Open-BIO
Opening bio-based markets via standards, labelling and procurement

Work package 5

In situ biodegradation

Deliverable N° 5.2:
Round robin test on freshwater biodegradation

Public summary

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List of abbreviations, acronyms and used standards

ASTM	American Society for Testing and Materials
ASTM D5271	Standard Test Method for Determining the Aerobic Biodegradation of Plastic Materials in an Activated-Sludge-Wastewater-Treatment System
ASTM D5864	Standard Test Method for Determining Aerobic Aquatic Biodegradation of Lubricants or Their Components
ASTM D6139	Standard Test Method for Determining the Aerobic Aquatic Biodegradation of Lubricants or Their Components Using the Gledhill Shake Flask
ASTM D6731	Standard Test Method for Determining the Aerobic, Aquatic Biodegradability of Lubricants or Lubricant Components in a Closed Respirometer
ASTM E1720	Standard Test Method for Determining Ready, Ultimate, Biodegradability of Organic Chemicals in a Sealed Vessel CO2 Production Test
ATU	AllylThioUrea
AUA	Agricultural University of Athens
BOD	Biological Oxygen Demand
CEN	European committee for standardisation (Comité Européen de Normalisation)
DOC	Dissolved organic carbon
EN 14047	Packaging – Determination of the ultimate aerobic biodegradability of packaging materials in an aqueous medium – Method by analysis of evolved carbon dioxide
EN 14048	Packaging – Determination of the ultimate aerobic biodegradability of packaging materials in an aqueous medium – Method by measuring oxygen demand in a closed respirometer
EN 14987	Plastics – Evaluation of disposability in waste water treatment plants. Test scheme for final acceptance and specifications
ISO	International Organization for Standardization
ISO 7827	Water quality – Evaluation of the “ready”, “ultimate” aerobic biodegradability of organic compounds in an aqueous medium – Method by analysis of dissolved organic carbon (DOC)
ISO 9408	Water quality – Evaluation of ultimate aerobic biodegradability of organic compounds in aqueous medium by determination of oxygen demand in a closed respirometer
ISO 9439	Water quality – Evaluation of ultimate aerobic biodegradability of organic compounds in aqueous medium – Carbon dioxide evolution test

ISO 10707	Water quality – Evaluation in an aqueous medium of the “ultimate” aerobic biodegradability of organic compounds – Method by analysis of biochemical oxygen demand (closed bottle test)
ISO 10708	Water quality – Evaluation in an aqueous medium of the ultimate aerobic biodegradability of organic compounds – Determination of biochemical oxygen demand in a two-phase closed bottle test
ISO 14593	Water quality – Evaluation of the ultimate aerobic biodegradability of organic compounds in aqueous medium – Method by analysis of inorganic carbon in sealed vessels (CO_2 headspace test)
ISO 14851	Determination of the ultimate aerobic biodegradability of plastic materials in an aqueous medium – Method by measuring the oxygen demand in a closed respirometer
ISO 14852	Determination of the ultimate aerobic biodegradability of plastic materials in an aqueous medium – Method by analysis of evolved carbon dioxide
ISO 17088	Specifications for compostable plastics
ISO 18606	Packaging and the environment - Organic recycling
KBBPPS	Knowledge Based Bio-based Products’ Pre-Standardization
LDPE	Low Density PolyEthylene
OECD	Organisation for Economic Co-operation and Development
OECD 301A	Ready Biodegradability - DOC Die-Away
OECD 301B	Ready Biodegradability - CO_2 Evolution (Modified Sturm Test)
OECD 301C	Ready Biodegradability - Modified MITI Test (I)
OECD 301D	Ready Biodegradability - Closed Bottle
OECD 301E	Ready Biodegradability - Modified OECD Screening
OECD 301F	Ready Biodegradability - Manometric Respirometry
OECD 310	Ready Biodegradability - CO_2 in sealed vessels (Headspace Test)
PBSe	PolyButylene Sebacate
PBSeT	PolyButylene Sebacate-co-butylenTerephtalate
PHB	Poly(3-HydroxyButyrate)
prEN 16807	Liquid petroleum products - Bio-lubricants - Criteria and requirements of bio-lubricants and bio-based lubricants

$ThCO_2$	Theoretical carbon dioxide production
ThOD	Theoretical Oxygen Demand
TC	Total Carbon
TOC	Total Organic Carbon
TS	Total Solids (= dry matter = dry solids)
TSS	Total Suspended Solids
VS	Volatile Solids
VSS	Volatile Suspended Solids

1 Public summary

Open-Bio is a research project funded by the European Commission within FP7 (*7th Framework Programme for Research and Technological Development*). The goal is to investigate how bio-based products can be integrated into the market, using standardisation, labelling and procurement. Work Package 5 of Open-Bio investigates biodegradability test methods for bio-based products in several natural environments: soil, freshwater and marine environment. The objective of task 5.1 "Developing additional biodegradability test data" of the Open-Bio project is the collection of a series biodegradation data in freshwater, while in task 5.3 "Biodegradability in freshwater test refinement" the objective is the development of a testing scheme for a broad category of bio-based products. For several bio-based products (e.g. detergents, rinse-off cosmetics, plastic protection layers for detergents for dishwashers and washing machines, lubricants, textiles, flushables, etc.) biodegradability in freshwater is an interesting characteristic as such products will anyway be disposed in freshwater.

This work is a follow-up of work carried out earlier in Work Package 6 of European project KBBPPS, in which the focus was mainly on lubricants. The literature study performed in KBBPPS (Deliverable N° 6.1: Report on current relevant biodegradation and ecotoxicity standards) showed that a broad range of freshwater biodegradation test methods is available on OECD, ISO, CEN and ASTM level. These test methods are based on different measurement techniques (dissolved organic carbon, CO_2 production, O_2 consumption, dissolved oxygen or inorganic carbon) and they are developed on a vertical level per product category (e.g. organic compounds, plastics, packaging, lubricants, etc.). An example is given below.

OECD	•Chemicals: OECD 301 A up to F and OECD 310
ISO	•Plastics: ISO 14851 (O_2 consumption) & ISO 14852 (CO_2 production) •Organic compounds: ISO 7827, ISO 9408, ISO 9439, ISO 10707, ISO 10708, ISO 14593, etc.
CEN	•Plastics : EN ISO 14851 (O_2 consumption) & EN ISO 14852 (CO_2 production) •Packaging materials: EN 14047 (CO_2 production) & EN 14048 (O_2 consumption)
ASTM	•Organic chemicals: ASTM E1720 •Plastics: ASTM D5271 •Lubricants: ASTM D5864, ASTM D6139 and ASTM D6731

In order to evaluate the biodegradation in freshwater of a (bio-based) product, it is consequently not required to develop a completely new test methodology with a new measurement technique only for bio-based products. The existing measurement techniques can be used in order to develop a horizontal test methodology for a broad range of bio-based products. Taken into account that a bio-based product can be a lubricant, a plastic, a paperboard, an organic compound, etc. test methods with measurement techniques, which can determine the biodegradability of a broad category of products, were selected. Certain measurement techniques were immediately excluded (e.g. dissolved organic carbon as this technique is not suitable to determine the biodegradability of insoluble bio-based products or dissolved oxygen as this technique is not suitable to determine the biodegradability of lubricants which might stick to the probe) as they are not applicable for a broad range of products. Only test methodologies based on carbon dioxide production or oxygen consumption are suitable for a broad range of products. Two methodologies were developed: (1) *Bio-based products - Determination of aerobic biological degradation of bio-based products in an aqueous solution - Test method based on O_2 consumption*, which is based on OECD 301F, ISO 9408, ISO 14851, EN 14048 and ASTM D6731, and (2) *Bio-based products - Determination of aerobic biological degradation of bio-based products in an aqueous solution - Test method based on CO_2 production*, which is based on OECD 301B, ISO 9439, ISO 14852, EN 14047, ASTM D5864 and ASTM D6139. The horizontal freshwater test methodologies incorporate the principles of the test methods on which they are based and include some improvements in order to increase the repeatability of the test methodologies (e.g. only one inoculum source is allowed instead of several inoculum sources, in the test methodology based on oxygen consumption an extra option is added to determine simultaneously the carbon dioxide production by measuring the carbon dioxide captured in the absorbent as a kind of double-check, etc.).

During the revision of the available biodegradation test methodologies on OECD, ISO, CEN and ASTM level, it became clear that almost in all test methodologies results of interlaboratory tests are missing. No or little information is available related to the performance of these freshwater test methodologies. In order to overcome this deficiency, interlaboratory tests were performed in the Open-Bio project using the horizontal test methodologies. Originally, it was the intention to perform the validation of the freshwater biodegradation methodology in collaboration with several invited recognized laboratories. However, due to the fact that the performance of such freshwater biodegradation test requires a large testing capacity and a significant amount of work during at least 2 months, no testing laboratories were found that were willing to participate to the validation work without receiving any financial compensation. Therefore, only Open-Bio partners Agricultural University of Athens, Novamont and OWS participated to the interlaboratory testing.

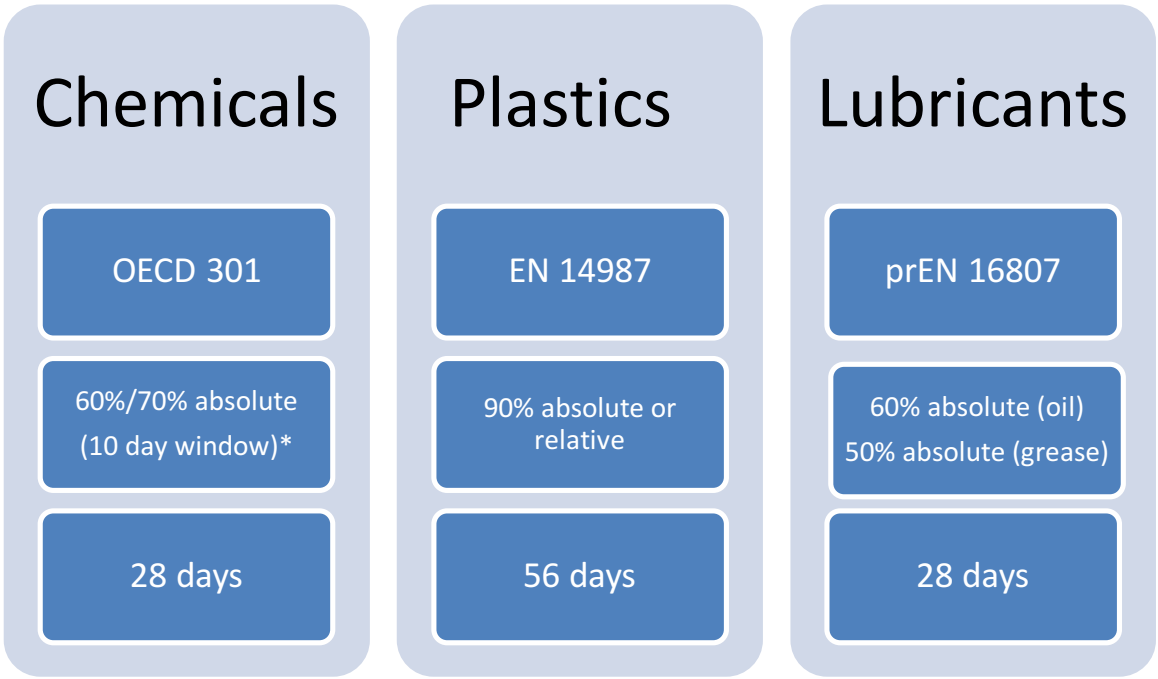
Similar to the biodegradation validation testing performed in soil and in the marine environment, polymers LDPE (Low Density Polyethylene; negative reference material), PHB copolymer (Poly(3-HydroxyButyrate)), PBSe (PolyButylene Sebacate) and PBSeT (PolyButylene Sebacate co butylene Terephtalate) were used for the interlaboratory test.

In general, it can be concluded that no significant anomalies were observed. For the major part of the performed tests no significant overproduction of carbon dioxide was observed (almost no biodegradation percentages > 100%) and the carbon dioxide production in the negative reference (= LDPE) reactors was comparable to the blank reactors. Moreover, in spite of the fact that different inoculum (= sludge) sources were used collected in different waste water treatment systems in different countries, the carbon dioxide production in the blank reactors was rather comparable amongst the different laboratories. Furthermore, it was also noticed that the difference in biodegradation between the different polymers was identical in all laboratories. Following biodegradation trend was observed: cellulose >/= PHB > PBSe > PBSeT > LPDE. Only for PBSeT it was noticed that the biodegradation behaviour varied significantly between laboratories and even between different replicates in the same laboratory. This was also observed in the marine pelagic biodegradation testing, but not in the soil biodegradation testing. This polymer probably needs a specific bacterial consortium in order to start the biodegradation.

The results of the performed interlaboratory freshwater biodegradation tests could be useful input for standardisation groups working on freshwater biodegradation of (bio-based) polymers (e.g. ISO/TC 61/SC 5 Physical-chemical properties, which was responsible for the development of ISO 14851 and ISO 14852). During the BioPlastics symposium of ISO/TC 61 meeting in Berlin (September 19, 2016), the interlaboratory research was already presented to ISO/TC 61. Moreover, the modifications (e.g. related to inoculum source, carbon dioxide double-check in test method based on oxygen consumption, etc.) that were incorporated in the developed horizontal test methodology: *Bio-based products - Determination of aerobic biological degradation of bio-based products in an aqueous solution - Test method based on O_2 consumption* based on research performed during the KBBPPS and Open-Bio project, were forwarded to ISO/TC 61/SC 5 Physical-chemical properties when ISO 14851 (test method for plastics based on oxygen consumption) was opened for revision. The ISO test method for plastics based on carbon dioxide production (ISO 14852) was not opened for revision during the project. Similar improvements could be incorporated in ISO 14852. Moreover, ISO/TC 61/SC 5 should also consider to revise the test item quantity as prescribed by ISO 14852 as the performed tests illustrate that biodegradation of cellulose tested in a 100 mg TOC/l concentration proceeds slower when compared to cellulose tested in a 20 mg TOC/l concentration. The biodegradation pattern observed for cellulose was not in line with the normal behaviour of positive reference materials. Most probably this is caused by the fact that the microorganisms and nutrients concentrations are too low for 100 mg TOC/l.

Besides the observation that freshwater biodegradation test methods were developed per product category, it was also observed during the KBBPPS literature study that pass levels

were historically developed per product category (an illustration is shown below). This leads to different pass levels and different allowed test durations, which might cause confusion in the market. It would be better to harmonise the pass levels or at least to explain why a deviation is made from the already existing documents. Moreover, the existing criteria could be improved from an environmental point of view by requiring that when blends of different constituents are tested (e.g. lubricants, which contain base oils and additives, or plastics, which might contain different polymers and additives, etc.), test constituents which are present in a concentration between 1% and 10% should individually be tested on biodegradation in order to avoid that not biodegradable constituents are accumulated in nature. This would be in line with criteria for compostable materials as prescribed by ISO 17088 and ISO 18606.



* The pass levels for ready biodegradability are 70% removal of DOC and 60% of ThOD or $ThCO_2$ production for respirometric methods. These pass values have to be reached in a 10-d window within the 28-d period of the test. The 10-d window begins when the degree of biodegradation has reached 10% DOC, ThOD or $ThCO_2$ and must end before day 28 of the test.

The two methods were improved following the interlaboratory study and the texts are presented in this report. Because of the detail in this deliverable, a separate, more concise report that only presents the resulting draft standards can be used beyond CEN or ISO. Deliverable D5.4 can be provided to specific standardization development organizations or labs that can use it as basis of their standards or further work.

Website: www.open-bio.eu