



# **Open-BIO**

## **Opening bio-based markets via standards, labelling and procurement**

**Work package 5**  
**In situ biodegradation**

### **Deliverable N° 5.6:**

## **Validation of lab and mesocosm tests against field experiments**

### **Public Summary**

Version: 1

Novara, October 2016

#### **prepared by:**

Novamont S.p.A.; HYDRA; Agricultural University of Athens (AUA); Organic Waste Systems (OWS);  
Lettinga Associates Foundation (LeAF); Wageningen UR Food, Biobased Research (DLO-FBR);

M. Tosin, M. Pognani & F. Degli Innocenti (Novamont S.p.A.); M. Weber, C. Lott, D. Makarow & B.  
Unger (HYDRA); D. Briassoulis, A. Mistriotis & A. Pikasi (AUA); B. De Wilde & N. Mortier  
(OWS nv) E. Schuman & M. van Eekert (LeAF); M. van der Zee (DLO-FBR)

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

#### **e-mail:**

maurizio.tosin@novamont.com; m.weber@hydra-institute.com; briassou@aua.gr;  
nike.mortier@ows.be; miriam.vaneeekert@wur.nl; maarten.vanderzee@wur.nl

#### **Project website:**

[www.open-bio.eu](http://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.*

*The sole responsibility for the content of this publication lies with the authors. It does not necessarily reflect the opinion of the European Communities. The European Commission is not responsible for any use that may be made of the information contained therein.*



## 1 Public summary

Open-Bio is a research project funded by the European Commission within FP7 (7<sup>th</sup> Framework Programme for Research and Technological Development). The goal is to investigate how bio-based products can be integrated into the market, using standardization, labelling and procurement.

One part (WP5) of the project was the pre-normative research to develop testing methods and specifications on the biodegradability of bio-based polymers in soil, freshwater and the marine environment. One of the objectives of WP5 was to focus on marine biodegradation, the development of a new test methods (included the preparation of protocols), the testing and the validation of the results. The three deliverables (D 5.5; D 5.7 part 1 and 2 and D 5.8), that anticipated this deliverable 5.6, presented a detailed review of current methods and standards relevant to marine degradation (D 5.5), the new test methods developed to assess the biodegradation at laboratory level (D 5.7 part 1) and the new test methods developed to assess the disintegration at mesocosm scale (D 5.7 part 2), and at field scale (D 5.8). This deliverable (D 5.6) aims to validate the main results obtained from laboratory and mesocosm tests by field test results.

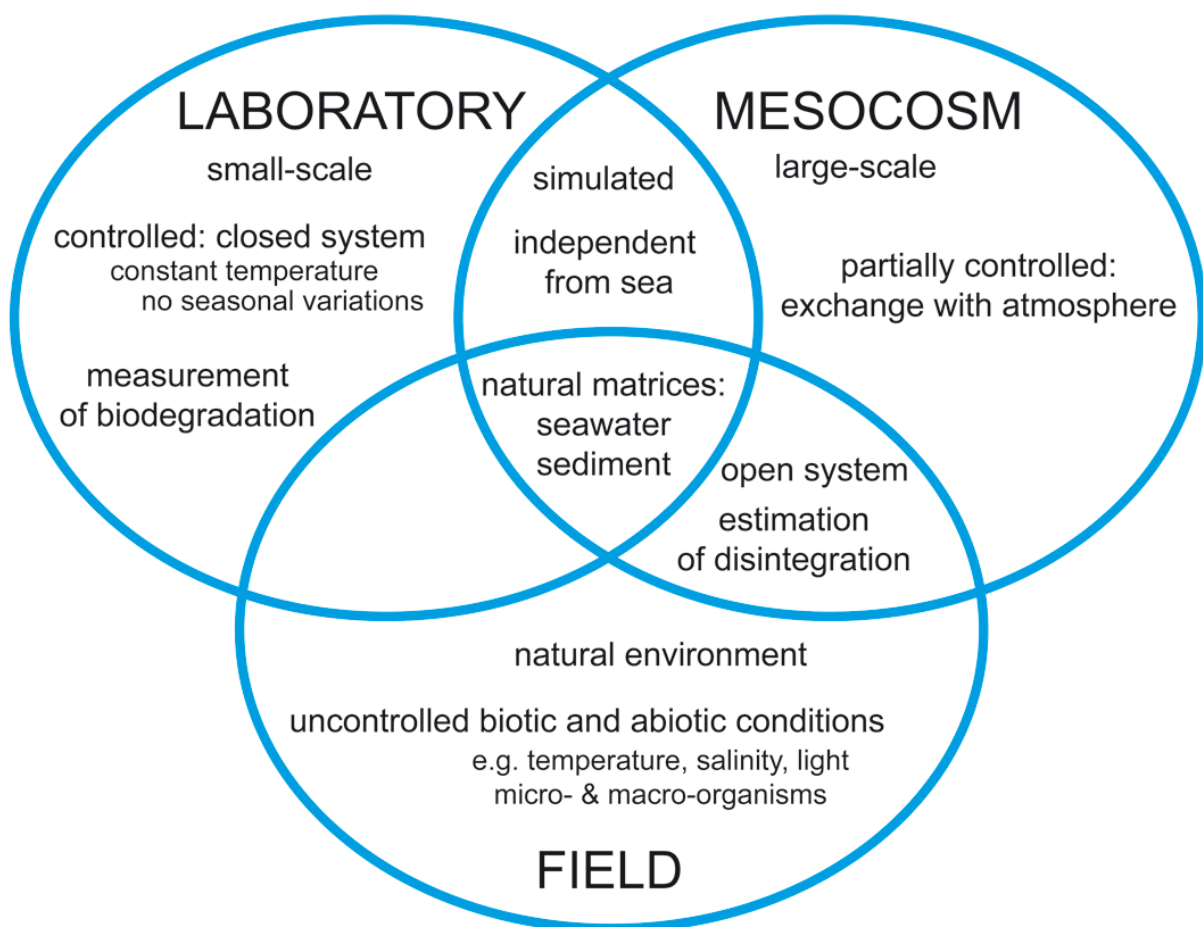
A three-scale approach allows to assess marine biodegradation with environmental relevance. The practical strategy adopted in the project was to simulate the biodegradation under marine conditions at three levels (Figure 1): laboratory, mesocosm and field. The laboratory scale is the simulation in low volume (0.25 – 4 L) with very stringent control of the environmental parameters and the possibility to determine the mineralization of bio-based plastic materials, measured as the amount of organic carbon in the test material transformed to CO<sub>2</sub>. The laboratory tests are performed under optimum conditions (e.g. temperature, nutrient content, etc.), thus these tests are “accelerated” tests. In parallel the disintegration of the test materials was evaluated in a closed-circuit system open to atmospheric exchange at mesocosm scale (volume: 400 L), and in open field tests in order to validate the laboratory biodegradation tests.

Two different Mediterranean locations (Elba Island, Italy and Salamina Island, Greece) were considered and three different habitats were selected for the studies. The eulittoral habitat represented the intertidal beach scenario, where the polymer is buried in sand being occasionally wet or dry. The benthic habitat represented the sublittoral seafloor, where the plastic is laying on the sediment surface. The pelagic habitat represented the water column scenario, where the polymer is neutrally buoyant in the free water. Each habitat (for every location) was also used as a source for natural inocula to perform the tests at laboratory and mesocosm scale. Three different biodegradable polymeric test materials (PBSe, PBSeT and PHB as positive control) were used during two cycles of tests (year 1 and year 2). As a negative control a not biodegradable LDPE test material was used.

Laboratory test methods were developed during the first year by a consortium of five partner laboratories and improved during the second year of testing. The final laboratory test systems were proven to be suitable to assess the biodegradability of the test materials with inocula/matrices obtained from three habitats from the two Mediterranean locations. Notwith-

standing the generally satisfactory results, further refinements are needed to reduce the variability and improve the reproducibility of the biodegradation results (see D 5.7 part 1).

Mesocosm tests were conducted to assess the disintegration capacity of the test materials under partially controlled conditions. Mesocosm tests were performed only at Elba Island using the inocula from this location. Two repetitive cycles of disintegration tests were conducted (year 1 and year 2). The developed test systems for all three habitats have been proven technically reliable and suitable to follow the disintegration of test materials for up to 10 months. The results of the disintegration of the tested polymers however were highly variable.



**Figure 1. Description of the environmental and technical conditions of the lab tests (measurement of biodegradation), the mesocosm and field tests (estimation of disintegration), and their interconnectedness.**

Field tests were performed in both Mediterranean locations for two consecutive cycles (year 1 and year 2) with the main goal to follow the disintegration of test items under natural environmental conditions. With the experience of the multiple application of the test systems, and refinement at some of the habitats in one location, field test systems for the tree habitats can be proposed as technically reliable and suitable to assess the performance of biodegradable polymers in the marine environment. However, the results of the disintegration ex-

periments in two repetitions, at two locations and in three habitats showed a high degree of variability, that should be assessed further.

**Natural variability:** Laboratory results do not match all mesocosm and field results. Laboratory tests permitted to determine the inherent biodegradability of the test materials with inocula from all three habitats and both field locations. Laboratory results were in accordance with the results from tests at the Salamina field location (Greece) where strong disintegration of the test items was obtained during both years and for all the three habitats. On the contrary, at the Elba Island field location only a low level of disintegration of the test materials was observed during the planned test duration of 10 months. However, after the extended exposure time of 22 months a substantial disintegration of the test materials was measured. These largely differing results from similar field experiments in the same climate show the natural variation that has to be taken into account when laboratory results are to be evaluated for ecological relevance. The laboratory tests were performed under optimized conditions with the addition of nutrients. The field tests at Salamina Island that produced similar results were conducted using the fish cage structures of an aquaculture facility. Here, elevated nutrient concentrations were reported. In contrast, the tests at Elba Island were carried out at an offshore island in a marine park with nutrient concentrations at or below detection level. This might explain the different disintegration and the match of the Salamina field results with the laboratory results. Another aspect to consider is that test materials exposed to the pelagic habitat were characterized by the growth of a dense layer of organisms that was suspected to play a role for disintegration. At the Elba field location fouling probably caused a substantial delay of the disintegration while at the Salamina field location disintegration was slowed down less.

The heterogeneity that is intrinsic to the complex marine ecosystem made it difficult to define the typical habitat and the typical environmental conditions where the highest disintegration activity might occur.

#### **Concluding remarks:**

- Inherent biodegradability of materials can be demonstrated using laboratory tests. There was no clear effect of sampling location or sampling time of the natural inocula sediment and seawater on the biodegradation rates
- Biodegradation was observed in all habitats, and the maximum biodegradation rate per polymer type varied between the habitats.
- The mesocosm tests developed by one partner are technically ready to be evaluated in a round robin test, in order to agree upon a standardized test system.
- The field tests for all three habitats were technically developed to a stage that can be now universally applied, and finally evaluated in a RR test, and approved to become a standardized set of methods. Different nutrient levels in the water were likely to be responsible for highly different disintegration rates. The formation of fouling in the pelagic zone might have reduced the disintegration of polymers.



- Biodegradation assessed in laboratory tests was in accordance with the disintegration results observed in the field test at Salamina (elevated nutrients) but was not matching with the disintegration shown at the Elba field location (low nutrients).
- Biodegradation cannot be directly assessed in mesocosm and field tests.
- The difference in outcome between the laboratory and the mesocosm/field test can be explained by:
  - Mechanical impact: absent in laboratory and mesocosm.
  - Physical factors: constant in laboratory and mesocosm.
  - Nutrient availability: nutrient addition accelerates biodegradation in the lab.
  - The microbial consortium present: starting point may be the same for all three (lab/meso/field) scales but development of population over time will be very different.
  - Density of microbial population: development is different in closed as opposed to open systems.

**Impact:** The three interconnected test schemes provide a toolset to assess the biodegradation and disintegration of bio-based and other materials in an environmentally relevant manner under marine conditions. The tests allow to verify the claim of marine biodegradability as an inherent material property and thus will lead to better information for producers, procurers, consumers and policy makers.

**Outlook:** The test systems are technically well developed and give usable results. There are some refinements needed to enhance reproducibility, to further confine variability and to accelerate the processes in the controlled systems. Following that, methods and specifications can be proposed for standardization and be inserted into the normation process.

**Future work:** Some aspects still need further development and investigation. The effect of fouling organisms on the biodegradation process in natural settings is not sufficiently understood. Also, generally different ecological settings as e.g. marine biodegradation in the lack of oxygen were not considered here, but may play an important role in the overall assessment of plastic in the seas. This is also true for other habitats like the deep-sea floor which accounts for half of the Earth's surface.

Project website: [www.Open-Bio.eu](http://www.Open-Bio.eu)

