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

Work package 5

In situ biodegradation

Deliverable N° 5.5:
Review of current methods and standards relevant to marine degradation

Public

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1 Publishable summary

Open-Bio project and background of this deliverable

Open-Bio is a research project funded by the European Commission within FP7. 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 is research on the biodegradability of bio-based polymers in soil, freshwater and the marine environment. The marine realm is the largest ecosystem on our planet. If the fate and possible effect of plastic in the world's oceans shall be addressed, or the effect of the marine environment on plastic, one needs to know the framework of conditions that shape this ecosystem. A solid testing scheme for the biodegradation of polymers in the marine environment is not existing yet. The aim of this deliverable is to provide the background knowledge needed for the definition of an improved and substantiated testing scheme from literature and relevant existing standards.

The marine litter problem and the use of resources

The accumulation of plastic debris in the ocean, including a growing amount of micro-plastic particles, has been identified as an environmental problem of global scale and policy makers become increasingly aware of the issue. It is broadly accepted that the absolute reduction and the elimination of plastic waste streams affecting the marine environment should be a key policy objective. This in turn requires the development of a drastically improved system of international waste governance, involving the participation of a diverse set of international stakeholders. Separate collection, reduction of use of plastics, information about these problems in the first years of school and to the wider public, and raising awareness in general are actions to be put in the field to reduce the problem. However, these actions are no guarantee that plastics will not ultimately end up in the marine environment. Although littering of (bio-) plastics should be avoided at any possible time and at all means, the development and increased adoption of preferably bio-based materials with improved properties in terms of biodegradation in marine environments may represent an important option for mitigating the negative impact of plastic waste in the sea. Even in the long term, certain residual waste streams, resulting for instance from marine-based industries such as fishing or shipping, are to be expected, indicating the long-term importance of such biodegradable bio-materials. In general multiple actions should be applied to avoid plastics from reaching and remaining in the oceans.

Although the use of plastic should be reduced and avoided at the best, for humankind a future life without plastic is not realistic anymore. 8% of the petroleum production is used for the production of plastic. This resource is not renewable. Therefore European policy makers announced the strategy to advance the bio-based economy in order to promote the sustainable use of our resources.

Bio-based and biodegradable plastics in the marine environment

The development of alternatives, such as the substitution of classical polymers by biodegradable plastics is increasing. However biodegradability is still difficult to predict in the marine environment. The ability to biodegrade can vary a lot and depends on the quality of the item and on the environmental conditions of the ecosystem of interest. Bio-based polymers are not biodegradable *per se* and biodegradability needs to be tested 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 under different environmental settings. The aim is to develop new tests, to adapt existing tests and to summarize research needs to set up a standard test scheme that ideally is relevant for all marine habitats.

Biodegradation standard tests for the marine ecosystem

There are considerably less tests available for marine than for freshwater systems and further investigations are needed to cover differences between the various marine habitats. Currently five test methods for the biodegradation in the marine environment are available: one from OECD (Organisation for Economic Co-operation and Development), one from ISO (International Organisation for Standardisation) and three from ASTM (American Society for Testing and Materials). No European CEN (European Committee for Standardisation) test method has been developed so far.

All of the limited number of biodegradation tests available are dedicated to the degradation under aerobic conditions. One standard only addresses disintegration and is not suited to measure biodegradation (ASTM D7473). The only standard specification that addresses disintegration, biodegradation and environmental impacts under the marine conditions of aerobic water or anaerobic sediment (ASTM D7081 in combination with ASTM D6691), has been withdrawn and is currently under revision. In early 2015 the Belgian private non-profit agency Vinçotte introduced the certification scheme for the “OK biodegradation MARINE label” based on the narrow criteria of ASTM D7081.

So far, biodegradation tests for polymers in the marine environment are very specific and standardised only to a little extent. Most of the guidelines have not been designed for the biodegradation of solid polymers and thus have to be adapted or developed anew.

As compared to freshwater, soil and compost conditions, the marine environment is considered less aggressive from an aerobic biodegradation point of view because e.g. the number of bacteria in seawater is relatively low. Although weathering, disintegration and degradation rates in the marine environment proceed much slower than on land, tests could be developed based on existing methods for freshwater and soil.

Missing aspects for standard test development

In order to better understand the great variation within the entire marine ecosystem, a set of marine habitats needs to be characterised according to their physical, chemical and biotic properties to obtain a baseline for conditions as natural as possible to be applied in standardised tests.

In Open-Bio we are currently working as a first approach on tests that represent a selected set of marine conditions: warm seawater with high oxygen and low nutrient levels. Novelties are tests in the intertidal sediment and at the water-sediment interface, also with high oxygen and low nutrients and organics in a temperature range of 20-25°C. Tests are currently being developed for the biodegradation in the sandy eulittoral (intertidal) (figure 1), and in the sublittoral zone in the free water and at the water-seafloor interface (figure 2). This is an extension beyond the OECD and ASTM standards and new tests are developed.

Figure 1. Field tests at the eulittoral (intertidal zone) in a) Greece and b) Italy.

Generally, the laboratory tests (figure 3) should mimic optimal conditions for biodegradation and disintegration, and thus conditions in the field may deviate from the laboratory. Complementary field (figure 1 and 2) and mesocosm tests (figure 4) provide the basis to assess and evaluate such deviations. The comparison of the results obtained in mesocosm and field tests and the results of tests carried out in the laboratory will help to set up the standardized test set for these three marine habitats.

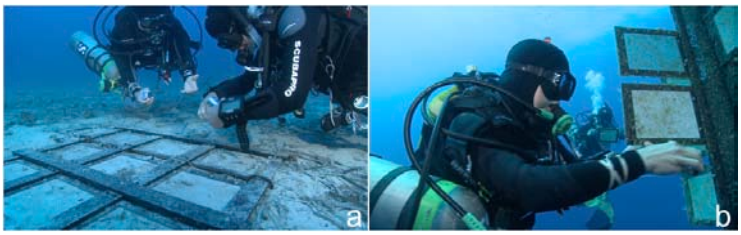


Figure 2. Field tests a) at the water-sediment interface and d) in the pelagic (water column) in Italy.

Figure 3. A) Closed bottles of the sublittoral water-sediment interface lab test, measuring the development of CO_2 and b) Oxytop incubation bottles of the pelagic (water column) lab test to measure O_2 consumption.

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. These sediments are mostly low in organics and therefore low in microbial activity. 70% of plastic waste sinks to the seafloor and the biggest sink for microplastic is the deep-sea sediment. Some coastal areas have increased nutrient and organic

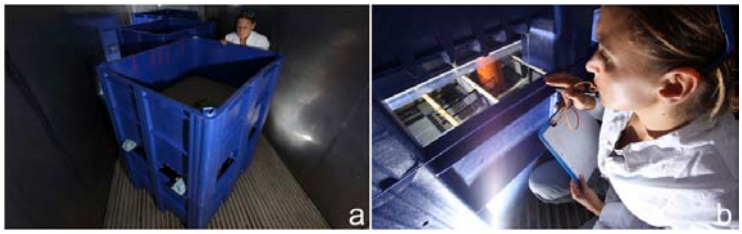


Figure 4. Mesocosm system testing under controlled conditions of a) the eulittoral (intertidal), b) sublittoral (water-sediment interface) and the pelagic (water column) habitats.

concentrations, and high microbial activity. Along heavily urbanised coasts plastic debris is ubiquitous in shallow water ecosystems.

After reviewing the current available studies on (bio)degradation of bio-based and fossil-based plastic in the marine environment we have identified several issues:

I. Results of many reported studies are difficult to compare due to variations in methodology (focus on deterioration, fragmentation or assimilation) and test conditions (field trials or laboratory tests, duration);
II. Biodegradation can only be proven by the analysis of direct measures in the laboratory, but laboratory test schemes can only mimic natural conditions within a narrow range, and some, by design, do not allow for the analysis of direct measures (e.g. flow-through systems);
III. Biodegradation in important marine habitats such as the deep-sea water column and seafloor, and also shallow water sedimentary seafloor, is poorly studied;
IV. The understanding of the abiotic impact on plastic, and the interplay of abiotic and biotic factors of degradation is limited;
V. The understanding of the degradation potential of single microbial strains and communities, as they occur in nature, is limited, and
VI. The understanding of the degradation of various polymer types and products is limited.

Recommendations

We recommend research in the following topics for the development of a standardized test scheme for the marine environment.

I. Define and include the most representative marine habitats in the test development;
II. Develop suitable indirect measures and intercalibrate them through a combination of laboratory, mesocosm and field tests with direct measurements of biodegradation;
III. Determine the single and synergistic effects of abiotic factors such as light, temperature, water movement, pressure, nutrient and organic content, etc., and their interplay with biotic factors;
IV. Study the fouling community and their microhabitat, including the effect of specific organisms and the community on degradation, and
V. Include the representative plastic types and products found in the sea as well as all products containing biodegradable materials in the degradation studies. That could allow to identify which products could be replaced and by what.

To reflect a wider range of conditions in the marine environment, the following points need to be considered for further standard test development:

- Effects of different levels of nutrients and organic contents;
- marine degradation under anaerobic (hypoxic/anoxic) conditions;

- marine degradation under high pressure and low temperature;
- degradation in and on mud and fine-grained sand.

For the the development and implementation of a standardised test for the biodegradation of bio-based polymers in the marine environment we recommend a gradual six-step scenario following an ecological approach:

1. Conduct pilot field tests to obtain baseline information on natural conditions, and to define test conditions for mesocosm and laboratory tests, then conduct field, mesocosm and lab tests. Use the mesocosm data as a link to the field data and to validate the laboratory data, and subsequently define improvements for the lab test;
2. Investigate the role of fouling in order to understand the ecological and biogeochemical conditions at the polymer surface;
3. Define the methodological toolset for the laboratory tests;
4. Study the ecotoxicology of the test materials with relevance for marine biota;
5. Propose test schemes according to the synthesis of the technical and scientific knowledge;
6. Verify the feasibility and reliability of the developed testing schemes by round robin tests. Conclusively and ideally, a choice of representative polymers that have been proven to be biodegradable under laboratory conditions should be tested in the field under natural conditions to validate the results and their environmental relevance.

In the progress of Open-Bio the first one-year series of lab tests (four partners), mesocosm tests (one partner) and field tests (two partners) is completed. The analysis of the data is on-going, whereas first adaptations for the next one-year test have been decided. The second round of tests will start soon or have started already. The preliminary validation of the lab tests based on the data gained from the field and mesocosm test is planned by the end of 2015 and will be finalized by summer 2016. The critical judgement of this approach will be available by the end of the Open-Bio project. The results of Open-Bio will be fed directly into the current standardisation processes of CEN/TC411. The socio-economic impact of the work within Open-Bio can be assessed after the further development of the standard test scheme and labels, which are needed for the advances of the bio-based economy.

Video of sampling at field test sites (Elba, Italy): URL https://youtu.be/Dl6w6wzB3aQ

2 Glossary

AFM	Atomic Force Microscopy
ANFOR	Association Française de Normalisation
ASTM	American Society for Testing and Materials
Aerobic	Requiring the presence of air or free oxygen or requiring air or free oxygen for life or survival, used especially to refer to aerobic bacteria or pertaining to respiration occurring in the presence of oxygen, as aerobic respiration.
Anaerobic	Not requiring, or capable of occurring, in the absence of air or free oxygen or relating to, the lack of molecular oxygen.
Anoxic	Free of oxygen
Benthic	The area of the sea floor
Bio-based	Derived from biomass (EN 16575)
Biofragmentation	Biological aspect of fragmentation, falling into pieces due to the activity of microorganisms.
Biomass	Material of biological origin, excluding material embedded in geological formations and/or fossilised (EN 16575).
BOD	Biological Oxygen Demand
CEN	European Committee for Standardisation
Deep sea	The area which is the lowest in the ocean and where little or no light reaches. From the marine biological perspective it is the marine zone, where no photosynthesis is possible but organisms depend on sunken organic matter or chemosynthesis.
DOC	Dissolved Organic Carbon
DSC	Dynamic Scanning Calorimetry
EN	European Standard
Eulittoral zone	The coastal zone between the spring high and low tide line. It is the intertidal zone.
EU	European Union

FITR	Fourier transform infrared spectroscopy
Fragmentation/ Disintegration	Breaking-up of a material into smaller pieces by physical, chemical or biological processes, or a combination of those.
GPC	Gel Permeation Chromatography
Hypoxic	Reduced in oxygen content.
JISC	Japanese Industrial Standards Committee
MW	Molecular Weight
Neritic zone	The shallow part of the sea from the waterline to the continental shelf at approximately 200 meters depth. From the biological perspective it is the illuminated marine environment, where sunlight reaches the sea floor.
NMR	Nuclear Magnetic Resonance spectroscopy
NOAA	National Oceanic and Atmospheric Administration
Oceanic	The parts of the sea deeper than approximately 200 meters. It is the region beyond the edge of the continental shelf and includes 65% of the sea's open water.
OD	Optical density of a liquid, with index 600, 650 etc. giving the wave-length of observation in nm.
OECD	Organisation for Economic Co-operation and Development
OPPTS	Former US Office of Prevention, Pesticides and Toxic Substances, now called “OCSPP” (Office of Chemical Safety and Pollution Prevention).
Oxic	In which oxygen is present, usually at atmospheric concentration.
PAH	Polycyclic aromatic hydrocarbon
PBS	Polybutylene succinate
PBST	Polybutylene succinate terephthalate
PBAT	Polybutyrate adipate terephthalate
PCL	Poly(ε-caprolactone)
PEG	Polyethylene glycol

Pelagic	The space of the water column, from the surface to the greatest depths.
PET	Poly (ethylene terephthalate)
PGA	Polyglycolic acid
PHA	Polyhydroxyalkanoates
PHB	Polyhydroxybutyrate
PHBV	Poly(β-hydroxybutyrate-co-β-hydroxyvalerate)
PLA	Poly(lactic acid)
PLLA	Poly(L-Lactic acid)
SEC	Steric Exclusion Chromatography
SEM	Scanning Electron Microscopy
STAP	Scientific and Technical Advisory Panel, an arm of the Global Environment Facility and part of the UN Family of organizations.
Sublittoral zone	The benthic zone, permanently covered by water extending from the low tide mark to the outer edge of the continental shelf at approximately 200 m depth.
Supralittoral zone	The coastal area above the spring high tide line, which is regularly splashed during storms. This area is never submerged by seawater.
ThOD	Theoretical Oxygen Demand
TIC	Total Inorganic Carbon
TOC	Total Organic Carbon
UNEP	United Nations Environmental Programme
UNESCO	United Nations Educational, Scientific and Cultural Organisation
XPS	X-ray photoelectron spectroscopy
XRD	X-ray diffraction

3 Introduction

3.1 Open-Bio project and background on this deliverable

Open-Bio is a research project funded by the European Commission within FP7. 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 is research on the biodegradability in soil, freshwater and the marine environment. The marine realm is the largest ecosystem on our planet. However, to most people "the sea" is an infinite mass of water hidden from their direct view by its shimmering surface. If the fate and possible effect of plastic in the world's oceans shall be addressed, or the effect of the marine environment on plastic, it is needed to know the framework of conditions that shape this ecosystem. This holds especially true for the development of substantiated testing schemes for the biodegradability of bio-based solid materials. A solid testing scheme for the marine environment does not exist yet. The aim of this deliverable is to provide the background knowledge needed for the definition of an improved and substantiated testing scheme from literature and relevant existing standards. A collection and critical analysis of information on the subject needed to base the subsequent activities on a knowledge background, was executed.

The focus for this deliverable was on the degradation of biodegradable bio-based **solid** materials (e.g. plastics) in the sea. Plastic is "biodegradable" if it will be fully converted to CO_2 or CH_4, water and biomass (i.e. re-mineralised) through the action of naturally-occurring microorganisms such as bacteria, fungi, and algae (modified after ASTM D883-00) [12]. “Bio-based” materials are derived from biomass of biological origin excluding material embedded in geological formations and/or fossilised (EN 16575)[1] [203], i.e. bio-based materials are not inherently biodegradable.

3.2 EU policy

To successfully increase the European share capacity on bio-based materials, the European Union’s 2020 strategy has outlined a vision for a resource-efficient, greener and more competitive European economy. It recognises that generating future employment and economic growth will increasingly depend on using resources efficiently, while reducing dependence on non-renewable resources [94]. This requires a fundamental transformation in multiple areas, including agriculture, industry, energy and transport systems [92]. While tackling these societal challenges requires far-reaching efforts from all sectors of society, they also yield important opportunities. Well-designed and ambitious measures to promote more sustainable patterns of consumption and production have the potential to stimulate lead markets for innovative products and technologies, which serve as reference points for other markets around the world [89, 153].

[1] For explanation on used abbreviations, terms and acronyms refer to the glossary page 11

Building on the Lead Market Initiative for bio-based products launched in 2007, it has also proposed a set of measures to enable the development of a competitive, Europe-wide market for bio-based products [90, 93]. The development of European sustainability CEN standards represent a key component of this strategy. As outlined in the Commission's Integrated Industrial Policy, standards are a key vehicle to facilitate harmonisation and economies of scale within the context of a European single market [91]. Moreover, the early development of corresponding standards at the European level not only offers important opportunities for developing a harmonised market for bio-based products and a basis for possible regulatory initiatives by the European Commission. It also promises to establish European CEN standards as a reference for other key markets. In this way, innovative products developed in Europe can more easily diffuse to external markets, offering important advantages to producers based in the EU [90].

Above all, standards can play an important role in supporting the public acceptance of bio-based products by ensuring, verifying and visualising key sustainability aspects. As debates on "food versus fuel" with respect to biofuels have shown [282], addressing these sustainability concerns and ensuring that environmental claims are credible represents a key factor for the uptake of bio-based products in the market. The degradation of bioplastics in the marine environment represents one such concern. To enable the development of markets for these bio-based materials, the ability to make credible claims to consumers and society at large regarding their biodegradability in marine environments is crucial. This in turn requires corresponding test methods and standards [201]. Sufficient standardised tests for the biodegradability under marine conditions are currently missing and their development is needed.

3.3 Marine litter problem

Generally, waste reaches the sea from the coast, by rivers, airborne transport, storm events and tsunamis, or directly from ships [252, 318]. Three quarters of it are synthetic materials and 10% is estimated to be fishing gear [293]. One of the largest contributors is discarded products from recreational and commercial seafaring [76, 124, 294, 298].

A review of Watkins et al. (2015) and the study by Hall (2000) [126, 312] showed that the costs for coastal communities resulting from marine litter, including all types of materials, are immense and manifold. They reported damages to fishing gear, increased cleaning efforts for beaches, aquaculture plants and harbours, the hindering of the navigation of vessels, the blocking of filters of industrial plants, and the decrease of tourism. Further effects on the environment include the entanglement of animals, the mechanical impairments by ingested plastic mistaken as food, the accumulation of persistent organic pollutants (POPs), and the transport of harmful algal and invasive species [194].

Fragmentation by mechanical forces and UV-light lead to micro-plastic particles (< 5 mm). Micro-plastic is also directly introduced to the ocean as discarded cosmetics, or via abrasion of tyres, clothes and other consumables, which pass the waste water treatment system in

sewage plants [43]. The small fragments are mistaken for food and are taken up by organisms such as pelagic predatory fish [52] or plankton [284].

Plastic in the marine environment is found at beaches near populated areas [43] but also on uninhabited oceanic islands [22], accumulated in surface waters [173] and in the deep-sea [109, 222], or in the arctic [27]. Waste is laying on the seafloor and is found entangled in plants such as mangroves [70] or buried in the sediment [301]. Predictions calculated a possible 250-fold increase of plastic litter on some beaches in East Asia within the next decade [158]. The accumulation of plastic debris in the ocean, including a growing amount of micro-plastic particles, has been identified as an environmental problem of global scale with policy makers becoming increasingly aware of the issue. It is broadly accepted that the absolute reduction and the elimination of plastic waste streams affecting the marine environment should be a key policy objective. This in turn requires the development of a drastically improved system of international waste governance, involving the participation of a diverse set of international stakeholders [276]. Although littering of all bioplastics should be avoided at any possible time and at all means, the development and increased adoption of preferably bio-based materials with improved properties in terms of biodegradation in marine environments represents an important option for mitigating the negative impact of plastic waste in the ocean [47]. Even in the long term, certain residual waste streams, resulting for instance from marine-based industries such as fishing or shipping, are to be expected, indicating the long term importance of such biodegradable bio-materials.

The production of plastics has increased over the past 50 years with an average of 8.7% per year, reaching a worldwide production of 299 million tons in 2013 [224]. Jambeck et al. (2015) [151] calculated that, in 2010, 192 coastal countries had produced 275 million metric tons of plastic waste of which 4.8 to 12.7 million metric tons had entered the sea. The authors published estimations on mismanaged plastic waste and how much plastic waste ended up as marine debris. Southeast Asian countries, such as China, Indonesia, Philippines and Vietnam rank highest. The 23 European Union coastal countries rank 18th and the United States 20th. Even if in some cases the municipalities organise an efficient differentiated collection of waste in order to recycle plastics, an estimated 10%, mainly plastic bags and bottles, reaches the marine environment [283]. Jambeck et al. (2015) [151] estimate that the amount of plastic entering the sea will increase by an order of magnitude if improvements in waste management systems fail. The study took also into account that 23% of the world's population (~1.2 billion people) live within 100 km of the coast [272], a number, which is likely to rise up to 50% by 2030 [3].

Therefore it is important to apply multiple actions to avoid plastics reaching the oceans. Separate collection, reduction of use of plastics, information about these problems in the first years of the school and to the wider public, and raising awareness in general are actions to

put in the field to reduce the problem. However, these actions are no guarantee that plastics will not ultimately end up in the marine environment.

3.4 Bio-based and biodegradable plastics

Besides the efforts to reduce the use of plastic and its littering into the environment, and to improve and intensify the recycling efforts, the development of alternatives, such as the substitution by biodegradable plastics is increasing and could be helpful [135, 295], although it should be clear that currently biodegradability is still difficult to predict in the marine environment. A lot of the work currently carried out within the framework of the Open-Bio project is dedicated to get more insight in how to deal with biodegradability issues of bio-based polymers in the different environmental compartments [12]. It is important to know that bio-based polymers are not biodegradable *per se* and that the biodegradability needs to be tested for each product. The ability of microorganisms to biodegrade can vary a lot and depends on the environmental conditions of the test ecosystem of interest. So far, available biodegradable polymers were developed to divert biological waste from landfill. The production of biodegradable polymers is predicted to increase from 571,000 tons in 2012 to over 1,126,000 tons in 2018 [31]. Worldwide the share of the bio-based polymers (including non-biodegradable products) production capacity is predicted to be 55% by Asia, 18% by South America, 14% by Europe and 13% by North America [204]. In 2011 it was 52% by Asia, 20% by Europe, 15% by North America and 13% by South America, showing that Europe is slower in developing its capacities than Asia and South America. Besides the capacity, numbers on market share and published numbers on recycling success are interesting to compare between the five continents, however data are not publicly available yet.

3.5 Set-up of the deliverable

This deliverable aims to provide the background necessary to develop sound standardised testing schemes to assess the potential biodegradability of bio-based products that end up in the different environments of the marine realm. In this deliverable the following issues are addressed and reviewed:

- Introduction into the field of marine biodegradation: (current) environmental situation and EU 2020 strategy (this chapter)
- Summary of materials that have been included in studies towards biodegradation under marine conditions; What can be deduced from the data? (Chapter 4);
- Summary of available standardised test methods (Chapter 5);
- Compilation of missing aspects for the development of a sound standardised testing scheme (Chapter 6);

- Possible adaptations on existing (standardised) test methods, which could be applied from existing soil and freshwater tests (Chapter 7);
- Suggestions for next steps, including research topics that need to be investigated in order to substantiate the knowledge in the further development of tests (Chapter 8);
- Concluding remarks and recommendations by presentation of a road map to describe how to proceed with the test development (Chapter 9).

4 Tested materials for their biodegradation in marine environments

4.1 Introduction and used definitions

Biodegradable material is what is completely converted to CO_2, water and biomass, "re-mineralised" mainly by microbes such as bacteria, fungi, and algae (ASTM D883-00) [12], and should be referred to a specific environment (soil, compost, freshwater, seawater). Degradation in the environment can be divided in two categories: abiotic or non-biological (e.g. chemical hydrolysis and photodegradation) and biotic or biological degradation (e.g. enzymatic and inside or outside the cell membrane) [179]. In biotic degradation, the polymers usually break down in several steps catalysed by different microbial enzymes, and this starts at the surface of the material. First, the molecule needs to be depolymerized and this process is mainly carried out by extracellular enzymes excreted by microorganisms. After the uptake of the so formed smaller (monomer) units by microorganisms, mineralisation may occur within the microorganisms [88].

The abiotic parameters that can affect the ability of the polymeric materials to biodegrade include mechanical stress, radiation, thermal stress and the attack by chemical substances that lead to transformations of the polymer structure and/or changes in properties of the polymers when they are exposed to environmental conditions (i.e. sunlight, humidity and/or burying). Abiotic degradation usually precedes biotic degradation [179].

The chemical structure of biodegradable polymers is intrinsically biodegradable. That means that their chemical structure enables direct enzymatic degradation (e.g. starch, cellulose, chitin, etc.) [271]. Biodegradation comprises different microbial processes: biodeterioration and/or disintegration, biofragmentation (both dissimilatory processes) and assimilation, without neglecting the possible effects of abiotic factors.

Biodeterioration, as described by Lucas et al. (2008) [179], is considered to be the change in the mechanical, physical and chemical properties of a given material which is caused by the action of microorganisms by physical, chemical and/or enzymatic action at the surface or/and inside the material. Furthermore, the process implies the formation of smaller particles, thus increasing the surface area available for (microbially induced) further breakdown.

(Bio)fragmentation is a lytic phenomenon necessary for the subsequent event called assimilation, however it does not necessarily imply complete biodegradation to CO_2. The cleavage of chain bonds of polymers by microorganisms is necessary in order to obtain a mixture of oligomers and/or monomers. Microorganisms secrete specific enzymes or generate free radicals so that biofragmentation can occur by enzymatic hydrolysis or by enzymatic oxidation or by free radicals. A polymer is considered as chemically fragmented when low molecular weight molecules are found within the medium. However such data do not clarify if the fragmentation was biological or abiotic.

Assimilation is the process by which part of the available nutrients (carbon, nitrogen and others) taken up by the microorganisms are used for the synthesis of biomass. These bio-

logical processes can only take place if the environmental conditions are suitable for the functioning of the exoenzymes and the microorganisms involved.

Polymers can be classified as being bio-based (note: the term bio-based refers to 100% bio-based but also to partially bio-based materials) or petroleum-based depending on their origin, i.e. from biomass and from non-renewable sources (e.g. fossil oil). The origin of the building blocks is not indicative for the biodegradability of the polymer. Bio-based biodegradable polymers are produced from natural bio-based resources (plants, animals or microorganisms) containing macromolecules such as polysaccharides, proteins and lipids. This category also includes natural rubber and certain polyesters either produced by microorganisms or plants (e.g. polyhydroxyalkanoates (PHA) and poly-3-hydroxybutyrate (PHB)) or synthesized from bio-derived monomers (e.g. polylactic acid (PLA)). Petroleum-based biodegradable polymers such as aliphatic polyesters (e.g. polyglycolic acid (PGA), polybutylene succinate (PBS) and polycaprolactone (PCL)), aliphatic-aromatic copolyesters (e.g. polybutyrate adipate terephthalate (PBAT)), polybutylene succinate terephthalate (PBST) and poly (vinyl alcohol)) are produced by synthesis from monomers derived from petrochemical refining, which in some cases also possess certain degrees of inherent biodegradability. Also, some of the polymers mentioned here can be made partly (PBST, PBAT) or even fully bio-based (PBS). Many commercial formulations of biodegradable polymers combine materials from both classes to reduce cost and/or enhance performance [274].
The fact that the materials are bio-based does not imply biodegradability. The biodegradability of a compound/material is related to the chemical composition irrespective of the origin of the molecules, which may be petrochemical or (partly) bio-based, and the properties thereof. Furthermore biodegradability does not automatically include all environments. For example the same material may be readily biodegradable under oxic conditions but hardly degrade in the absence of oxygen.

Different materials have been tested for their biodegradation in marine environments in various studies. This range of materials includes: aliphatic polyesters, microbial polyesters, polyethylene glycols etc. In the sections below a summary of these studies is presented.

4.2 Methods used to determine biodegradation

Different methods may be used to determine whether biodegradation actually occurs. Besides mineralisation tests, other methods are used to track material changes. Shah et al. (2008) [262] published a comprehensive review on the biological degradation of biodegradable and conventional synthetic plastics, including the explanation of the current standard testing methods. Visual analysis by scanning electron microscopy (SEM) and atomic force microscopy (AFM) are used to describe the roughening of the surface, formation of cracks, fragmentation, changes in colour and formation of biofilms. To investigate changes of the polymer molecular structure Fourier transform infrared spectroscopy (FTIR), differential scanning

calorimetry (DSC), nuclear magnetic resonance spectroscopy (NMR), X-ray photoelectron spectroscopy (XPS), X-ray diffraction (XRD), contact angle measurements and water uptake are used. Analyses of changes in mechanical properties or molar mass are often used in degradation tests because it is relatively easy. To obtain good results the differences need to be large. This test cannot be applied when the test polymer is starting to disintegrate. Then weight loss measurements are often used because it is also an easy analysis. The analysis becomes difficult when powders or small fragments have to be recovered. A simple semi-quantitative test is the detection of a clear-zone, for example on an agar plate. This test shows that the used microbes are at least able to depolymerize the test substance, which is the first step of biodegradation. Tests on enzymatic degradation are used accordingly. All the so far mentioned analyses can give a first indication of a possible microbial attack, but no direct proof of biodegradation.
For that, tests measuring CO_2 evolution or O_2 consumption are used. They are useful for aerobic test conditions. Anaerobic digesters are used for conditions without oxygen. The best proof for biodegradation possible is by using stable isotope or ^{14}C radiolabelled polymers. Problematic is here that the radiolabelled material and its disposal are expensive. Alternatively, also other, cheaper, methods like stable isotope labelling are possible.

4.3 Overview of results obtained in lab studies

Studies showing, with direct measurements, that marine microbes degrade plastic polymers are still scarce and we recommend filling this gap of knowledge with suitable experimental approaches. The references in Shah et al. (2008) [262] give a first overview on the state of the art. In the following text some further examples are described and commented.

4.3.1 Studies involving bacterial transformation of plastics

Various studies have been executed on the biodegradation of polyhydroxyalkanoates (PHAs) in terrestrial environments and many terrestrial microorganisms that degrade these compounds have been characterised. However, little knowledge exists as far as the degradation of polyhydroxyalkanoates in marine environments is concerned. Only a few species of marine bacteria have been identified as PHA degraders. Leathers et al. (2000) [175] identified a new degrading species *Pseudoalteromonas* from a tropical marine environment as responsible for the biodegradation of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV). The testing method used by the researchers included the preparation of growth media by amending the defined minimal medium with small amounts (0.3%) of PHBV or (2% of) glucose. Then the minimal media were amended with a defined quantity of agar per litre to generate solid media. The working bacterial stock cultures were maintained on a solid complex medium and liquid culture pre-inocula were inoculated from working stocks. Experimental cultures were inoculated to a calculated defined initial OD_{600}. The PHBV depolymerase activity was measured as the decrease in OD_{650} against substrate buffer blanks. *Pseudoalteromonas* sp.

NRRL B-30083 was isolated as the prevalent PHBV degrader from the marine environment. Although the strain produced distinct zones of clearing on solid medium containing PHBV as a sole carbon source, its poor growth and lack of PHBV depolymerase activity make it a difficult organism to fully characterise in liquid cultures grown on PHBV. The researchers suggest that further research, on the action of marine microorganisms on PHAs, has yet to be done [175].

Mabrouk and Sabry (2001) [184] reported the degradation of poly(3-hydroxybutyrate) (PHB) and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) as a sole carbon source by the marine bacterium *Streptomyces* SNG9 using the clear zone test. They were able to repress the PHB polymerase by adding simple soluble carbon sources, which could be an indication that the presence of other carbon sources also represses the transformation of these kinds of compounds in the marine environment.

Most of the articles available in the published literature deal with short term immersion (less than 100 days). Deroiné et al. (2014) [75] aimed to establish a baseline for Poly(β-hydroxybutyrate-co-β-hydroxyvalerate) (PHBV) lifetime prediction in a marine environment. They exposed PHBV pellets at 4, 25 and 40°C in the laboratory and continuously renewed and filtered natural seawater. Further samples were exposed in Lorient harbour (France). Both experiments ran over a period of 360 days. The samples were characterized measuring the water uptake, surface roughness, SEM, tensile tests, molecular weight measurements by SEC and thermal properties by DSC. The authors report that increasing the temperature in the laboratory promoted the water uptake and caused hydrolysis. They concluded that due to its morphology (pellets), hydrolysis of PHBV in natural seawater is quite slow, and samples were observed to undergo preferentially an enzymatic surface degradation. Because the two degradation mechanisms occurred in parallel (enzymatic degradation and chain scission), the choice of test conditions is critical, and the lifetime of PHBV in a marine environment is difficult to predict accurately.

Deroiné et al. (2014) [74] also investigated the ageing of PLA over a period of 180 days comparing different aqueous environments. Samples were immersed in distilled water at different temperatures (25 ºC, 30 ºC, 40 ºC and 50 ºC) in order to evaluate the influence of temperature on PLA degradation kinetics and to predict lifetime. Then, samples were immersed in seawater both in the laboratory and in the field, in order to compare the effects of environment, marine organisms and salt. The degradation was followed by gravimetry, tensile tests, SEM, SEC and DSC. In this study an effort to establish a baseline for degradation mechanisms and degradation kinetics in order to make lifetime predictions of polylactide behaviour in seawater was executed. The authors conclude that the data acquired during this study do not yet allow such predictions to be made, as the limited temperature range which allows acceleration without introducing new damage mechanisms does not allow a correlation with marine conditions. Further work is underway to extend the study, in particular by the use of thin film specimens which should enable more rapid saturation to be attained and core/surface differences to be limited.

In another study by Le Duigou et al. (2009) [174] the long-term durability (3 months at 20 and 40°C) of composites of flax fibres and Poly(L-lactic acid) (PLLA) in the marine environment was assessed. Several techniques were used to examine how the composite behaviour changes in seawater: weighing to quantify changes due to water ingress, GPC (gel permeation chromatography) and DSC to measure molecular weight and characteristic transitions, and tensile tests to determine mechanical properties. Acoustic emission has been used in some tests to characterise damage onset, and scanning electron microscopy (SEM) was employed to visualise this damage. The study revealed a decrease of almost 50% of molecular weight after 3 months at 40°C in seawater. The authors interpret that the results indicated that flax/PLLA composites undergo permanent changes after immersion in seawater. And further that the absorption of water results in several degradation mechanisms such as hydrolysis of the matrix, revealed by reduction in molecular weight, structural changes, degradation of the fibre/matrix interface (de-bonding, pull-out), differential swelling at the fibre–matrix interface, and degradation of fibres. These mechanisms result in a reduction in mechanical properties. They conclude that the stiffness of unreinforced PLLA is hardly affected by water, but the bio-composites lose tensile stiffness and strength progressively as water entered and weakened the material [174].

In 1998 Rutkowska et al. [245] tested polycaprolactone (PCL) with four processing additives in a harbour of the Baltic Sea for a period of up to two months. And for comparison reasons they incubated samples in a buffered salt solution for ten weeks in the laboratory. Weight loss and changes in mechanical properties let the authors state that the seawater environment is favourable for the degradation of PCL and that complete biodegradation (as evidenced by disappearance) occurred within 2 months. Disappearance of a material however is not a suitable measure to assess the processes that made the matter “disappear”.

Vila et al. (2015) [308] published a review on bacterial polycyclic aromatic hydrocarbon (PAH) degradation in marine and terrestrial habitats. Therein studies on the microbial community structure and their dynamics, as well as enzyme activity and genetic profile are listed. The authors write that the progress in optimizing these natural biological processes relies on the identification of the underlying microbial actors and on deciphering their interactions at molecular, cellular, community, and ecosystem level. Novel advances are built on a progressive approach that span from pure cultures to environmental communities, illustrating the complex metabolic networks within a single cell, and their further implications in higher complexity systems. Understanding these processes will provide new tools to assess biodegradation occurrence and, as a final outcome, predict the success of bioremediation thus reducing its uncertainties.

The aerobic biodegradation of polyethylene glycols (PEGs), of different molecular weights ranging from 250 to 57800 Da, in fresh and seawater was systematically investigated by Bernhard et al. (2008) [28]. Inocula used were obtained from municipal wastewater and sea-

water aquarium filters. The biodegradation tests were carried out in freshwater and in artificial seawater using marine microorganisms acquired from filters taken out of a seawater aquarium. The biodegradation tests were performed using two different test systems. One was the CO_2 Evolution Test (modified Sturm Test) according to the guidelines OECD 301 B "Aerobic Biodegradation" [209] and ISO 9439 "Evaluation of ultimate aerobic biodegradability of organic compounds in aqueous medium -- Carbon dioxide evolution test" [142] which determines the ultimate biodegradability of organic compounds by aerobic microorganisms in water, using a static aqueous test system. The standards used to assess the biodegradability in artificial seawater were ISO 16221 "Water quality – Guidance for determination of biodegradability in the marine environment" [145] and OECD 306 "Biodegradability in seawater" [210]. Specific analyses using liquid chromatography mass spectrometry (LC–MS) and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) showed that aerobic biodegradation of PEGs in artificial seawater using marine microorganisms is possible but there are differences compared to freshwater media. In seawater media with marine microorganisms, PEGs up to 7400 Da are entirely biodegradable whereas PEGs having higher molecular weights (MWs) are only partially degradable and persistent to microbial attack. The results also showed that under marine conditions the level of biodegradation decreases, while the lag time increases with increasing MW. Further studies for freshwater media may examine if PEGs with higher MW than 58000 Da show a decrease in biodegradability when MW increases. Under both environmental conditions it appeared that the time required for degradation generally increases with increasing MW. The authors [28] propose that future studies may investigate if the microorganisms involved in the biodegradation in freshwater media are different from those in seawater although the degradation pathway is interpreted to be similar. The results are of selective use as the PEGs with low molecular weight were liquids. High molecular PEGs have not yet been measured in marine habitats so further research is proposed in this area.

Webb et al. (2010) [314] exposed poly(ethylene terephthalate) (PET) samples in laboratory incubations with natural seawater in the dark (a) and in the presence of light (b) with an assumed (a) heterotrophic and (b) photoautotrophic bacterial community. Chemical analysis of the polymer surface by X-ray spectroscopy showed changes on the molecular level in samples from the dark incubation with a decrement of ester groups, an increase of carboxyl groups and the generation of hydroxyl groups that were completely absent in the original polymer. Nanotopography assessed by atomic force microscopy showed differentiated surface roughness of both the samples from light and dark incubations. This appears to the authors as a consequence of the bacteria-surface interaction followed by bacterial attachment and plastic degradation.
Oberbeckmann et al. (2014) [208] reported that FTIR measurements of plastic bottles exposed in the field did not reveal any difference in PET surface structure which would have indicated degradation or deformation of PET.

Sudhakara et al. (2008) [279] tested whether the two marine bacteria *Bacillus sphericus* and *B. cereus* degrade not pre-treated, thermally pre-treated LDPE and HDPE and not pre-treated starch-blended LDPE. Degradation was determined indirectly by measuring weight loss and FTIR for all samples. The highest weight loss was measured for the starch-blended LDPE.
Rutkowska et al. (2002) [244] reported that the changes measured by weight loss, tensile strength, optical reflection and transmission microscope depend on the quantity of starch in polyethylene blends and the conditions of the natural environment, and they reported that for pure PE "there were no visible weight changes".
Murata et al. (2004) [198] reported data on technical PE degradation by increasing pressure (corresponding from 0 to 100m water depth) and increasing temperature (410-440°C). They measured the rate of volatilization, the rate of double bond formation and the distribution of degradation products, and concluded that their procedure can be a potential alternative to control the product distribution in a process converting waste plastics into liquid hydrocarbons. If the degradation is similarly influenced in the marine environment when exposed to higher pressures and possibly to higher temperatures remains to be studied.

4.3.2 Studies involving fungal transformation of plastics

Kathiresan et al. (2003) [161] conducted a one month lab test with five bacteria and two fungi in broth media and measured the weight loss of polyethylene (PE) plastic bags and cups. PE bags lost between 20.54 and 7.75% weight, when exposed to the bacteria and 28.8 and 17.35% when exposed to the fungi. The cups lost between 0.56 to 8.16% respectively 5.54 and 7.26%. When exposed 9 months in the field, buried 5 cm into mangrove soil, bags had lost 3.77 to 4.21% and cups 0.17 to 0.25%. The authors conclude that bacteria of the genus *Pseudomonas* and the fungus *Aspergillus glaucus* degrade PE plastic bags and cups in mangrove soil. Also Devi et al. (2015) [77] reported that *Aspergillus tubingensis* and *Aspergillus flavus* degrade HDPE in lab tests under marine conditions. Both studies used weight loss as a measure, and Devi et al. (2015) also FTIR as an indication for degradation, whereas direct measurements of degradation were not applied.
The role of marine fungi as saprotrophs is not well understood. Most probably they have similar capacities as fungi from terrestrial ecosystems, where they degrade complex natural polymers and recycle nutrients [235], and according to Islam and Datta (2015) [139] attack man-made polymers. The selected references in the following text are chosen because the studied fungi, although for example from soil, are also known from marine environments. That means they are interesting because they indicate a potential of marine fungi in the process of degradation in the sea and point towards future research topics.
Devi et al. (2015) [77] reported that the marine *Aspergillus tubingensis* and *Aspergillus flavus* degrade HDPE in lab tests. After one month of exposure of HDPE as a sole carbon source the weight loss was 6.02 and 8.51%. Besides weight loss they used FTIR as an indication for degradation, whereas direct measurements of degradation were not applied. Webb et al. (2000) [315] reported about *Aureobasidium pullulans* successfully growing on plasticised PVC

as the only carbon source exposed on land. In further 6 week long lab tests *A. pullulans* could degrade the plasticiser dioctyl adipate, produced extracellular esterase, and caused a weight loss of pPVC between 3.4 to 3.7%. In 2012 Ali et al. [4] isolated three fungal species from soil (*Aspergillus niger, Phanerochaete chrysosporium and Aspergillus sydowii*) able to grow on self-made PVC polymers (from Aldrich powder) as the only carbon source. The CO_2 evaluation in a Sturm test with mineral salt medium was after 4 weeks 7.31 g/L (control 4.9 g/L) from *P. chrysosporium* and 6.02 g/L (control 3.7 g/L) from *A. niger*.

Researchers from Novamont laboratories isolated *Aspergillum versicolor* and *A. sydowii* from the surface of bio-based biodegradable plastics samples exposed in marine sediment (currently under study and data not published, pers. comm. Degli Innocenti). The samples had holes and were very brittle or had disappeared, which means that they were in the active degradation phase. From pilot studies the researchers have indications that *A. sydowii* is more active (pers. comment Tosin).

The study of Matavuly and Molitoris (2009) [188] revealed that from 134 strains of marine fungi only 4%, and from 143 terrestrial fungi strains 55% were able to degrade BIOPOL™ (a commercial poly-3-hydroxyalkanoate) in a clear zone test that directly measures depolymerization of polymers. Why there were less marine fungi capable to degrade PHA the authors could not explain. They report the indication that rather physiological properties are important for the degradation capability and not the systematic or ecological grouping. The authors of this review highly recommend further research on marine fungal degradation of biodegradable polymers.

Devi et al. (2014) [78] had isolated 45 marine fungi of which 9 showed clear zones when exposed to PHB. The highest enzyme production was detected at 30°C and a purified enzyme revealed 90% depolymerization of PHB films within 4 days.

4.4 Overview of results obtained in the field

The biodegradation of three aliphatic polyesters in the deep sea was examined by Sekiguchi et al. 2011 [259]. This research group examined poly(ε-caprolactone) (PCL), poly(β-hydroxybutyrate/valerate) (PHB/V), and poly(-butylene succinate) (PBS). The experiments of this research group were performed by immersing fibres from the above mentioned materials in water tanks where deep-sea water was pumped up from three different locations in Japan. The water intake temperature and depth was 5°C at 350m, 2°C at 321m and 10°C at 612 meters, rates were 5000 l/h, 3500 l/h and 137 l/h for the three different water tanks corresponding to the three different locations. The incubation period lasted up to 12 months. The mechanical properties and the surface morphology of the incubated fibres were examined. From the results of this study, the authors reported that PCL and PHB/V were degradable in deep-sea water, despite the low temperatures. PBS fibres were found to be degradable only

at the location with 10°C water. Five PCL-degrading bacteria were isolated and characterised. The isolates were found to belong to the genera *Pseudomonas*, *Alcanivorax,* and *Tenacibaculum*. The growth of the isolates of *Pseudomonas* was found to be acclimated to conditions of low temperature (4ºC) and high hydrostatic pressure. Research on more materials, and on microbes which can degrade plastics under deep-sea conditions needs to be further intensified.
In the sea plastic debris found at greater depths is mostly exposed in habitats being cold and dark compared to the sea surface. Which abiotic and biotic factors are likely to have most affects, either in the deep sea or in the sublittoral and neritic zone needs to be studied further. Long-term studies should be conducted to confirm the period required for total degradation in a range of marine habitats. Work is also needed to confirm whether there are adverse effects from the fragmentation of plastic bags into numerous small pieces (e.g. into microplastics) and to quantify any substances that are released to the environment as a consequence of this breakdown.

O'Brine and Thompson (2010) [206] investigated the breakdown of two oxo-degradables, a compostable and a standard PE plastic bag, at 0.6 meters in coastal waters off Plymouth, UK, for 40 weeks. The degradation was quantified by tensile strength and surface area loss measurements. From the compostable plastic no remains could be recovered after 24 weeks. After 40 weeks less than 2% of the surface area of the three other materials was lost.

An experimental study on the degradation of blended starch - poly(β-hydroxybutyrate-co-β-hydroxyvalerate) (PHBV) formulations under marine conditions was executed by Imam et al. (1999) [137]. In this research, which included field tests, degradation of the polymer formulations was monitored for one year at four stations in coastal water southwest of Puerto Rico. Two stations were within a mangrove stand. The other two were offshore; one of these stations was on a reef, and the other was at a location in deeper water. The degradation of these blends of polymers was determined by weight loss and deterioration of tensile properties. The relation to microbial degradation was made by counting the microbes by standard spread plate methodology using three different media. The authors reported that the degradation at the station in deeper water exhibited an initial lag period, after which degradation rates were comparable to the degradation rates at the other stations. They interpreted that significant biodegradation occurred only after colonisation of the plastic, and this was dependent on the resident microbial populations. Therefore, they assumed that extended degradation lags would occur in open ocean water where microbes are sparse. According to Imam et al (1999) [137], it is possible that the degradation of polyhydroxyalkanoates (PHAs) occurs relatively rapidly in coastal marine mud, in which the microbial populations and metabolic activities are high. Another factor determining the degradation of the starch-PHBV formulations is the amount of starch present in the formulae. The higher the weight percentage (w-%) the more is removed. Despite the fact that the above mentioned study is a contribution to the research regarding marine degradation of PHAs and the marine performance of blends

of these compounds, the reports regarding marine degradation of either starch or PHBV are limited and the degradable nature of starch-PHBV plastics has to be validated [137].

Tsuji and Suzuyoshi (2002a) [289] tested poly(ε-caprolactone) (PCL), poly[(R)-3-hydroxybutyrate] (R-PHB), and poly(L-lactide) (PLLA) in static seawater by polarizing optical microscopy, gravimetry, GPC, DSC, and tensile testing. The change in weight loss, tensile strength, and Young's modulus, a measure for elasticity, revealed that the biodegradability of the aliphatic polyesters in the controlled seawater decreased in the order: PCL>R-PHB>PLLA. The results of gravimetry, GPC, and DSC showed that the biodegradation of PCL and R-PHB films proceeds via surface erosion mechanisms. The same polymers were also tested in natural dynamic seawater for a period of 5 weeks [290]. The authors reported that the gravimetry and tensile testing showed that the mechanical stresses and strains in the natural dynamic seawater caused mechanical destruction or degradation of the films, resulting in seemingly accelerated (bio)-degradation of all the films compared with that in the controlled static seawater. And when PCL and R-PHB had pores and hydrophilic surfaces these had enhanced the biodegradation in seawater [291].

Recently several studies highlighted that the bacterial consortia that individually develop on the solid surface of plastic marine debris are significantly different from the average bacterial composition of the sea water surrounding this kind of inert environment [208, 234, 327], which might be explained by the type of habitat (benthic vs. pelagic, biofilm vs. planktonic lifestyle, and linked concentration effects).
On the surface of plastic items Zettler et al. (2013) [327] identified microbes of the groups *Phormidium*, *Pseudoalteromonas*, Hyphomonadaceae (metylotrophs), Myxococcales, Chloroflexi, *Haliscomenobacter* (associated to hydrocarbon contaminated soils), *Devosia* (known from diesel contaminated soils) and Oceanospirillales (associated to the sites of the environmental disaster of the Deepwater Horizon oil spill). To the authors the microbial population of this special habitat, they name the "plastisphere", appears to have a characteristic "core" set of microbial taxa. From morphological and genetic results they deduce the possibility that microbes play a role in the degradation of hydrocarbon polymers.
Harrison et al. (2014) [129] showed that after 14 days the genera *Arcobacter* and *Colwellia* dominated on LDPE samples exposed on three marine sediments in mesocosm experiments. These bacterial groups are known from hydrocarbon-rich sites and have been affiliated with hydrocarbon-degrading mineralisation in cold ecosystems.
Guzman et al. (2011) [125] reported that the growth of the unicellular alga *Phaeodactylum tricornutum* (diatom) is positively stimulated by a commercial bag from a Carrefour shop, based on thermoplastic starch. The growth of this diatom was followed *in vivo* measuring the chlorophyll content by spectrophotometric techniques.
Reisser et al. (2014) [234] analysed the biofilm community on millimetre-sized plastic particles by scanning electron microscopy to consist of diatoms, coccolithophores, dinoflagellates, bacteria and fungi, and also invertebrates such as barnacles and bryozoa. They also found

deformations of the polymer surface with grooves and pits conforming to the shape of microorganisms. The authors suggest that these biota might play an important role in plastic degradation.
Flemming (1998) [98] states that the deterioration of polymeric materials is caused by adhering microorganisms that colonise their surfaces forming biofilms. He lists different mechanisms with specific effects, e.g.: coating the surface, increasing the leaching of additives and monomers, enzymatic attack, and penetration of the polymer matrix with microbial filaments of the plastic polymers.
A recent study by Eich et al. (2015) [85] compared the initial biofilm formation on PE and Mater-Bi, a starch-based biodegradable polymer. They found significant differences in the initial fouling community between the two polymers and between samples exposed in the free water and at the seafloor. How this complex ecosystem of the biofilm actually affects the degradation of the plastic is not understood and more research into that is needed.

4.5 Conclusions

After reviewing the current available studies on (bio)degradation of bio-based and fossil-based plastic in the marine environment we have identified several issues:

- Results of many reported studies are difficult to compare due to variations in methodology (focus on deterioration, fragmentation or assimilation) and test conditions (field trials or laboratory tests, duration);
- Biodegradation can only be proven by the analysis of direct measures in the laboratory, but laboratory test schemes can only mimic natural conditions within a narrow range, and some, by design, do not allow for the analysis of direct measures (e.g. flow-through systems);
- Biodegradation in important marine habitats such as the deep-sea water, seafloor and also shallow water sedimentary seafloor, is poorly studied;
- The understanding of the abiotic impact on plastic, and the interplay of abiotic and biotic factors of degradation is limited;
- The understanding of the degradation potential of single microbial strains and communities, as they occur in nature, is limited, and
- The understanding of the degradation of various polymer types and products is limited.

Therefore we recommend research on the following topics:

- Develop a standardized test scheme for the marine environment.
- Develop suitable indirect measures and intercalibrate them through a combination of laboratory, mesocosm and field tests with direct measurements of biodegradation;
- Define and include the most representative marine habitats in the test development;
- Determine the single and synergistic effects of abiotic factors such as light, temperature, water movement, pressure, nutrient and organic content, etc., and their interplay with biotic factors;

- Study the fouling community and their microhabitat, including the effect of specific organisms and the community on degradation, and
- Include the representative plastic types and products found in the sea as well as all products containing biodegradable materials in the degradation studies. This could allow to identify which products could be replaced and by what.

5 Biodegradation standard tests for the marine ecosystem

5.1 Introduction

Initially biodegradation test methods in an aqueous medium were developed in order to predict biodegradability in freshwater ecosystems and wastewater treatment plants. An overview of biodegradation test methods is given in deliverable 6.1 of the KBBPPS project [69]. It's outcome was that a sufficiently broad range of test methods exist for freshwater, but they need further optimisation. For marine systems considerably less tests are available than for freshwater systems and further investigations are needed to cover differences between the different habitats of the sea.

Due to the growing awareness of the need to protect the marine environment against the increasing loads of waste, methods on biodegradation were also developed for the marine environment. Generally speaking the biodegradation of test compounds in the environment is performed by a mixture of microorganisms that are naturally present. Biodegradation in a marine aerobic environment differs from biodegradation in a freshwater aerobic environment due to differences with regard to:

(1) the microbial population;
(2) the chemical characteristics of the water (salt content, nutrient content, etc.).

Currently biodegradation test methods for a marine environment are developed on OECD (Organisation for Economic Co-operation and Development) level, ISO (International Organisation for Standardisation) level and ASTM (American Society for Testing and Materials) level (Table 1). No European CEN (European Committee for Standardisation) or JISC (Japanese Industrial Standards Committee) test method has been developed so far. An overview of the most important parameters of the existing methods is given in this chapter.

Table 1. Overview of biodegradation methods in marine ecosystems.

Biodegradation test method	Title English	Suitability	Ref.
OECD 306 (1992)*	Biodegradability in Seawater	DOC measurement: not suitable OD: suitable, Nitrification could cause problems, when tests exceed 28 days	[210]
ISO 16221 (2001)	Water quality – Guidance for determination of biodegradability in the marine environment	DOC measurement: not suitable OD: suitable, Nitrification could cause problems, when tests exceed 28 days CO_2: suitable	[145]
ASTM D 6691 – 09	Standard Test Method for Determining Aerobic Biodegradation of Plastic Materials in the Marine Environment by a Defined Microbial Consortium or Natural Sea Water Inoculum	CO_2: suitable	[15]
ASTM D 6692 – 01	Standard Test Method for Determining the Biodegradability of Radiolabelled Polymeric Plastic Materials in Seawater	CO_2 of radiolabelled C: suitable for homo-polymers, not for blends	[13]
ASTM D 7473 - 12**	Standard Test Method for Weight Attrition of Plastic Materials in the Marine Environment by Open System Aquarium Incubations	Weight loss: suitable in combination with ASTM D 6691 Remark: it is not a biodegradation measurement	[17]

* First version.

** This test method does not determine biodegradation, but weight attrition.

5.2 OECD guideline

5.2.1 Description

One OECD guideline with regard to the evaluation of the biodegradation of chemicals in seawater is available: OECD 306 “Biodegradability in Seawater” (first version: Adopted 17 July 1992) [210].

The results of this test cannot be taken as indications of ready biodegradability, but are to be used specifically for obtaining information about the biodegradability of chemicals in the marine environment. If the results are positive (> 70 % DOC (dissolved organic carbon) removal or > 60 % ThOD (theoretical oxygen demand)), it may be concluded that there is a potential for biodegradation in a marine environment. The guideline emphasises that it is no simulation test as nutrients are added and the test concentration of the substance is much higher than the concentration that would be present in the sea. If a more definitive value would be required for the degree of biodegradation in seawater, other methods need to be used (e.g. simulation test in seawater using a test item concentration closer to the likely environmental concentration). It must be noticed that also in other biodegradation test methods (in freshwater, soil, etc.) nutrients are added and the test concentration is also higher when compared to actual concentrations in nature.

In this guideline two test methods are described:

(1) the shake flask method;

(2) closed bottle method.

An overview of the main parameters of these methods is given in Table 2, while the amount of replicates as prescribed by OECD 306 is given in Table 3.

Table 2. Overview of the main parameters as described in the shake flask method and the closed bottle method (OECD 306).

Parameter	Shake flask method	Closed bottle method
Suitable test items	Min. water solubility: 25-40 mg C/l Not volatile No adsorption onto glass	Min. solubility: 2 mg/l (less soluble can also be tested)
Inoculum	Natural seawater (after filtration – aging is allowed) to which nutrients are added (phosphate buffer, $CaCl_2$, $MgSO_4.7H_2O$ and $FeCl_3.6H_2O$) $DOC_{seawater}$ < 20% $DOC_{test\ mixture}$	Natural seawater (after filtration – aging is allowed) to which nutrients are added (phosphate buffer, $CaCl_2$, $MgSO_4.7H_2O$ and $FeCl_3.6H_2O$)
Characterisation of inoculum	Heterotrophic microbial colony count (optional) Dissolved nitrate, ammonium and phosphate Salinity DOC	Heterotrophic microbial colony count (optional) Dissolved nitrate, ammonium and phosphate Salinity DOC
Temperature	15-20°C	
Reference material	Sodium benzoate, sodium acetate or aniline*	
Measurement technique	DOC	Dissolved oxygen
Amount of test item	5-40 mg DOC/l	2-10 mg test substance/l
Duration	60 days (recommended, but extension is possible)	28 days** Can be extended on condition that the blank BOD values remain within the 30 % limit of the O_2 in the test vessel (if this is not the case, results are not reliable due to interferences as wall growth and nitrification)
Validity	Reference substrate: comparable to results of ring test	Biodegradation reference substrate comparable to results of ring test ($\geq$ 60 % (short time span)) Blank respiration < 30 % O_2 test vessel

* Reference materials are suitable when testing organic pure chemicals, but they are not suitable as reference material for more complex substances like bio-based plastics.

** The ring test showed that if the test was extended beyond 28 days no useful information could be gathered due to severe interferences caused by wall growth and nitrification.

Table 3. Amount of replicates in a typical run as prescribed by OECD 306.

Method	Blank series	Reference series	Test series	Abiotic sterile control	Toxicity control
Shake flask method	2	1	2	1 (optionally)	1 (optionally)
Closed bottle method	> 8	> 8	> 8		6

5.2.2 Suitability of the OECD method for bio-based plastics?

As the shake flask method is based on DOC measurements, this method is not suitable in order to evaluate the biodegradation of solid or poorly water soluble test materials. The closed bottle method, which is based on dissolved oxygen measurements, is more suitable for plastics. However, as indicated in OECD 306, the closed bottle method could be affected by nitrification (oxidation of ammonia) and is therefore less suitable in order to evaluate biodegradation during periods of > 28 days.

5.3 International standard

5.3.1 Description

One ISO standard with regard to biodegradation of organic compounds in a marine environment is available: ISO 16221 (2001) “Water quality – Guidance for determination of biodegradability in the marine environment” [145].
This standard is based on OECD 306, but a few modifications are made with regard to the measurement techniques and the inoculum. The measurement techniques are based on established aerobic freshwater tests. The main parameters of the test method are given in Table 4. The amount of replicates is a function of the used measurement technique (Table 5). For methods in which entire sampling containers have to be sacrificed for the measurements (ISO 10707 [141] and ISO 14593 [143]), the number of test vessels depends directly on the number of intended measurements and is accordingly high.

5.3.2 Suitability of the ISO method for bio-based plastics?

The method based on DOC measurements (ISO 7827) is not suitable in order to evaluate the biodegradability of solid or poorly water soluble test materials. The other proposed methods will be applicable to (bio-based) plastics. Although it must be noticed that methods based on oxygen consumption (ISO 10707 and ISO 10708) could be affected by nitrification (oxidation of ammonia) falsifying the oxygen recording. They are therefore less suitable in order to evaluate biodegradation during long periods when compared to methods based on carbon dioxide production (OECD 306 prescribes that tests based on oxygen consumption with a duration of > 28 days are characterised by a high variability of the oxygen measurements

due to nitrification and vessel wall growth). Therefore, the test methods based on carbon dioxide production (ISO 9439 or ISO 14593) can be selected as most suitable methods in order to evaluate biodegradability of bio-based plastics.

Table 4. Overview of the main parameters as described in ISO 16221.

Parameter	Description
Inoculum	Natural seawater (after filtration – preconditioning is possible up to 1 week) with nutrients (phosphate buffer & $FeCl_3.6H_2O$) or artificial seawater
Characterisation of inoculum	Salinity & DOC Colony-forming heterotrophic bacteria (plate count with marine agar) (recommended) [Suitable concentration = 105 cells/ml]
Temperature	15-25°C
Reference material	Sodium benzoate or aniline*
Measurement technique	DOC die-away test (ISO 7827) (DOC measurements) [146] Closed bottle test (ISO 10707) (BOD measurements) [141] Two-phase closed bottle test (ISO 10708) (BOD measurements) [140] CO_2 evolution test (ISO 9439) (CO_2 measurements) [142] CO_2 headspace test (ISO 14593) (TIC measurements) [143]
Amount of test item	5-40 mg DOC/l (ISO 7827) [146] 2-10 mg substance/l (ISO 10707) [141] 100 mg ThOD/l (ISO 10708) [140] 20 mg TOC/l (ISO 9439) [142] 20-40 mg TOC/l (ISO 14593) [143]
Duration	60 days
Validity	Biodegradation reference material > 60 % (respirometric measurements) or > 70 % (DOC measurements) after 14 days

* Reference materials are suitable when testing organic pure chemicals, but they are not suitable as reference material for more complex substances like bio-based plastics.

Remark: The addition of nutrients to the seawater is not identical when compared to OECD 306. OECD 306 mentions that 1 ml phosphate buffer, 1 ml $CaCl_2$ stock solution, 1 ml $MgSO_4.7H_2O$ stock solution and 1 ml $FeCl_3.6H_2O$ stock solution should be added per litre of seawater, while ISO 16221 prescribes that 10 ml phosphate buffer and 1 ml $FeCl_3.6H_2O$ stock solution should be added in order to prepare 1 litre test medium. The addition of $CaCl_2$ stock solution and $MgSO_4.7H_2O$ stock solution is not prescribed in ISO 16221.

Table 5. Minimum amount of replicates as prescribed by ISO 16221.

Method	Blank series	Reference series	Test series	Abiotic sterile control	Toxicity control
ISO 16221	2	1	2	1 (optionally)	1 (optionally)

5.4 American standards

5.4.1 Description

An overview of the American standards with regard to biodegradation and weight attrition in a marine environment is given in Table 6.

ASTM D6691 [15] can be described as a Tier 1 test, while ASTM D7473 [17] is a Tier 2 test, closer to real-life conditions. In ASTM D 6691 the sample is cryogenically milled to increase the surface area and biodegradability (CO_2 production) is determined, while plastics are tested as such in ASTM D7473 and weight loss is measured. As weight loss (= disintegration = physically fallen apart into smaller pieces) is measured, this standard cannot be used for demonstrating biodegradation (= complete mineralisation to H_2O, CO_2 and biomass).

ASTM D6692 [13] is designed in order to determine the degree of aerobic biodegradability of polymeric compounds utilised in plastic materials by determining the level of respiration of such radiolabelled carbon compounds to radiolabelled carbon dioxide.

The main principles of the American standard test methods D6691 and D6692, which determine the biodegradability of plastic materials in marine environments, are given in Table 7.

Table 6. Overview of the ASTM standards with regard to biodegradation in aerobic marine environment.

Standard	Description	Ref.
D6691 - 09	Standard Test Method for Determining Aerobic Biodegradation of Plastic Materials in the Marine Environment by a Defined Microbial Consortium or Natural Sea Water Inoculum	[15]
D6692 - 01	Standard Test Method for Determining the Biodegradability of Radiolabelled Polymeric Plastic Materials in Seawater	[13]
D7473 - 12	Standard Test Method for Weight Attrition of Plastic Materials in the Marine Environment by Open System Aquarium Incubations	[17]

Table 7. Overview of the main parameters as described in ASTM D6691 - 09 and ASTM D6692 - 01.

Parameter	ASTM D6691 - 09	ASTM D6692 – 01
Inoculum	Synthetic seawater with pre-grown population of at least 10 aerobic marine micro-organisms OR Natural seawater with inorganic nutrients (0.5 g/l NH_4Cl & 0.1 g/l of $KH_2(PO_4)$)	Natural sea water with inorganic nutrients (0.5 g/l NH_4Cl & 0.1 g/l of $KH_2(PO_4)$) Marine sediment can be added to increase the microbial diversity
Temperature	30 ± 2°C	Constant temperature (no specific temperature is mentioned)
Reference material	Cellulose, chitin or Kraft paper (control for activity of the inoculum) Sodium bicarbonate and sodium sulfite (control for CO_2 sensors)	Glucose or starch (uniformly labelled by ^{14}C)
Measurement technique	Respirometer to measure the CO_2 production	Measurement of amount of radioactive polymer that had been mineralised to $^{14}CO_2$ at various time points. Bottles are sacrificed at measurement. Before measuring the produced CO_2, the pH of the samples is brought to 2.5-3 followed by 6 hours shaking. During this period the $^{14}CO_2$ is trapped in a filter paper wick with an appropriate CO_2 trapping agent.
Amount of test item	20 mg per bottle (125 ml bottles) containing 75 ml natural seawater with inorganic nutrients or synthetic seawater	5-10 mg uniform ^{14}C radiolabelled polymer per bottle (120 ml bottles) containing 50 ml natural seawater with inorganic nutrients (Specific activity > 0.1 µCi/mg and < 5-10 µCi/mg)
Duration	Normally 10 - 90 days	Several days to several weeks
Amount of replicates	Triplicate	At least 6 bottles per series
Validity	Reference > 70 % biodegradation	No validation criteria

Remark: The addition of nutrients to the seawater is not identical when compared to OECD 306 and ISO 16221. The difference is even very significant (especially for ammonium chloride). The amount of inorganic nutrients per reactor is given in Table 8 together with the amount of nutrients in a typical freshwater biodegradation test (OECD 301).

Table 8. Overview of the concentration of inorganic nutrients in the test medium (mg/l).

Method	KH_2PO4	K_2HPO_4	$Na_2HPO_4.2H_2O$	NH_4Cl	$CaCl_2$	$MgSO_4.7H_2O$	$FeCl_3.6H_2O$
OECD 306	8.5	21.75	33.3	0.5	27.5	22.5	0.25
ISO 16221	85	217.5	334	5	-	-	0.25
ASTM D6691	100	-	-	500	-	-	-
ASTM D6692	100	-	-	500	-	-	-
OECD 301	85	217.5	334	5	27.5	22.5	0.25

In spite of the fact that ASTM D7473 is no standard test method with regard to biodegradability, the main principles of this standard are also discussed in this chapter due to the relationship with ASTM D6691. ASTM D7473 is used to measure the weight loss as a function of time for non-floating plastic materials under continuous flow (open system) aquarium conditions. The conditions as simulated in this test are representative for aquatic environments near the coasts and near the bottom of a water body in absence of sunlight. According to ASTM D7473 aquarium testing is considered as a more realistic approach of a marine environment when compared to a closed flask test as an aquarium test allows flushing, exposure to a diverse population of microbes, removal of metabolic end products, re-supply of oxygen, exposure to anoxic conditions in sediment and exposure to seasonal temperature variation of incoming seawater and natural concentration of macro- and micronutrients.
ASTM D7473 can only be applied on materials, which achieve at least 30 % mineralization in test method ASTM D6691. If the mineralisation of a test material does not reach 30 %, according to ASTM D6691, it shall be considered non-biodegradable in the marine environment. The main parameters of test method ASTM D7473 are given in Table 9.

Table 9. Overview of the main parameters as described in ASTM D7473 - 12.

Parameter	ASTM D7473 – 12
Inoculum	(1) Continuous fresh supply of natural seawater (= oxygenated seawater) (2) Continuous fresh supply of natural seawater (= oxygenated seawater) & surface marine sediment (anaerobic processes can play a role for films placed on the sediment)
Temperature	Temperature of the natural seawater is recorded at zero time and at each sampling point. Seasonal temperature fluctuations and mesophilic and psychrophilic microbes will play a role.
Reference material	-
Measurement technique	At selected time intervals, samples (triplicate) are removed from the aquarium box. The samples are rinsed and the weight of the rinsed samples is determined after drying to constant weight (35-40°C). The samples are also inspected visually (for example: blackening of the undersides of the sample). Correction is made for soluble components. Weight loss is also calculated per unit area of film.
Sample bottle	Plastic boxes
Amount of test item	0.5 by 0.5 inch pieces
Duration	180 days
Amount of replicates	3 replicates per weight determination / 5 weight determinations per test
Validity	-

5.4.2 Suitability of the ASTM methods for bio-based plastics?

The method based on the determination of evolved CO_2 of radiolabelled materials (ASTM D 6692) can be suitable for the determination of the biodegradability of homopolymers, but it is certainly not suitable in order to determine biodegradation of blends. Plastics are often composed of different main compounds and several additives. Consequently, it is not feasible to radiolabel each constituent of the plastic. Moreover it must be noticed that ASTM D 6692 is an expensive method. ASTM D 6691 can be used in order to determine biodegradation of plastics.

5.5 Standard specifications for the marine ecosystem

5.5.1 Description

ASTM D7081 “Standard Specification for Non-Floating Biodegradable Plastics in the Marine Environment” is the only standard specification [14], which encompasses criteria (including disintegration, biodegradation and environmental impacts with regard to aquatic toxicity, metals and other toxic substances) for non-floating plastics that are designed to be biodegradable under the marine environmental conditions of aerobic marine waters or anaerobic marine sediments. This standard however, was withdrawn in 2014 and is currently under revision.

This standard specification is intended to establish the requirements for labelling materials and products, as “marine disposable” or “biodegradable in marine waters and sediments”. An overview of the specific requirements is given in Table 10.

Table 10. Overview of the requirements for biodegradable plastics in marine waters and sediments as prescribed by ASTM D7081 - 05.

Parameter	Requirement as prescribed by ASTM D7081 – 05
Density	Minimum 1.05 g/cm³
Disintegration	Monomaterial: Maximum 30 % remains in the > 2 mm fraction after 12 weeks (ASTM D6691 under mesophilic or psychrophilic conditions)
Biodegradation	Minimum 30 % relative biodegradation (ASTM D6691) within 180 days at 30 ± 2°C for the plastic and substrate Minimum 90 % biodegradation in an active environment (e.g. compost) (ASTM D5338) (alternative methods: ISO 14851, ISO 14852 and ISO 14855) Satisfactory rate of biodegradation in test method ASTM D 5338 within 180 days or 365 days (for radiolabelled materials): 1. Homopolymer: minimum 60 % relative biodegradation OR 2. Other polymers and substrates: minimum 90 % relative biodegradation OR 3. Products (> 1 polymer): each polymer in a concentration > 1 %: 60 % relative biodegradation Remark: Plastics used as coating or binder, need to be tested separately and need to reach 90 % biodegradation
Toxicity	Products shall not adversely impact on the survival of marine organisms nor adversely affect the ecosystem using one of following methods: 1. Polytox (microbial oxygen absorption) 2. Microtox (microbial bioluminescence) 3. Fish acute toxicity (OPPTS 850.1075) 4. Daphnia acute toxicity (OPPTS 850.1010) 5. Static algal toxicity (OPPTS 850.5400) Heavy metals < 25 % of those prescribed in the country where the product is sold
Others	Compliance to ASTM D6400

Remarks:
- Only a disintegration criterion for monomaterials is mentioned in this standard specification (minimum 70 % disintegration). No criterion is mentioned in order to evaluate disintegration of final products. Disintegration should be evaluated on the final product and an appropriate disintegration criterion should be specified.

- Only 30 % biodegradation is required in an aquatic marine environment for the plastic and substrate. Consequently, if a plastic contains a component A (biodegradable in compost, but not in the marine environment) in a 40 % concentration and a component B (biodegradable in compost and in the marine environment) in a 60 % concentration, the 30 % biodegradation criterion will be easily reached in spite of the fact component A will never biodegrade in a marine environment.

- In order to evaluate the toxicity towards aquatic organisms, several test methods are mentioned, but no test item concentration is prescribed and it is not specified when it can be concluded that no toxic effect is observed (= no pass level). Moreover, in aquatic tests it is normally specified that the test item should not exceed the limit of solubility in the dilution water. How this problem should be handled when evaluating toxicity of plastic materials (= solid materials) is not specified in the standard specification. In other specifications for biodegradable plastics (for example compostable plastics or biodegradable mulch films) ecotoxicological effects are evaluated after the biodegradation phase. Possibly this is also a better approach for the evaluation of the environmental safety in the marine environment. In this way the biodegradation residuals could be evaluated. However, when evaluating the environmental safety after the biodegradation phase, marine species should be selected in order to evaluate the toxicity (freshwater species are currently specified).

5.5.2 Suitability of the ASTM method in order to define if bio-based plastics are biodegradable under marine conditions

The current American standard specification contains all necessary items (biodegradation, disintegration, environmental safety towards aquatic organisms and limits for heavy metals and toxic substances) in order to define criteria for bio-based plastics biodegradable under marine conditions, but it is not severe enough with regard to disintegration and biodegradation. Moreover, the evaluation of the environmental safety is described too vague in order to evaluate environmental safety correctly.
This specification was withdrawn in April 2014 in accordance with section 10.6.3 of the Regulations Governing ASTM Technical Committees, which requires that standards shall be updated by the end of the eighth year since the last approval date.

5.6 OK biodegradable MARINE

Recently (2-3-2015) the OK biodegradable MARINE certification scheme (Doc.nr: TS-OK12-e) [309] has been introduced by Vinçotte, an accredited private Belgian Agency. This certification scheme comprises the certification of the claim on biodegradability and the communication about the certification. The label can be granted to all raw materials, all components and/or intermediate products, and all finished products provided they are non-floating (i.e. have a density higher than 1.05 g/cm^3).

The certification scheme follows ASTM D7081 [14], but the pass level in seawater is set at 90% for biodegradation and disintegration. Further details are given in Table 11.

Table 11. Overview of the requirements for OK biodegradable MARINE label

Parameter	Requirement as prescribed by OK biodegradable MARINE label
Density	Minimum 1.05 g/cm³
Disintegration	Test must specify thickness and density. The test item will be under the form of pieces of 2 × 2 cm for raw materials. The temperature is maintained at 30 ± 2°C and the content of the vessels is shaken during the test. Test is executed in 3 replicates. Incubation of 84 days is required. The test item is considered to meet the disintegration requirement if no more than 10% of its original dry weight remains after sieving on a 2.0 mm sieve [309]
Biodegradation	Minimum 90 % (relative or absolute) biodegradation within 6 months; Otherwise as ASTM D7081
Toxicity	Concentration tested shall be 1% of dry mass basis. Incubation of 3 months is required. Less than 90% of the tested microorganisms should be affected at the tested concentration.
Chemical characteristics	Heavy metals and fluorine requirements of EN13432 should be met. Co < 38 ppm
Others	Compliance to ASTM D7081

5.7 Conclusions

Initially, biodegradation test methods in an aqueous medium were developed in order to predict biodegradability in freshwater ecosystems and wastewater treatment plants, but for marine systems considerably fewer tests are available.
Currently, biodegradation test methods for a marine environment are developed on OECD (Organisation for Economic Co-operation and Development) level, ISO (International Organisation for Standardisation) level and ASTM (American Society for Testing and Materials) level:

- OECD 306 “Biodegradability in Seawater”: the shake flask method is based on DOC measurements and therefore not suitable in order to evaluate the biodegradation of solid or poorly water soluble test materials. The closed bottles method (based on oxygen measurements) is more suitable for plastics but care should be taken with longer term tests (>28 days).

- ISO 16221 “Water quality - Guidance for determination of biodegradability” is based on OECD 306 and can be used to determine the biodegradation of organic compounds. The biodegradation is assessed through the CO_2 production which enables the use for (solid) plastics.

- ASTM D6691-09 “Standard Test Method for Determining Aerobic Biodegradation of Plastic Materials in the Marine Environment by a Defined Microbial Consortium or Natural Sea Water Inoculum” can be used to determine the biodegradability of plastics in the aerobic marine environment.

- ASTM D7801 “ Standard Specification for Non-Floating Biodegradable Plastics in the Marine Environment” is the only standard specification, which encompasses criteria (including disintegration, biodegradation and environmental impacts with regard to aquatic toxicity, metals and other toxic substances) for non-floating plastics that are designed to be biodegradable under the marine environmental conditions of aerobic marine waters or anaerobic marine sediments. This standard however, was withdrawn in 2014 and is currently under revision.

Recently a new certification scheme, based on ASTM D7801, was introduced by Vinçotte “OK biodegradable MARINE” for labelling of non-floating raw material, components and/or intermediate and finished products.

6 Missing aspects for standard test development

6.1 Introduction

The knowledge on biodegradation of plastics in marine habitats is still limited and research is needed for the development of standard tests. This paragraph aims to summarise and explain missing aspects (Figure 1) by taking into account the conditions in the marine habitats where plastic is ending up, and the fact that plastic itself constitutes a micro-habitat. In order to better understand the great variation within the entire marine ecosystem, it needs to be categorised into certain spaces (compartments) which need to be characterised according to their physical, chemical and biotic properties (factors), thus defining a set of marine habitats. The compilation of the diversity of habitats and their environmental parameters highlights that a series of representative habitats should be included in the test development.

Addressed in this review:

- Formulation of the final goal for standardised marine biodegradation tests
- Plastic accumulation in specific habitats
- The diversity of marine habitats
- The range of a given set of physical, chemical and geo-(morpho-)logical conditions in marine habitats to account for e.g. highly dynamic conditions or very stable ones

Touched in this review, further research is needed:

- Global climatic and small-scale regional differentiation
- The presence of characteristic communities of organisms, with local, seasonal and depth-related variations
- The diversity of fouling microorganisms and their metabolisms
- Interactions of polymer, organisms and surroundings
- Interactions/synergistic and antagonistic effects of polymers, organisms and surroundings, including the influences of different habitats on the biodegradation of a bioplastic
- Polymer test material composition, structure, surface properties, size and amount
- The range of available microbial degradation strategies/metabolisms
- Fouling: its effect on weight of the polymer when measuring weight loss and the oxygen consumption when using an oxymeter for measuring biodegradation
- Needed accelerators for degradation processes
- Environmental safety towards aquatic organisms and limits for heavy metals and toxic substances in order to evaluate environmental safety correctly

Addressed within Open-Bio experimental work:

- Time lag between optimal lab conditions and field conditions
- Potential „bottle effects“ due to a long time duration of the test
- Limitations for degradation: e.g. nutrients, oxygen, temperature, light

Figure 1. Summary of the currently missing aspects needed for the development of a set of standards for marine biodegradation.

6.2 The diversity of marine ecosystems – factors determining physical, chemical and geo-(morpho-)logical conditions

6.2.1 Marine habitats

The definition of marine habitats follows an ecological approach and is based on the presence of characteristic communities of organisms that thrive within a range of a given set of physical, chemical and geo-(morpho-)logical conditions. Global climatic and small-scale regional effects cause transitions from one habitat to the other creating a mosaic of adjacent habitats all over the planet from polar to tropical, from coastal to open ocean and the deep sea. There are two general groups of habitats: The habitats of the seafloor, termed benthos or benthic domain, and the habitats of the free water, the pelagic domain. The highest diversity of benthic habitats is found along the coasts with often distinct boundaries between them. The pelagic domain is less easily separated into distinct habitats although major ocean currents, eddies or layers of different densities can sharply separate water masses both horizontally (e.g. Gulfstream) and vertically (e.g. surface water vs. deep water in the Strait of Gibraltar). The link between all marine habitats is the water mass that covers about 71% of the Earth's surface and comprises 96.5% of all water globally.

6.2.2 Physical properties

Water has a high specific heat capacity and stores a large amount of energy from solar radiation. Temperatures range from the freezing point of sea water at about -2°C to 35°C and higher in coastal lagoons. Another important abiotic factor is water movement either as an oscillation by wind-driven waves or as unidirectional flow by gravity-driven currents. Hydrostatic pressure increases with depth at a rate of 1 bar per 10 meters and has an influence e.g. on the solubility of gasses and on metabolic processes [170].

6.2.3 Chemical properties

Seawater is a solution of different salts with an average content of 3.5%, defined as salinity. It also contains dissolved gasses (like oxygen, nitrogen and carbon dioxide), organic molecules of natural origin (e.g. amino acids, lipids, humic substances) and anthropogenic contaminants of locally varying concentrations. The pH of seawater is usually between 7.5 and 8.4 but can change drastically with certain geological and biological events. This is also true for seawater in sediments or in the vicinity of living organisms that exchange gasses and other dissolved substances during metabolic processes (e.g. respiration).

6.2.4 Substrate

Whereas the free water body of the pelagic domain is relatively homogenous, benthic habitats greatly depend on the nature of the seafloor. Rock, gravel, sand or mud offer different

conditions for the settlement of bottom-dwelling organisms. Rock provides a stable long-lasting surface whereas sand maybe turned over continually, be washed away or reshaped completely during a storm event. Sediment bottoms account for 85% of the shelf habitats. Apart from newly generated rocky seafloor at spreading zones the deep-sea floor is dominated by sediment.

6.2.5 Microbes

Microbial life dominates the world's oceans since roughly 4 billion years and microbes are the most important drivers of biogeochemical processes. Microbes are found everywhere in liquid water where temperatures are moderate. But marine microbial life is also possible at higher temperatures; in hyperthermophilic conditions microbes have been detected at temperatures up to approximately 113°C. Thus a sample of natural seawater and sediment should be considered a mixed culture of many thousands of different types of microorganisms. Typical concentrations of bacteria in seawater are 10 million cells per millilitre for coastal lagoons and 100000 cells for oceanic surface water, and about 10^{12} per g sediments [303], with local, seasonal and depth-related variations occurring. Amaral-Zettler et al. (2010) [6] reviewed the number of microorganisms in different marine compartments and state that the average cell concentration in the open ocean is even 10^6 cells per millilitre of seawater, and that marine surface sediments may contain 10^8–10^9 cells per gram and at greater depth 10^5 cells per gram. Moreover, some habitats (microbial mats) contain up to 10^{12} cells per millilitre, which is close to the count of viable cells that has been reported for highly engineered systems like granular sludge from a Upflow Anaerobic Sludge Blanket reactor, namely 10^{12}-10^{14} cells per gram of volatile organic solids [217]. Nearly all organic matter is recycled by microorganisms and global element cycles are governed by bacteria, archaea and fungi [10, 21, 95, 160, 278].

6.2.6 Food web

One very special set of conditions is found within the body of organisms. The internalisation of food into dedicated organs (gills, mouth, and gut) with all its physical and chemical agents and the transport within the different trophic levels of a food web can be considered a habitat on its own.

6.2.7 The photic zone

By far the most important abiotic factor that shapes marine habitats is solar radiation. Light is absorbed by the surface water itself and stored as heat which is distributed all over the globe by a system of large-scale currents. In the range of 400 - 700 nm wavelength light is used by bacteria, protists, algae and plants to perform photosynthesis, the primary production of biomass from the inorganic molecules water and carbon dioxide, and the formation of molecular

oxygen as a waste product. The penetration depth of the seawater for this photosynthetically active radiation (PAR) limits the depth distribution of light-dependent photosynthetic organisms. It varies locally and may reach over 100 metres. In the twilight zone below the depth of efficient photosynthesis down to depths of about 1000 metres there is still light to see, although less than 1% of the surface light is measurable. The deeper parts comprise 90% volume of the marine realm and are in permanent darkness.

6.2.8 Stability in depth

At the interface between sea surface and atmosphere physical, chemical and as a consequence biological conditions fluctuate within a wide range. Wind stirs up the water surface creating waves. Temperature gradients and evaporation initiate currents, mixing of water masses occur. Gasses exchange between water and atmosphere. With depth most of these surface effects lose power and fade out. More than half of the Earth's surface is covered by deep-sea (abyssal) plains where the absence of light, low temperatures, little water movement, lower oxygen and higher nutrient concentrations (SiO_2, NO_3^-, PO_4^{3-}) prevail. Apart from certain hotspots food and organic matter are scarce. The deep sea depends on what sinks down from the productive surface layer. There are fluctuations with seasons and large-scale currents but the range of variation is usually low.

6.2.9 High dynamics in shallow-water

Some of the commonly best-known marine habitats are in very shallow water. Tropical coral reefs are built by billions of carbonate-forming animal colonies and algae, and face a daily change in light. In the tropics, water temperature is relatively stable at around 25°C with minor seasonal excursions. Water movement can range from tidal currents and small surface waves to tropical storms that destroy the structures of hundreds of years of coral growth and shift millions of tons of sediment within a few hours. During long periods of calm weather the water can heat up and become too hot for corals and their algal symbionts, eventually leading to a bleaching or even mass die-off. The European Wadden Sea which is falling dry every day faces variable environmental conditions over a high range. In winter the sandflats can be ice-covered, in hot summer the seafloor can be heated up to 40°C and more. Storms resuspend the sediment and cover organic matter such as algal or seagrass debris several centimetres deep. This highly dynamic system is reflected by the typical community of organisms consisting of many borrowing animals that can retreat deeply into the seafloor.

6.2.10 Organisms shape the habitat

Clams, worms and crustaceans turn a flat sediment bottom into a three-dimensional maze of surfaces by ventilating seawater through tube-like burrows, some to a depth of up to 1 metre. Corals build their own habitat and create a tropical reef as a hard structure so rich in micro-

habitats that it harbours some hundred thousands of other species. Marine flowering plants as seagrasses in warm-temperate regions or large algae as the giant kelp in cold-temperate waters (macrophytes) dominate their habitats and serve as the main structuring element and home for thousands of other species.

6.2.11 Interaction with other man-made factors

Plastic in the marine environment contributes only a part to the whole complex of anthropogenic pollution. In areas of highest contamination with plastic often a co-contamination with waste water rich in dissolved organic matter, liquid pollutants and other solid materials occur. Often these heavily polluted areas, as e.g. estuaries close to coastal mega-cities have little in common with known natural marine habitats. Physical and chemical conditions will form a complex mix of natural and man-made factors, and biotic conditions may be reduced to the presence of a specialised microbial community. Regarding the biodegradation of plastic in such a heavily altered marine environment synergistic and antagonistic processes are likely to occur and should be classified as special cases.

6.3 Plastic accumulating in the marine habitats and factors affecting its biodegradation

6.3.1 Plastic in the Sea - Choosing the model habitats for testing biodegradation

An overwhelming number of studies have shown that plastic as by today has arrived in the entire marine environment. Transported to the sea by rivers or wind from inland, directly introduced from the coast and from ships, or lost from fisheries plastic items of different scale can be found in almost any survey by sampling or video monitoring. Macro-plastic and micro-plastic particles float at the ocean's surface, are bound in sea-ice, buried in deep-sea sediment and washed ashore at remote oceanic islands as well as in mangrove forests or tidal marshes all over the world. Plankton trawls from far below the sea surface bring up plastic. All coastal habitats contain a measurable amount of plastic foil, lines, containers and fragments. From deep-sea studies it can be assumed that most of the ocean floor contains plastic remains (references in Figure 2).

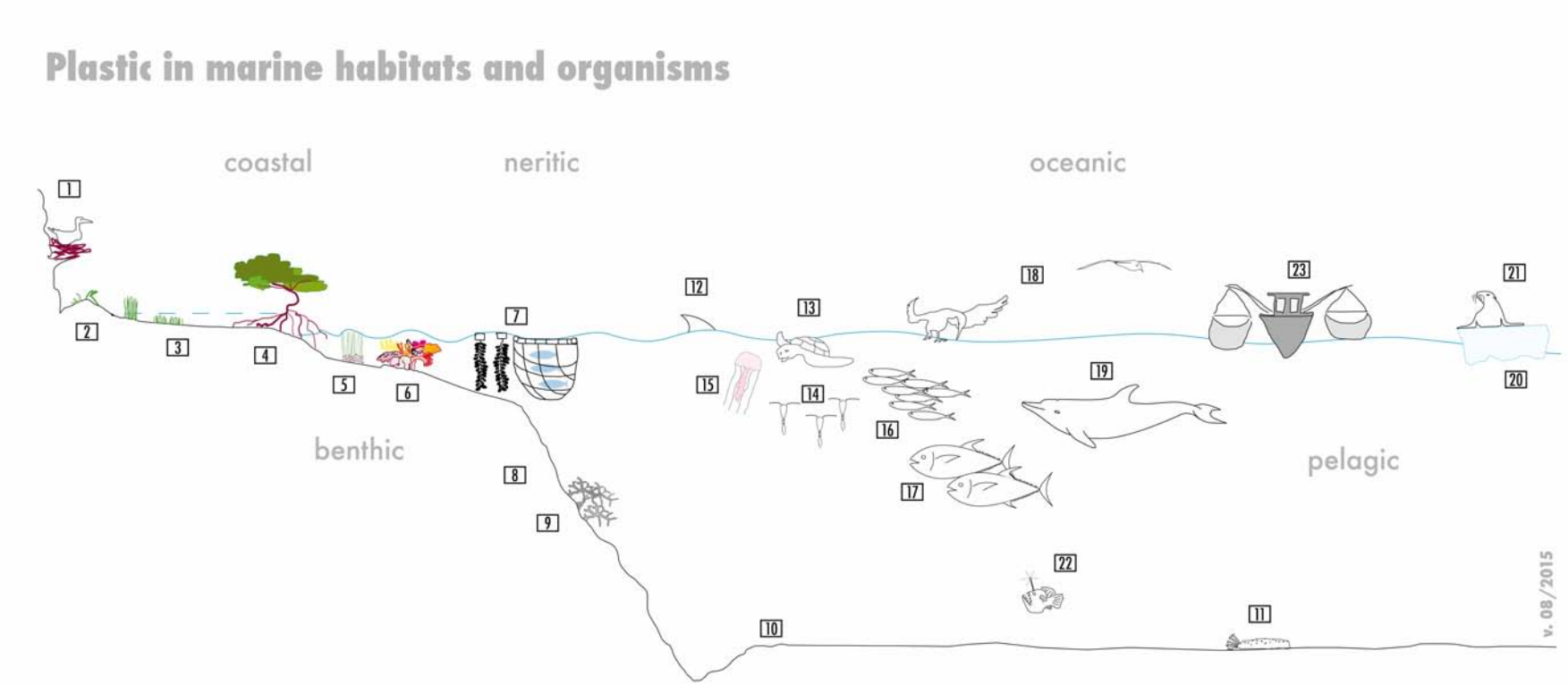


Figure 2. Overview of plastic in the marine environment. References for each item on next page.

References for Figure 2:

1 **Sea birds use plastic debris as nesting material, young get entangled, ingest plastic** [2, 18, 34, 46, 57, 112, 166, 168, 171, 172, 247, 257, 275, 306]

2 **Sand dunes and beaches accumulate plastic from land and sea. Tourism is a source of plastic** [22, 25, 42, 49, 51, 54, 55, 63, 66, 70, 71, 73, 76, 83, 84, 86, 96, 97, 102, 121, 122, 130, 131, 134, 149, 152, 155, 162, 167, 176, 180, 187, 189, 192, 200, 214, 216, 225, 242, 243, 251, 253, 254, 263-268, 273, 281, 286, 287, 297, 299, 317]

3 **Salt marshes and coastal lagoons are traps for floating plastic in the intertidal zone** [64, 131, 226, 292, 296, 307]

4 **Mangroves and coastal forests accumulate plastic from rivers and from sea** [64, 70, 148, 166, 193]

5 **Macrophytes like seagrass and kelp act as sediment traps and may accumulate microplastics** [105]

6 **Coral reefs are richly structured and act as traps, polyps take up microplastics** [1, 82, 127]

7 **Artificial habitats like mussel and fish farms rely on particulate feed, microplastic is taken up by bivalves and fish** [41, 44, 55, 181, 300, 310]

8 **Plastic may be transported from the continental shelf over the continental margin by deep-sea canyons** [106, 108, 110, 114, 195, 215, 221, 230, 256]

9 **Deep-sea coral reefs are richly structured and can trap plastic debris** [166, 319]

10 **Plastic is widely distributed in deep-sea habitats and regularly found in scientific samples** [9, 27, 72, 111, 138, 191, 222, 231, 256, 301, 313, 319]

11 **Deposit- and sediment-feeding animals are reported to take up microplastic particles** [29, 39, 45, 53, 118, 128, 177, 181, 300, 320]

12 **Great pelagic predators ingest plastic** [26, 50, 52, 56, 107, 112, 166]

13 **Plastic ingestion by sea turtles is considered a major threat to their survival** [23, 48, 65, 107, 112, 116, 117, 119, 123, 132, 166, 168, 183, 196, 197, 255, 258, 304]

14 **Microplankton like copepods ingest microplastic particles** [60, 159, 181, 261, 321]

15 **Macroplankton like jellyfish and salps take up plastic** [60]

16 **Plankton feeding fish like sardines, anchovy and herring, and large filter feeders take up microplastic directly or via plankton** [32, 99, 100, 112, 115, 136, 181, 182, 321]

17 **Pelagic predators like tuna eat smaller plankton-feeding fish and may take up plastic this way** [52, 99, 154, 186]

18 **Sea birds ingest plastic debris from the sea surface, get entangled in lines and derelict fishing gear** [2, 19, 20, 33, 35, 36, 57-59, 61, 81, 103, 104, 112, 120, 123, 156, 166, 168, 169, 171, 178, 185, 190, 227-229, 241, 246, 248-250, 275, 280, 285, 302, 305, 323, 325, 326]

19 **Whales and dolphins eat plastic, get entangled in plastic and abandoned fishing gear** [24, 67, 80, 107, 112, 117, 150, 166, 168, 169, 269, 270]

20 **Microplastic trapped in sea ice is released as Artic ice cover is melting** [207]

21 **Seals and penguins ingest, and become entangled in plastic debris** [5, 37, 38, 101, 112, 166, 168, 169, 218, 233, 277, 311]

22 **Mid-water and deep-sea fish are found to have ingested plastic** [52]

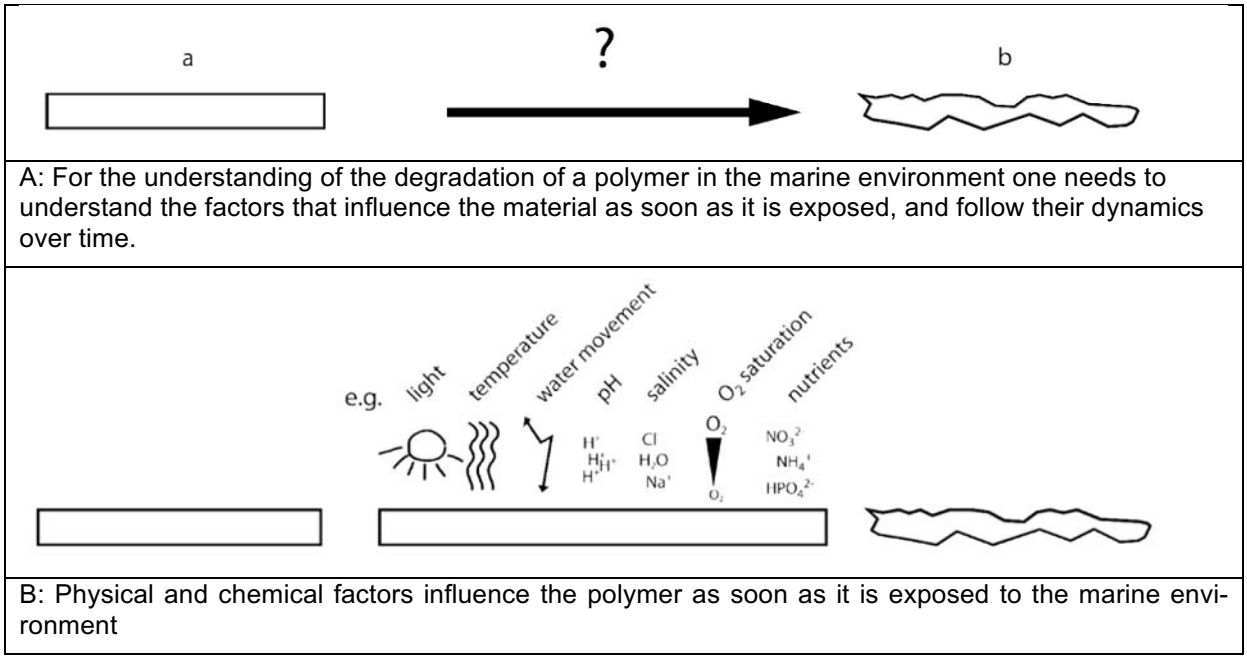
23 **Plastic debris is clogging nets of commercial fishermen; fish, shellfish and shrimp for human consumption contain microplastic which might leach sorbed chemicals to the organism** [55, 113, 133, 136, 163, 164, 199, 202, 236-240, 260, 300]

6.3.2 Degradation rate and environmental parameters

Very generally speaking, the marine environment is considered less aggressive from an aerobic biodegradation point of view as compared to freshwater, soil and compost conditions [69], because e.g. the number of bacteria in seawater is relatively low. Weathering, disintegration and degradation rates proceed in the marine environment much slower than on land, although the main abiotic parameters involved are the same [220].
Being submerged accelerates the rate of degradation of some types of plastics. The exposure at lower temperatures than on land may slow down the degradation, and the microbes may be limited by the shortage of N- and P-compounds [232, 322]. Biodegradable plastics could be a carbon and nutrient source for the degrading microorganisms.

6.3.3 Plastic as microhabitat

Polymers exposed to the marine environment (Figure 3A) form a microhabitat experiencing the effect of physical, chemical and biotic factors that will eventually alter the material (Figure 3B). An important aspect is that surfaces exposed to the marine environment, allow the development of a zone of concentrated nutrients by adsorption that favours the formation of a microbial biofilm [79, 328]. The surface tension also allows diatoms to shape the biofilm [62]. This way plastic serves as a substrate and after the rapid colonisation of micro-organisms, macro-organisms follow. This process is called "fouling".
The material properties themselves affect the fouling processes. A comparative study on diatoms of biofilms formed on fibreglass and glass coupons revealed significant differences in density and diversity [219]. Also on different polymers, comparing LDPE with the starch based biodegradable polymer MaterBi from shopping bags, community differences were recorded in the early fouling [85]. Jones et al. (2007) [157] showed that two different solid surfaces (stainless steel and polycarbonate) submerged in seawater determined the rapid growth of bacterial biofilm. During the first week the microbial population characterising the biofilm was similar in both materials but after some weeks each material started to develop an own microbial biofilm population diverging from the other material. These results highlighted that different materials promote different microbial species in the biofilm composition. The fouling is a dynamic succession, where formative factors are for example competition for space, biochemical conditions and intra and inter-specific competition. The occurrence of certain cyanobacteria, dinoflagellates, sponges and bryozoa can lead to the production of toxic secondary metabolites within the plastic microhabitat. Some of these substances are known as potent toxins and can locally change the conditions fundamentally, e.g. by killing parts of the bacterial community.



A: For the understanding of the degradation of a polymer in the marine environment one needs to understand the factors that influence the material as soon as it is exposed, and follow their dynamics over time.

B: Physical and chemical factors influence the polymer as soon as it is exposed to the marine environment

Figure 3. Factors that control degradation of polymers in marine environments

Within weeks, several hundred organisms are in ecological interactions (Figure 4). The fouling of the polymer gradually alters the biogeochemical conditions at its surface (Figure 5A). The biofilm is acting as a filter towards the environment. The physiological processes of the community eventually dominate the conditions at the polymer surface that may differ greatly from the surrounding environmental conditions. The conditions reflect the interplay of the physico-chemical factors and the biological processes in the microhabitat on the surface of the polymer (Figure 5B). The polymer-biofilm interface is where the critical conditions are found, and under which the biodegradation occurs. The detection of the organisms, the biochemical processes and functions will allow conclusions on mechanisms enhancing or delaying the biodegradation. How this knowledge will help to optimise the conditions of a standard lab test, needs to be tested in future projects.

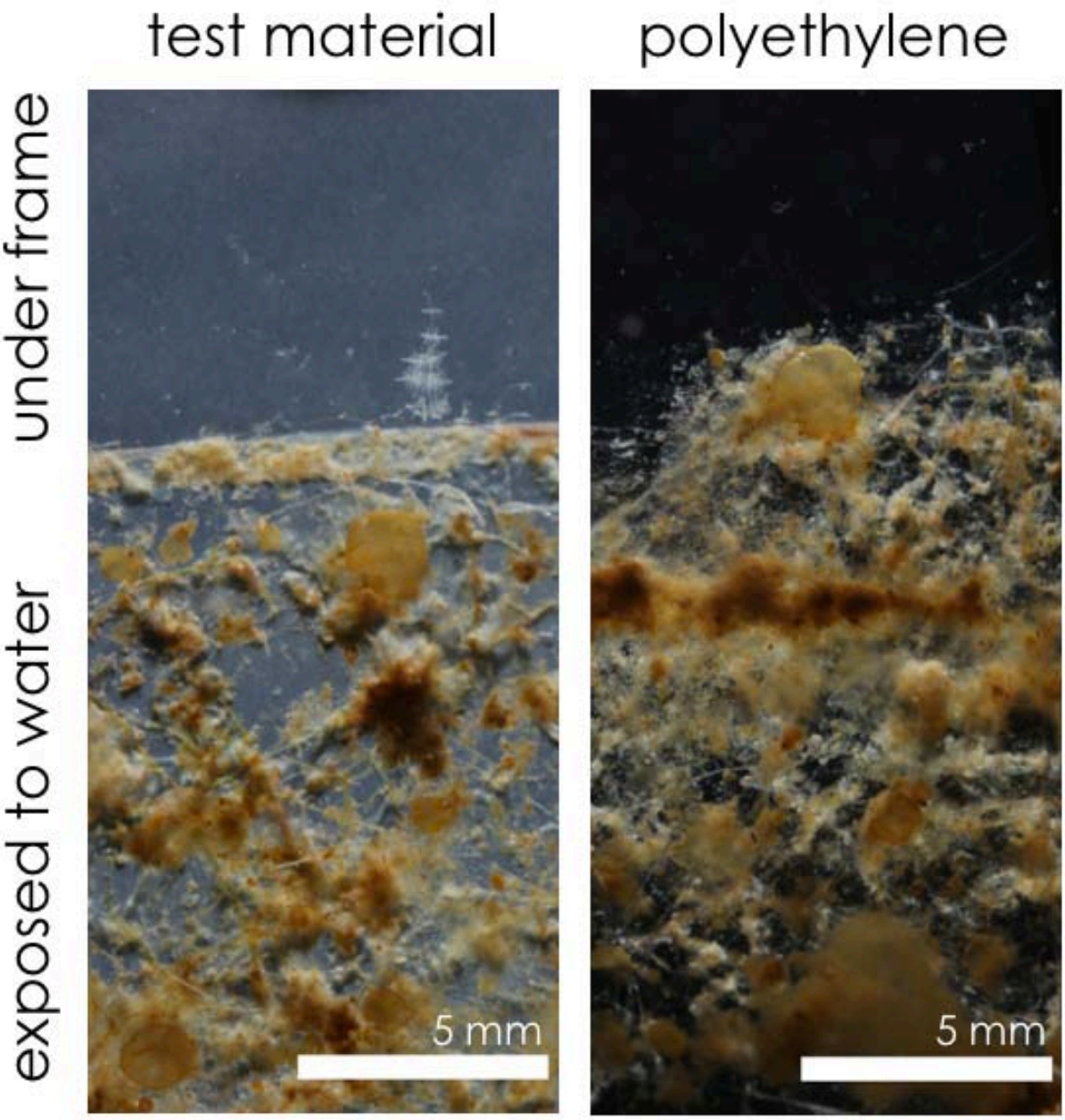
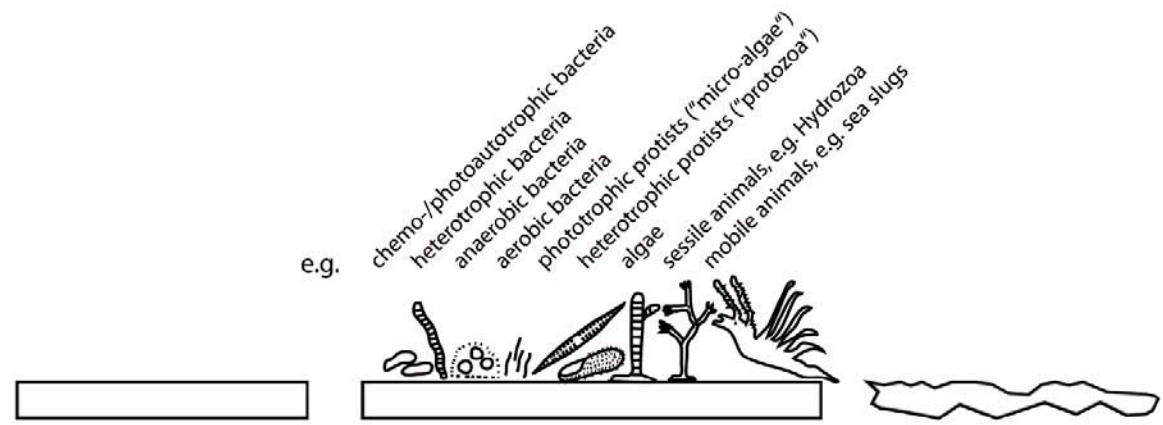
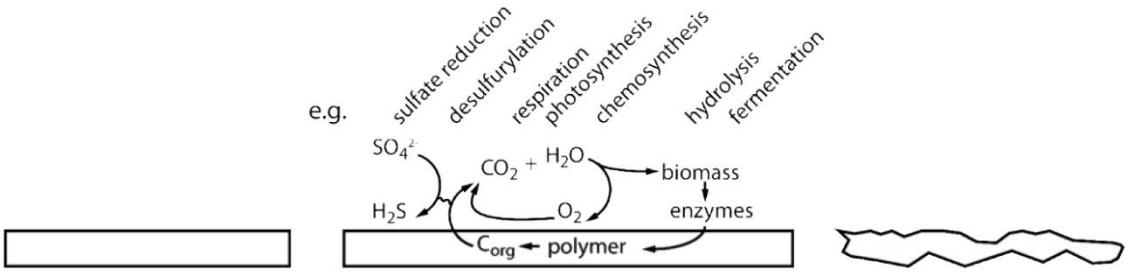


Figure 4. Two examples of a plastic surface after 11 weeks exposure in the sea. The upper part of the specimens has been covered by a holder which practically inhibited the settlement of macroscopic organisms.



A: Microbial biofilm formation starts within hours of exposure to seawater. Within days protists arrive and within weeks algae and invertebrates as hydrozoans and bryozoans may settle. Mobile animals as sea slugs and shrimp use this micro-habitat as grazers and micro-predators.



B: The metabolic activities of the organisms that form a biofilm greatly influence the biogeochemical conditions at the plastic surface and the exchange of substances with the surrounding.

Figure 5. Fouling of plastics surfaces.

6.3.4 Degradation rate and fouling

Fouling on the solid surface of polymeric substrates placed into seawater does not necessarily mean that the microbes involved are effectively able to bio-degrade it using the polymeric carbon for their metabolism. It can just be a physical support.

Changes of the external environmental factors however also lead to changes of the chemical conditions in the microhabitat (Figure 6). For example oxygen can be consumed but also produced within the fouling community, if the polymer is exposed to light. From natural biofilms it is known that under high light exposure oxygen becomes highly over-saturated and free oxygen radicals can occur. These may strongly enhance the degradation process chemically. At night or in low light conditions at greater depth the consumption of oxygen can result in hypoxic or anoxic conditions within the microhabitat. Typical anaerobic metabolic processes such as hydrolysis, fermentation, desulfurylation and sulfate reduction are likely and may impact the degradation processes of the polymer.

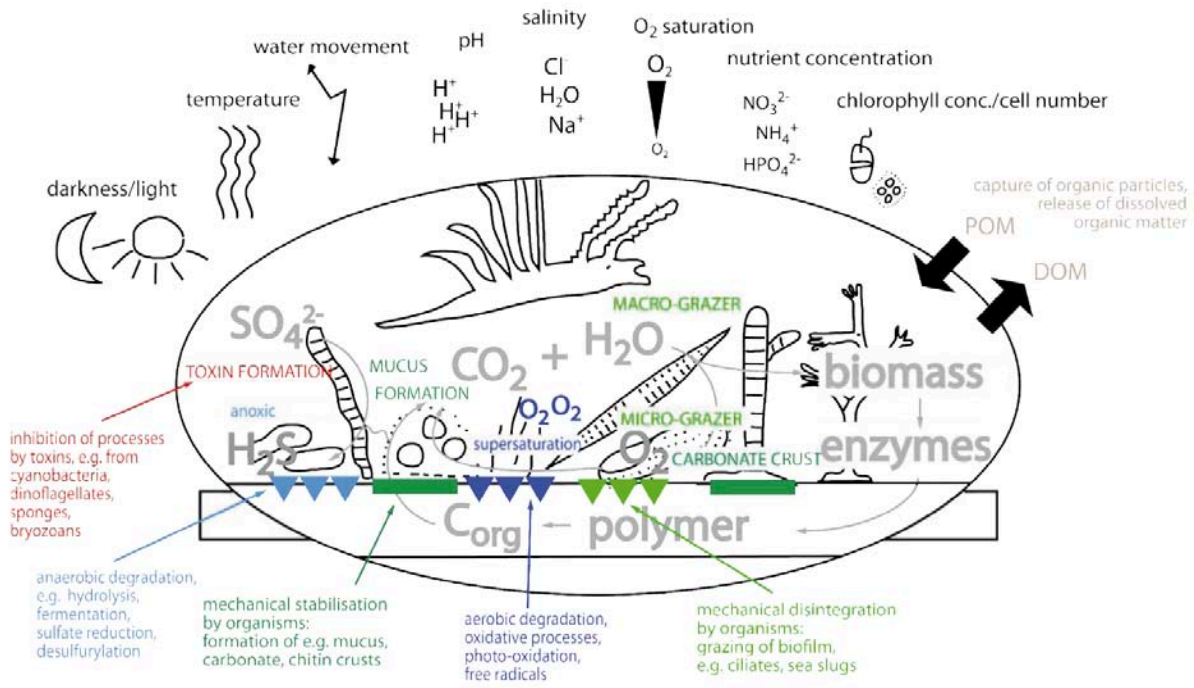


Figure 6. A matured plastic surface in the sea is a complex microcosm on its own. Now the organisms that form the biofilm dominate the biogeochemical conditions at the plastic surface and the exchange of substances with the surrounding. The fouling community acts as a filter between the external environmental conditions and the plastic material.

Biofilms are constituted of extracellular polymeric substances (EPS, „mucus“), which are organic polysaccharides and proteins. These organic macromolecules are produced and secreted from bacteria, fungi and protozoa in the biofilm [98]. EPS, crusts from red algae or chitin skeletons of bryozoa can shield the polymer surface from the surroundings and may delay or inhibit the degradation process. Physical impact on the material itself can occur through the activity of higher organisms. Single-celled protists such as ciliates feed on bacteria and scrape them from surfaces (micro-grazers). Algae-feeding sea slugs (macro-grazers) possess a rasping organ and can mechanically impact the surface structure of the polymer. The action of the feeding apparatus can roughen or even puncture the surface and thus increase the structural disintegration.
It remains to be studied whether the fouling community at the plastic surface accelerates [7] or delays (Weber, personal comm.) biodegradation.

6.3.5 Degradation rate and benthic habitats

The progressive fouling increases the density of the plastic item causing it to sink [324]. 80% of the seafloor surface is covered with sediment. Sediments exist in a variety of types. The most abundant sediment is mud, second most abundant is fine-grained sand. These sediments are by the majority low in organics and low in microbial activity. 70% of plastic waste

sinks to the seafloor and the biggest sink for micro-plastic is the deep-sea sediment [319] where it is exposed to mud with low activity under high pressure and in cold temperatures.
On the other hand Wu et al. (2008) [322] highlighted that the microbial communities living in marine sediments are able to degrade a series of complex polymeric compounds, such as cellulose, lignin, xylene, chitin, and catechol. Since for marine compartments other than the pelagic domain the number of bacteria may be very different, tests adopted for the benthic compartment could have very different outcomes compared to the seawater tests.
Depending on the sediment grain size and the hydrodynamic conditions, the upper few centimetres of the sediment are oxygenated, compared to the deeper sediment [316]. The microbial activity and diversity is higher in the aerobic sediment layer [165]. So far, it can only be speculated whether under marine conditions plastic material is better degraded in the more active oxygenated upper sediment layer, or under anaerobic conditions.
Ratto et al. (2001) [232] support the hypothesis that the direct contact of the plastic material with the sediment surface will promote the biodegradation process. In tank experiments the weight loss was higher and occurred faster when the samples were in thanks with sediment compared to seawater only. Also Tosin et al. [288] report of highest disintegration at the interface sediment-water, when they exposed polymers in tanks with sedment and seawater.
First results from research within Open-Bio show that polymers buried in sediment in the eulittoral (tidal zone) disintegrate fastest. Tensile property measurements however revealed that the polymers exposed in the sublittoral sediment-water interface show the highest reduction in material strength. Furthermore the results show that in mesocosms where controlled conditions occur the polymers disintegrate faster than in the field, showing that the disintegration of polymers does differ depending on the environmental conditions (Figure 7). Further studies are needed to understand details when, why and how disintegration and biodegradation do occur.

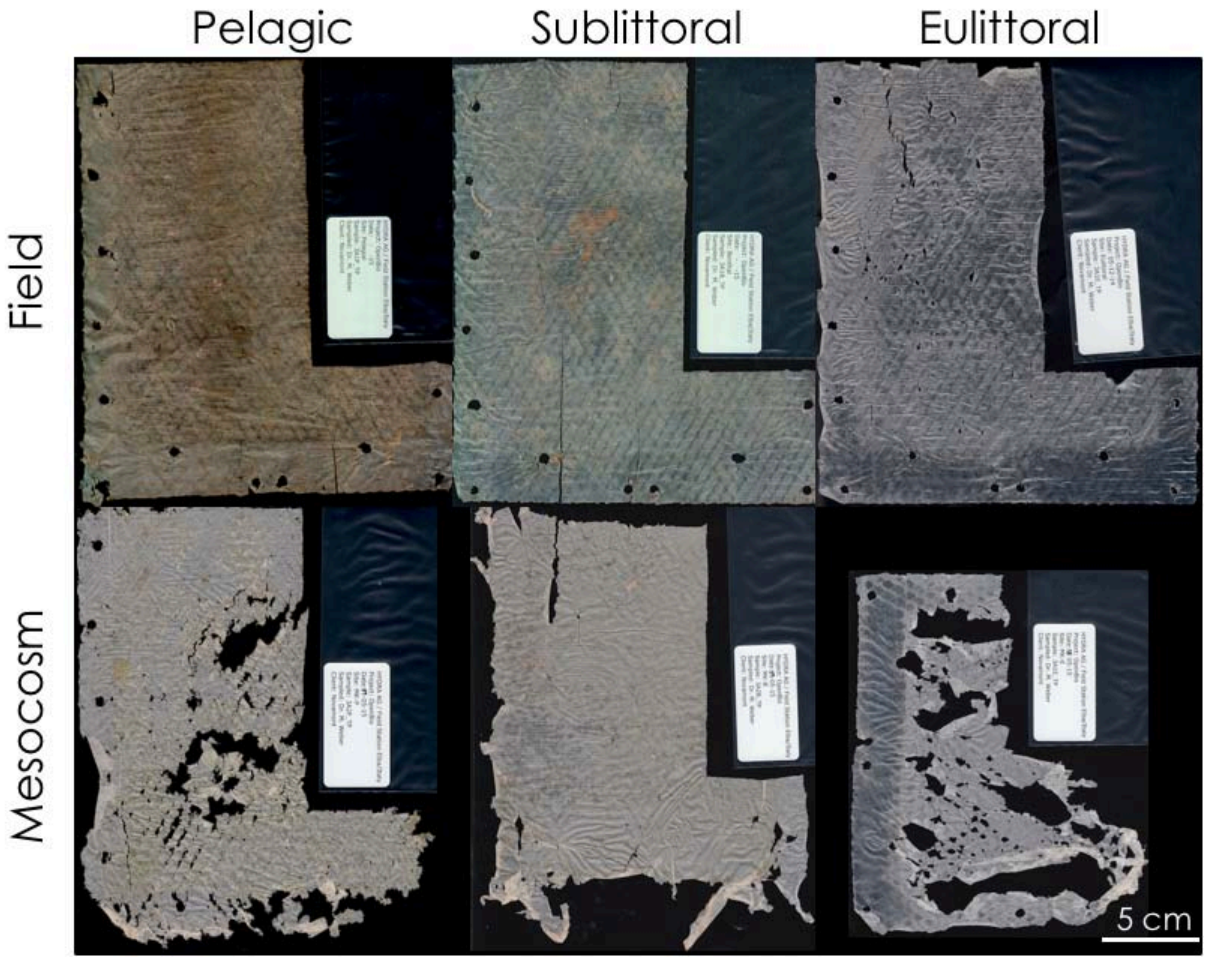


Figure 7. The disintegration of PBSeT polymers exposed for 7.5 months in the pelagic (water column), at the sublittoral sediment-water interface and the eulittoral (intertidal) zone. The upper line was exposed in the field and the lower line in large mesocosm tanks under controlled environmental conditions.

7 Possible adaptation of existing soil and freshwater standard tests

7.1 Introduction

For some of the marine zones as depicted in Figure 8 tests have been or could be developed based on existing methods for freshwater and soil.

Very generally speaking, the marine environment is considered less aggressive compared to freshwater, soil and compost conditions [69]. So far, biodegradation tests for polymers in the marine environment are very specific and standardised only to a little extent [87]. Most of the guidelines have not been designed for solid polymer biodegradation and thus have to be adapted for the special requirements or be developed anew.

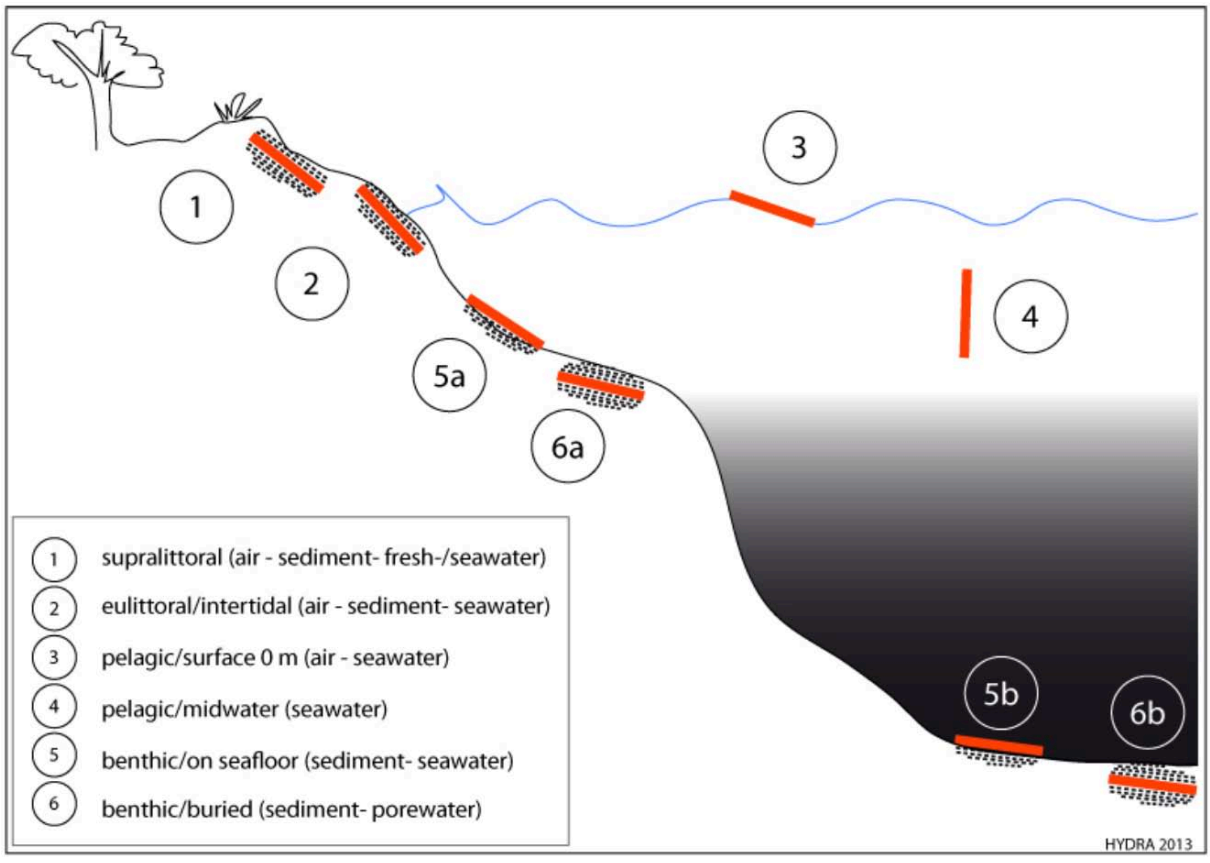


Figure 8. Major types of marine habitats where plastic has been found; 1. (Partially) buried in sediment/sand without exposure to the tides (supralittoral zone); 2. (Partially) buried in sediment/sand with regular humidification by tidal water (eulittoral zone); 3. Floating on the sea surface; 4. Open ocean water, free floating (pelagic zone); 5. Lying on the bottom a) of the sublittoral zone or b) of the deep sea; 6. Buried a) in the sublittoral sediment or b) in the deep sea sediment.

7.2 Pelagic environment

Tests from ASTM (D5271 withdrawn 2011 [11]) and ISO (14851 [144]) are available as a basis for setting up a test to determine the biodegradability in the pelagic domain under aerobic conditions. ASTM D5271 tests the degradation when 2.5 g/L mixed-liquid volatile suspended solid activated-sludge biomass is present. In ISO 14851 also an inoculum from activated sludge, compost or soil should be used. Prepared for testing biodegradability in marine environment, also ASTM D6691 [15] foresees an inoculation of minimum nine microorganisms known to degrade various biodegradable polymers, starches, cellulose and bacterial polyesters. All these standards should be considered for defining an inoculum to use in marine tests at laboratory conditions.

Anaerobic conditions do not occur when bio-based materials are free floating except in those cases when biofilms or other biological material would adhere to the materials. This could lead to local anaerobic micro-niches and their effect on the degradation speed needs to be elucidated and evaluated in the near future. Furthermore, it needs to be clarified how to transfer the biofilm effect to a lab test system. Lab tests are usually kept in the dark and that is why little biofilm develops, and no phototrophic organisms will grow in it. This is a discrepancy to the real world, where a lot of phototrophs grow on the polymers (Figure 4). Therefore, besides the laboratory scale batch tests, other setups have been proposed to assess the biodegradability at a somewhat larger scale and/or with more different specimens at once. One example is the aquarium set up proposed by Tosin et al (2012) [288]. Breslin and Li (1993) [40] describe a similar method to determine the effect of seawater on (bio)plastic films. Frames with the testing compounds were placed in a flow-through table that was continuously flooded with seawater pumped (via a screen to avoid inflow of large particles) directly from an estuarine marsh onto the samples. Currently in the Open-Bio project a closed-circuit mesocosm is tested. In this system the environmental parameters light, temperature, water flow and the nutrient regime are fully controlled.

7.3 Benthic environment

Not many biodegradation tests have been developed for the non-pelagic marine compartments although some existing tests could be adjusted for tests in sediments. E.g., ASTM D6692 [13] (refer to Chapter 5 for details on methods) allows the addition of marine sediment to increase microbial diversity in the test. The protocol foresees an aerobic test so this could be a model for a test to assess the biodegradability of a bio-based material buried in sediment under aerobic water. The continuous test method ASTM D7473 – 12 [17] (refer to Chapter 5 for details on methods) would be even more suitable (but is probably also more difficult to carry out) because the amount of sediment present promotes the formation of anaerobic zones deeper in the sediment.

7.4 Supralittoral zone: in sediment

At the moment there is no method available to determine the biodegradability of solid polymers in the supralittoral zone where the samples are exposed to a sandy soil with mostly low moisture content. Aerobic conditions prevail in such environments and occasional flooding occurs. This can occur with freshwater by rain or sea water from storms, which is difficult to simulate in a laboratory environment. The supralittoral zone has some features in common with the unsaturated (vadose) zone in sandy soils. A lot of research has been dedicated towards the development of a test method to determine the biodegradability of xenobiotic compounds in such environments (example [223]), but in those cases the methods deal with (semi) volatile compounds, which is entirely different from the (often) solid substrates currently under study for this deliverable.
Compost or soil test methods, such as ASTM D 5988 “Standard Test Method for Determining Aerobic Biodegradation of Plastic Materials in Soil” [16], may be applicable although the nutrient content, especially in compost, is obviously much higher. Nevertheless, test methods of solid substrates for composting are available and may be adopted [69]. Besides the nutrient content (in the systems) also the temperature during the test may be an issue. Temperatures reached (or imposed) during compost tests (as a result of carbon breakdown) will be relatively stable and much higher (50-60°C) than encountered in the supralittoral zone (<0 to >40°C). Moreover, during composting there is no day-night rhythm.

7.5 Eulittoral zone: in sediment

A test method to assess the degradation of plastic samples partially buried in the sand and kept wet by the tides (eulittoral zone) was applied by Tosin et al. (2012) [288]. In this test plastic samples were placed in a box filled with wet sand, and during the test moisture was amended. However the tides were not simulated. To mimic the eulittoral more pre-testing with the moistening regime, dynamic temperatures and different sandy sediments is needed. Tests developed to assess the biodegradation in saturated soil could be suitable (or modified) to determine the biodegradability in intertidal marine sediment. Current aerobic tests to determine the biodegradation of plastics in terrestrial soil are available on OECD, ISO, AFNOR and ASTM level [69]. In most cases fresh soil mixtures are used to ensure enough microbial activity at fixed temperatures and moisture (usually defined as a fixed percentage of the water-holding capacity or as pore water pressure). Nutrients are only added to a desired C:N ratio and oxygen consumption and/or carbon dioxide formation are determined as a measure for biodegradation. ISO 175562012 [147] permits the use of a standard soil consisting of industrial quartz sand, clay, natural soil and mature compost, specific salts and water. Most methods assess biodegradability under aerobic conditions.

7.6 Sublittoral and deep-sea zone: on and in sediment

7.6.1 Aerobic conditions

As described above for the eulittoral zone, tests developed to assess the biodegradation in saturated soil could be also suitable (or modified) to determine the biodegradability in sediment/seawater (benthic environment/sublittoral) interface. Basically such a test does not exist yet, but as stated above ASTM D6692 could also be modified for this purpose. Another possibility for the adaptation of an existing test could be OECD 308, which defines the ready (i.e. without adaptation) aerobic and anaerobic transformation in aquatic sediments. This method has been developed for fresh water sediments but could also be adopted for estuarine or marine sediments [87, 212]. The transformation of a test substance is assessed in aquatic sediments with an aerobic top layer and anaerobic conditions deeper into the sediment layer. The method is based on OECD 301 thru 306. Transformation rates in both the water and sediment phase can be determined. The method as it is now (OECD 308) is only applicable for test substances for which a specific analytical method is available.
While the Open-Bio project is running two ISO/DIS test methods were developed for the sublittoral zone: I) Determination of aerobic biodegradation of non-floating plastic materials in a seawater/sediment interface — Method by measuring the oxygen demand in closed respirometer (ISO/DIS 18830) was opened for voting until 08.08.2015, and II) determination of aerobic biodegradation of non-floating plastic materials in seawater/sediment interface – Method by analysis of evolved carbon dioxide (ISO/DIS 19676) was opened for voting until 19.08.2015.
For the deep sea no test is available and pre-tests are definitely needed, because most of the plastic waste sinks to the deep sea [324].

7.6.2 Anaerobic conditions

OECD 307 is designed to determine the anaerobic biodegradability as well. In that case terrestrial soil is pre-incubated under aerobic conditions and thereafter subjected to anaerobic conditions by flooding the soil with 1-3 cm of water and flushing the headspace of the flask with an inert gas (N_2 or argon) [211].
The biodegradation in anaerobic sediment or in anaerobic seawater could be tested in a set-up similar to setups used in anaerobic microbiology and environmental technology for studying e.g. microbial activity or degradation of (xenobiotic) organic compounds under anaerobic conditions. In general, sludge or sediment is mixed with a water phase/medium/wastewater in closed bottle systems and product formation (or substrate loss) is followed in time (after the headspace has been flushed to guarantee optimal anaerobic conditions). Examples of set-ups for activity or biomethane potential tests are given by Angelidaki et al. (2009) [8] for digesters and e.g. high-salinity wastewaters ([30] and references therein). Also, standard test methods developed for anaerobic digestion could be modified for this purpose replacing the inoculum in those tests by anaerobic deep-sea sediments covered with a layer of seawater

while imposing anoxic conditions in the headspace of the flasks. Test material could be buried in the sediment. These methods have been reviewed recently in Open-Bio project deliverable 6.6 [68].

The high pressure in the deep-sea is an important factor determining the fate of the materials that end up in that environment. Generally, it should be possible to apply such pressures (while varying the temperatures) to assess the physical effect on the test material. To assess the potential of microbial biodegradation on the remains of the test material should be possible, but is probably also very costly.

8 Next steps for the development of marine biodegradation standardised tests

Ideally, tests in the marine environment should cover all possible habitats/habitat conditions in which plastic can end up. Realistically, there can be a complementary set of a few tests that cover most of the environmental scenarios in terms of plastic abundance and habitat importance on a global scale (Figure 8). The interpretation of the results obtained by these standardised tests under optimal conditions includes indications to extrapolate the results to different environmental conditions.
The choices for a certain testing protocol have to be based on solid evaluations. And a compromise is to develop tests under optimal conditions for each marine compartment. This implicates that the results will deviate from the results in situ. Therefore it is important to assess the degradation behaviour under real conditions to become capable to validate the lab tests.

There are specific aspects that need to be addressed while assessing the biodegradability of test compounds. Biodegradation of test compounds is carried out by (a mixture of) micro-organisms that are naturally present in the environment that the polymer entered. The most important factors determining whether or not any test compound is degraded are given below:

- **Physical environmental conditions**: Care has to be taken to impose the correct physical environmental conditions. Because chemical and biological reactions occur optimally only in a specific range. The optimal microbial activity depends for example on pH, temperature, salinity, and pressure. High pressures prevail in the deep-sea, the largest zone of the world's ocean. Many existing tests are developed for open-water conditions whereas most of the plastics will end up IN and/or ON sand or sediment, where the physical conditions are different.

- **Chemical composition of the water**: The chemical composition of the water**,** such as the presence of co-substrates and nutrients (macro-nutrients like ions of nitrogen, phosphorous, magnesium and other bivalent cations, but also trace elements) determine the efficient growth of microbes. In the majority of the tests mineral medium is used. This medium contains nutrients (N, P, Na, K, Mg, Ca, Fe) in sufficient concentrations (which may differ between tests) to sustain biodegradation or the test compounds and/or growth (depending on the test) while repressing other disturbing processes (e.g. nitrification) if necessary [69]. Sometimes (e.g. OECD 309: simulation biodegradation test) the test is carried out in surface water [213]. In the marine tests on the one hand artificial seawater is used (as an analogue for the mineral medium) (ISO16221), which mimics the average seawater composition. In most test methods however, natural seawater is used as a matrix (OECD 306, ISO16221), but sometimes additional nutrients are added (OECD 306, ISO16221) to create optimal conditions for biodegradation. Many tests have been developed for aerobic conditions whereas material may also end up in an environment where

anaerobic conditions prevail. Here care has to be taken to know if for example sulfidogenic or methanogenic conditions occur, because they involve completely different microorganisms.

- **Concentration of carbon and nutrients**: the concentrations of carbon, nitrogen and phosphorous compounds and the ratio of total solids and volatile solids (TS/VS) should be monitored, at least at the beginning and the end of the test, ideally also during the test.
- **Quality and amount of inoculum:** Special care should be attributed to the inoculum quality and quantity, because they are defining the outcome of the test to a large extent. The presence of the appropriate microorganisms has a major influence on the test outcome. The strategies needed to degrade a pure material or compound depend on the polymer. A substance that is introduced anew into an environment may be readily degraded by the microorganisms present or only after adaptation, or co-metabolically (in the presence of a cosubstrate). Furthermore the substance may be degraded by a sole microorganism or by a consortium of microorganisms. One could debate whether seawater (which is often used) is the best way to inoculate the tests and mimic marine biodegradation, because of the differences in composition and microbial abundance between different locations. In a ring test by Nyholm and Kristensen (1992) [205] it was shown that differences in outcome could be attributed to site-specific differences in microflora in the different seawaters and differences in applied concentrations. It was concluded that a positive result indicates that the substance will most likely degrade rapidly, whereas a negative result does not confirm that biodegradation does NOT take place (because environmental conditions, e.g. contaminant concentration, are different in situ) [205]. This is critical because a positive result cannot be extrapolated to other habitats. The first results from Open-Bio show that the disintegration (field and mesocosm test) and the biodegradation (lab tests) is fastest in the tests with sediment, for example. And the two field sites tested reveal that the disintegration on the sedimentary seafloor is faster at the Greek site than at the Italian site under study (Weber, personal comment). The same effects of the variations of the inoculum are known from soil and fresh water tests and this knowledge should be used for the development of the marine tests.

  Halophilic microorganisms are different from freshwater microorganisms or microorganisms from wastewater treatment plants (that are often used for freshwater tests). In those cases one could argue that the seawater should be amended with extracts of sediment or aerobic marine microorganisms, e.g. after extraction from sea aquarium filters and pre-aeration (as was done by Bernhard et al. (2008) [28]) as the only aim of those tests is to determine biodegradability under *optimal* conditions.

  For freshwater tests a variety of inocula may be used like activated sludge, effluent from wastewater treatment plants, surface water, soil extract, or mixtures thereof depending on the test applied (“ready biodegradability”, “inherent biodegradability” or “simulated biodegradation”). In the marine tests often seawater (approximately 10^5 CFU/ml is used as an inoculum (OECD 306, ISO16221). In some cases the methods describe that the

seawater should/could be amended with pre-grown aerobic marine microorganisms (ASTM6691) or marine sediment (ASTM6692). For the marine tests more research is needed to define the appropriate inoculum. Further molecular methods should be used to assess the composition of inoculum added.

- **Time scale:** The time scale can be a major downside of many test procedures, because they are designed for testing periods of 28 to 100 days [87], whereas the degradation of the test materials may take longer. Therefore it may be difficult to adapt existing methods developed for other environments to marine conditions. Also because the influence of the microorganisms to be added to the test is not to be underestimated. Care has to be taken not to change too many environmental parameters, like temperature, salinity, pH, UV light or by addition of special enzymes, a primer compound, or (adapted) microorganisms, because this would be too far off from the real-life in situ conditions for ready biodegradability [6] On the other hand specialised microbial consortia may develop in situ if similar (biodegradable) polymers have been in the environment for longer periods of time and biodegradation is possible.
- **Properties and concentration of polymer materials:** The properties (e.g. solubility, molecular structure and physico-chemical parameters) and the concentration of the test compound have to be considered. Usually concentrations encountered in the environment are lower than applied in the laboratory (for analytical reasons). The amount on test substances added is usually in milligram per litre range, which is rather high, but justifiable. The products to be tested (i.e. plastics) are in most cases not easily dissolved, which means that they will not occur in the environment as highly diluted substrates, but more likely in solid form, thus higher concentrations than microgram per litre range that would normally be applied for dissolved substances to mimic in situ conditions. Also, the solid state of test polymers may serve as a carrier material for the microorganisms, which would be a case for a higher concentration of microbial biomass in the tests.

9 Conclusions and recommendations: Test development within Open-Bio and beyond

9.1 Conclusions

The focus for this deliverable was on the degradation of biodegradable bio-based **solid** materials (e.g. plastics) in the sea and standardized tests that need to be developed to assess biodegradability of these materials. Such tests and test schemes have been developed in the past for plastics and other solid materials, but in most cases they are solely applicable in freshwater ecosystems and wastewater treatment plants. For marine systems considerably fewer tests are available and these tests are mostly dedicated to the degradation in aerobic environments of the shallow water column. Disintegration in marine conditions is also very limitedly addressed in existing test schemes.

The marine environment is only partially made up of aerobic water, and plastics do end up in other habitats. In order to assess the biodegradability and disintegration of plastics in all representative marine environments, laboratory and mesocosm tests need to be developed that reflect the required conditions (anaerobic or aerobic, presence of sediment, availability of nutrients etc). The tests shall mimic optimal conditions for biodegradation and disintegration, and so conditions in the field may deviate from the laboratory. Field and mesocosm tests are the bases to assess and evaluate such deviations. Comparing results obtained in mesocosm and field tests and results of tests carried out in the laboratory may assist in setting up the standardized test set for the marine environment.

9.2 Recommendations

In Open-Bio we are currently working on standard tests that represent a selected set of marine conditions: warm seawater with high oxygen and low nutrient concentrations available. Novelties are the tests in the intertidal sediment and at the sediment-water interface, also with high oxygen and low nutrients and organics at 20-25°C. Tests are currently being developed for the biodegradation in the sandy eulittoral (intertidal) and sublittoral zones, such as the benthic sand-seawater interface and in neritic seawater (shallow pelagic zone). It is an extension beyond the OECD and ASTM standards. In nature there are several more important sets of conditions: a lot of areas are hypoxic or without oxygen (anoxic), vast regions are covered with very fine sediment and are cold, and some coastal areas have increased nutrient and organic concentrations. These regions are for example urbanised coastal areas (50% of the world's population live within 60 km of the sea, 3/4 of all large cities are located on the coast), or shallow water mangroves and seagrass ecosystems at the coasts. The deep sea covers about 60% of our planet, has an average temperature of 2-3°C, and is covered mostly by fine sediments of low organic content. 71% of the earth surface is the marine ecosystem. From that surface 80% is covered with sediment. Sediments exist in a variety of types. The most abundant sediment is mud; second most abundant is fine-grained sand.

These sediments are low in organics and therefore low in microbial activity. 70% of plastic waste sinks to the seafloor and the biggest sink for microplastic is, newly published, the deep-sea sediment [319].

To reflect more representative regions in the marine ecosystem, the following crucial conditions need to be further considered for standard test development:

- A lot of plastic ends up in areas where no oxygen is present: tests under anaerobic conditions should be developed
- A lot of plastic accumulate in the deep sea: test under high-pressure and low temperature should be developed
- Most of the seafloor is covered by fine-grained sand or muddy (hypoxic/anoxic) sediment: tests with fine sediments under low oxygen conditions are needed.
- Effects of different levels of nutrients and organic contents should be considered to develop all the standard tests.

Littered or lost at sea are final products, rarely raw materials in the form of films or grains. These final products should be tested on their biodegradability in larger mesocosm systems. In mesocosms the real world is well representable and more similar to lab systems, and the physical and chemical conditions are very stable, controllable and measurable. In the field environmental conditions are variable and a test system with the possibility to vary the conditions is needed for a comprehensive standard test set.
We recommend to extract the representative habitats from nature and translate their conditions into a set of standard laboratory and mesocosm tests to be developed and implemented, representing the most important regions in our oceans.

9.3 Challenging the ecological complexity: taming natural variability into a feasible laboratory test scenario - a step-by-step approach

An ecological approach to investigate the degradation of polymers in the marine system is suggested. In order to start the complex task while focussing on the development of a standardised test for the bio-degradation of bio-based polymers in the marine environment, a gradual six-step scenario is recommended:

1) Laboratory tests are currently being performed by four Open-Bio partners under test conditions based on preliminary tests. The tests simulate three environmental scenarios: free water, water-sediment interface and in sediment. All tests are aerobic at temperatures between 20 and 25°C and carried out in the dark. Mesocosm tests are being performed by one partner in tanks in a temperature-controlled laboratory at 20 °C and a 12:12 h light:dark rhythm. One test mimics the burial of polymers in the intertidal beach with a tidal rhythm of 6 hours, the two other tests simulate free water and the water-sediment interface. In the field tests the selected polymers are exposed in three marine habitats with

relatively easy access, a coastal beach setting, pending in the water column and laying on the seafloor. At specified time intervals the samples are retrieved and material tests conducted. The most important abiotic environmental parameters as e.g. temperature, light, salinity, and pH are measured at least at each sampling interval, ideally recorded continuously. This will give a solid overview over the general habitat conditions and will render them comparable to other habitats and known literature values. The results from the material tests will give a first insight into how fast degradation takes place under natural marine conditions in the chosen habitats. First correlations to environmental metadata are possible. At this stage the biotic factors resulting from the biofilm and the fouling community will not be addressed, but treated as a black box model (Figure 3A). On the basis of the data from field tests, controlled mesocosm (tank) tests and laboratory (flask) tests can be refined. The mesocosm tests will be used as a link to the field tests and will allow the validation and subsequent improvements of the laboratory tests. In this first approach, Open-Bio is testing in three shallow water habitats. In future projects further representative habitats, for example the deep sea, should be included.

2) In the second step we briefly address the ecological perspective, which is as an in-depth study clearly a task for future projects. We recommend to choose key processes of the biogeochemical interactions on the polymer and to describe them for each test habitat. The processes at the surface of the polymer and its fouling community should be analysed over a one-year cycle. The measurement of the chemical parameters such as pH, oxygen and sulfide, directly at the polymer surface, for example with micro-electrodes, will allow to characterise the most important biogeochemical processes and will help to describe the real conditions during the degradation process. The knowledge of this step is needed for defining the final settings of the standardised lab test set.
3) In the third step we define a streamlined methodological tool set for the laboratory tests which should comprehensively allow to determine biodegradation under the controlled laboratory conditions that best match the natural conditions in the complex marine environment. This work will need a team discussion of experts of standardisation, biodegradation, material development and marine science to define the details. Tasks are to rule out redundancy, excluding the similar outcomes of the field tests from representative habitats and to extract which lab tests under which conditions are needed to evaluate the degradability in the marine environment. Further details need to be discussed, for example whether the used media (matrices) such as water and sediment should be natural and sampled at a specific place and time of the year from the field or whether artificial seawater/sediment spiked with a known inoculum of microbes should be used. The latter would allow avoiding seasonal and/or local effects and would provide standardised comparable media (matrices). On the other hand artificial media and inocula could cover a too narrow range of possible natural agents. Both approaches have their advantages and disadvantages and both possibilities should be tested within the pre-normative approach in order to propose the best testing scheme for marine conditions. In Open-Bio first results

will be presented for lab tests simulating the three habitats, the eulittoral zone (intertidal), sublittoral pelagic (water column) and the benthic zone (water/sediment interface).

4) As a fourth step the ecotoxicological effects on the environment need to be discussed for the test polymers, which are claimed to be biodegradable under marine conditions. Moreover, tests for all representative marine habitats should be developed in future projects and should cover *all* marine trophic levels from microbes to vertebrates and consider effects from sub-cellular (genetic, endocrine) level to whole organisms (fitness, fertility), with an extrapolation to population and ecosystem level by mathematic modelling in the case of detected effects.
5) The fifth step will consist in the synthesis of the accumulated technical and scientific knowledge. From that, there will result the determination of methods and conditions suitable for the definition of one or more testing schemes. Distinct tests will be proposed to be evaluated and eventually refined in the laboratory.
6) The sixth and final step will be to verify the feasibility and reliability of the developed testing schemes by round robin tests. These should be done for several different classes of (pure) polymers and also products to prove the suitability of the method for the materials currently on the market. Conclusively and ideally, a choice of representative polymers that have been proven to be biodegradable under laboratory conditions should be tested in the field under real conditions to validate the results and their environmental relevance.

We recommend to elucidate the following questions (Figure 9) and to develop further research projects in order to answer them as soon as possible.

Define the relevant marine habitats

On a global scale, in which marine habitats does accumulate most of the plastic?
Should the shallow and deep-sea sublittoral habitats be considered, as an estimated 70% of plastic sinks to the seafloor [319]?

Define the suitable/optimal analytical test method/measure of degradation for each habitat, its pass level for biodegradation and whether a negative result does confirm that biodegradation does not take place.

Oxymeter, weight loss, etc. (see also [179])
Set the fail/pass criteria for degradation/disintegration.
Which percentage of degradation is needed in each test system?

Should the optimal or environmentally relevant conditions be used?

Oxygen, pH, temperature, salinity, light, nutrients, pressure (deep-sea vs. shallow water), presence of co-substrates, etc.

Define the substrates/media needed

Should coastal surface water be taken from a remote beach or in a harbour?
Should coastal sand or mud be added?
Is deep-sea mud needed?
How much of macro-nutrients (for example nitrogen, phosphorous, magnesium, other bivalent cations, trace elements) need to be present, or should be added ?

Find the optimal inoculum composition and whether the same suits for all test polymers and habitats.

Which marine microorganisms do degrade solid plastics?
What are the degradation strategies of these microorganisms?
But also, are the conditions ideal for the inoculum (for example aerobic, anaerobic, sulfidic, etc.)?

Find the optimal cell number/inoculum quantity for each test

Should a mixed inoculum be used, to avoid a too special microbial community?
Could this be a mix of natural samples of sediment/water, taking into account that most microorganisms are not cultivatable?

Clarify if accelerators should be added/used in the tests

Are there co-substrates /co-factors to trigger the degradation in the lab? Which could that be?

Find the optimal test material amount and physical structure and define criteria for blends

Does the polymer‘s physical form and amount play a role in the degradation process?
Is ground polymer degraded quicker?

Clarify the effect of fouling on the degradation process

How are the conditions at the polymer surface during the fouling process?
Do they change, from oxic to anoxic, to acidic, etc.?
Does the fouling differ by polymer type?

Find the optimal duration for a lab test

Is there a „bottle effect“ if the test takes „too long“? Are there synergistic and antagonistic effects of other anthropogenic factors and/or other metabolic processes (e.g. nitrification).

Assess the deviations to results from the field

How long is the time lag between ideal lab conditions and field conditions for the degradation process?

Define the marine ecotoxicity tests needed

Which toxic effects on organisms do occur?
How should indirect effects (e.g. carrier function: Adsorption of persistent contaminants, such as PCBs) be addressed?

Define and evaluate the effects of degrading plastic on the marine ecosystem

How does the degradation of polymers affect the marine environment?
Does there oxygen depletion and/or eutrophication occur locally (fertiliser effect)?

Figure 9. The most important factors (in bold) determining if a test compound will be degraded that should be considered when developing new, or adapting standardised tests from other environments, such as soil and freshwater. Examples of questions to be answered to define the factor are given in italics.

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