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

Work package 5
In situ biodegradation

Deliverable N° 5.7:

Marine degradation test of bio-based materials at laboratory and mesocosm scale assessed

Public summary

Delft, November 2016

prepared by:
Novamont S.p.A.; Organic Waste Systems (OWS); HYDRA Institut für Meereswissenschaften AG (Hydra); Agricultural University of Athens (AUA); Lettinga Associates Foundation (LeAF); Institut des Sciences Analytiques (ISA); Wageningen UR Food, Biobased Research (DLO-FBR); BASF

M. Tosin, M. Pognani & F. Degli Innocenti (Novamont S.p.A.); B. De Wilde & N. Mortier (OWS nv) D. Briassoulis, A. Mistriotis & A. Pikasi (AUA); E. Schuman & M. van Eekert (LeAF); E. Biedermann (BASF); M.van der Zee (DLO-FBR); P. Jame & A. Bulete (ISA)

Novamont S.p.A.
Via G. Fauser 8, 28100 Novara Italy
Tel. +39 (0)321.699.611

HYDRA Institut für Meereswissenschaften AG
Seestraße 8, D-80802 München, Germany
Tel.: +498913060131 and +390565988027

Email:
maurizio.tosin@novamont.com; nike.mortier@ows.be; m.weber@hydra-institute.com; briassou@aua.gr; miriam.vaneekert@wur.nl; eynat.biedermann@basf.com; maarten.vanderzee@wur.nl; patrick.jame@isa-lyon.fr

Partner website:
www.novamont.com; www.ows.be; www.hydra-institute.com; www.aua.gr; www.leaf-wageningen.nl; www.isa-lyon.fr; www.wageningenur.nl/fbr; www.basf.com

Project website:
www.open-bio.eu

The research leading to these results has received funding from the European Union Seventh Framework Programme (FP7/2007-2013) under grant agreement n° KBBE/FP7EN/613677.

General

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. One part of the project (WP5: In situ biodegradation) deals with research on the biodegradation behaviour of bio-based polymers in natural environments: soil, freshwater and the marine environment.

The biodegradation of materials is still difficult to predict in the marine environment. The ability to biodegrade can vary a lot and depends on the properties of the materials and on the (local) environmental conditions of the marine ecosystem. Bio-based polymers are not biodegradable per se and biodegradation needs to be assessed for each product. A lot of the work currently carried out within Open-Bio, is dedicated to get more insight in how to deal with biodegradability issues of bio-based polymers in different environmental settings. A solid testing scheme for the biodegradation of plastics in the marine environment does not exist so far. There are considerably less test methods available in the literature for marine than for freshwater or soil environments and further investigations are needed to explain the differences observed between the various marine habitats.

Currently, few test methods for the assessment of the biodegradation of materials in the marine environment are available from ISO (International Organisation for Standardisation) and ASTM (American Society for Testing and Materials). No European CEN (European Committee for Standardisation) test method has been developed so far. The available test methods concern the biodegradation under aerobic conditions. One test method only addresses disintegration and it is not suited to measure biodegradation (ASTM D7473-12). The only standard specification defining requirements concerning disintegration, biodegradation and environmental impacts in marine conditions (ASTM D7081-05 in combination with test method ASTM D6691-09), has been withdrawn and is currently under revision. This standard specification was targeting biodegradability in aerobic seawater. Early 2015, the Belgian private non-profit agency, Vinçotte, introduced the certification scheme for the “OK biodegradation MARINE label” based on the criteria of ASTM D7081-05.

So far, biodegradation test methods for polymers in the marine environment are very specific (only natural seawater was considered) and poorly standardised. As compared to: freshwater, soil and compost conditions, the marine environment, especially seawater, is considered less aggressive from a biodegradation point of view. A major difference between marine environment and soil is the biofouling (colonization by micro and macroorganisms) effect on the biodegradation process which has not been studied in depth in the marine environment and its effects remain practically unknown.

In order to better understand the great variation within the entire marine ecosystem, a set of well-defined marine habitats needed to be identified and characterised according to their physical, chemical and biotic properties, to obtain a baseline for conditions as natural as possible to be applied for each habitat standardised tests. These conditions and the possible modifications needed to obtain relevant test schemes are reviewed in deliverable 5.5

produced within the framework of the Open-Bio project[1]. As a result within the Open-Bio project new testing methods are currently being developed for the biodegradation in the sandy eulittoral (intertidal beach) zone, in the sublittoral (benthic) zone at the water/seafloor interface and in the pelagic (free water column) zone.

In nature there are several more sets of conditions that are important: many marine areas are very low in oxygen (hypoxic) or free from oxygen (anoxic), vast regions are covered with very fine sediment (mud) and are cold. Notwithstanding the high diversity of different marine habitats, the study of all of them is out of the scope of the project. The main goal of this deliverable (5.7) was to develop new foreground knowledge by defining new testing schemes based on the adaptation of existing test methods for relevant environments in order to be able to present the new laboratory, mesocosm and field test results and compare them comprehensively. A secondary aim was to improve the new developed laboratory test methods through on inter-laboratory tests and on the results of parallel field and mesocosm tests in order to obtain better and more robust testing schemes for the three marine habitats (eulittoral, benthic and pelagic) considered in the project.

<u>Laboratory scale testing</u>

The overall goal of this task was to develop laboratory methodologies and dedicated testing schemes for marine biodegradation of plastics under three distinct marine environments. To reach this, four test materials: LDPE (Low Density Polyethylene; reference material), PBSe (PolyButylene Sebacate), PBSeT (PolyButylene Sebacate co butylenTerephtalate) and PHB (Polyhydroxyalkanoate Copolymer) were tested at laboratory scale, following three different newly developed test methods (under evaluation). The biodegradation in Benthic (interface sandy sediment/seawater) and Eulittoral condition (intertidal beach sandy sediment) was measured according to two new methods proposed in the project, while the biodegradation in Pelagic condition (free seawater) was determined based on a modified version of ASTM D6691 (lower nutrient content). Five laboratories carried out the biodegradation tests measuring the CO_2 production or the O_2 consumption using seawater and sediment coming from Salamis Island (Greece) and Elba Island (Italy). The tests were repeated for two consecutive years.

During the first year of testing some problems were identified and improvements were implemented following their evaluation. The proposed test methods were proven suitable to measure the rate of biodegradation of plastics in the three different marine conditions but optimization of the test parameters (e.g. amount and shape of test item, amount of inoculum, addition of nutrients, etc) is required to shorten test times and improve the reproducibility.

In general, from the test materials point of view, PHB was biodegraded in all conditions. It is therefore considered a good positive control. On the contrary, as expected, LDPE remained completely intact up to the end of the test. The polyesters PBSe and PBSeT showed a steadily increasing biodegradation with the time of their exposure to Eulittoral and Benthic conditions while inhomogeneous trends were observed under Pelagic conditions.

[1] Deliverable 5.5: Review of current methods and standards relevant to marine degradation. Downloadable from www.biobasedeconomy.eu

Concerning the inocula: the benthic sediment, in general, showed a high organic content indicating high microbial activity, leading to clearly distinguishable biodegradation but also a tendency of an overproduction of CO_2 especially during the first year. Furthermore, this high biological activity in the case of a readily biodegradable material as PHB combined with the low diffusivity of air in the water led to a formation of anaerobic zones on the surface of the sediment (in the lab). The Eulittoral sediment having low organic content produced generally reliable results even if the biodegradation rate was rather low. Finally the free seawater (pelagic) showed reliable results for PHB but not for the polyesters. The reason of this is probably the very low concentration of microorganisms in this environment.

Concerning the test method point of view it appeared that the Benthic method was characterized by not clear and unequivocal results due to CO_2 overproduction. Progress was made during these two years to improve the Benthic test method, but the test methodology still needs further improvement. Further research is advised, following the suggestions reported in this deliverable (such as administering test items in powder form, adding additional nutrients to the test medium, increasing the test item concentration, refining the sediment pre-treatment phase, etc.). Regarding the Eulittoral method fewer modifications are needed. The main problem observed was attributed to the low biodegradation rate. The use of powdered test samples in conjunction with additional nutrients appeared to result in an increase of the biodegradation rate, but this also needs to be further investigated. The Pelagic test method also seems be promising as similar biodegradation trends were obtained in both years. Only the biodegradation results obtained with aliphatic aromatic copolyesters were not reproducible, probably due to the low microorganism concentration. Further research is needed to improve the Pelagic test method.

Mesocosm scale testing

The goal of the second part of this task was to develop a stand-alone mesocosm test to assess the degradation of polymers under partially controlled marine conditions. A closed-circuit tank system which mimicked the same three shallow-water habitats as in the laboratory tests, namely eulittoral (intertidal beach scenario), pelagic (water column scenario) and benthic (sublittoral seafloor scenario, sediment-water interface) within a single system was developed. The mesocosms were placed in a climate chamber where light, temperature, water movement, tides and water quality could be controlled complementary to laboratory tests. The volume of several hundred litres per habitat, and the use of natural sediment and seawater provided experimental conditions that were closer to the natural environment, and thus also allowed a link to field tests. Three independent mesocosm tank systems were run in parallel, two times consecutively, for the duration of one year each. The same polymers as in the laboratory tests (PHB, LDPE, PBSeT and PBSe) were tested.

The developed mesocosm system was well suited for the intended tests. Its simple construction and low technical effort proved to be reliable and efficient. Generally, all tested polymers, except the negative control LDPE, showed disintegration, with a differentiation by habitat and polymer type. However, there was a high variability in the rate of disintegration between replicates and between the experiments in year 1 and 2. Part of this heterogeneity could be explained by inhomogeneities in e.g. water movement, illumination and fouling, or slight differences in the system between the years, e.g. sediment grain size. Another part of the heterogeneity could not be explained *ad hoc*, and also be attributed to natural variations of the matrices water and sediment, and the microbial community therein. The observation of this high variability in a partially controlled test system and the analyses of the possible causes provided important information for the validation of laboratory and field tests.

The biodegradation of polymers, defined as the remineralisation to CO_2 (and/or CH_4) and water, and the conversion to biomass can only be directly measured in closed test systems where either CO_2 development or O_2 consumption is monitored, but not in the open tanks of the mesocosms. Therefore, material disintegration of polymer samples was estimated by the determination of lost area % over time. This technique provided a simple method to assess disintegration of polymer films, but had some intrinsic inaccuracies. The method was based on the visible lack of material and thus could only produce results once advanced disintegration had led to the perforation of the film. To assess the polymer degradation independently from eventual fragmentation there were also analytical methods applied to assess polymer disintegration on a macromolecular level like GPC and MALDI-TOF, but the results obtained did not show their suitability.

Methods that determined the mechanical properties of the tested materials at different exposure times gave satisfying results in case of slow degradation, but could no longer be applied for samples at an advanced stage of disintegration. Linked to the reliable determination of degradation is another question that could not be sufficiently addressed. Up to now no method could be applied that allowed to directly link the polymer biodegradation and specimen disintegration in the lab test to the disintegration of the same polymer in the mesocosm tests. Such a methodological link would be useful for a calibration of the tests in the laboratory and in mesocosms, and furthermore also for field tests, and should be developed in further projects.

One disadvantage of the mesocosm tests performed was the relatively slow disintegration achieved at the applied temperature of 21 °C, which extended the necessary experiment duration to up to 1 year. Slight modifications of the conditions within a natural range, e.g. higher temperatures or the addition of nutrients could accelerate the disintegration and render the tests more practical.

The outcome of this part of the project is the proposal of a mesocosm test system suited to be applied independently from direct access to the sea with relatively low technical and financial effort. The mesocosm system can fill the gap of knowledge on the performance of biodegradable polymers under environmentally relevant marine conditions, in three of the most important coastal habitats. It can be developed into an additional test method to link the series of laboratory tests to field tests in the sea.

Final conclusion

The ensemble of tests will open the possibility for materials and products to be tested under marine conditions in a reproducible environmentally relevant manner, and help society, producers and policy makers to verify claims of biodegradability. Obviously they would still require further interlaboratory assessment in ISO, CEN and ASTM and by having more labs installing the techniques and practicing with them.