion conditions (ISO 15985). This method is an optimized simulation of an intensive anaerobic digestion process in which biodegradation and disintegration of a plastic material can be determined. An overview of the main parameters of this method is given in Table 61.

Table 59. Overview of the different international test methods for the determination of anaerobic biodegradation of materials.

Standard	Description
ISO 11734 (1995)	Water quality – Evaluation of the "ultimate" anaerobic biodegradability of organic compounds in digested sludge – Method by measurement of the biogas production
ISO 14853 (2005)	Plastics – Determination of the ultimate anaerobic biodegradation of plastic materials in an aqueous system – Method by measurement of biogas production
ISO 15985 (2004)	Plastics – Determination of the ultimate anaerobic biodegradation and disintegration under high-solids anaerobic-digestion conditions – Method by analysis of released biogas

Table 60. Overview of the main parameters as described in ISO 11734 (1995) and ISO 14853 (2005).

Parameter	ISO 11734 (1995)	ISO 14853 (2005)
Inoculum	Washed sludge (preferably anaerobically digested for 5 days) from a sewage treatment plant treating predominantly domestic sewage is added to a mineral medium in order to obtain a total solids concentration of 1 g/l to 3 g/l in the vessels (IC test solution < 10 mg/l). Medium contains also resazurin.	Washed sludge (preferably anaerobically digested for 5 days) from a sewage treatment plant treating predominantly domestic sewage is added to a mineral medium in order to obtain a total solids concentration of 1 g/l to 3 g/l in the vessels (IC test solution < 20 mg/l). Medium contains also resazurin.
Temperature	35°C ± 2 °C	35°C ± 2 °C
Reference material	Sodium benzoate, phenol or polyethyleneglycol 400	Poly-β-hydroxybutyrate, cellulose or polyethyleneglycol 400 (Figure 22)
Measurement technique	Measurement of gas production (CH_4 & CO_2) with a pressure meter connected to a syringe needle during the test and measurement of inorganic carbon (= evolved CO_2 which is transformed to hydrogen carbonate or carbonate) at the end of the test.	Manometric or volumetric measurement of gas production (CH_4 & CO_2) during the test and measurement of inorganic carbon (= evolved CO_2 which is transformed to hydrogen carbonate or carbonate) at the end of the test.
Bottle volume	0.1 l – 1 l	0.1 l – 1 l
Amount of test item	20 – 100 mg organic C/l	20 – 200 mg organic C/l
Replicates	Triplicate for specimen bottles & blanks and singular for reference compound and inhibition control	Triplicate for specimen bottles & blanks and singular for reference compound and inhibition control
Duration	60 days	Normal duration: 60 days Maximum duration: 90 days
Validity	Anaerobic biodegradation reference > 60 % of the theoretical maximum. Bottles containing oxidized (pink) resazurin should be discarded. Gas production in inhibition vessels shall be at least equal to that in the vessel containing only reference substrate.	Anaerobic biodegradation reference > 70 % of the theoretical maximum. Bottles containing oxidized (pink) resazurin should be discarded. Gas production in inhibition vessels shall be at least equal to that in the vessel containing only reference substrate.

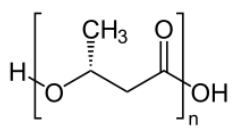
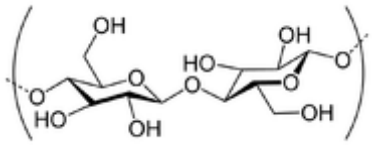
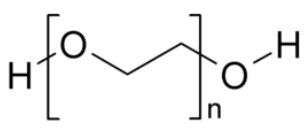
Poly-β-hydroxybutyrate	Cellulose	Polyethyleneglycol 400
		

Figure 22. Structural formula of the reference compounds as described in ISO 14853 (2005).

Table 61. Overview of the main parameters as described in ISO 15985 (2004).

Parameter	ISO 15985 (2004)
Inoculum	Methanogenic inoculum (> 20 % TS) from anaerobic digesters (preferable: digester under dry conditions, although it is also acceptable to derive it from wet fermentation on condition that the sludge is dewatered) operating only on pre-treated household waste. Digester shall have a maximum retention time of 30 days under thermophilic conditions and a minimum gas-production yield of at least 15 ml/g dry solids/day for at least 30 days. Inoculum must undergo a post fermentation (7 days). Inoculum is added as such (no dilution). Requirements: pH: 7.5 – 8.5, VFA < 1 g/kg, NH_4^+-N: 0.5 – 2 g/kg
Temperature	52°C (± 2°C) (thermophilic conditions)
Conditions	Static non-mixed
Reference material	Cellulose (particle size < 20 µm)
Measurement technique	Measurement of total carbon in gas (CO_2 and CH_4): produced gas volume is measured and optionally CO_2 and CH_4 concentration in the produced gas is measured.
Bottle volume	Minimum 750 ml
Amount of test item	15-20 g volatile solids test item per 1000 g wet weight inoculum (dry solids inoculum > 20 %) For evaluation of disintegration: maximum surface area = 2 cm × 2 cm
Replicates	Triplicate for all series
Duration	15 days or longer until a plateau in biodegradation has been reached
Validity	Cellulose > 70 % biodegradation (after 15 days) Difference between % biodegradation of the reference material in the different vessels is < 20 % of the mean value

5.1.4 American standards

ASTM has developed 2 standards with regard to the anaerobic biodegradation in municipal sewage sludge (Table 62). These standards are representative for the anaerobic part of a wastewater plant. ASTM E 1196 is developed in order to evaluate the anaerobic biodegradation of organic chemicals, while ASTM D 5210 is developed for more complex products (synthetic plastic materials including formulation additives). An overview of the main parameters of these two methods is given in Table 63. The measurement techniques as prescribed by ASTM D 5210 are more progressive when compared to ASTM E 1196.

The other methods (ASTM D 5511 and ASTM D 5526) are referring to anaerobic degradation under dryer conditions. ASTM D 5511 has been developed to permit the determination of the rate and degree of anaerobic biodegradability of plastic products when placed in a high-solids anaerobic digester for the production of digestate from municipal solid waste. Biodegradation of plastic materials is an important phenomenon as if affects the decomposition of other waste materials enclosed by the plastic and it influences the quality and the appearance of the digestate. ASTM D 5526 refers to anaerobic biodegradation of plastic materials in an accelerated-landfill test environment. This method resembles landfills in which the generated gas is recovered and/or actively promoted (by inoculation, moisture control and temperature control). An overview of the main parameters of these methods is given in Table 64.

Table 62. Overview of the different American test methods for the determination of anaerobic biodegradation of materials.

Standard	Description
E 1196 – 92	Standard Test Method for Determining the Anaerobic Biodegradation Potential of Organic Chemicals
D 5210 – 92	Standard Test Method for Determining the Anaerobic Biodegradation of Plastic Materials in the Presence of Municipal Sewage Sludge
D 5511 – 12	Standard Test Method for Determining Anaerobic Biodegradation of Plastic Materials Under High Solids Anaerobic Digestion Conditions
D 5526 – 12	Standard Test Method for Determining Anaerobic Biodegradation of Plastic Materials Under Accelerated Landfill Conditions

Table 63. Overview of the main parameters as described in ASTM E 1196 - 92 and ASTM D 5210 - 92.

Parameter	ASTM E 1196 – 92	ASTM D 5210 - 92
Inoculum	Sieved sludge (fresh sludge is recommended) from an anaerobic sludge digestor (total organic solids: 1 to 2 %) is added in a 10 % concentration to a mineral medium, which contains also resazurin (= oxidation / reduction indicator).	Sieved sludge (anaerobically digested for 7-14 days in order to reduce the background activity) from an anaerobic sludge digestor (total organic solids: 1 to 2 %) is added in a 10 % concentration to a mineral medium, which contains also resazurin (= oxidation / reduction indicator).
Temperature	35°C ± 2 °C	
Reference material	Ethanol (Figure 23)	Cellulose or starch (Figure 23)
Measurement technique	Measurement of gas production (CH_4 & CO_2) with a pressure transducer or syringe	Measurement of gas production (CH_4 & CO_2) with a pressure transducer or syringe, SOC and residual polymer weight at the end of the test
Bottle volume	160 ml	
Amount of test item	50 mg organic C/l	Not specified
Replicates	Triplicate (specimen bottles, blanks and controls)	
Duration	56 days	Until gas evolution of test compound has stopped (= two weeks without significant gas production in excess of that in the blank).
Validity	Anaerobic biodegradation reference > 50 % of the theoretical maximum. Bottles containing oxidized (pink) resazurin should be discarded.	Anaerobic biodegradation reference > 70 % (on basis of CO_2 and CH_4). Bottles containing oxidized (pink) resazurin should be discarded.

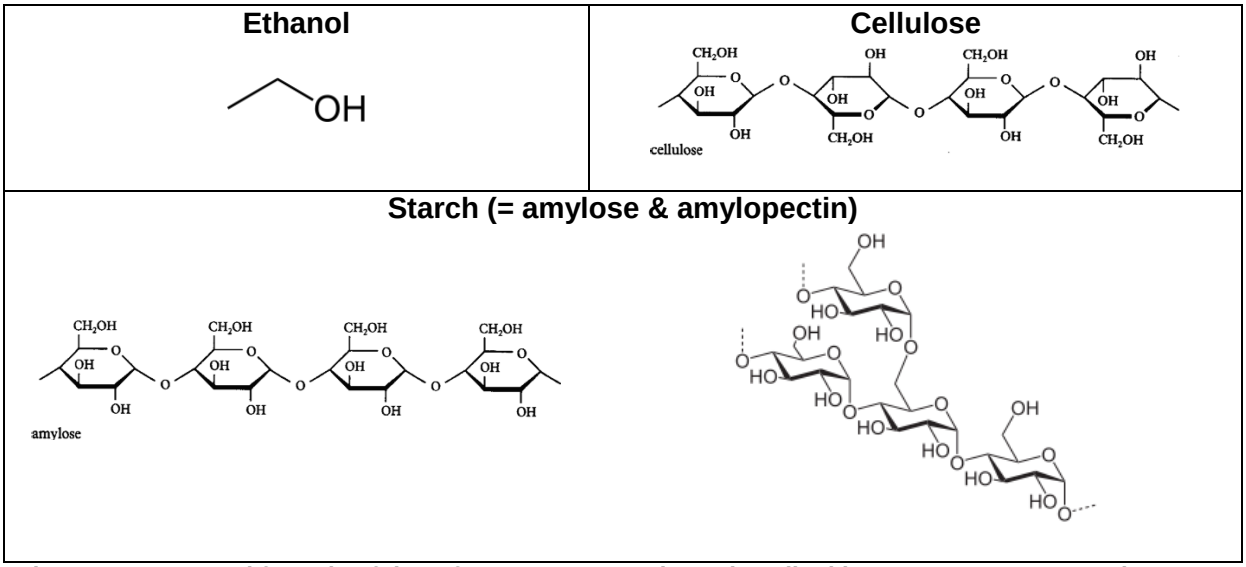


Figure 23. Structural formula of the reference compounds as described in ASTM E 1196 – 02 and ASTM D 5210 - 92.

Table 64. Overview of the main parameters as described in ASTM D 5511 - 12 and ASTM D 5526 - 12.

Parameter	ASTM D 5511 -12	ASTM D 5526 - 12
Inoculum	Methanogenic inoculum (> 20 % TS) from anaerobic digesters (preferable: digester under dry conditions, although it is also acceptable to derive it from wet fermentation on condition that the sludge is dewatered) operating only on pre-treated household waste. Digester shall have a maximum retention time of 30 days under thermophilic conditions and a minimum gas-production yield of at least 15 ml/g dry solids/day for at least 30 days. Inoculum must undergo a post fermentation (7 days). Inoculum is added as such (no dilution). Requirements: pH: 7.5 – 8.5, VFA < 1 g/kg, NH_4^+-N: 0.5 – 2 g/kg	Methanogenic inoculum (> 30 % TS) from anaerobic digesters operating only on pre-treated household waste. Digester shall have a max. retention time of 30 days under mesophilic conditions and a minimum gas-production yield of at least 15 ml/g dry solids/day for at least 7 days or batch digester with a gas production > 1 l/kg waste/day and a CH_4 concentration > 60 %. Inoculum must undergo a post fermentation (7 days). Inoculum is mixed with pretreated-household waste (aerobically stabilised over 2-4 weeks by aeration and maintaining a dry-matter content of 50 ± 5% and a temperature of 55 ± 10°C). Requirements: pH: 7.5 – 8.5, VFA < 1 g/kg, NH_4^+-N: 0.5 – 2 g/kg
Temperature	37°C (± 2°C) (mesophilic conditions) 52°C (± 2°C) (thermophilic conditions)	35°C (± 2°C)
Conditions	Static non-mixed	
Reference material	Cellulose Polyethylene (optional as a negative control)	
Measurement technique	Measurement of total carbon in gas (CO_2 and CH_4): produced gas volume is measured and CO_2 and CH_4 concentration in the produced gas is measured.	Measurement of total carbon in gas (CO_2 and CH_4): produced gas is measured (based on pressure) and CO_2 and CH_4 concentration in the produced gas is measured.
Bottle volume	2 l wide mouth Erlenmeyer flask	Glass vessel (4 l – 6 l)
Amount of test item	15-100 g volatile solids test item per 1000 g wet weight inoculum (dry solids inoculum > 20 %)	60-100 g dry matter test item per 600 g dry matter pretreated household waste and 100 g dry weight mesophilic anaerobic inoculum or 150 g dry weight anaerobic inoculum from batch digester at 3 dry matter contents (35 % - 45 % and 60 %)
Replicates	Triplicate	
Duration	15-30 days	Until no significant gas production in excess of the blank during 1 week
Validity	Cellulose > 70 % biodegradation (after 30 days) Deviation among cellulose replicates < 20 % of the mean	Cellulose > 70 % biodegradation

5.2 Toxicity

When substances are accidentally spilled in the environment, they can also reach anaerobic environments. In a wastewater treatment system anaerobic sludge is present in the secondary settlement tank and in the natural environment chemicals can reach anaerobic sediments in bays, estuaries and the sea. Due to their physical properties (low solubility in water, high adsorption on suspended solids, etc.) some chemicals will preferably reach such anaerobic zones.

It is desirable that chemicals, which might enter in the environment, are biodegradable under both aerobic and anaerobic environments and it is essential that such chemicals do not inhibit the activity of the microorganisms in either zone. OECD and ISO have developed guidelines in order to measure the inhibition on the gas production of anaerobic bacteria.

5.2.1 OECD guidelines

Currently OECD has developed a guideline for testing of chemicals towards anaerobic bacteria: OECD 224 “Determination of the inhibition of the activity of anaerobic bacteria – reduction of gas production from anaerobically digesting (sewage) sludge” (Adopted: 8 January 2007). This guideline provides useful information for predicting the effect of a substance on gas production in anaerobic digesters. However, it must be noticed that only longer tests can indicate whether adaptation of the microorganisms to the test substance can occur or whether substances, which are adsorbed onto the sludge, can build up to a toxic concentration.

In this test a mixture of anaerobically digesting sludge (20 g/l to 40 g/l total solids) and a degradable substrate solution (= nutrient broth, yeast extract and D-glucose) is incubated alone and simultaneously with a range of concentrations of the test substance (500 mg/l, 250 mg/l, 125 mg/l, 62.5 mg/l, 31.2 mg/l and 15.6 mg/l) in sealed vessels up to 3 days at 35°C ± 2°C. The gas production is measured by monitoring the pressure increase in the bottles. The percentage gas inhibition is calculated from the amounts of produced gas in the control bottles and the test bottles (EC_{50}).

This guideline also allows the use of inocula from other anaerobic sites (muds, saturated soils and sediments). A few modifications are required when using these inocula (e.g. incubation temperature can be adapted at the temperature of the sample site in order to minimise the disturbance of the methane-producing consortia of bacteria, etc.).

5.2.2 International standards

Two ISO standards were developed towards the effect of substances on anaerobic bacteria: ISO 13641-1 (2003) “Water quality – Determination of inhibition of gas production of anaerobic bacteria – Part 1: General test” and ISO 13641-2 (2003) “Water quality – Determination of inhibition of gas production of anaerobic bacteria – Part 2: Test for low biomass concentrations”. The first method uses undiluted sludge and the second method uses one hundredth diluted sludge to represent muds and sediments having low bacterial populations. The principles of these tests are comparable OECD 224.

5.3 Standard specifications

No standard specifications were found for products, which may occur in an anaerobic environment due to accidental spills. Also no standard specifications are developed towards biodegradable biopolymers, which might be disposed in an anaerobic digester.

5.4 Labelling

No labelling systems were found for products, which may occur in an anaerobic environment due to accidental spills. Also no labelling systems are developed towards biodegradable biopolymers, which might be disposed in an anaerobic digester.

5.5 Discussion and critical review

Based on the review on the existing ***biodegradation standards*** in an anaerobic environment, it can be concluded that there exists a sufficiently broad range of standards in order to determine the anaerobic biodegradation in aquatic environments, high-solids anaerobic-digestion environments and landfill environments.

Anaerobic aquatic environments (in wastewater treatment plants) can indeed be suitable in order to evaluate anaerobic biodegradability of lubricants and solvents, but high-solids anaerobic-digestion environments and anaerobic landfill environments are normally not considered as environments in which lubricants and/or solvents are disposed. These standards are more suitable for biopolymers.

The methods developed in order to determine anaerobic biodegradability of organic compounds in an aquatic anaerobic environment (OECD 311, ISO 11734 and ASTM E 1196) can be used in order to evaluate the anaerobic biodegradability for lubricants and solvents.

The American standard (ASTM D 1196) is mainly based on respirometric techniques. However, as part of the produced CO_2 dissolves in the test medium, the anaerobic biodegradation will be underestimated if only the gas production is taken into account. In order to calculate the anaerobic biodegradation more correctly, the recent guidelines (OECD 311 and ISO 11734) are all based on biogas production and inorganic carbon determination. These test methods are more appropriate in order to determine the biodegradation percentage.

Not all standards give guidance with regard to the interpretation of the results of the anaerobic biodegradation tests in aquatic environments. According to ASTM E 1196 a “high” biodegradability result in this test method is a good evidence that the test substance will be biodegradable in wastewater treatment plants, anaerobic digesters and in many natural anaerobic environments (e.g. sediments, swamps,…). No further specifications are given with regard to the interpretation of a “high” biodegradability result. OECD 311 has filled this gap by stating that complete anaerobic biodegradation can be assumed to occur if 75 % to 80 % of theoretical gas production is achieved.

One useful method was developed in order to determine ***toxicity*** towards anaerobic bacteria (OECD 224 or ISO 13641). This standard can be used for lubricants and solvents. The guidance documents towards the sample preparation and the interpretation of the results of toxicity tests for difficult substances (OECD), poorly water soluble substances (ISO 14442) and lubricants (ASTM D 6081) should be taken into account.

For biopolymers, which are degradable in anaerobic digestors, it might be necessary to evaluate if toxic residuals remain present in the produced digestate. This could be evaluated after an aerobic stabilisation period of the digestate (= composting phase). The produced compost can be checked on toxicity using plants or earthworms. Further research is needed in order to determine how this should be done.

Currently no ***standard specifications nor labelling systems*** are developed for products which are biodegradable in an anaerobic digester (e.g. biopolymers). This is mainly caused

by the fact that there exists a wide variation in the construction and the operation of anaerobic digestion systems. The construction and operation systems can be divided into categories based on two parameters: (1) temperature (mesophilic and thermophilic) and (2) dry solids content (wet systems and dry systems). This makes it difficult to develop one set of criteria. A standard specification should be developed, which includes criteria per operation system. Such specifications should encompass criteria with regard to anaerobic biodegradation, but also environmental safety of the produced digestate should be checked. Further investigation is required in order to develop such standard specification. This standard specification should form the basis for a new labelling system.

The labelling systems for lubricants (Chapter 3.4) do not refer to anaerobic environments in order to evaluate biodegradability and environmental safety. However, taken into account that a high percentage of ultimately aerobically biodegradable components need to be present in the major part of the labelled products, it is expected that the labelled products will already be degraded before they come in contact with anaerobic environments.

6 Soil environment

The review of the soil environment has been executed by the Agricultural University of Athens.

Biodegradable in soil plastics are defined those degradable plastics in which the degradation results from the action of naturally (i.e. in real soil conditions) occurring micro-organisms such as bacteria, fungi and algae. Most of the available international standards for biodegradable materials are designed for testing biodegradation under various conditions in a variety of media (including composting conditions), but not for testing specifically biodegradation under real soil conditions, especially in agricultural soil which is used for the production of food. This topic remains a highly controversial issue.

Organic chemicals may be introduced into the soil both intentionally and accidentally, after which they may, or may not, degrade biologically. For chemicals which do degrade, the rate of degradation can vary considerably, depending not only on the molecular structure of the chemical, but also on soil conditions such as temperature, water and oxygen availability which influence microbial activity. The activity of microorganisms often plays a major role in degradation processes. It is necessary to have laboratory tests available to estimate the rate and extent of biodegradation and thereby the persistence of organic chemicals in soil. Numerous laboratory methods are available for the estimation of aerobic biodegradation based on different specific circumstances, for example, soil type, temperature and exposure times.

6.1 Biodegradation

In this section the currently available norms and standards or, non-standardised testing methods, on testing plastics for biodegradation in soil are analysed with the aim to identify and clarify the constraints, gaps and the limitations of existing relevant testing methods, and propose needed modifications especially with regard to simulating soil conditions. This updates the relevant information presented earlier by *Briassoulis and Dejean, 2010.*

Table 65 presents an overview of the most important standard test methods for assessing biodegradation of plastics and chemicals in soil up to the year 2012.

Table 65 Overview of Standard testing methods for determining biodegradability of materials in soil.

American Society for Testing and Materials International (ASTM)		
Current versions of standards	**Previous versions of standards**	**Title**
ASTM D 5988-12	***ASTM D 5988-96/ 2003**	Standard test method for determining aerobic biodegradation of plastic materials in soil *(Previous title: Standard test method for determining aerobic biodegradation in soil of plastic materials or residual plastic materials after composting)*
International Organization for Standardization (ISO)		
Current versions of standards	**Previous versions of standards**	**Title**
ISO 17556-2012	***ISO 17556-2003**	Plastics--determination of the ultimate aerobic biodegradability in soil by measuring the oxygen demand in a respirometer or the amount of carbon dioxide evolved
ISO 11266-1994		Soil quality-Guidance on laboratory testing for biodegradation of organic chemicals in soil under aerobic conditions
French Normalisation Organisation (AFNOR)		
NF U52-001 February 2005		Biodegradable materials for use in agriculture and horticulture-mulching products-Requirements and test methods
OECD Guidelines		
304A		Inherent Biodegradability in Soil
307		Aerobic and Anaerobic Transformation in Soil

*Standard superseded by the 2012 version

6.1.1 ASTM Standards for testing biodegradability of plastics in soil

There is only one ASTM standard testing method (ASTM D 5988-12) for testing biodegradability of plastic materials in soil. No relevant ASTM standard exists for organic chemicals.

ASTM D 5988 (2003) *"Standard test method for determining aerobic biodegradation in soil of plastic materials or residual plastic materials after composting"* refered to the determination under laboratory conditions of the degree and rate of aerobic biodegradation of plastic materials, including formulation additives that may be biodegradable, in contact with soil, or a mixture of soil and mature compost. This test method was designed to measure the biodegradability of plastic materials relative to a reference material (e.g. cellulose or starch) in an aerobic environment. In the revised version of this specification - ASTM D 5988-12 - the title has been altered into *"Standard test method for determining aerobic biodegradation of plastic materials in soil"* and the following changes have been made with regard to the provisions of the previous version:

- In the used apparatus, the case of using vessels for testing a compost containing residual plastic material, has been erased and in the darkened chamber or cabinet, a range of allowed temperature is described between (20-28)^{o}C±2^{o}C instead of the 21±2^{o}C of the previous method.

- The soil used in the new method should be natural and fertile collected from the surface layers of fields and forests. A laboratory mixture is made of equal parts (by weight) of soil samples obtained from at least 3 diverse locations (for example, an agricultural field, a forest, and a pasture or meadow). It is advisable to avoid soil that has been exposed to pollutants that cause significant perturbations of the microbial population. The soils are preferably used fresh from the field to assure active microbiota. Air-dried or frozen soils must be reactivated before use in this test. It is preferable to use fertile soil classified as "sandy loam" in accordance with USDA classification, or "silty sand" in accordance with the German DIN classification.

- It is also acceptable in the revised version of the test that the test matrix is a mixture of natural soil and mature compost such as obtained at the end of Test Method D 5338 (ratio 1 g compost to 25 g soil).

- Validation criteria in the revised version have been expanded and are analytically described in the corresponding paragraph that follows.

Technical characteristics

- Measuring the evolved carbon dioxide as a function of time of exposure.
- Technical specifications: room temperature (e.g. 20-28˚C±2˚C); soil medium conditions: pH 6-8, moisture content to 80 to 100% of the moisture-holding capacity (MHC) of the soil (if the MHC is determined in accordance with Test Method D425; if in accordance with Test Method D2980 then 50 to 70% MHC is appropriate), C:N ratio (assuming it refers to the test-sample) is adjusted to a value between 10:1 and

20:1 by weight (e.g. with ammonium phosphate solution). The soil is sieved so that soil particle size is less than 2 mm.

Validation criteria:

- A control substance known to biodegrade (starch or cellulose) has also to be tested, in order to check the activity of the soil. If after six months less than 70% theoretical CO2 evolution is observed for the control substance, the test has to be regarded as invalid and should be repeated using fresh soil.
- The amounts of carbon dioxide evolved from the blanks (or the BOD values for the alternative measurement of oxygen consumption) shall be within 20% of the mean at the plateau phase or at the end of the test. If not, the test must be regarded as invalid and must be repeated using fresh soil.

Applicability:

- All plastic materials that are not inhibitory to the bacteria and fungi present in soil.

Equivalence:

- This test method is equivalent to ISO 17556.

6.1.2 ISO Standards for testing biodegradability of plastics and chemicals in soil

- There is one ISO Standard testing method (ISO 17556:2012) for testing biodegradability of plastic materials in soil and another one (ISO 11266:1994) for testing biodegradability of organic chemicals in soil.

ISO 17556:2012

ISO 17556:2003 with the title *"Plastics -- Determination of the ultimate aerobic biodegradability in soil by measuring the oxygen demand in a respirometer or the amount of carbon dioxide evolved"* (see for details Briassoulis and Dejean, 2010) was used to determine the optimum degree of biodegradation of plastics by adjusting the humidity of the test soil. The new version of the standard is the ISO 17556:2012. This second edition cancels and replaces the first edition (ISO 17556:2003), which has been technically revised. Main technical changes include:

- A definition of the term "total organic carbon" has been added.
- The temperature of the test environment has been changed: constant to within ± 2°C in the range between 20°C and 28°C, preferably 25°C (instead of; constant temperature to within 1°C, preferably between 20°C and 25°C).
- The specifications for the analytical instrument for determining the amount of carbon dioxide evolved have been revised: according to the standard, this equipment consists of any suitable apparatus with sufficient accuracy, e.g. a carbon dioxide IR analyser or DIC analyser or apparatus for titrimetric determination after complete absorption in a basic solution or apparatus for the gravimetric determination of carbon

dioxide in accordance with ISO 14855-2 (the previous version of the standard presented only the use of carbon dioxide or DIC analyser or apparatus for Titrimetric determination after complete absorption in a basic solution).

- The description of the preparation of the test material has been revised. More specifically, this text has been rephrased and additionally it has been recommended that the test samples may be reduced in size by means of cryogenic milling.
- The description of the collection and sieving of soil has been revised. Some points of the text have been rephrased and the appropriate size of the particles of the sieved soil has been changed into 5 mm giving a preference of a size less than 2 mm (in the previous version, it was recommended particles of the soil to have size less than 2mm).
- The use of a standard soil is permitted as an alternative to natural soil. More analytically, the method describes a standard soil that constitutes of industrial quartz sand, clay, natural soil and mature compost. According to the standard, the use of this soil is very useful in determining the biodegradability of plastic materials in bulky soils (loamy or clayey soils), reducing handling and aeration problems. Also, specific salts are added to the soil preferably when adjusting the water content.
- The description of the start-up and execution of the test has been revised.
- A new annex giving examples of long-term tests has been added.
- A new annex giving the results of round-robin testing has been added.

Technical characteristics

- Measuring the oxygen demand in a closed respirometer or the amount of carbon dioxide evolved.
- The test period should typically not exceed six months. If significant biodegradation is still observed and the plateau phase has not been reached after this length of time the test may be extended up to 24 months.
- The amount of test material shall be sufficient to outweigh any variations in the background oxygen consumption or any carbon dioxide evolved from the test soil: 100-300 mg of test material to 100-300 g of soil is usually adequate. The maximum amount of test material is limited by the oxygen supply to the test system. The use of 200 mg of test material with 200 g of soil is recommended unless the soil contains an excessively large amount of organic matter.
- room temperature: constant to within ± 2°C in the range between 20°C and 28°C, preferably 25°C.
- ratio C / N: 40 / 1 for organic C of test or reference material to nitrogen in the soil.
- optimum water content of soil between 40 – 60 % of total water-holding capacity .
- pH: 6 - 8.

Validation criteria:

- The degree of biodegradation of the reference material (microcrystalline-cellulose powder, ashless cellulose filters or poly(-hydroxybutyrate)) is more than 60 % at the plateau phase or at the end of the test.
- The BOD values of, or amounts of carbon dioxide evolved from the three blanks are within 20 % of the mean at the plateau phase or at the end of the test.

If these criteria are not fulfilled, the test must be repeated using another pre-conditioned or pre-exposed soil.

Applicability:

- Natural and/or synthetic polymers, copolymers or mixtures of these
- Plastic materials which contain additives such as plasticizers or colorants
- Water-soluble polymers
- It does not necessarily apply to materials which, under the test conditions, inhibit the activity of the microorganisms present in the soil.

Equivalence:

- This test method is equivalent to ASTM D 5988.

Interpretation of the results:

- Information on the toxicity of the test material may be useful in the interpretation of test results showing a low biodegradability.

ISO 11266-94

Correspondingly, ISO 11266-1994 provides guidance on the selection and conduct of appropriate test method for the determination of biodegradation of organic chemicals in aerobic soils. Usually, during the laboratory testing, a radiolabelled compound is used allowing the determination of the rate of disappearance of the test compound and the formation of metabolites, carbon dioxide, other volatiles and non-extractable residue. The metabolites should be identified using appropriated analytical methods. The disappearance of the test compound can also be followed by specific analysis.

Technical characteristics

- If practicable, soil selected should come directly from the site where chemical contact is anticipated. However, if it is not possible to obtain samples owing to contamination which has already been introduced, the soil selected should have comparable properties. Also, the field history of the soil should be considered and recent amendments, such as tillage practices and pesticide applications should be noted.
- Substances to be tested should be pure compounds (chemical purity > 98 %). The influence of any carriers or formulation ingredients should also be considered.

- The test chemical may be added in water (depending on the solubility in water), in organic solvents, or directly as a solid (e.g. mixed in quartz sand).
- The incubation is usually carried out in the dark. However, if the contribution of algae to biodegradation needs to be considered appropriate lighting conditions should be selected.
- Incubation temperature: 25-35^{o}C is the range of maximum microbial activity in soil. For soils from temperate zones temperature between 10-25^{o}C is adequate and more representative of natural conditions.
- The water content of the soil should be appropriate for the specific goals of the study. It is usually expressed as pore-water pressure. Generally, microbial activity in soil is optimal at between -0.01MPa and -0.031MPa (measurement based on ISO 11274). Alternatively, waterholding capacity (WHC) may be used although it does not give comparable measurements between different soil samples. Maximum microbial activity is found between 40-60 % of the WHC.
- Test duration: there is no recommended minimum length of a test but, as microbial activity in soil decreases during long incubation periods, it is recommended that tests should not be continued for longer than 120 days.
- If no biodegradation is observed, the likely reasons may be:
    - the test substance is toxic
    - the test substance does not biodegrade
    - the microbial activity of the soil is zero

6.1.3 Testing method based on French norm for testing biodegradability of agricultural films in soil

The French Norm NF U52-001 (2005) determines the biodegradability of agricultural films in soil (Briassoulis and Dejean, 2010). It includes the description of the testing method, specifications and labelling of the tested film that meets the requirements set. In this section the NF U52-001 (2005) testing method for biodegradability in soil is presented.

Technical characteristics:

- C:N should be adjusted to 10:1 up to 20:1 of organic carbon in the sample to total N in the soil (by addition of monohydrate of ammonium phosphate to the soil).
- pH: 6-8; water content at 80 % of saturation; natural soil, sieved < 2 mm with organic C < 2%.
- Sample containing between 200 mg - 1g of organic carbon in 500 g of soil substrate; sample is added as fragments (with a length of 1-2 cm) or as powder.

Validation criteria for soil biodegradability testing:

- Degree of biodegradation of microcrystalline cellulose (reference material) in the soil is more than 70 % at plateau phase or at the end of a six month period.
- Replicate between the tests of the same material should not present more than 20 % relative variation.

Applicability:

- Biodegradable mulching films for agriculture and horticulture

6.1.4 OECD guidelines for testing biodegradability in soil

OECD guideline No 307 describes a method designed for evaluating aerobic and anaerobic transformation of chemicals in soil. The experiments are performed by using ^{14}C-labelled material to determine the rate of transformation of the test substance, and the nature and rates of formation and decline of transformation products to which plants and soil organisms may be exposed. Such studies are required for chemicals which are directly applied to soil or which are likely to reach the soil environment.

Another method is presented in OECD Guideline No 304A where the evaluation of the mineralisation rate of a ^{14}C-labelled compound in soil is done. The method is applicable to volatile or non-volatile, soluble or insoluble compounds which are not inhibitory to micro-organisms.

Both test methods are described in detail in the following tables:

Table 66. Description of the OECD test methods for the measurement of the biodegradability and the transformation rate of chemicals in soil.

Guideline	Description
OECD 307	Soil samples are treated with the test substance and incubated in the dark under controlled laboratory conditions (at constant temperature 20 ± 2 °C for exposure in temperate climates, or 10 ± 2 °C in the case of colder climates and soil moisture of between 2.0 and 2.5 pF*). After appropriate time intervals, soil samples are extracted and analysed for the parent substance and for transformation products. Volatile products are also collected for analysis using appropriate adsorption devices. Using ^{14}C-labelled material, the various mineralisation rates of the test substance can be measured by trapping evolved $^{14}CO_2$ and a mass balance, including the formation of soil bound residues, can be established. The rate and pathway studies should normally not exceed 120 days. Where necessary to characterise the decline of the test substance and the formation and decline of major transformation products, studies can be continued for longer periods (e.g. 6 or 12 months). Longer incubation periods should be justified in the test report and accompanied by biomass measurements during and at the end of these periods.

Guideline	Description
OECD 304A	*Basic test:* A small sample of soil is treated with the ^{14}C-labelled test chemical in a biometer flask apparatus in temperature 22°C ± 2°C. Release of $^{14}CO_2$ from the test chemical is measured by means of alkali absorption and liquid scintillation counting. *Optional experiments include the following tests:* *Evaporation test*: When testing chemicals of a vapour pressure higher than 0.0133 Pa, a polyurethane foam plug is placed into the biometer flask apparatus to absorb the labelled volatile part of the parent compound and volatile metabolites for liquid scintillation counting. *Residue test*: At the point of 50 % mineralisation, the test soil may be extracted. The extractable portion of the compound, and its metabolites remaining in the soil, may be determined by liquid scintillation counting. Furthermore, data on the bound residue part may be obtained by measuring the $^{14}CO_2$ released after combustion of the soil.

* pF expresses the force with which soil particles hold water: It is a function of volumetric moisture content.

Table 67. Validity criteria of the test methods for the measurement of the biodegradability and the transformation rate of chemicals in soil.

Guideline	Validity criteria
OECD 307	*Recovery* Extraction and analysis of, at least, duplicate soil samples immediately after the addition of the test substance gives a first indication of the repeatability of the analytical method and of the uniformity of the application procedure for the test substance. Recoveries for later stages of the experiments are given by the respective mass balances. Recoveries should range from 90% to 110% for labelled chemicals and from 70 % to 110 % for non-labelled chemicals.
OECD 304A	-

6.2 Toxicity

6.2.1 OECD guidelines

An overview of the guidelines referring to the ecotoxicity of chemicals in soil medium as developed by OECD is given in Table 68.

Table 68. Overview of the OECD guidelines with regard to ecotoxicity of chemicals in soil.

Guideline	Adopted	Description
OECD 207	4 April 1984	Earthworm, Acute Toxicity Tests
OECD 208	19 July 2006	Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test
OECD 222	13 April 2004	Earthworm Reproduction Test (*Eisenia fetida/Eisenia Andrei*)
OECD 317	22 July 2010	Bioaccumulation in Terrestrial Oligochaetes

The principle of each ecotoxicity test presented in the above table as well as a brief summary of the test procedure is recorded in Table 67. Correspondingly, Table 68 gives the conditions for the validity of each method.

Table 69. Description of the OECD test methods for the measurement of the ecotoxicity of chemicals in soil.

Guideline	Description
OECD 207	It includes two kinds of tests for the determination of the toxicity of chemicals to earthworms: *a paper contact toxicity test (optional)*: indicates those substances likely to be toxic to earthworms in soil and requires further testing in an artificial soil. Exposure of the earthworms to test substances on moist filter paper to identify potentially toxic chemicals to earthworms in soil. Test temperature: 20°±2°C. Tests are done in the dark and for a period of 48 hours with a further optional mortality assessment after 72 hours. *artificial soil test:* gives toxicity data more representative of natural exposure of earthworms to chemicals. Earthworms are kept in samples of a precisely defined artificial soil to which a range of concentrations of the test substance has been applied. Mortality is assessed 7 and 14 days after application. In both tests, one concentration resulting in no mortality and one resulting in total mortality should be used. The mortality in the controls should not exceed 10 per cent at the end of either test.
OECD 208	Assessment of the effects on seedling emergence and early growth of higher plants following exposure to the test substance in the soil. Seeds are evaluated for effects following usually 14 to 21 days after 50 % emergence of the seedlings in the control group. Endpoint: Visual assessments of seedling emergence, dry or fresh shoot weight or height, visible detrimental effects on parts of the plant. Comparison to those of untreated control plants. The emerging plants should be maintained in controlled environment chambers, phytotrons, or greenhouses. Recommended conditions for greenhouse testing: temperature: 22 ^{o}C ± 10 ^{o}C, humidity: 70 % ± 25 %, photoperiod: minimum 16 hour light, light intensity: 350 ± 50 µE/m^2/s.
OECD 222	Assessment of the effects of chemicals in soil on the reproductive output (and other sub-lethal end points) of the earthworm species Eisinia fetida. Adult worms are exposed to a range of concentrations of the test substance. The range of concentrations is selected to cause both sub-lethal and lethal effects within 8 weeks. Mortality and growth effects on the adult worms are determined after 4 weeks of exposure. The adults are then removed from the soil and effects on reproduction assessed after a further 4 weeks by counting the number of offspring present in the soil. The reproductive output of the worms exposed to the test substance is compared to that of the control(s) by using a regression model.

Guideline	Description
OECD 317	Measurement of the bioaccumulation of a substance in terrestrial oligochaetes. Applicable to stable, neutral organic chemicals, which tend to adsorb to soils, to metallo-organic compounds, metals and other trace elements. Two phases: *uptake (exposure) phase*: replicated groups of worms are exposed to soil containing the test substance. Groups of control worms are also held under identical conditions without the test substance. The dry weight and lipid content of the test organisms are measured. Measurements are made at sampling times up to 14 days (enchytraeids) or 21 days (earthworms) until the steady-state. *elimination (post-exposure) phase:* the worms are transferred to a soil free of the test substance. This phase is always required unless uptake of the test substance during the exposure phase has been insignificant. An elimination phase provides information on the rate at which the test substance is excreted by the test organisms. Measurements are made at sampling times during 14 days (enchytraeids) or 21 days (earthworms) unless earlier analytical determination showed 90% reduction of the test substance residues in worms. The concentration of the test substance in/on the worms is monitored throughout both phases of the test.

Table 70. Validity criteria of the test methods for the measurement of the ecotoxicity of chemicals in soil.

Guideline	Validity criteria
OECD 207	The mortality in the controls series should not exceed 10 per cent at the end of the paper contact toxicity as well as the artificial soil test
OECD 208	For the control series: - the seedling emergence ≥ 70 % - the seedlings should not exhibit visible phytotoxic effects (e.g. chlorosis, necrosis, wilting, leaf and stem deformations) and the plants should exhibit only normal variation in growth and morphology for that particular species - the mean survival of emerged control seedlings ≥ 90 % for the duration of the study - environmental conditions for a particular species must be identical and growing media should contain the same amount of soil matrix, support media, or substrate from the same source.
OECD 222	For the control series: - each replicate (containing 10 adults) to have produced ≥ 30 juveniles by the end of the test - the coefficient of variation of reproduction to be ≤ 30 % - adult mortality over the initial 4 weeks of the test to be ≤ 10 %. Where a test fails to meet the above validity criteria the test should be terminated unless a justification for proceeding with the test can be provided. The justification should be included in the report.
OECD 317	For both controls and treatments: - At the end of the test, the overall mortality during uptake and elimination phase should not exceed 10 % (earthworms) or 20 % (enchytraeids) of the total number of the introduced worms. - For *Eisenia fetida* and *Eisenia andrei*, the mean mass loss as measured at the end of the uptake and at the end of the elimination phase should not exceed 20% compared to the initial fresh weight at start of each phase.

6.2.2 International standards

Table 71 presents the currently available international standards that are relative to the measurement of the ecotoxicity of chemicals in soil media:

Table 71. Overview of the international standards with regard to ecotoxicity of chemicals in soil.

Standard	Description
ISO 11269-2:2012	Soil quality -- Determination of the effects of pollutants on soil flora -- Part 2: Effects of chemicals on the emergence and growth of higher plants
ISO 11268-1:2012	Soil quality -- Effects of pollutants on earthworms (Eisenia fetida) -- Part 1: Determination of acute toxicity using artificial soil substrate
ISO 22030:2005	Soil quality -- Biological methods -- Chronic toxicity in higher plants

ISO 11269-2:2012 describes a method that is applicable to the determination of possible toxic effects of solid or liquid chemicals incorporated in soil on the emergence and early stages of growth and development of a variety of terrestrial plants. It does not give an indication of damage resulting from direct contact of seedlings with the chemical in the vapour or liquid phase outside the soil environment. The method is also applicable to the comparison of soils of known and unknown quality.

ISO 11268-1:2012 is based on placing adult earthworms in a defined substrate containing the test substance in different concentrations and determining the percent mortality after 7 days and 14 days. It is not applicable to volatile substances, i.e. substances for which Henry's constant or the air/water partition coefficient is greater than 1, or for which the vapor pressure exceeds 0,0133 Pa at 25°C. It does not take into account the possible degradation of the test substance.

ISO 22030:2005 describes a method for determining the inhibition of the growth and reproductive capability of higher plants by soils under controlled conditions. Two species are recommended: a rapid-cycling variant of turnip rape and oat. The duration of test should be sufficient to include chronic endpoints that demonstrate the reproductive capability of the test plants.

By using natural test soils, e.g. from contaminated sites or remediated soils, and by comparing the development of the test plants in these soils with reference or standard control soils, the test is applicable to assess soil quality, especially the function of the soil as a habitat for plants. This method can be modified to allow use of the chronic plant assay for the testing of chemicals incorporated into soil. By preparing a dilution series of a test substance in standard control soils, the same endpoints can be measured to assess the chronic toxicity of chemicals.

6.2.3 American standards

The American standard ASTM E 1676 – 04 “Standard Guide for Conducting Laboratory Soil Toxicity or Bioaccumulation Tests with the Lumbricid Earthworm Eisenia Fetida and the Enchytraeid Potworm Enchytraeus albidus” covers procedures for obtaining laboratory data to evaluate the adverse effects of contaminants (for example, chemicals or biomolecules) associated with soil to earthworms (Family Lumbricidae) and potworms (Family Enchytraeidae). The methods are designed to assess lethal or sublethal toxic effects on earthworms or bioaccumulation of contaminants in short-term tests (7 to 28 days) or on potworms in short to long-term tests (14 to 42 days) in terrestrial systems.

6.3 Standard specifications

Table 72 presents an overview of standard specifications for plastics, which are biodegradable in soil. No standard specifications were developed on European or on international level.

Table 72. Overview of specifications standards for determining biodegradability in soil.

American Society for Testing and Materials International (ASTM)	
ASTM WK29802 (under development)	Standard Specification for Aerobically Biodegradable Plastics in Soil Environment
French Normalisation Organisation (AFNOR)	
NF U52-001 February 2005	Biodegradable materials for use in agriculture and horticulture - Mulching products-Requirements and test methods
Other Specifications	
Belgium Royal decree (9/09/2008) effective in July 2009	Decree specifying the norms that the products should meet to be compostable or biodegradable

6.3.1 ASTM Standards specifications for aerobically biodegradable plastics in soil

The ASTM specifications for biodegradability of plastics in soil are under development (since 2010; http://www.astm.org/DATABASE.CART/WORKITEMS/WK29802.htm). This specification covers plastics and products made from plastics that are designed to biodegrade aerobically when in contact with soil with no adverse impact on the environment by the plastics or its degradation products. Also plastics, which need the pre-treatment of light or heat to facilitate biodegradation are within the scope of this new standard specification.

The properties in this specification are those required to determine if products (including packaging) will biodegrade to predetermined acceptable limits under controlled test conditions. Further, the properties in the specification are required to assure that biodegradation of these materials will not diminish the value or utility of the soil resulting from the biodegradation process (= environmental safety).

As stated in the scope of this draft standard, although results may indicate that the tested plastic material will biodegrade under the after-mentioned test conditions at a certain rate, it is cautioned that the results of any laboratory exposure in this specification cannot be directly extrapolated to soil environments at the actual site of use or disposal since soil properties, local temperatures, and humidity ranges shall be considered as they vary widely with geography. Real-world testing is required to establish a correlation with laboratory methods.

As this draft Standard is not public yet, no specifications may be provided. Currently, the testing method ASTM D 5988-12 is used in combination with the specifications for biodegradability of plastics in soil set by other organisations of labelling schemes (see chapter 6.4: VINÇOTTE Certest Products - OK biodegradable SOIL - Initial acceptance tests).

6.3.2 Specifications defined based on French norm for biodegradable agricultural films in soil

In the framework of this specification, a classification of the products is defined based on their expected life time. Moreover, requirements with regard to biodegradability and environmental safety were developed.

Biodegradability testing requirements & criteria:

- The tests can be done in three media: water, soil, compost
- Minimum biodegradation percentage (%): 90 (water), 60 (soil), 90 (compost)
- Time (months): 6 (water), 12 (soil), 6 (compost)
- Rate of biodegradation should be reached at a minimum for two of the three media for validation of the biodegradability of the mulching film. It is not required though that the soil medium is necessarily one of the two media to be tested for the validation of its biodegradability in soil.

Environmental safety testing requirements & criteria:

- Threshold limits in heavy metals, fluorine, PCB (polychlorinated biphenyl) and PAH (polycyclic aromatic hydrocarbon) contents
- Ecotoxicity tests:
    - Emergence and growth of 1 mono and 1 dicotyledonous plants (ISO 11269-2)
    - Acute earthworm toxicity test ((FD X 31-251)
    - Growth inhibition test with Pseudokirchneriella subcapitata (NF T 90-375)

Requirements with regard to labeling:

On the Packaging label should be indicated:

- Product conforms to NF U 52-001
- Name and address of producer of the product
- Commercial name or reference of the product
- Composition: Families of the three principle components
- Length
- Width
- Thickness
- Apparent density
- Class of material: A, B, C, D, E
- Final disposal: burying / composting
- Fabrication date and lot No
- Storage condition in original packaging (temperature, humidity, light etc)

NOTE: The expiration date of the product under optimal storage conditions and in the original packaging (month, year) may be mentioned

On the Mulching Film:

The commercial name or the reference number of the material should be printed on the seam if possible otherwise it should be printed on the tube around which it is rolled.

NOTE: The expiration date of the product under optimal storage conditions (month, year) may be mentioned

6.3.3 Belgian Royal Decree for Acceptance of Compostable and Biodegradable Plastic Materials

As analytically reported in the work of Briassoulis et al. 2010, in Belgium a royal decree became effective in July 2009 that defines three properties of a product depending on its end-of-life management option: compostable, home compostable and biodegradable. This Belgium decree determines the requirements and standards that have to be fulfilled by each category of product.

Biodegradability testing requirements & criteria:

The materials should conform to the French specification NF U 52-001 for biodegradability of agricultural films in soil as described later in section. Also, the biodegradation should be at minimum 90 % absolute or relative (reference material: microcrystalline cellulose) within 24 months.

Environmental safety testing requirements & criteria:

The following ecotoxicity test is required: OECD 208 test (*Terrestrial Plant Test 208: Seedling Emergence and Seedling Growth Test)* also in combination with the ecotoxicity tests described in EN 13432 norm.

6.4 Labelling

6.4.1 VINÇOTTE Certest Products - OK biodegradable SOIL - Initial acceptance tests

The Belgian certification institute *AIB-VINÇOTTE International S.A./N.V. has established a certificate for* awarding and use of the OK biodegradable SOIL conformity mark on bioproducts. A number of normative references have been used and specific requirements have been set evaluating the biodegradation and the environmental safety of the bioproducts under test.

Biodegradability testing requirements and criteria:

- Biodegradability: 90 % biodegradation (absolute or relative to a suitable reference substrate) within a maximum period of 2 years.
- Test method: ISO 17556, ISO 11266 or ASTM D 5988.
- Biodegradability must be determined for the complete product/material or for each organic constituent present in more than 1 % of dry weight of the material.
- The total proportion of organic constituents, not tested on biodegradability, may not exceed 5 %.
- Materials of natural origin are exempted as specified in EN 13432.
- All constituents in the maximum concentrations as specified on the positive list (TS-OK-10) are regarded as fulfilling the biodegradation requirements.

Environmental safety testing requirements and criteria:

- The concentration of the test material (constituent) must be tested in soil is always at least one hundredth of the concentration in which the constituent is added to the final product (soil) on wet mass basis. No assessment of ecotoxicity is necessary for constituents accounting for less than 0.1% of the dry weight of the material of product provided that the total percentage of these constituents does not exceed 0.5% of the dry weight of this material or product. All food additive approved ingredients are regarded as fulfilling the compost quality requirements. Constituents that appear on the (candidate) list of substances of very high concern are not accepted. This must be verified for all constituents that are not tested for ecotoxicity, do not appear on the positive list and are not food additive approved ingredients.

Applicability:

- All raw materials
- All components and constituents also known as intermediate products
- All finished products
- The use of the OK biodegradable SOIL conformity mark is only allowed on finished products for horticultural and agricultural application that have a function in the same environment (soil) where they are meant to biodegrade.

6.5 Discussion and critical review

Despite that several different international and national norms and standards have been developed for testing the biodegradability of plastic materials or plastic products, only a few of them are applicable to testing biodegradability in soil and only a national one for testing biodegradability of agricultural plastic films in soil (*Briassoulis and Dejean, 2010*).

According to the few norms, testing methods, specifications and certification schemes identified for soil medium the biodegradability level of the plastic material or product should be:

- 60 to 90 %.
- obtained in a range of temperatures from 20°C to 28°C
- in a time period from 6 to 12 months (long term biodegradation: 90 % reached in 24 months)

The chosen by all norms parameter recorded to quantify the biodegradability is the evolved carbon dioxide. The lowest accepted rate of biodegradation is 60 %. The compliance requirements of the few standards dedicated to testing biodegradability of polymers in soil, including the corresponding “validity of test” requirements, adapted from (Briassoulis and Dejean, 2010), are summarised in Table 73.

Table 73. Standards Compliance Requirements for biodegradability of plastic and chemicals in soil

Standard Test Method								Standard Specification
Standard Test Method	**Method**	**Test Validity**	**Temperature (°C)**	**C:N Ratio (by weight)**	**pH of soil**	**Sample quantity Water Content (% MHC)**	**Soil Quality**	**Biodegradation Requirement/ mineralisation (%) time frame to achieve biodegradation requirements (months)**
ASTM D 5988-12	Analysis of evolved carbon dioxide; successive titrations	- more than 70% theoretical CO_2 evolution is observed for a reference material (starch or cellulose) - CO_2 evolved from the blanks (or	(**20 - 28**) ± 2	**10:1 up to 20:1** Amend the soil with nitrogen to give a C:N of between 10:1 and 20:1 (by	**6-8** (pH measured on a 5 parts of soil in 1part distilled water)	*Sample Quantity:* - 200-1000 mg C for 500 g soil *Water content:* - 80-100 % of moisture-holding	-Natural, fertile collected from the surface layers of fields and forests. -Laboratory mixture: made of equal parts (by weight) of	This test method is used in association with specifications for biodegradability in soil (e.g. Vinçotte Certest roducts: Biodegradation 90 %

		BOD values) shall be within 20% of the mean at the plateau phase or at the end of the test		weight) to the added carbon in the test specimen by adding the appropriate volume of ammonium phosphate solution.		capacity (MHC) of the soil (by D425) or - 50 to 70 % (by D2980)	soil samples obtained from at least 3 diverse locations (e.g. an agricultural field, a forest, and a pasture or meadow). -Avoid soil exposed to pollutants - Soil particle size less than 2 mm	absolute or 90 % relative (EN 13432) in 24 mo)
ISO/17556-2012	Oxygen demand or evolved carbon dioxide.	*a)* Degree of biodegradation is more than 60% of reference material at the plateau phase or at the end of the test & b) Amounts	(**20 - 28**) ± 2 (Preference in 25°C)	**40:1** Organic C of test or reference material to Nitrogen in the soil (N in soil adjusted with	**6-8** pH adjusted between 6-8 (not specified how)	*Sample Quantity:* - 100-300 mg of test material to 100-300 g of soil is usually adequate. - 200 mg of test material	-"Standard" bulky soil , which is made of a predefined mixture containing industrial quartz sand, clay, natural	This test method is used in association with specifications for biodegradability in soil (e.g. Vinçotte Certest roducts:

		of carbon dioxide evolved or oxygen consumed from the three blanks are within 20% of the mean at the plateau phase or at the end of the test Test duration: 6 - 24 [2]		ammonium chloride or other from a list) N should be similarly adjusted in reference material. N adjustment for soil used in blank is not mentioned		with 200 g of soil recommended *Sample conditioning:* Sample should be pulverised or small size (size affects rate) *Water content:* 40-60% of the total water holding capacity of the soil	soil (16%) and mature compost. - Natural soil collected from the surface layers of fields and forests. is sieved with particles<5mm - preferably less than 2 mm	Biodegradation 90 % absolute or 90 % relative (EN 13432) in 24 mo)
ISO 11266-1994	Determination of oxygen demand in a	*Test duration:* There is no recommende	Max microbial activity: 25-35°C (±2°C), for soils from	Properties found in natural soil	pH found in natural soil	Test substance: concentration depends	If practicable, soils selected for testing should be	This test method is used in association

	closed respirometer (based on ISO 9408). Use of radiolabelled compound to determine rate of test compound disappearance and formation of metabolites, CO_2 other volatiles, non-extractable residue. Metabolites identified using analytical methods. Test compound disappearanc	d min length for a test but, as microbial activity in soil decreases during long incubation periods, it is recommended that tests should not be continued for longer than 120d.	temperate zones: 10-25 °C ±2°C)			on the experimental objectives. Water content of the soil: Pore-water pressure: microbial activity in soil is optimal between -0,01MPa & -0,031 MPa. WHC: optimal water content between 40-60% of the WHC	natural coming from the site where chemical contact is anticipated, or the soil selected should have comparable properties	with specifications for biodegradability in soil (e.g. Vinçotte Certest roducts: Biodegradation 90 % absolute or 90 % relative (EN 13432) in 24 mo)

	e can also be followed by specific analysis							
NF U52-001 February 2005 **(Part on testing method for biodegradation in soil)**	Analysis of evolved carbon dioxide.	Degree of biodegradation of microcrystline cellulose (reference material) in the soil is more than 70% at plateau phase or at the end of a six month period Replicate between the tests of the same material should not present more than 20% relative	**28**±2 (biodegradation in soil)	**10:1** up to **20:1** of organic carbon in the sample to total N in the soil. (soil N adjusted by addition of monohydrate of ammonium phosphate) Same soil should be used in the	**6-8** (measured one part of soil in 5 parts of water)	*Sample Quantity:* Sample containing between 200 mg -1 g of organic carbon in 500 g of dry soil substrate *Sample conditioning:* Sample film cut to pieces 1-2 cm or powder	- Natural soil: - Extraneous mater removed (leaves, wood, stones) sieved < 2mm - organic C < 2%	This Standard includes specifications: Biodegradation in soil **≥ 60 %** in **12** mo Reach the threshold rate of biodegradation in at least two out of three media: a) at least 60% of the maximum degradation of cellulose in soil b) at least 90%of maximum degradation of

		variation.		reference and blank test		*<u>Water content:</u>* - 80% of saturation		cellulose in compost or in water medium
Vinçotte Certest Products	ISO 17556, ISO 11266 or ASTM D5988-96	*a)* Degree of biodegradation is more than 70% (ASTM D5988) or 60% (ISO 17556) of reference material at the plateau phase or at the end of the test & b) Amounts of carbon dioxide evolved or oxygen consumed from the three blanks are within	(**20 - 28**) ± 2	According to the corresponding standard	**6-8**	According to the corresponding standard	According to the corresponding standard	This certification scheme includes specifications: Biodegradation **90 %** absolute or **90 %** relative (EN 13432) **in 24** mo

		20% of the mean at the plateau phase or at the end of the test						
Belgium Royal decree (July 2009)	Oxygen demand or evolved carbon dioxide (ISO 17566, ISO 14851, ISO 14852 or ISO 14855)	According to the correspondin g standard	**20–30**	According to the correspondin g standard	According to the correspondin g standard	According to the correspondin g standard	According to the correspondin g standard	This certification scheme includes specifications: **90** % absolute or **90** % relative Or 60 % absolute within 12 months at 20°C – 30°C & 90% absolute or relative within 6 months at 58°C in **24** mo
OECD 304A	Evaluation of the mineralisatio n rate of a ^{14}C-labelled		22°C±2°C	Range of values depending on the type	Range of values depending on the type	*Sample Quantity:* 50 g of soil and 100 ml	Several types of soil used	N/A

	compound in soil			of soil	of soil	of the radioactive test solution *Water content:* 40 % maximum water capacity.		
OECD 307	Evaluation of aerobic and anaerobic transformation of chemicals in soil using ^{14}C-labelled material	Recoveries should range from 90 % to 110 % for labelled chemicals and from 70 % to 110 % for non-labelled chemicals	20°C±2°C for temperate climates and 10°C±2°C	Organic carbon content of 0.5 - 2.5 % and a microbial biomass of at least 1 % of total organic carbon recommended	pH of 5.5-8.0	*Sample Quantity:* 50-200 g of soil (substance can be dissolved in water or, when necessary, in minimum amounts of acetone or other organic solvents in which the test substance is sufficiently	A sandy loam or silty loam or loam or loamy sand	N/A

						soluble and stable) *<u>Water content:</u>* pF between 2.0 and 2.5		

([1]) ASTM WK29802 is under development

([2]) The test period should typically not exceed six months. If significant biodegradation is still observed and the plateau phase has not been reached after this length of time the test may be extended up to 24 months.

6.5.1 Biopolymers

The major category of commercial biodegradable in soil materials concerns agricultural applications. A major consideration for biodegradable agricultural plastics, mainly biodegradable mulching films, is the fact that the end of the life management of these products will be done at the farm to reduce the management of the waste and the associated cost. So, at the present time it seems that two streams are possible for biodegradation at the farm: biodegradation in soil and biodegradation in farm yard composting. In both cases it is important that the tests should be run in media simulating the conditions of the end of life management of these materials, that is to say in soil or in farm yard composting and with a time duration corresponding to a year, maximum. If this frame of parameters and media can be achieved at the laboratory, the corresponding tests will approach the reality of the practices at the farm.

The applicability of ISO 17556:2012, as well as of ASTM D 5988-2012 and NF U52-001-2005, for biodegradable plastics in real soil conditions (e.g. agricultural biodegradable bio-based films) remains questionable because of several concerns:

- Transferability of results: there is no way to predict same results in different soils
- Validation of test (positive reference)
- Prerequisites for soil

According to De Wilde B. (2002), as far as acceptance of biodegradable plastics in soil is concerned, two main categories of testing requirements concern:

1) Biodegradation:
   - 90 %
   - Duration: depending on the application (under mesophilic conditions the material shall be biodegraded to at least 90 % during the time of maximum 24 months. If the material fails to reach 90 % but reaches 60 % (absolute) this is considered to be a proof that the material is potentially biodegradable).

2) Soil quality:
   - Chemical: heavy metals
   - Ecotoxicity

These proposed specifications for biodegradation in soil are analogous, to some extent, with those of the French Norm NF U52-001 (2005) (apart for the optional use of the soil medium by NF U52-001), extended over a longer reference period to determine long-term effects. As mentioned by *Briassoulis and Dejean, 2010* analogous provision has been adopted by SP Technical Research Institute of Sweden concerning the requirements and associated test methods to certify polymeric materials and products: the ultimate aerobic biodegradability in soil (by measuring the oxygen demand in a respirometer or the amount of carbon dioxide evolved) is determined by the requirement of biodegradation ≥ 90 % within 24 months. The time frame and biodegradation rate requirements of NF U52-001 do not guarantee the achievement of the long term biodegradation in soil behaviour as proposed by De Wilde B. (2002).

Furthermore, as pointed out in (*Briassoulis and Dejean, 2010*), it should be required that the soil medium is necessarily one of the two media to be tested for the validation of the biodegradability of a biodegradable bio-based product in soil. This requirement is missing in the present version of the norm NF U52-001, and should be added, and explicitly specified as such, in a future revised version of this norm.

6.5.2 Lubricants and solvents

The only standard test method for testing biodegradability of organic chemicals (pure compounds (chemical purity > 98 %) in aerobic soil is, ISO 11266-1994.

OECD guidelines 304A and 307 describe special test methods for the determination of the mineralisation rate of a ^{14}C-labelled compound in soil and the degree of aerobic and anaerobic transformation of chemicals in soil correspondingly. However, these guidelines are very specialized and not easily applicable as they require highly specialised laboratories and the use of specially produced radiolabeled samples of the substances to be tested.

There are no standard test methods or specifications for biodegradation of bio-lubricants and bio-solvents in soil available up-to-date.

6.5.3 Major discrepancies in the biodegradation test methods

Concerning the three main standard testing methods for biodegradation in soil, ISO 17566, ISO 11266 and ASTM D5988, some major discrepancies are identified:

6.5.3.1 Soil Medium

ASTM D 5988 standard

- Soil should be natural and fertile collected from the surface layers of fields and forests.
- Laboratory mixture made of equal parts (by weight) of soil samples obtained from at least 3 diverse locations
- A mixture of natural soil and mature compost is also acceptable
- Soil pH should be 6-8. No suggestion about the case when pH is measured outside this range

ISO 17566 standard

- Recommends that the test samples may be reduced in size by means of cryogenic milling.
- The use of a “standard soil” is permitted as an alternative to natural soil that constitutes of industrial quartz sand, clay, natural soil and mature compost. This soil is considered very useful in determining the biodegradability of plastic materials in loamy or clayey soils, reducing handling and aeration problems.
- Specific salts may be added in the soil preferably when adjusting the water content.
- Soil pH should be 6-8; pH should be adjusted to meet this requirement.

ISO 11266 standard

- If practicable, soils selected for testing should be natural, coming from the site where chemical contact is anticipated, or the soil selected should have comparable properties.
- pH value is the pH found in the natural soil.

NF U52-001 standard

- Recommends that the test samples film is cut to pieces 1-2 cm or powder
- Biodegradation in soil using natural soil with organic C < 2%
- Soil pH should be 6-8; pH should be adjusted to meet this requirement

The ISO procedures may enhance or alter biodegradation of polymers drastically, but they lead to a rather controlled biodegradation process in an artificial soil that in many cases may be not representative of biodegradation of non-pulverised polymers under real soil conditions. In contrast the ASTM standard leads to biodegradation results closer to real soil conditions, which however may still be very different from some specific categories of soil types and real soil conditions. As this standard allows the test specimens to be in the form of films, pieces, fragments, powder or formed articles, biodegradability of the same material will be affected by the test specimens form. If not tested in its real form, measured biodegradability will not be realistic. Also, since the size (surface area exposed to the soil) of the sample affects the rate of biodegradation and ASTM D 5988-12 requires evolution of 70 % of the $ThCO_2$ in 6 months for the reference material, the biodegradability validation criteria may be affected by the surface area of the sample.

6.5.3.2 Ratio C/N:

ASTM D5988 standard

- C:N ratio adjusted to a value between 10:1 and 20:1 *by weight to the added carbon in the test specimen* (note defined explicitly; presumably for the C:N of test sample; needs clarification though).

ISO 17566 standard

- The ratio C:N is at least 40:1 for the sample organic C to the soil N

ISO 11266 standard

- Value as found in the natural soil

NF U52-001 standard

- The ratio C:N 10:1 up to 20:1 for the sample organic C to the soil N

Average C:N ratios of soils vary from region to region depending on the predominant soil type and the prevailing conditions (e.g. farming, climate etc). However, as a C:N ratio value between 8 and 17 is typical (Alistair F. Pitty, Geography and soil properties, Taylor & Francis, 1979), the recommendation of the ASTM standard is in better agreement with the real soil

conditions in that respect provided that the sample organic C to N (if this provision interpreted correctly) remains in the same range and so it will not affect significantly this ratio. The provision of NF U52-001 defines this ratio in the same range but for organic C over the total sample-soil N. On the other hand, the provision of the ISO standard for a ratio of the organic C of the sample to the soil N of 40:1 "*so as to ensure good biodegradation*" follows the general direction of this method for enhancing artificially biodegradation in soil. It may also be noticed that N is necessary for the biodegradation of C. Therefore high C:N may not ensure good biodegradation.

6.5.3.3 Round Robin Tests:

The most recent version of the standard ISO 17556 – 2012 provides in its Annex G the results of a Round Robin Test, which was organized among six laboratories in 2009. The objective of this test was to prove the suitability of a "standard" soil proposed in the standard for tests of biodegradation in soil. However, the presented data also demonstrate the difficulties involved in this testing method. Throughout this RR test, a test material (starch/poly(butylene adipate-co-butylene terephthalate) blend) and a reference (microcrystalline cellulose) were used following the procedure of the standard. Not all trials reached the plateau phase. Two different testing methods were considered, namely the free airflow method and the method using flasks. Only one lab used the flask option, while six labs used the free airflow method.

In order to reduce variability, only the results obtained by the free airflow method were considered in the following analysis. The results show that the degree of biodegradation of the reference material is approximately equal to 72 % with a standard deviation 18. Figure 24 shows that the results concerning the biodegradation of the reference material exhibit a typical experimental variability which follows the normal distribution (green columns).

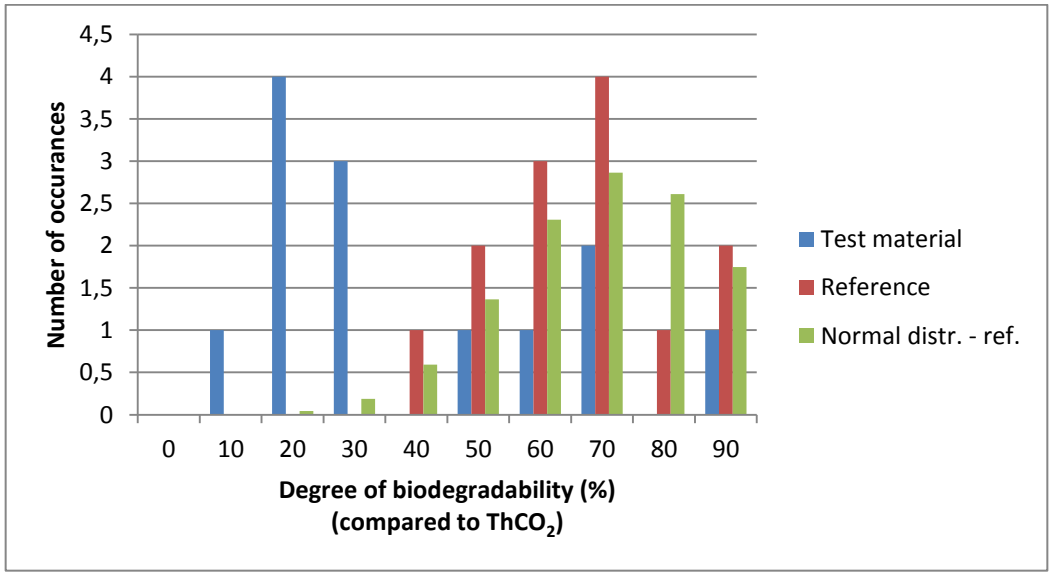


Figure 24. Statistical analysis of results obtained in six different labs for the biodegradation in soil of a test material using the Standard ISO 17556.

On the contrary, the values obtained for the test material present an erratic, almost random behavior. No explanation was offered for this high variability. Biological or morphological characteristics of the different types of soil used in this RR test may be responsible for this discrepancy. These results indicate that the current test method provides results with variable quality depending on the tested material. Therefore, further research is necessary for assessing the validity and the possible reasons of failure of the currently used testing method for a wide range of test materials.

Figure 24 presents all the data obtained through the RR test including cases where the plateau phase was not reached. However, the same statistically erratic data also appear if the results are restricted to the cases where the plateau phase was reached.

In Annex G of ISO 17556:2012 it is concluded that standard soil can help in standardizing the test procedure, as it makes use of a standard matrix, with a standard texture and particle size.

7 Industrial composting environment

The review of the industrial composting environment has been executed by the Agricultural University of Athens.

Nowadays, the terms “biodegradation”, “biodegradable materials”, “compostability” etc. are very common but frequently misused and source of misunderstanding Compostability is not only related to biodegradation, but 4 characteristics need to be taken into account (Figure 25).

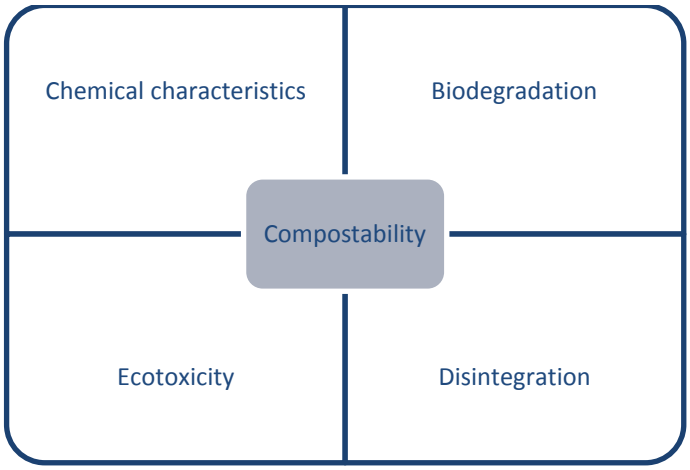


Figure 25. Overview of the main characteristics required for industrial compostability.

Two main characteristics are related to environmental safety (= compost quality): chemical characteristics and ecotoxicity. The two other characteristics are related to degradation: biodegradation and disintegration. Disintegration is the physical falling apart of the plastic material, or more precisely of the product that has been made from it, into fine visually indistinguishable fragments (dimensions < 2 mm) at the end of a typical composting cycle, while biodegradation is the complete breakdown to mineral end products (De Wilde (2002)).

Existing norms and standards on testing plastics with regard to biodegradability in industrial composting environments and compostability are reviewed in this chapter. The disintegration methods will not be discussed in detail in this study. This updates the relevant information presented earlier by Briassoulis et al. 2010.

7.1 Biodegradation

Table 74 presents a synthesis of the most important tests for assessing biodegradability in industrial composting environments and compostability of plastics up the year 2012. The norms presented in this table are all about plastic materials or packaging.

Table 74. Overview of standard testing methods for determining biodegradability of materials in compost

American Society for Testing and Materials International (ASTM)		
Current versions of standards	**Previous versions of standards**	**Title**
ASTM D5929-96 (Reapproved 2009)		Standard Test Method for Determining Biodegradability of Materials Exposed to Municipal Solid Waste Composting Conditions by Compost Respirometry
ASTM D5338-11	***ASTM D5338-98 (2003)**	Standard test method for determining aerobic biodegradation of plastic materials under controlled composting conditions Incorporating thermophilic temperatures
ASTM D6340-98 (2007)		Standard test method for determining aerobic biodegradation of radiolabeled plastic materials in an aqueous or compost environment.
	****ASTM D5509-96**	Standard practice for exposing plastics to a simulated compost environment
	****ASTM D5512-96**	Standard practice for exposing plastics to a simulated compost environment using an externally heated reactor
	****ASTM D5951-96 (2002)**	Standard practice for preparing residual solids obtained after biodegradability standard methods for toxicity and compost quality testing—fate & effect testing
International Organization for Standardization (ISO)		
Current versions of standards	**Previous versions of standards**	**Title**
ISO 14855-1:2012	***ISO 14855-1:2005**	Determination of the ultimate aerobic biodegradability of plastic materials under controlled composting conditions—Method by analysis of evolved carbon dioxide—Part 1: General method
ISO 14855-2:2007/Cor 1:2009	**ISO 14855-2:2007**	Determination of the ultimate aerobic biodegradability of plastic materials under controlled composting conditions—Method by analysis of evolved carbon dioxide—Part 2: Gravimetric measurement of carbon dioxide evolved in a laboratory-scale test

European committee for standardization (CEN)		
Current versions of standards	**Previous versions of standards**	**Title**
EN 14046:2003		Packaging—Evaluation of the ultimate aerobic biodegradability of packaging materials under controlled composting conditions—Method by analysis of released carbon dioxide.
EN ISO 14855-1:2007 + AC: 1:2009 (to be replaced by FprEN ISO 14855-1-2012)	***EN ISO 14855:2004**	Determination of the ultimate aerobic biodegradability and disintegration of plastic materials under controlled composting conditions—Method by analysis of evolved carbon dioxide Part 1: General method: identical ISO 14855-1:2005/Cor 1:2009 Identical (ISO 14855-1:2005 + Cor 1:2009)
EN ISO 14855-2:2009	***EN ISO 14855:2004**	Determination of the ultimate aerobic biodegradability and disintegration of plastic materials under controlled composting conditions—Method by analysis of evolved carbon dioxide Part 2: Gravimetric measurement of carbon dioxide evolved in a laboratory scale test (ISO 14855-2:2007, including Cor 1:2009)
Deutsches Institut fur Normung (DIN)		
DIN V 54900-2		Testing of compostability of plastics—Part 2: testing of the complete biodegradability of plastics in laboratory tests.
Japanese industrial standard		
***JIS K 6953-2000**	**JIS K 6953-2011**	Determination of the ultimate aerobic biodegradability of plastic materials under controlled composting conditions—Method by analysis of evolved carbon dioxide

*Standard superseded by its updated version

** Withdrawn standard, no replacement

7.1.1 ASTM Standards for testing biodegradability of biodegradable materials under municipal and industrial composting conditions

There are several ASTM Standard testing methods for testing biodegradability of plastic materials under industrial composting conditions. No relevant ASTM standard exists for organic chemicals.

ASTM D 5929-09

The ASTM D 5929-96 (2009) standard covers the biodegradation properties of a material by reproducibly exposing materials to conditions typical of municipal solid waste (MSW) composting. A material is composted under controlled conditions using a synthetic compost matrix and determining the acclimation time, cumulative oxygen uptake, cumulative carbon dioxide production and percent of theoretical biodegradation over the period of the test.

Technical characteristics:

- The reactors are operated for a period of 45 days; maintenance of temperature at 40^{o}C. The nitrogen content of the synthetic MSW should be adjusted if the C/N ratio is greater than 40:1.
- At the end of the run of the experiment, the total weight of the compost material and the dry solids concentration is determined. Also, the pH is measured and if it is below 7, the volatile fatty acids (VFA) content of the compost is determined.
- The total oxygen uptake and carbon dioxide produced are compared with the theoretical values obtained from the elemental analysis, and a percentage of biodegradation is generated. Possible negative effects of the material are evaluated by observing the acclimation time of the synthetic MSW and evaluating the oxygen uptake rate.

Validation criteria:

- If the VFA is > 2 g/kg, the reactors have soured and the results are invalid.
- To ensure an active and viable inoculum, the total oxygen uptake for the control reactors should exceed 80 g. If this is not observed over the 45 days then the test must be regarded as invalid and should be repeated with new inoculum.

Applicability:

This test method is applicable to any material that is designated to be disposed in municipal solid waste composting facilities

ASTM D 5338-03

The ASTM D 5338-98 (2003) standard that determines the degree and rate of aerobic biodegradation of plastic materials on exposure to a controlled-composting environment under laboratory conditions, at thermophilic temperatures, was updated into ASTM D5338-11. This latest version clarifies that the thermophilic temperatures are most readily achieved in large-scale, professionally-managed facilities. However, these temperatures may also be

reached in smaller residential composting units, frequently referred to as “backyard” or “home” composting. This test method is designed to yield reproducible and repeatable test results under controlled conditions that resemble composting conditions, where thermophilic temperatures are achieved.

Technical characteristics:

- Controlled-composting environment under laboratory conditions, at thermophilic temperatures of 58°C±2°C.
- Test substances are exposed to an inoculum that is derived from compost from municipal solid waste (2 – 4 months old).
- The aerobic composting takes place in an environment where temperature, aeration and humidity are closely monitored and controlled.
- Biodegradation percentage of the test item is obtained by determining the percentage of carbon in the test substance that is converted to CO_2 during the duration of the test.The net production of CO_2 is recorded relative to a control containing only mature compost.

Validation criteria:

- If more than 2 g of volatile fatty acids per kilogram of dry matter in the composting vessel is formed, the test must be regarded as invalid.
- If sufficient biodegradation - a minimum of 70 % for cellulose within 45 days - is not observed with the positive reference, the test must be regarded as invalid and should be repeated, using new inoculum.
- If the deviation of the percentage of biodegradation of the positive reference is greater than or equal to 20 % at the end of the test, then the test shall be regarded as invalid.

Applicability:

- All plastic materials, which are intended to be composted in facilities that achieve thermophilic temperatures.

Equivalence:

This test method is equivalent to ISO 14855.

ASTM D 6340-07

The ASTM D 6340-98 (2007) standard reported in Table 74 that is relevant to testing the biodegradability of plastic materials in several compost environments is briefly presented in the research work of Briassoulis et al. 2010. ASTM D 6340-98 (2007) determines the rate and degree of biological oxidation of carbon in radio-labelled plastic materials when placed in a composting environment containing simulated municipal solid waste or an aqueous environment under laboratory conditions.

Technical characteristics:

- For the case of using of compost environment: Compost for this testing may be made up with either municipal solid waste (MSW) or woody compost, or obtained from an active municipal solid waste or yard waste composting center. So, the compost, designed to stimulate MSW organic matter should contain the biochemical ingredients found in MSW (lignified cellulose, protein, natural inoculum, soluble carbohydrates and buffering capacity sufficient to maintain a neutral to slightly basic pH).
- The particle size of the components of the compost mix are sized to pass through a 6 mm screen. Moisture content should be adjusted to 55-60 % for testing.
- Addition of 200-300 mg of plastic (in pieces of ~2 cm^2) in approximately 300 g of compost.
- Controlled-composting environment in the testing chamber: temperature of 58°C±5°C.
- Requirement: the target component of the plastic material be synthesized using the radioactive isotope carbon-14. Depending upon the objective, either a portion of the components of the plastic or all of the carbon can be uniformly labeled with carbon-14. The test method will determine how that labeled portion will be metabolized and biologically oxidized by the microorganisms in the system tested.

Validation criteria:

- $^{14}CO_2$ measured in this test method is a direct indication of the oxidation of the sample. However, the extent and the rate of oxidation are related to the compost mixture made for that individual test and the form of the sample. Although different batches of compost can produce different results, the compost formula for the simulation of MSW in this test method will generally give repeatable results. This is due to the selection of common feed ingredients that are standardized in the trade and tend to have a consistent composition.
- Depending upon the objectives of the test, it is generally wise to include within each test series a standard preparation of known degradation rates and to make test comparisons.
- Early days of the composting process: monitoring of the viscosity of methoxyethyl amine by observing the bubble flow.
- Occasionally, two phases develop due to larger quantities of water trapped in the methoxyethyl amine, and these 2 phases can be blended into one by addition of several ml of methanol. During prolonged composting trials (over 20 days) the sampling interval can be extended depending upon the objectives of the experiments.
- In order to test whether the oxidation of the carbon in the plastic could occur chemically under the composting conditions, it may be necessary to use a sterile control. This is not necessary if the chemistry of the compound being tested is well documented and it is known that chemical oxidation does not occur under these composting conditions.

Applicability:

The test method applies to plastics the biodegradation rate of which is slow and requires test periods of as long as 365 days.

7.1.2 ISO Standards for testing biodegradability of plastics under municipal and industrial composting conditions

There are several ISO standard testing methods for testing biodegradability of plastic materials under aerobic industrial composting conditions.

ISO 14855-1:2012

ISO 14855-1:2005 that is analytically described in the work of Briassoulis et al. 2012, was revised into ISO 14855-1:2012 and specifies a method for the determination of the ultimate aerobic biodegradability of plastics, based on organic compounds, under controlled composting conditions by measurement of the amount of carbon dioxide evolved and the degree of disintegration of the plastic at the end of the test. The method is designed to simulate typical aerobic composting conditions for the organic fraction of solid mixed municipal waste.

Technical characteristics:

- The test material is exposed to an inoculum which consists of stabilized mature compost derived, if possible, from composting the organic fraction of solid municipal waste.
- Temperature, aeration and humidity are closely monitored and controlled for a test period not exceeding 6 months.
- Testing enclosure or room is maintained at a constant temperature 58°C±2°C and is kept free from vapours inhibitory to microorganisms. Monitored and controlled humidity should be kept at around 50 %, and proper air circulation to maintain the oxygen concentration above a minimum of 6 %.
- The C:N ratio for the test mixture should preferably be between 10 and 40.
- The biodegradation percentage is given by the ratio of the carbon dioxide produced from the test material to the maximum theoretical amount of carbon dioxide that can be produced from the test material. The biodegradation percentage does not include the amount of carbon converted to new cell biomass which is not metabolized in turn to carbon dioxide. Additionally the degree of disintegration and the loss in mass of the test material can be determined at the end of the test.

Alternative method:

The use of mature compost can lead to some difficulties in interpreting the results: (1) “priming effect”: the polymer-induced degradation of the organic matter present in large amounts in the mature compost, affects the measurement of the biodegradability, (2) biomass determination is not possible and (3) quantification of residual polymeric material left in the bed.

Therefore a variant was developed. This alterantive uses a mineral bed (vermiculite) inoculated with thermophilic microorganisms obtained from compost with a specific activation phase instead of mature compost in order to eliminate the difficulties associated with the use of mature compost. This variant can be used to measure the biodegradation in terms of CO_2 evolution and the rate of conversion, to quantify and analyse the biomass and the residues of polymeric material left in the solid bed at the end of the test, and to perform a complete carbon balance. This variant is not sensibly affected by the priming effect and can, therefore, be used to assess materials known to cause this problem with mature compost.

Validation criteria:

The test is considered as valid if:

- The degree of biodegradation of the reference material is more than 70 % after 45 days.
- The difference between the percentage biodegradation of the reference material in the different vessels is less than 20 % at the end of the test.
- The inoculum in the blank has produced more than 50 mg but less than 150 mg of carbon dioxide per gram of volatile solids (mean values) after 10 days of incubation.

Applicability:

This part of ISO 14855 specifies a method that is designed to determine ultimate aerobic biodegradability of plastics, based on organic compounds, under controlled composting conditions

Equivalence:

This test method is equivalent to EN 14046.

ISO 14855-2:2007/Cor 1:2009

ISO 14855-2:2007 standard testing method presents a method for determining the ultimate aerobic biodegradability of plastic materials under controlled composting conditions (humidity, aeration ratio and temperature) by gravimetric measurement of the amount of carbon dioxide evolved. The degradation rate is periodically measured by determining the mass of the evolved carbon dioxide using an absorption column filled with soda lime and soda talc on an electronic balance. The standard was revised into ISO 14855-2:2007/Cor 1:2009 with a technical Corrigendum 1.

The test material is mixed with an inoculum derived from mature compost and with an inert material such as sea sand. The sea sand plays an active part by acting as a holding body for humidity and micro-organisms.

Technical characteristics:

- It is recommended to adjust the compost to a C/N ratio of 15 and a C/P (carbon/phosphorous) ratio of 30. Water content equal to 65 %. The three previous parameters may also be adjusted to other values, determined by experience,

depending on seasonal variations and climatic differences. pH adjustment between 7 and 9.

- The relation between the total dry solids of the inoculum and the total dry solids of the test material should preferably be about 6:1. If added, inert material is not considered in this relationship. The test mixture should have the same water content as the inoculum. The water content of the test mixture should be set at 80 % to 90 % of the water-holding capacity (WHC) of the test mixture. The same amount of inoculum by total dry solids should be placed in each test vessel.

Validation criteria:

- The degree of biodegradation of the reference material is more than 70 % after 45 days.
- The difference between the percentage biodegradation of the reference material in the different vessels is less than 20 % at the end of the test.
- The pH should be measured at regular intervals, as at the start of the test. If the pH is found less than 7, biodegradation could be inhibited due to acidification of the compost by the rapid degradation of an easily degradable test material. In this case, measurement of the volatile fatty acid spectrum is recommended to check for souring of the contents of the composting vessel. If more than 2 g of volatile fatty acids per kilogram of total dry solids has been formed, then the test shall be regarded as invalid due to acidification and the resultant inhibition of microbial activity. To prevent acidification, more compost should be added to all the test vessels or the test should be repeated using, for example, less test material, more compost or pre-exposed compost.

If these criteria are not fulfilled, the test must be repeated using pre-conditioned or pre-exposed compost.

Applicability:

- Natural and/or synthetic polymers and copolymers, and mixtures of these
- Plastic materials that contain additives such as plasticizers or colorants
- Water-soluble polymers
- Materials that, under the test conditions, do not inhibit the activity of micro-organisms present in the inoculum

Equivalence:

This test method is equivalent to EN 14046 that describes correspondingly a method for the evaluation of the ultimate aerobic biodegradability of packaging materials based on organic compounds under controlled composting conditions by measurement of released carbon dioxide at the end of the test. This method is designed to resemble typical aerobic composting conditions for the organic fraction of mixed municipal solid waste. The test method is designed to yield a percentage and rate of conversion of carbon of the test material to released carbon dioxide.

7.1.3 European Norms for testing biodegradability of plastics under municipal and industrial composting conditions

There are several EN standard testing methods for testing biodegradability of plastic materials under aerobic industrial composting conditions which are equivalent to the corresponding ISO standard test methods.

EN 14046:2003

The standard EN 14046:2003 *'Packaging—evaluation of the ultimate aerobic biodegradability of packaging materials under controlled composting conditions—Method by analysis of released carbon dioxide'* was developed under the directive area of ''Packaging and packaging waste 94/62/EC'', by CEN/TC 261. This standard is equivalent to the standard ISO 14855 described in the previous section (EN ISO 14855:2004; *Determination of the ultimate aerobic biodegradability and disintegration of plastic materials under controlled composting conditions—method by analysis of evolved carbon dioxide*).

EN ISO 14855-2:2009, EN ISO 14855-1:2010, EN ISO 20200:2005

- EN ISO 14855-2:2009 is identical to ISO 14855-2:2007 incorporating Corrigendum 1:2009
- EN ISO 14855-1:2007 + AC:2009 is identical ISO 14855-1:2005/Cor 1:2009

Further analysis and critical review of these standards can be found in Briassoulis et al. 2010.

7.1.4 Other Norms for testing biodegradability of plastics under municipal and industrial composting conditions

Some national standards are also presented in Table 74 for illustrative purposes.

7.2 Toxicity

The compost obtained at the end of the composting process can be adversely influenced by the addition of compostable plastics or packaging at start of the composting process. In order to verify if this is the case, ecotoxicological tests with compost with and without test material can be executed.

As mature compost is used in order to increase the soil quality, the residuals of the compostable materials will finally reach the soil environment. Consequently, compost quality can be checked by mixing the compost with artificial soil. This mixture can then be used in order to executed toxicity tests. The toxicity in the test mixture can be compared with the toxicity in the blank mixture. To assess toxicity associated with compost applications, plastics can be tested on both plant and animal species, as required by the corresponding norms and/or chosen by the interested parties (*Briassoulis et al, 2010*). Rudnik et al, 2007 mentions that the methods for the evaluation of the ecotoxicity of compostable polymer materials are mainly based on the use of plants, soil fauna (earthworms), aquatic fauna (Daphnia), algae (green algae), microbes (luminescent bacteria).

With regard to plant phytotoxicity testing, while a product may not negatively impact plant growth in the short term, over time it could become phytotoxic along plant development due to the build-up of inorganic materials within determined parts of the plant, which could potentially lead to a reduction in soil productivity (*Biodegradable Plastics, 2002*). To test for such a potential effect plant phytotoxicity testing can be used on the finished compost that contains degraded polymers. However, in order to simulate the accumulation a test item during several years, the test item should be added in a high concentration at start of the pilot-scale composting test. Both acute plant toxicity and chronic plant toxicity tests can be executed on the obtained compost/soil mixtures.

Animal testing is generally carried out using earthworms (soil organisms) and Daphnia (aquatic organisms) (*Biodegradable Plastics, 2002)*. The Daphnia toxicity test is used to establish whether degradation products present in liquids pose any problem to surface water bodies. Earthworms are used because they feed on soil and they are very sensitive to toxicants. Earthworms are exposed to several mixture ratios of compost and soil mixtures. Following 14 days of exposure, the number of surviving earthworms is counted and weighed and the survival rate is calculated (test of acute toxicity within 14 days (mortality) (OECD 222); chronic toxicity (reproduction) 56 days (OECD 207)). Compost worms (Eisenia fetida) are used for testing the toxicity of biodegradable plastic residues. These worms are very sensitive to metals such as tin, zinc, heavy metals and to high acidity (Rudnik, 2008)]. For this test worms are cleaned and accurately weighed at intervals over 28 days.

An overview of toxicity tests in water and soil is given in Chapter 3.2 and Chapter 6.2, respectively.

Concentrations of heavy metals of the compostable material must be below the limits set by the standards (*Briassoulis et al, 2010)*. The corresponding provisions differ between the various standards as presented in Table 75 (adapted from *(Briassoulis et al, 2010))*.

Table 75. Maximum heavy metal content for compostable materials according to various standards

Element (mg/kg d.w.)	US ASTM D 6400 ASTM D 6868	Canada BNQ P 9011-911-5	Europe/Australia EN 13432 AS 4736	Japan (Biodegradable plastic Society)
Zn	1400	925	150	150
Cu	750		50	60
Ni	210	90	25	30
Cd	17	10	0.5	0.5
Pb	150	250	50	10
Hg	8.5	2.5	0.5	0.2
Cr			50	50
Mo		10	1	
Se	50	7	0.75	
As	20.5	37.5	5	5
F			100	
Co	75	75		

7.3 Standard specifications

Table 76 presents the standard specifications for the compostability under municipal and industrial aerobic conditions for different product categories (plastics, packaging, etc.). These standards on all based on the same criteria (biodegradation, disintegration and environmental safety including ecotoxicity tests and chemical characterization).

Table 76. Overview of specifications standards for determining compostability of plastics.

American Society for Testing and Materials International (ASTM)		
Current versions of standards	**Previous versions of standards**	**Title**
ASTM D 6400-12	***ASTM D 6400-04**	Standard Specification for Labeling of Plastics Designed to be Aerobically Composted in Municipal or Industrial Facilities (Previous title: Standard specification for compostable plastics)
	****ASTM D 6002-96 (2002)**	Standard guide for assessing the compostability of environmentally degradable plastics
ASTM D 6868-11		Standard Specification for Labeling 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
International Organization for Standardization (ISO)		
Current versions of standards	**Previous versions of standards**	**Title**
ISO 17088:2012	***ISO 17088:2008**	Specifications for compostable plastics
ISO 18606:2013		Packaging and the environment - Organic recycling
European committee for standardization (CEN)		
EN 13432-2000		Packaging—Requirements for packaging recoverable through composting and biodegradation—Test scheme and evaluation criteria for the final acceptance of packaging
EN 14995-2006		Plastics—Evaluation of compostability—Test scheme and specifications
Deutsches Institut fur Normung (DIN)		
DIN EN 13432-2000		Requirements for packaging recoverable through composting and biodegradation—Test scheme and evaluation criteria for the final acceptance of packaging.

DIN EN 14995-2007	Plastics—Evaluation of compostability—Test scheme and specifications.
DIN V 54900-1998	Testing of the compostability of plastics
Italian Norm (Italian Unification Agency) (UNI)	
UNI EN 14995-2006	Plastics—Evaluation of compostability—Test scheme and specifications
Other Specifications	
Belgium Royal decree (9/09/2008) effective in July 2009	Decree specifying the norms that the products should meet to be compostable or biodegradable
Australian standard	
AS 4736-2006	Biodegradable plastics—Biodegradable plastics suitable for composting and other microbial treatment

*Standard superseded by its updated version

** Withdrawn standard, no replacement

7.3.1 ASTM Standard specifications for characterising compostable plastic products and materials

There are several ASTM standard specifications for defining compostability of plastics under municipal and industrial composting conditions.

ASTM D 6400-04

ASTM D 6400-04 establishes the requirements for identifying plastics and products made from plastics that will compost satisfactorily in industrial and municipal aerobic composting facilities. During 2012, the standard was expanded into and superseded by the new version ASTM D 6400-12, which covers plastics and products made from plastics that are designed to be composted under aerobic conditions in municipal and industrial composting facilities, where thermophilic conditions are achieved. The properties in this specification are those required to determine if end items (including packaging), which use plastics and polymers as coatings or binders, will compost satisfactorily, in large scale aerobic municipal or industrial composting facilities. The standard establishes the requirements for labeling of materials and products, including packaging made from plastics, as “compostable in aerobic municipal and industrial composting facilities”. This later version also highlights that maximum throughput is a high priority to composters, and the intermediate stages of plastic disintegration and biodegradation should not be visible to the end user for aesthetic reasons.

The composting specifications refer to requirements and criteria towards biodegradability, disintegration and environmental safety.

Biodegradation testing requirements & criteria:

- Method for the measurement of biodegradation: the test is carried out using the test methods ASTM D 5338, ISO 14855-1 or ISO 14855-2.

- A plastic product must demonstrate a satisfactory rate of biodegradation by achieving the following ratio of conversion to carbon dioxide within 180 days:
  - 90 % of the organic carbon in the whole item or for each organic constituent, which is present in the material at a concentration of more than 1 % (by dry mass), shall be converted to carbon dioxide by the end of the test period when compared to the positive control or in the absolute.
  - Organic constituents present at levels between 1 to 10 % shall be tested individually.
  - Organic constituents which are present at concentrations less than 1 % do not need to demonstrate biodegradability. However, the sum of such unproven constituents shall not exceed 5 %.

Disintegration testing requirements & criteria:

- Method for the measurement of disintegration during composting: the test is carried out in accordance with ISO 16929 with a minimum vessel volume of 35 l, or ISO 20200 under thermophilic aerobic composting conditions.
- A plastic product is considered to have demonstrated satisfactory disintegration if after twelve weeks (84 days) in a controlled composting test, no more than 10 % of its original dry weight remains after sieving on a 2.0 mm sieve.

Environmental safety testing requirements & criteria:

- The plastic material shall have concentrations of regulated metals less than 50 % of those prescribed for sludges or composts in the country where the product is sold. In the case of United States and Canada there are specific regulated metal concentrations.
- The germination rate and the plant biomass of the sample composts shall be no less than 90 % that of the corresponding blank composts for two different plant species following OECD Guideline 208 with specific modifications found in Annex E of EN 13432. Sample composts generated in accordance with ISO 20200 should not be used for ecotoxicity testing unless the concentration of the test items at the start of testing is in accordance with the requirements of ISO 16929.

Applicability:

- Plastics and products made from plastics that are designed to be composted under aerobic conditions in municipal and industrial aerobic composting facilities, where thermophilic conditions are achieved.

Equivalence:

- This test method is equivalent to ISO 17088-2012

ASTM D 6868-11

Correspondingly, ASTM D 6868-11 specification covers end items that include plastics or polymers where plastic film/sheet or polymers are incorporated (either through lamination,

extrusion or mixing) to paper and other substrates and the entire end item is designed to be composted under aerobic conditions in municipal and industrial composting facilities, where thermophilic temperatures are achieved.

It is intended to establish the specifications for labeling of end items which use plastics or polymers as coatings or binders, as “compostable in aerobic municipal and industrial composting facilities”.

The properties in this specification are those required to determine if end items (including packaging) which use plastics and polymers as coatings or binders will compost satisfactorily, in large scale aerobic municipal or industrial composting where maximum throughput is a high priority and where intermediate stages of plastic biodegradation should not be visible to the end user for aesthetic reasons.

The composting specifications refer to requirements and criteria towards biodegradability, disintegration and environmental safety.

Biodegradation testing requirements & criteria:

- A level of biodegradation for the plastic coatings and additives shall be established by tests under controlled conditions.
- An end item having a plastic coating or additives are considered to have achieved a satisfactory level of biodegradation if the following criteria are met or exceeded:
    - The plastic coating or polymeric additives must meet a satisfactory rate of biodegradation by achieving the following ratio of conversion to carbon dioxide within 180 days (ASTM D 6400):
        - 90 % of the organic carbon in the whole item or for each organic constituent, which is present in the material at a concentration of more than 1 % (by dry mass), shall be converted to carbon dioxide by the end of the test period when compared to the positive control or in the absolute.
        - Organic constituents present at levels between 1 to 10 % shall be tested individually.
        - Organic constituents which are present at concentrations less than 1 % do not need to demonstrate biodegradability. However, the sum of such unproven constituents shall not exceed 5 %.
    - Substrates used in the end item must individually satisfy the following requirements:
        - 90 % of the organic carbon is converted to CO_2 using Test Method D 5338 within 180 days at 58±2°C when compared to the positive control (alternative test methods: ISO 14851:1999, ISO 14852:1999, ISO 14855:1999)
        - End items made of ligno-cellulosic substrates are assumed to be biodegradable by showing that over 95% of their carbon comes from biobased resources using D 6866.
        - Polymers or additives derived from biobased resources that are blended with ligno-cellulosic substrates shall separately demonstrate

that they meet the requirements of subsection 6.3 of specification D 6400, if they are more than 1 % of the dry weight of the end item.

- Any organic constituent present in more than 1 % of the dry weight of the end item shall fulfill the biodegradation requirements of subsection 6.3 of specification D 6400.
- The total portion of organic constituents or additives that do not fulfill the requirements of subsection 6.3 of specification D 6400 shall not exceed 5 % of the end item by weight.

Disintegration testing requirements & criteria:

- An end item will disintegrate during composting such that any remaining residuals (plastic, polymer, or substrate) are not readily distinguishable from the other organic materials in the finished product. Additionally, the material or product must not be found in significant quantities during screening prior to final distribution of the compost. An end item is considered to have demonstrated satisfactory disintegration if after 12 weeks in a controlled composting test, no more than 10% of its original dry weight remains after sieving on a 2mm-sieve.

Environmental safety testing requirements & criteria:

- The end item shall have concentrations of heavy metals less than 50% of those prescribed in the Government Standard "*40 CFR Part 503.13: Standards for the Use or Disposal of Sewage Sludge*".
- The germination rate and the plant biomass of the sample composts shall be no less than 90 % that of the corresponding blank composts for 2 different plant species following OECD guideline 208 with the modifications found in Annex E of EN 13432.

7.3.2 ISO Standard specifications for characterising compostable materials

ISO 17088:2012

ISO 17088 is a standard specification for identification of plastic products and materials as compostable under municipal and industrial composting conditions. The specification ISO 17088:2008 is intended to establish the requirements for the identification and labelling of plastic products and materials, including packaging made from plastics, as "compostable" or "compostable in municipal and industrial composting facilities" or "biodegradable during aerobic composting" (Briassoulis et al. 2010). The labelling will, in addition, have to be conform to all international, regional, national or local regulations (e.g. European Directive 94/62/EC). It has been revised into the ISO 17088:2012. This second edition cancels and replaces the first edition (ISO 17088:2008).

The composting specifications refer to requirements and criteria towards biodegradability, disintegration and environmental safety (negative effects on the finished compost & maximum concentration of regulated metals in compost).

Biodegradation testing requirements & criteria:

- The aerobic biodegradation test should be carried out in accordance with ISO 14855-1, ISO 14855-2 or ASTM D 5338.
- The test period shall be no longer than 180 days.
- The aerobic biodegradability shall be determined for the whole material or for each organic constituent. For organic constituents which are present in the material in a concentration between 1 % and 10 % (by dry weight), the level of biodegradation shall be determined separately. Constituents which are present in a concentration < 1 % do not need to demonstrate biodegradability. The sum of such constituents shall not exceed 5 %.
- The plastic product under test is considered to have demonstrated a satisfactory rate and level of biodegradation if 90 % of the organic carbon (relative to a positive-control reference material) shall have been converted to CO_2 by the end of the test period. Both the positive control and the test sample shall be composted for the same length of time and the results compared at the same point in time after the activity of both has reached a plateau. As an alternative, 90 % (in absolute terms) of the organic carbon shall have been converted to carbon dioxide by the end of the test period.

Disintegration testing requirements & criteria:

- The disintegration test should be carried out in accordance with ISO 16929, ISO 20200, ISO 14855-1 or ASTM D 5338 under thermophilic composting conditions without the CO_2-trapping equipment.
- A plastic product is considered to have demonstrated satisfactory disintegration if, after 84 days in a controlled composting test, no more than 10 % of its original dry mass remains after sieving through a 2.0 mm sieve.

Environmental safety testing requirements & criteria:

- The concentrations of regulated metals and other toxic substances in the plastic product or material shall be less than 50 % of those prescribed for sludges, fertilizers and composts in the country where the final product will be placed on the market or disposed of.
- The plastic product or material shall contain a minimum of 50 % of volatile solids.
- The seedling germination rate and the plant biomass in the test compost shall be no less than 90 % of that of corresponding blank compost to which no test or reference material was added at the start of testing, determined in accordance with OECD Guideline 208 with the modifications specified in Annex E of EN 13432:2000.

Applicability:

- Plastics and products made from plastics that are suitable for recovery through aerobic composting.

Equivalence:

This test method is equivalent to ASTM D 6400-2012

Need for revision of this standard:

Although the biodegradation test includes the conversion of the polymers into biomass and humic substances in addition to carbon dioxide, no recognized standard test methods or specifications exist for the quantification of these conversion products. When such tests and specifications become available, this International standard might be revised.

ISO 18606:2013

In January 2013 another ISO standard was published: ISO 18606 "Packaging and the Environment-Organic Recycling". While ISO 17088 only mentions specifications for plastics, ISO 18606 covers a broader range of products as it encompasses criteria for packaging (= plastic packaging, paper packaging, food packaging, packaging from bagasse, etc.). Packaging is considered as recoverable by organic recycling only if all the individual components meet the requirements. Therefore, packaging is not considered recoverable by organic recycling if only some of the components meet the requirements of ISO 18606:2013. However, if the components can be easily, physically separated before disposal, then the physically separated components can be individually considered for organic recycling.

ISO 18606:2013 is applicable to organic recycling of used packaging but does not address regulations regarding the recoverability of any residual packaged goods.

ISO 18606:2013 does not provide information on requirements for the biodegradability of used packaging which ends up in the soil environment as litter, because littering is not considered as a recovery option. It is also not applicable to biological treatment undertaken in small installations by householders.

For each of the packaging components the following four aspects are addressed: (1) biodegradation, (2) disintegration during biological waste treatment process (i.e. composting), (3) negative effects on the biological process and (4) negative effects on the quality of the resulting compost, including the presence of high levels of regulated metals and other substances hazardous to the environment.

Biodegradation testing requirements & criteria:

- Chemically unmodified packaging materials and constituents of natural origin, such as wood, wood fibre, cotton fibre, starch, paper pulp, bagasse or jute shall be accepted as biodegradable without testing.
- The aerobic biodegradation test should be carried out in accordance with ISO 14855-1 or ISO 14855-2. (As an alternative also ISO 14851 and ISO 14852 are allowed.)
- The test period shall be no longer than 180 days.
- The aerobic biodegradability shall be determined for the whole material or for each organic constituent. For organic constituents which are present in the material in a concentration between 1 % and 10 % (by dry weight), the level of biodegradation shall be determined separately. Constituents which are present in a concentration < 1 % do not need to demonstrate biodegradability. The sum of such constituents shall not exceed 5 %.

- A constituent or the material is considered to have demonstrated a satisfactory rate and level of biodegradation if 90 % of the organic carbon (relative to a positive-control reference material) shall have been converted to CO_2 by the end of the test period. Both the positive control and the test sample shall be composted for the same length of time and the results compared at the same point in time after the activity of both has reached a plateau. As an alternative, 90 % (in absolute terms) of the organic carbon shall have been converted to carbon dioxide by the end of the test period.

Disintegration testing requirements & criteria:

- The disintegration test should be carried out in accordance with ISO 16929. Alternatively also a lab-scale test ISO 20200 can be used. In case of differing results, ISO 16929 shall prevail. Also a full-scale industrial composting testing can be used as long as it is well defined and uses equivalent test duration, sample concentration and analytical evaluation of disintegration. In contrast to ISO 17088, ISO 14855-1 and ASTM D 5338 under thermophilic composting conditions without the CO_2-trapping equipment are not allowed anymore in order to determine the disintegration.
- Packaging is considered to have demonstrated satisfactory disintegration if, after 84 days in a controlled composting test, no more than 10 % of its original dry mass remains after sieving through a 2.0 mm sieve. The particles or pieces which do not differ from the compost for colour, structure, dimension, moisture feeling and brightness/gloss are considered to be compost.

Environmental safety testing requirements & criteria:

- The concentrations of regulated metals and other substances hazardous to the environment in the packaging shall not exceed the limits specific to the country where the final product will be placed on the market or disposed (US = 50 % of those prescribed in 40 CFR 503.13, Canada = BNQ 9011-911-I/2007, EU = EN 13432, etc.).
- The packaging or packaging components shall contain a minimum of 50 % of volatile solids.
- The seedling germination rate and the plant biomass in the test compost shall be no less than 90 % of that of corresponding blank compost to which no test material was added at the start of testing, determined in accordance with OECD Guideline 208 with certain modifications. Compost to be used for plant toxicity tests shall be prepared according to ISO 16929 using a 10 % sample input concentration.

The procedure for applying ISO 18606:2013 is contained in ISO 18601. The relevance of this recently presented standard to the biodegradation and compostability of packaging will become clear during the next years.

7.3.3 European Norms specifications for characterising compostable packaging, plastic products and materials

There are two European norms available for identification of plastic products and packaging materials as compostable under municipal and industrial composting conditions.

EN 13432:2000, EN 14995-2006

The European norm EN 13432:2000 with the title *"Packaging—requirements for packaging recoverable through composting and biodegradation—test scheme and evaluation criteria for the final acceptance of packaging"* defines the characteristics a material must own in order to be claimed as "compostable" and, therefore, recycled through composting or organic solid waste.

The European norm EN 13432:2000 is a reference point for all European producers, authorities, facility managers and consumers. Unlike the ASTM standards, this standard can be applied to any packaging or packaging component, and is not limited to plastic materials. Correspondingly, EN 14995-2006 also refers to the evaluation of the compostability of plastics.

The scope of testing materials under EN 13432-2000 and EN 14995-2006 is to ensure that a compostable packaging or plastic material, respectively, must have the following characteristics:

Biodegradation testing requirements & criteria:

- Biodegradability tested according to ISO 14855
- Percentage of biodegradation shall be at least 90 % in total or 90 % of the maximum degradation of reference material after a plateau has been reached for both test and reference materials over a period of 6 month, when biodegradation is defined according to Specifications Standard EN 13432.

Disintegration testing requirements & criteria

- Tested in a controlled pilot-scale test or in a full-scale treatment facility
- The test material is degraded, together with organic waste, for 3 months. After this time, the compost is sieved with a 2 mm sieve. The residues of the tested material with dimensions higher than 2 mm are considered as not having disintegrated. This fraction must be less than 10 % of the initial mass.

Environmental safety testing requirements & criteria:

- Low levels of heavy metals and other toxic and hazardous substances and a minimum of 50 % of volatile solids.
- No negative influence on the composting process is permitted. Chemical-physical parameters by which the compost quality shall be defined are: volumetric weight (density), pH, salinity, total dry solids, volatile solids, total N, ammonium N, P, Mg and K.
- Moreover, ecotoxic effects on 2 higher plants shall be determined by comparing compost produced with and without addition of test material (OECD test 208, modified). The germination rate and plant biomass of the sample composts of both plant species should be more than 90 % of those from the corresponding blank compost.

Need for revision of this standards:

There are different opinions on this and suggestions to amend the EN 14046 standard to 90 % biodegradation within 90 days; A proposal was made also to amend the EN 13432 standard to 90 % disintegration within a maximum of 12 months. As a compromise, extension of the timescale could be accompanied by a reduction of the temperature at which the tests are carried out – from 58°C to 38°C [Perchard D (2005) CEEES workshop, Biodegradable Polymers – Where are the Limits, 3 November 2005; CEEES-Confederation of the environmental engineering societies http://www.ceees.org/auxiliary/biopolymer051103.pdf Accessed 16 April 2009].

7.3.4 Belgian Royal Decree for Acceptance of Compostable and Biodegradable Plastic Materials

As analytically reported in the work of Briassoulis et al. 2010, in Belgium a royal decree became effective in July 2009 that defines three properties of a product depending on its end-of-life management option: compostable, home compostable and biodegradable. This Belgium decree determines the requirements and standards that have to be fulfilled by each category of product:

Biodegradation testing requirements & criteria:

- Not chemically modified materials from natural origin do not need to be tested for biodegradation.
- Not significant organic components on condition that their cumulative percentage is < 5 % and their individual share is < 1 % do not need to be tested for biodegradation.
- Test methods ISO 14588, ISO 14851, ISO 14852 or ISO 17566 shall be used.
- Pursuant to the EN 13432 standard, at least 90 % absolute or 90 % relative (referring to microcrystalline cellulose) material have to be broken down by biological action within 6 months

Disintegration testing requirements & criteria:

- Test method ISO 16929 or a test in a full-scale treatment facility shall be used.
- Less than 10 % of the plastic remains on a 2 mm screen within 84 days is required.

Environmental safety testing requirements & criteria:

- Minimum volatile solids content: 50 % on dry weight basis.
- Restrictions with regard to heavy metals (Zn, Cu, Ni, Cd, Pb, Hg, Cr, Mo, Se & As) and fluorine.
- Substances, which are dangerous for humans or the environment (KB 17/7/2002), may not be used in the product.
- No negative influence on the composting process is permitted. Chemical-physical parameters by which the compost quality shall be defined are: volumetric weight (density), pH, salinity, total dry solids, volatile solids, total N, ammonium N, P, Mg and K.

- Moreover, ecotoxic effects on 2 higher plants shall be determined by comparing compost produced with and without addition of test material (OECD test 208, modified according to EN 13432). The germination rate and plant biomass of the sample composts of both plant species should be more than 90 % of those from the corresponding blank compost.

7.3.5 Australian Standard AS 4736:2006

According to the Australian Standard AS 4736:2006 with the title *"Biodegradable plastics-Biodegradable plastics suitable for composting and other microbial treatment*", it is demonstrated that compostable plastics must meet the following criteria:

General requirements:

- Minimum volatile solids content: 50 %
- Heavy metals content < limit values

Biodegradability testing requirements & criteria:

- Test methods ISO 14855, ISO 14851 or ISO 14852 shall be used.
- The test material shall degrade at least 90 % in total or of the maximum degradation of a suitable reference material within 180 days (after a plateau has been reached).

Disintegration testing requirements & criteria:

- ISO 16929 shall be used.
- Less than 10% of the plastic remains on a 2 mm screen within 12 weeks is required.

Environmental safety testing requirements & criteria:

- Ecotoxic effects on two higher plants shall be determined by EN 13432 Appendix E.
- Germination rate and plant germination of the plants in the test composts shall be more than 90 % of those from the corresponding blank compost.
- Ecotoxic effect on earthworms shall be determined by ASTM E1676.
- The difference in the morbidity or mean weight of surviving earthworms between the test compost and the control compost shall not be larger than 10 %.

7.3.6 Other national specifications for characterising compostable packaging, plastic products and materials

There are several national specifications established for the identification of plastic products and materials as compostable under municipal and industrial composting conditions. Some illustrative examples are briefly presented.

In Italy a previous standard (UNI 10785), concerning the compostability of plastics, was replaced by its European version: UNI EN 14995-2006: plastics - evaluation of compostability - test scheme and specifications (Briassoulis et al. 2010).

Also a German standard with regard to compostability (DIN V 54900-1998) was developed.

7.4 Labelling

Labeling of qualified products is an important communication and promotional tool, e.g. from producers to other involved parties such as the consumers or municipal staff. Table 77 lists some of currently available labels, their certifying bodies, and the required standards. The OK COMPOST labeling system of AIB Vinçotte in described in detail in this chapter.

Table 77. Overview of certification schemes and labels for environmentally degradable polymeric materials and plastics.

Logo	Organization	Norm	Symbol
Seedling logo	European Bioplastics	EN 13432, ASTM D 6400, EN 14995 and ISO 17088	
OK compost logo	Vinçotte	EN 13432	
DIN-Geprüft Compostable logo	DIN CERTCO	EN 13432, ASTM D 6400, EN 14995, ISO 17088 and AS 4736	
Compostable logo	Biodegradable Products Institute (BPI)	ASTM D 6400 and ASTM D 6868	
Cedar Grove Composting logo	Cedar Grove	based on ASTM D6400 and ASTM D6868 with additionally mandatory full-scale test	
GreenPla logo	Japan Bioplastics Association (JBPA)	Green PLA certification scheme	

Logo	Organization	Norm	Symbol
Australian seedling logo	Australasian Bioplastics Association (ABA)	AS 4736	
National logo in Italy	Consorzio Italiano Compostatori (CIC)	based on EN 13432 with additionally mandatory full-scale test	
National logo in Finland	Jätelaitosyhdistys	EN 13432	
National logo in Sweden	SP Technical Research Institute of Sweden	SPCR 141	
National logo in Spain (Catalonia)	Departament de Medi Ambient i Habitatge	EN 13432 and EN 14995	

7.4.1 VINÇOTTE Certest Products - OK compost - Initial acceptance tests

AIB-VINÇOTTE International S.A./N.V. has established a set of specifications for identifying the compostability of products and materials under municipal or industrial composting conditions. These specifications are based on the European standard specification with regard to compostability EN 13432 (2000). To be eligible for “OK Compost” certification, materials or products shall obtain the following properties:

Biodegradation testing requirements & criteria:

- At least 90 % of the test material has to be biodegraded within 6 months.

Disintegration testing requirements & criteria:

- At least 90 % of the product should be able to pass through a 2 × 2 mm mesh sieve after 12 weeks.

Environmental safety testing requirements & criteria:

- Criteria of EN 13432 need to be fulfilled (heavy metals, fluorine, volatile solids and plant toxicity), but some clarifications are mentioned in the document of VINÇOTTE:
  - The concentration of test material to be added to the compost must be 10% on wet mass basis (of which 9% as powder or granulates) according to Standard ISO 16929 or Standard EN 14045.
  - An assessment of the negative effects (ecotoxicity) of constituents accounting for less than 0.1 % of the dry weight of a material or product does not have to be checked provided the total percentage of these constituents does not exceed 0.5 % of the dry weight of this material or product.
  - All constituents and their maximum concentrations as specified on the positive list (TS-OK-10) are regarded as fulfilling the compost quality requirements.
  - All food additive approved ingredients are regarded as fulfilling the compost quality requirements
  - Constituents that appear on the (candidate) list of substances of very high concern are not accepted. This must be verified for all constituents that are not tested for ecotoxicity, do not appear on the positive list and are not food additive approved ingredients.

7.5 Discussion and critical review

ASTM D 6400 (2012) is comparable or in harmony with standards in Europe, Japan, Korea, China, and Taiwan. The specifications set by the ASTM D 6400 (2012) standard along with the three standards to which it is referred to, are comparable (but not the same) to what has been developed by the European Committee for Standardization (CEN) in Europe, and in harmony with the relevant ISO standards for compostable plastics, moving the industry closer to global standards.

7.5.1 Biodegradability

The key specification of EN 13432:2000 is the requirement of at least 90 % biodegradation in total or at least 90 % biodegradation of the maximum degradation of the reference material after a plateau stage for both reference and test materials has been reached , as measured by ISO 14855-2012 (controlled composting) test method. The biodegradation test described in ISO 14855-1-2012 is similar to the test method in ASTM D 5338-2011, with a few differences. Thus, ISO 14855-1-2012:

- Does not require the negative control vessels; therefore, only 9 vessels are required instead of 12.
- Includes the determination of percentage of biodegradation based on weight loss as an optional result to support the value determined from carbon dioxide evolution.

Biodegradability is determined by measuring the amount of CO_2 produced over a certain period by the test material. The main point of differentiation between the various international standards is the percentage of biodegradation required for compliance. ASTM D 6400-12 and ISO 17088:2012 require both that 90 % absolute or relative biodegradation of the whole item or for each organic constituent, which is present in the material at a concentration of more than 1% (by dry mass) within 180 days. Moreover, all organic constituents present at levels between 1 % and 10 % shall also meet the 90 % biodegradation criteria. EN 13432 (2000) is less strict as organic constituents present at levels between 1 % and 10 % do not need to be tested separately. Furthermore there are on-going discussions on revising the European Norm EN 13432-2000 (and EN 14046-2003) so that it requires 90 % biodegradation within 90 days instead of 180 days (Perchard D, 2005). This is an important issue that is under discussion at ISO level. The compliance requirements for the key standards, adapted from (*Briassoulis et al, 2010*), are shown in Table 78.

Table 78. Standards Compliance Requirements under controlled aerobic composting conditions.

Standard Test Method							Standard Specification
Standard Test Method	**Method**	**Test Validity**	**Temperature (°C)**	**C:N Ratio (by weight)**	**Sample quantity and media**	**Water Content (% weight of water/dry weight)**	**Composting Requirement -Time frame to achieve biodegradation requirements (months)**
ASTM D5929-96 (Reapproved 2009)	Cumulative carbon dioxide evolved and/or oxygen consumed	-The total oxygen uptake for the control reactors should exceed 80g. If this is not observed over the 45 days then the test must be regarded as invalid and should be repeated with new inoculum. -After 45 days take 10 g of solids and dilute it in 50 ml of water. If pH<7 and VFA (volatile fatty acids method D2908) >2 g/kg dry sample then test is invalid	**40°** Compost temperature not to exceed 65° C. (If exceeded increase the compost aeration rate through higher recirculation)	**≤ 40:1** Synthetic MSW (simulated municipal waste media inoculated with active MSW compost) should be adjusted with urea not to exceed a C:N ratio of 40:1 if 35 g of C are added to this mixture	12g inoculum and quantity of test material required to obtain 50g of ThO_2 <u>Sample size</u> <3x3x12 cm <u>Sample per vessel:</u> Quantity of sample to contain 50 g of theoretical	Water is added through the buffer solution to reach: 102.8 % wgt/wgt of water to dry solid MSW media.	***May use applicable Specification Standards*** ***(ASTM D6400, D6868, ISO 17088, Vincotte, EN 13432)***

				(Organic C of test sample plus MSW; Total N of test mixture)	O_2 uptake Compost media per vessel: 269.37 g synthetic MSW, 12 g inoculum from active MSW unit, 276.92 ml buffered water		
ASTM D5338-11	Analysis of evolved carbon dioxide.	- ≥70 % biodegradation of the reference material after 45 days; - the difference between the % biodegradation of the reference material in the different vessels is less than 20 % at the end of the test;	**58**±2	**<40** Organic C for both the inoculum and test substance combined Total nitrogen content of the test mixture (if needed add ammonium chloride)	600 g dry solids of inoculum with 100 g of dry solids coming from the sample Inoculum quality: -Ash content <70%	Addition of distilled water so that the dry solids content in the mixture is ~ 50% of wet solids	***Based on ASTM D6400, D6868, ISO 17088*** *Disintegration:* after 84 days <10% of the original weight of a plastic product remains after sieving on a 2.0 mm sieve *Biodegradation:* 90% of the organic carbon (absolute or relative) shall be converted to CO_2 after 180 days ***Vincotte, EN 13432*** *At least 90% of the*

					- pH 7-8.2 - Total dry solids: 50 % - 55 % -To produced more than 50 mg but less than 150 mg of CO_2 per gram of volatile solids (mean values) after 10 days of incubation.		*materials have to be broken down by biological action within 6 months* *Time frame to achive biodegradation*: 45 days, but incubation time may be extended if significant biodegradation of the test substance is still being observed
ASTM D6340-07	Determination of rate and degree of biological oxidation of carbon in radiolabelled plastic materials in	$^{14}CO_2$ measured in this test method is a direct indication of the oxidation of the sample. The compost formula for the simulation of MSW in this test	**58**±5	Compost, designed to stimulate MSW organic matter Should contain the biochemical	200-300 mg of plastc in ~300 g compost	Moisture content should be adjusted to 55-60%	***May use applicable Specification Standards*** ***(ASTM D 6400, D 6868, ISO 17088, Vincotte, EN 13432)***

	composting or aqueous environment	method will generally give repeatable results. Depending upon the objectives of the test, include within each test series a standard preparation of known degradation rates and make test comparisons.		ingredients found in MSW (lignified cellulose, protein, natural inoculum, soluble carbohydrates and buffering capacity sufficient to maintain a neutral to slightly basic pH)			
ISO 14855-1:2012 **and** **EN 14046:2003**	Analysis of evolved CO_2.	- ≥70 % biodegradation of the reference material after 45 days; - the difference between the % biodegradation of the reference material in the different vessels is less than 20 % at	58±2	10-40 Organic C for both the inoculum and test substance combined Total nitrogen content of the test mixture (if needed	Two alternatives: <u>Inoculum:</u> Dry mass of inoculum: dry mass of test material = 6:1 <u>Activated Vermiculite:</u>	Test mixture shall have the same water content as the inoculum	***Based on ASTM D6400, D6868, ISO 17088*** *Disintegration:* after 84 days <10% of the original weight of a plastic product remains after sieving on a 2.0 mm sieve *Biodegradation:* 90% of the organic carbon (absolute or relative) shall be converted

		the end of the test; - the inoculum in the blank has produced more than 50 mg but less than 150 mg of CO_2 per gram of volatile solids (mean values) after 10 days of incubation.		addurea)	Dry mass of activated vermiculite: dry mass of test material = 4:1 <u>Inoculum quality</u>: -Volatile solids > 15 % of the wet solids -Total dry solids shall be between 50 % and 55 % of the wet solids -To produced more than 50 mg but less than 150 mg of CO_2 per gram of		to CO_2 after 180 days ***Vincotte, EN 13432*** *At least 90% of the materials have to be broken down by biological action within 6 months* *Time frame to achieve biodegradation:* 6* months

					volatile solids (mean values) after 10 days of incubation.		
ISO 14855-2:2007	Gravimetric measurement of the amount of CO_2 evolved	- ≥70 % biodegradation of the reference material after 45 days; - the difference between the % biodegradation of the reference material in the different vessels is less than 20 % at the end of the test; - if more than 2g volatile fatty acids per kg of total dry solides has been formed, the test is regarded as invalid	58±2	15	Total dry solids of the inoculum and the total dry solids of the test material should preferably be about 6:1.	Test mixture: 80-90 % of the WHC of the test mixture	***Based on ASTM D6400, D6868, ISO 17088*** *Disintegration:* after 84 days <10 % of the original weight of a plastic product remains after sieving on a 2.0 mm sieve *Biodegradation:* 90 % of the organic carbon (absolute or relative) shall be converted to CO_2 after 180 days ***Vincotte, EN 13432*** *At least 90% of the materials have to be broken down by biological action within 6 months*

(*) There are different opinions on this and suggestions to amend the EN 14046 standard to 90 % biodegradation within 90 days; A proposal was made also to amend the EN 13432 standard to 90% disintegration within a maximum of 12 months. As a compromise, extension of the timescale could be accompanied by a reduction of the temperature at which the tests are carried out – from 58°C to 38°C.

In both ISO 14855-1-2012 and ASTM D5338-2011 methods, the amount of CO_2 evolved due to biodegradation can be measured using acid–base titration, or by using a direct measurement such as infrared or gas chromatography. ISO 14855-2-2009, which measures mineralization of a polymer by a gravimetric method, is similar to 14855-1-2012 except for the method of CO_2 measurement: In 14855-2 method the system is capable of determining carbon dioxide directly from the change in mass of a carbon dioxide trap. The carbon dioxide trap consists of columns filled with soda lime, soda talc and anhydrous calcium chloride. An ammonia trap (dilute sulfuric acid) and a water trap (silica gel and calcium chloride) are required between the composting vessel and the carbon-dioxide-absorbing column.

In ISO14855-1 specification, the apparatus for the determination of carbon dioxide is designed to determine carbon dioxide directly or by complete absorption in a basic solution and determination of the dissolved inorganic carbon (DIC). If the carbon dioxide in the exhaust air is measured directly, for example with a continuous infrared analyser or a gas chromatograph, exact control or measurement of the air-flow rate is required.

In addition, inert materials such as sea sand or vermiculite can be used with the compost for providing better aeration and retention of moisture content. The mixture of compost and sea sand or vermiculite is periodically taken out from the closed system to turn or agitate to prevent channelling of air in the biodegradation vessel.

Furthermore, while the temperature profile is continuously at 58°C and the maximum test duration is 6 months in ISO 14855-1-2012, ISO 14855-2-2007, the CEN test procedures and ASTM D 5338-11 , a lower temperature of 40°C and a much shorter period of 45 days is applied.in the case of ASTM D 5929-09

7.5.2 Disintegration

Materials are tested for disintegration in the form in which they will be ultimately used. Either a controlled pilot-scale test or a test in a full-scale aerobic composting treatment facility can be used (*Briassoulis et al, 2010)*. The disintegration of the test material is evaluated on the basis of the total dry solids by comparing the retrieved fractions of the test material > 2 mm and the amount tested. Less than 10% in weight should remain on a 2 mm screen for most standards at the end of the test period. Disintegration tests cannot differentiate between biodegradation related disintegration and abiotic disintegration[14], but instead demonstrate that sufficient disintegration of the test materials has been achieved within the specified testing time (*Briassoulis et al, 2010)*. For disintegration, the European norm EN 13432 suggests testing the materials in controlled pilot-scale or full-scale tests, while the international standard ISO 17088 (2012) refers to ISO 16929, ISO 20200, ISO 14855-1 and ASTM D 5338 under thermophilic composting conditions without the CO_2-trapping equipment. ISO 18606 only refers to ISO 16929, ISO 20200 or a full-scale industrial composting test. ASTM D 6400-12 refers to ISO 16929 and ISO 20200 under thermophilic

[14] Abiotic degradation may be induced by UV or thermal in dry or humid conditions, water, salt solution etc. The ASTM D6954, Tier 1, BS 8472 and ISO 4611, ISO 4892-2 Standards define the requirements for abiotic degradations as follows:
Average molecular, weight Mw < 10 000, gel fraction < 5 %, elongation at break ≤ 5 % of the original value

aerobic composting conditions. But the rest is similar, i.e., the final compost is screened with a 2 mm sieve, and the material needs to pass the disintegration criterion (i.e., no more than 10% of the original dry weight is recovered after 12 weeks of composting according).

7.5.3 Industrial compostability

According to the review of *Briassoulis et al, 2010* ASTM and ISO standard guidelines are limited to the compostability evaluation (biodegradation, disintegration, compost quality) of plastic materials or a plastic material from a package; however, the standards EN 13432-2000 and EN 14995-2006 developed by the European Committee for Standardization (CEN) provide detailed guidelines for evaluation of biodegradability and compostability of packaging and packaging components, and plastics, respectively, based on their characterization, biodegradability, disintegration, and compost quality/ecotoxicity. For the compost quality or ecotoxicity test, physical and chemical parameters such as density, total dry and volatile solids, salt content, and pH, have to be determined to show that the tested packaging does or does not have negative effects on the compost quality. Only the plant growth test, based on OECD guideline 208, is included in EN 13432-2000 for ecotoxicity. The results (germination numbers and plant biomass) of the compost with the tested material and the blank compost are compared.

7.5.4 Home compostability

In spite of some debate on the value of home composting – some saying it is a valuable and sustainable way of waste reduction and management, others saying it is an important source of greenhouse gases if improperly managed while also hygienic aspects could be a concern – it is an important waste management option in several countries. The major difference with industrial composting is the temperature profile: although heat generation is the same, heat losses are much bigger because of smaller volume and maximum temperatures reached are much lower. For some biodegradable polymers, which need a thermal trigger to start hydrolysing, this makes a big difference.

So far, no international standards exist which regard to specifications for home compostability. Only in Australia a norm was published: AS 5810-2010 “Biodegradable plastics – Biodegradable plastics suitable for home composting”. This norm is largely inspired by the OK compost HOME programme of Vinçotte which was published earlier. In essence, the requirements are largely the same as for industrial compostability with as major difference the necessity to determine biodegradation at ambient temperature as well as disintegration. For the latter a qualitative or visual evaluation of disintegration is sufficient and a quantitative determination with calculation of a mass balance after sieving and retrieval is not needed if such information is already available for industrial composting.

8 Relation towards REACH

The regulation (EC) No 1907/2006 of the European parliament and of the council of 18 December 2006 describes an integrated system for Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH). This regulation is based on the principle that it is the task for the manufacturers, importers and downstream users to ensure that they manufacture, place on the market or use substances that do not adversely affect human health or the environment.

Chemical substances will be subject to registration followed by a defined procedure that will be applied by the European Chemicals Agency (ECHA) in Helsinki. This agency is responsible for the accepting or rejecting new chemical substances.

Registration is the essence of REACH: no data = no market. All substances, which are produced or imported in a quantity of 1 tonne or more per year shall be submitted for registration to the Agency. For some product groups a registration duty does not exist. The required information includes:

(1) General registrant information
(2) Identification of the substance
(3) Information on manufacture and use(s) of substance
(4) Classification and labelling
(5) Guidance on safe use
(6) Information on exposure
(7) Physicochemical properties of the substance
(8) Toxicological information
(9) Ecotoxicological information

The amount of required information depends on the production volumes. Standard requirements are outlined for the lowest tonnage level (1-10 tonnes) and every time a new tonnage level (10-100 tonnes, 100-1000 tonnes, > 1000 tonnes) is obtained, additional requirements are added.

As an illustration more information is given with regard to the required ecotoxicological information required per tonnage level in Table 79.

Table 79. Overview of the required ecotoxicological information per tonnage level.

Tonnage level	Required ecotoxicological information
1 – 10	Aquatic toxicity: Short-term toxicity testing on invertebrates Aquatic toxicity: Growth inhibition study with aquatic plants Biotic degradation: Ready biodegradability
10 – 100	Aquatic toxicity: Short-term toxicity testing on fish Aquatic toxicity: Activated sludge respiration inhibition testing Abiotic degradation: hydrolysis as a function of pH Fate and behaviour in the environment: adsorption/desorption

Tonnage level	Required ecotoxicological information
100 – 1000	Aquatic toxicity: Long-term toxicity testing on invertebrates Aquatic toxicity: Long-term toxicity testing on fish Biotic degradation: Simulation testing on ultimate degradation in surface water Biotic degradation: Soil simulation testing (substances with high potential for adsorption to soil) Biotic degradation: Sediment simulation testing (substances with high potential for adsorption to sediment) Biotic degradation: Identification of degradation products Fate and behaviour in the environment: Bioaccumulation in aquatic species Fate and behaviour in the environment: Further information on adsorption/desorption Effect on terrestrial organisms: Short-term toxicity to invertebrates Effect on terrestrial organisms: Effects on soil micro-organisms Effect on terrestrial organisms: Short-term toxicity to plants
> 1000	Effect on terrestrial organisms: Long-term toxicity testing on invertebrates Effect on terrestrial organisms: Long-term toxicity testing on plants Long-term toxicity to sediment organisms Long-term or reproductive toxicity to birds

The next step is the evaluation. There are two types of assessment: dossier evaluation and substance evaluation.

The final step is authorisation. For substances of very high concern a permission of the Commission is required. Substances of very high concern include substances with CMR (carcinogenic, mutagenic or toxic for reproduction) properties, PTB-substances (Persistent, Bioaccumulative and Toxic), vPvB-substances (Very Persistent and Very Bioaccumulative) and other substances, which have serious and irreversible consequences towards human health and the environment. Only if the risks can be controlled, a permission will be given. If this is not the case, the Commission will evaluate if the use of the substance has a high social and economic importance and if there exist alternatives. Based on these criteria, the Commission will decide if a permission is given.

9 Conclusion

Freshwater aerobic aqueous environment

Based on the literature review of the different biodegradation test methods in an aqueous aerobic freshwater environment it can be concluded that a sufficiently broad range of measurement techniques already exists. Not each test method is suitable to test bio-lubricants or bio-solvents due to the fact that bio-lubricants are often poorly water soluble and that bio-solvents are often volatile. Therefore specific biodegradation test methods need to be selected taking into account these characteristics. Special care should be given towards the addition of these substances (poorly water soluble or volatile) to the testing system and if required special addition techniques should be used. It also needs to be evaluated if the usual water-soluble reference materials (aniline, sodium benzoate, etc.) should be replaced by a poorly water soluble or a volatile alternative.

The review of the freshwater biodegradation test methods also revealed a few items, which should be further investigated in order to optimize the test methods. Among others influence of inoculum source (geographical variations, seasonal variations, etc.) on the biodegradability potential and variability of the results, interpretation of variability due to nitrification, necessity of the addition of a nitrification inhibitor, determination of the minimum amount of replicates, etc. should be further investigated.

With regard to environmental safety, it can also be concluded that a sufficiently broad range of testing methods towards freshwater organisms on different trophic levels (bacteria, algae, freshwater aquatic plants, crustacean and fish) already exists. For bio-lubricants and bio-solvents, additional attention is especially needed towards the addition of poorly water soluble bio-lubricants and volatile bio-solvents to the testing systems as this can influence the test results.

The review revealed that specifications need to be developed towards bio-lubricants and bio-solvents. A technical report with useful recommendations for a specification towards bio-lubricants (CEN/TR 16227) and the specifications for the EU Ecolabel for lubricants can be taken as a guideline in order to develop a specification towards bio-lubricants. This should also be developed for bio-solvents.

A labelling system for bio-lubricants has been developed by different organisations, but no European or international labelling systems especially towards bio-solvents is developed yet. This should be developed taking into account parameters like biodegradability, environmental safety, minimum bio-based content, etc.

Work with regard to the above mentioned aspects will be performed in the framework of task 6.2 of KBBPPS.

Marine aerobic aqueous environment

From the literature review on the biodegradation test methods in a marine aerobic environment, it can be concluded that there exist considerably less biodegradation test methods when compared to a freshwater environment. However, a sufficiently broad range of methods exists in order to determine the biodegradation in a marine environment. Not all

methods are suitable in order to evaluate the biodegradability of bio-lubricants and bio-solvents, but a suitable measurement technique can be selected taken into account the specific properties (volatility and/or solubility) of a bio-lubricant or a bio-solvent. Special care should be given towards the addition of these substances (poorly water soluble or volatile) to the testing system and if required special addition techniques should be used.

The review of the marine biodegradation test methods also revealed a few items, which should be further investigated in order to optimize the test methods. Among others the inoculum (natural seawater versus artificial seawater), the addition of nutrients, the difference between conditions in different parts of the sea (supralittoral, eulittoral, sublittoral benthic, deep sea benthic, pelagic & buried in the sediments), etc. should be further investigated.

With regard to marine environmental safety it can be concluded that less tests were developed when compared to the freshwater environment especially on OECD level. ISO and ASTM are already more progressive as more guidelines towards marine organisms were developed. The guidance documents towards the sample preparation and the interpretation of the results of toxicity tests for difficult substances, poorly water soluble substances and lubricants should be taken into account.

Currently no standard specifications towards more environmentally friendly alternatives for lubricants and solvents used in a marine environment are developed yet. These specifications need to be developed. This can be based on the EU Ecolabel for lubricants, which encompasses already marine applications.

Work with regard to the above mentioned aspects will be performed in the framework of task 6.2 of KBBPPS.

Anaerobic environment

Based on the review on the existing biodegradation standards in an anaerobic environment, it can be concluded that there exists a sufficiently broad range of standards in order to determine the degree of anaerobic biodegradation in aquatic environments, high-solids anaerobic-digestion environments and landfill environments. Suitable methods need to be selected for bio-lubricants and bio-solvents.

Lubricants and solvents can reach anaerobic aquatic environments in wastewater treatment plants, but high-solids anaerobic-digestion environments and anaerobic landfill environments are probably not considered as environments in which lubricants and/or solvents are often spilled or disposed. These standards are more suitable for biopolymers.

Toxicity towards anaerobic bacteria can be evaluated based on existing methods. These methods can be used for lubricants and solvents. The guidance documents towards the sample preparation and the interpretation of the results of toxicity tests for difficult substances, poorly water soluble substances and lubricants should be taken into account.

For biopolymers, which are degradable in anaerobic digestors, it might be necessary to evaluate if toxic residuals remain present in the produced digestate. Further research is needed in order to determine how this should be done.

Currently no standard specifications nor labelling systems are developed for products which are biodegradable in an anaerobic digester (e.g. biopolymers). This is mainly caused by the fact that there exists a wide variation in the construction and the operation of anaerobic digestion systems. The construction and operation systems can be divided into categories based on two parameters: (1) temperature (mesophilic and thermophilic) and (2) dry solids content (wet systems and dry systems). A standard specification should be developed, which includes criteria per operation system. This standard specification should form the basis for a new labelling system.

The labelling systems for lubricants do not refer to anaerobic environments in order to evaluate biodegradability and environmental safety. However, taken into account that a high percentage of ultimately aerobically biodegradable components needs to be present in the major part of the labelled products, it is expected that the labelled products will already be degraded before they come in contact with anaerobic environments.

Soil environment

A few international norms are available about testing biodegradability in soil. Their current weakness concerns their reliability in the cases of intentional incorporation of biodegradable materials in the soil under real conditions. Such a practice is widely used in agriculture and concerns the vast majority of applications where testing of biodegradation in soil is a key prerequisite with respect to both, environmental and food safety aspects. Considering the existing biodegradable plastics in agriculture and the effective life management of the plastics in use at the agricultural field, only few norms have suitable tests that could be adapted for testing biodegradability in soil under real field conditions. The standardised criteria, parameters and testing methodologies for the characterization, labelling and validation of the agricultural plastic waste streams with respect to possible biodegradation in soil suggest that some major revisions are needed, before a new (i.e. revised) universal norm and improved standard testing methods become available for testing agricultural plastics for biodegradation under real, and highly variable, soil conditions. Based on the analysis of the different norms and their content it appears necessary to incorporate provisions for transferability of results to different soils, validation of test through a positive reference and set prerequisites for soil media. Furthermore, terminology and technical specifications vary and need to be harmonised. Long term biodegradation in soil prediction is another open issue (*Briassoulis and Dejean, 2010*). It is clarified though that there is no need for new testing method for biodegradation in soil. However, the existing standards for biodegradation in soil have to be improved and adapted in order to take into account the need for transferability of results to different soils under real field conditions. This goal should be achieved in a way that the standard testing method allows for repeatability of results by various laboratories. Another issue raised recently concerns the possibility to measure the possible production of new cell biomass or incorporating it into the humus that is not “measured” through the current testing methods.

An improved revised universal norm should be based on testing method(s) that include a well-documented range of several typical soil types and a well-defined range of conditions bracketing the majority of soil types and prevailing conditions, for a specific region. A basic requirement to characterise a product as “biodegradable product” is the necessary time for

biodegradation. This is particularly important for agricultural applications where intentional incorporation in soil is the key motivation for the use of biodegradable products. Such a practice, if biodegradation rate is slow, may result into excessive accumulation of materials in the soil. The time use at the field will depend on the type of crop and on the farmer practices but it is common that the biodegradable plastics should have mineralised in the soil before the soil cultivation practices start for the next year crop. In addition, a range of grades for different biodegradation time under different latitudes or climates can be established, following the analogous procedure established by the standard for ageing of plastic films under different geographic areas (solar irradiance). For example in the French norm a set of grades is adopted to define the required biodegradation time of the different mulching films (*Briassoulis and Dejean, 2010*). In addition all relevant safety (e.g. heavy metals) and ecotoxicity requirements for soil biodegradable products should be met (as for example required by the French Norm, OK biodegradable SOIL).

Concerning bio-based lubricants, solvents etc, there is a need for an appropriate testing method that should be based on proper adaptation of testing methods for biodegradation of bio-based polymers in soil, combined with specifications and labeling analogous to those already available for biodegradable in soil plastics. Work in this direction will be performed in the framework of task 6.2 of KBBPPS and will be based on adaptation of existing standards for biodegradation in soil of biobased plastics.

Industrial composting environment

Many norms concerning testing of compostable plastics have been developed at national and international level. Some are about plastic materials others about products like packaging. The media and conditions of testing cover mainly the conditions designed for industrial composting facilities, and only a few concern home composting conditions (*Briassoulis et al. 2010)*. Also, only a few of the existing norms will be suitable, after appropriate revisions, to be adapted to testing biodegradable/compostable agricultural plastic products under farm composting conditions. Farm composting involves techniques not foreseen by the industrial or home composting methods. Farm composting is particularly relevant to biodegradation of biobased materials used in agriculture and specifications differ depending on the cultivations (e.g. organic farming requirements are more strict according to the relevant legislation).

The terminology and the biodegradability validation criteria under composting conditions, such as the threshold percentages of biodegradation and disintegration, the time and temperature, and the ecotoxicity, differ to some degree for the various norms and standard testing methods. Criteria for the establishment of a new integrative norm for compostable plastics used in agricultural applications need to be defined (*Briassoulis et al. 2010)*. Such a norm may include for example home composting and farm composting or only farm composting test methods and specifications where the last one may be based on the existing test methods adapted to practices and conditions of farm composting.

An industrial composting environment is probably not considered as an environment in which lubricants and/or solvents are often spilled or disposed. These standards are more suitable for biopolymers.

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