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

Work package 5

In-situ biodegradation

Deliverable N° D5.8:
Marine degradation test field assessment

Public summary

Version: 1

Munich, October 2016

prepared by:
C. Lott, M. Weber, D. Makarow, B. Unger (HYDRA)
A. Pikasi, D. Briassoulis, A. Mistriotis, C. Briassoulis (AUA)
M. Pognani, M. Tosin (Novamont)
A. Bulete, P. Jame (ISA)

HYDRA Institut für Meereswissenschaften AG
Seestrasse 8
D-80802 München
Germany
phone: +498913060131 and +390565988027
E-mail: ifm@hydra-institute.com
Partner website: www.hydra-institute.com

E-mails: m.weber@hydra-institute.com; briassou@aua.gr; maurizio.tosin@novamont.com;
Patrick.JAME@isa-lyon.fr

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.

1 Public summary

Open-Bio is a research project funded by the European Commission within the 7th Framework Programme for Research and Technological Development FP7. The goal was 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) was focussed on research on the biodegradation performance of bio-based polymers in different natural environments: soil, freshwater and the marine environment.

The biodegradation of materials in the marine environment is still difficult to predict. The ability to biodegrade can vary a lot and depends on the properties of the material and on the environmental conditions. Bio-based polymers are not necessarily biodegradable and biodegradation needs to be assessed for each material. A solid testing scheme for the biodegradation of plastics in the marine environment does not exist so far. In the past there was a standard specification with requirements for non-floating biodegradable plastics in the marine environment (ASTM D7081), but this standard specification is currently withdrawn for revision. There are considerably less test methods available in the literature for marine than for freshwater or soil environments, and further investigations are needed to understand the differences in the biodegradation of polymers observed between various marine habitats.

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. This should provide a baseline for conditions as natural as possible to be applied for each habitat in standardised tests. The conditions and the possible modifications needed to obtain relevant test schemes were reviewed in Open-Bio deliverable 5.5 [1]. Following this review new testing methods were developed within Open-Bio. Three coastal habitats were chosen for *in-situ* tests according to environmental relevance and accessibility: The eulittoral habitat where plastic is washed to the beach and covered by sandy sediment, the pelagic habitat where plastic is floating neutrally buoyant in the water column, and the benthic habitat where plastic is sunken to the seafloor.

A three-scale approach was applied in Open-Bio: Laboratory tests on relevant polymers allowed for the development and implementation of new test methods by the direct measurement of biodegradation (= mineralisation into CO_2 and water) as CO_2 production and/or O_2 consumption (D 5.7, part 1). Field tests with the same polymers were conducted to observe the degradation performence in nature. Mesocosm tests functioned as a link between the two (D 5.7, part 2). This report regards the field tests. All three parts were comparatively validated in D 5.6.

The main objectives of the field tests were to determine the disintegration of test materials in order to validate the environmental relevance of existing test conditions for lab and mesocosm tests, to verify lab and mesocosm results, to reflect events in nature, and to link field tests via larger-scale controlled experiments in mesocosms as an artificial but closer-to-

---

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

nature system with standardised small-scale laboratory tests (see D 5.6). Four test materials, LDPE (Low Density Polyethylene; negative reference material), PBSe (PolyButylene Sebacate), PBSeT (PolyButylene Sebacate co butylenTerephtalate) and PHB (Polyhydroxyalkanoate copolymer; positive reference material) were tested at two locations in the Mediterranean Sea, at Salamis Island (Greece) and at Elba Island (Italy). The polymers were tested in the form of films mounted in frames that were exposed in the three habitats. For the pelagic tests the specimens were kept pending in the water column from floats at 10 m (Salamis) and 20 m water depth (Elba). The benthic tests were performed by placing the holder frames on the sandy seafloor at 20 m (Salamis) and 40 m water depth (Elba). The eulittoral tests were performed at a natural beach (Salamis) and in bins filled with beach sand placed in a protected lagoon (Elba). The tests were repeated for two consecutive years.

The developed field test systems are technically reliable: The two sets of experiments conducted on the Islands of Elba and Salamis provided a wealth of results on various aspects of the fate and performance of biodegradable polymers in the marine environment, also on the technical aspects. The test systems were improved and the final experimental setup was technically reliable and allowed to monitor eventual disintegration of the test materials for long exposure times. Technical specifications can now be defined and used for a standardisation process.

Environmental site characterisation is recommended: As test conditions in the field are not controllable, the natural conditions need to be surveyed in order to rank and classify the obtained test results in comparison with tests that apply other methods (lab, mesocosm), or with field tests in other locations worldwide. The metadata collection should comprise at least a physical-chemical characterisation of the matrices seawater and sediment. For the water, temperature, pH, oxygenation, and salinity should be measured regularly. For both water and sediment porewater analyses regarding nutrients like physiologically available N, P, C compounds, and trace elements like Si, Fe, and Mn should be carried out seasonally. Sediment should be subjected to a standard analysis of granulometry, porosity (or water holding capacity), and permeability, and also for available nutrients and trace elements.

Degradation in the sea is heterogeneous: Degradation of the polymers did happen in all experiments at both locations, however in some experiments the degradation was too slow to be measured as disintegration. Especially in the pelagic tests on Elba the materials did not disintegrate substantially, but the mechanical properties were declining over the duration of the tests. The variable degradation rates in different habitats and locations were attributed to the environmental conditions such as the differences in nutrients, the assumed abundance of microorganisms, and also to seasonal and yearly climatic variations. Another part of the heterogeneity was explained with the natural variations of the biological settings in the respective habitat. The matrices water and sediment are naturally heterogeneous both temporally and spatially, e.g. in microbial abundance or seasonal nutrient content. If more knowledge on this is considered relevant, specific studies with experimental manipulations of single parameters are recommended. A tentative experiment towards the role of organismal growth on the polymers has been conducted at the Greek test location.

Biofouling does influence the degradation of plastic: Every surface exposed to the marine environment will be colonised by micro- and macro-organisms, and eventually covered with a biofilm. If conditions are favourable, especially if light and nutrients are available, a thick coating of algae and sessile animals, known as fouling is developing. It was assumed, that this fouling might play a crucial role in the degradation. At Salamis Island additional test specimens were covered in finer dark mesh (geotextile), in order to reduce light and the access of algal spores and animal larvae. The test materials exposed in this way disintegrated faster than the samples prone to fouling. Observational data from specimens of the tests at Elba indicate a differentiation between the polymer surface directly exposed to the environment, including fouling organisms, and the part of the material that has been covered by the holding frame. Not only were light and organism recruitment reduced, but also biogeochemistry was likely to have changed, e.g. the oxygen and pH regimes. This indicated that processes linked to fouling are rather complex and worthwhile to be studied in order to better understand biodegradation in marine conditions. Moreover, this knowledge will be useful to compare field observations and test results with results from standardised laboratory and mesocosm experiments, where fouling is usually low or negligible.

***In-situ* tests require rather long exposure times:** Disintegration under natural marine conditions was mostly low compared to other scenarios, like e.g. soil and fresh water, or laboratory marine tests with higher temperatures and nutrient addition. This implies long experiment durations of many months or even several years until substantial disintegration is measurable, even with thin polymer films. Linked to that is a higher economic effort and delay in the process of knowledge gathering, which also can delay product development, market implementation, and political decision making. Crucial to this is the question how much disintegration in field tests is enough to satisfactorily take the degradation of a specific test material or test item as proven. It has to be taken into account that open-system tests like in the field or in mesocosms *cannot* be used solely to test, measure and proof biodegradability, but have always to be preceded by a standardised lab test in a closed system that allows to directly assess biodegradation in the strict sense of complete mineralisation.

Analytical methods to assess polymer degradation *in situ* need improvement: There is still a methodological gap in the determination of degradation *in situ*. All measures applied in the tests reported here either had disadvantages or proved not suited at all to follow the state of physical and macromolecular integrity, or its decay over time. The simplest measure, loss of material as % area lost determined photogrammetrically, proved to produce the most reliable and valuable set of data. This method however has the drawback that disintegration can be measured only once the polymer film starts to be punctured and visibly shows holes or cracks. A similar approach is used in the weight attrition method, where the specimen’s weight is determined before and after the experiment (e.g. ASTM D7473, 2012). Because of possible errors by adhering sediment grains and biofilm in eulittoral and benthic tests, or fouling in pelagic tests this method was not considered suitable. The tensile property measurements conducted were useful at very low disintegration rates, and in the very early stage of degradation. This method was however jeopardised by production-related irregularities in the test films, and at a very early stage of disintegration by the formation of holes or cracks, and by fouling. At advanced disintegration or fragmentation this method could no longer be applied. Another factor that causes false positive results is the weakening of the polymer item by pure physical stress. One good example during the tests reported here was the decrease of mechanical strength of LDPE in the eulittoral test at a natural beach at Salamis Island. The tensile property tests suggested a degradation, but the loss in strength could be attributed to the occurrence of small cracks in the polymer, most probably caused by mechanical stress by water and particle movement within the sediment during stronger wave action. In the laboratory tests where no mechanical stress was present LDPE did not show biodegradation. The measurement of the film thickness often used to assess degradation was giving obviously erroneous results possibly due to swelling of the polymer by hydration, or thickening of the specimen by biofilm formation and fouling. The attempts to remove biofilm or fouling without massively affecting the test material did fail. More sophisticated methods such as Gel Permeation Chromatography (GPC) and Matrix-Assisted Laser Ablation Desorption/Ionisation Time of Flight mass spectroscopy (MALDI-TOF) could not detect differences in material properties at different times of exposure. Fourier transform infrared spectroscopy (FTIR) was carried out on samples from the Greek test location and gave well-differentiated spectra with gradual changes over exposure time. The observed spectral changes were attributed to hydrolysis of the polymer. This is a promising approach and should be substantiated by calibration data on samples of the same polymers retrieved from laboratory tests. If successful, this could also allow for the intercalibration of field disintegration data and laboratory biodegradation data via the monitoring of the alteration of the molecular structure of the polymers by FTIR. Further research is needed to develop a reliable feasible and economic method to monitor the decay of polymers during the biodegradation process in open systems. As an outlook, a yet to be found non-invasive high-resolution three-dimensional scanning method that differentiates the test material from fouling or adhering debris would allow for the volumetry of the material in question at any stage.

The tests covered important marine habitats, but other scenarios are still missing: Conclusively, the tested polymers showed different disintegration rates in different habitats and locations, and followed the intrinsic activity of each test site for each polymer. From the variability it can be clearly deduced, as expected, that there is not *one* marine environment. The marine realm provides a wealth of scenarios in which polymers that ended up there will eventually degrade at a higher or lower rate, or maybe even persist and become fossilised under certain conditions, as it is known for natural polymers (e.g. chitin, melanin etc.) [2]. There is a trade-off between providing a comprehensive toolset to assess biodegradation that covers literally all marine scenarios and the feasibility to conduct and finance such studies. With field tests in three shallow-water scenarios developed here already a good share of the marine coasts of the world is represented, if one applies these tests in different climate zones. By the selection of the corresponding sites the test systems could also be applied without major modifications in situations where no or only little oxygen is present, thus covering an important part of the oceans where fundamentally different microbial processes dominate. Although with great technological effort but still feasible, these test sys-

---

[2] Ehrlich H, Rigby JK, Botting JP, et al (2013) Discovery of 505-million-year old chitin in the basal demosponge Vauxia gracilenta. Sci Rep 3:3497.

tems could also be adapted to their use in the largest habitat on Earth, the deep sea floor. Deep-sea tests on a regular basis however are rather unlikely, but should be performed exemplarily on the most important polymers in order to gain the baseline knowledge needed.

In-situ field or mesocosm tests alone are not suited as a proof or disproof of biodegradation, but require beforehand as mandatory the proof of biodegradability of the material in question by laboratory tests. Mesocosm and field tests however serve to validate the results obtained by laboratory tests under controlled conditions (see Open-Bio D 5.6) and can give valuable insight if conducted in a complementary manner. Thus the three-scale approach for the assessment of biodegradation in the marine environment proposed here is highly useful.

Project website: http://www.open-bio.eu