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Bacterial degradation of synthetic plastics

SYNTHETIC PLASTICS ARE EMERGING ENVIRONMENTAL CONTAMINANTS THAT HAVE BEEN FOUND TO ACCUMULATE WITHIN MARINE WATERS WORLDWIDE. IN MARINE ENVIRONMENTS, MICROORGANISMS FUNCTION AS PIONEERING SURFACE COLONIZERS AND DRIVE CRITICAL ECOSYSTEM PROCESSES INCLUDING PRIMARY PRODUCTION, BIOGEOCHEMICAL CYCLING AND THE BIODEGRADATION OF ANTHROPOGENIC POLLUTANTS. THIS PAPER REVIEWS THE CURRENT KNOWLEDGE ON THE BIODEGRADATION OF SYNTHETIC PLASTICS BY MICROORGANISMS. THE MICROBIAL BIODEGRADATION OF PLASTIC MATERIALS IS A COMPLEX PHENOMENON THAT INCLUDES SEVERAL STEPS THAT ARE DESCRIBED HERE.

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Introduction

Colonization of plastic marine debris by microorganisms has been firstly reported in the 1970s, where authors mention diatoms and other microbes on the debris (Carpenter et al. 1972, Colton et al. 1974). In marine waters, plastic debris represent a novel ecological habitat for microorganisms since it entered the consumer arena less than 60 years ago, acting as new floating type of particles for microbial colonization and transportation. Plastic has become the most common form of marine debris and it presents a major and growing global pollution problem. In the North Western Mediterranean Sea, plastics were found at concentrations of up to 3.6 10^5 pieces/km^2 (Collignon et al. 2012), which is equivalent to what was found in the “great Pacific garbage patch” (5.0 10^5 pieces/km^2 were found in the North Atlantic Subtropical Gyre, Law et al. 2010). Particles may serve as a niche for microorganisms, offering a support for growth especially when it concerns organic aggregates, but also a protected area with limited predation. The presence of particles in aquatic systems is known to stimulate microbial productivity and respiration (Simon 2002, Ghiglione et al. 2009). However, detailed analyses on sorted plastic particles are scarce. So far, the only study dealing with the bacterial communities living in the so-called ‘plastisphere’ showed a high diversity composed of heterotrophs, autotrophs, predators, symbionts and also some opportunistic pathogens (Zettler et al. 2013).

The research on degradability of plastics began in the early 1980s and numerous papers provide information on the microbial biodegradation of a variety of plastics such as polyesters, polyhydroxybutyrate (PHB), polycaprolactone (PCL), polylactic acid (PLA), polyurethane PUR, polyvinyl alcohol (PVA), nylon, and polyethylene (PE) (Shimao et al. 2001).

The descriptor 10 (D 10) of the EU Marine Strategy Framework Directive (MSFD, 2008/56/EC) concerns marine litter. Started in 2011, a technical subgroup on marine litter (TSML) aims to provide scientific and technical background for the implementation of MSFD requirements with regard to D 10. With the excessive use of plastics and increasing pressure being placed on capacities available for plastic waste disposal, the need for biodegradable plastics and biodegradation of plastic wastes has assumed increasing importance in the last few years. Indeed, it is important to consider the microbial degradation of synthetic plastics in order to understand what is necessary for their biodegradation. This requires understanding of the interactions between materials and microorganisms and the biochemical changes involved.

This paper reviews the current research on the biodegradation of synthetic plastics by microorganisms. The microbial biodegradation of plastic materials includes several steps that are described here. Biodegradation is not disconnected from abiotic degradation, since several studies about biodegradation of some polymers show that the abiotic degradation (mechanical, light, thermal or chemical degradation) precedes microbial assimilation (Kister et al., 2000; Proikakis et al., 2006).

Different steps of plastic degradation by microorganisms

Several steps occur in the plastic biodegradation process (Figure 1) and could be identified by specific terminology (Lucas et al. 2008):

-**Bio-deterioration** defines the action of microbial communities and other decomposer organisms responsible for the physical and chemical deterioration that resulted in a superficial degradation that modifies the mechanical, physical and chemical properties of the plastic.

-Bio-fragmentation refers to the catalytic actions that cleave polymeric plastics into oligomers, dimers or monomers by ecto-enzymes or free-radicals secreted by microorganisms.

-**Assimilation** characterizes to the integration of molecules transported in the cytoplasm in the microbial metabolism.

-**Mineralisation** refers to the complete degradation of molecules that resulted in the excretion of completely oxidized metabolites (CO_2, N_2, CH_4, H_2O).

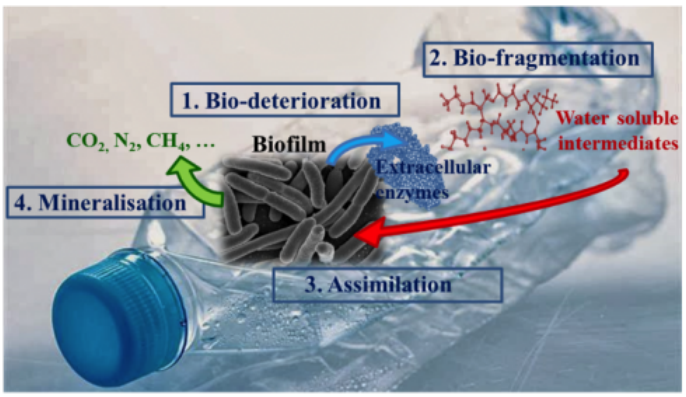

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Fig. 1. The different steps of plastic biodegradation by microorganisms

Herein are described the different degrees of the biodegradation process (bio-deterioration, bio-fragmentation, assimilation and mineralisation). The technical estimations adapted to each level of biodegradation are given in Table 1 and Table 2.

Bio-deterioration

Deterioration is a superficial degradation that modifies mechanical, physical and chemical properties of the plastic. In most cases, abiotic parameters contribute to weaken the polymeric structure (Helbling et al., 2006; Ipekoglu et al., 2007). Sometimes, these abiotic parameters are useful either as a synergistic factor, or to initiate the biodegradation process (Jakubowicz et al., 2006).

For more details on the role of pollutants attached to the plastic in the development of microbial biofilm, see Fotopoulou et al. in the present CIESM Workshop Monograph n°46.

The bio-deterioration seems to be triggered by the formation of a microbial **biofilm** growing on the surface and inside the plastic. The development of the biofilm is dependent on the composition and the structure of the plastic, but also on the environmental conditions (Lugauskas et al., 2003). Since plastic polymers such as PE and PS are hydrophobic, forming a stable biofilm requires that the plastic polymers such as PE and PS are hydrophobic, forming a stable biofilm requires that the bacterial surface will also be hydrophobic. For example, the biofilm of Rhodococcus ruber C208 formed on polyethylene showed high viability and even after 60 days of incubation adhered to the polyethylene without any supplementation of external carbon (Figure 2).

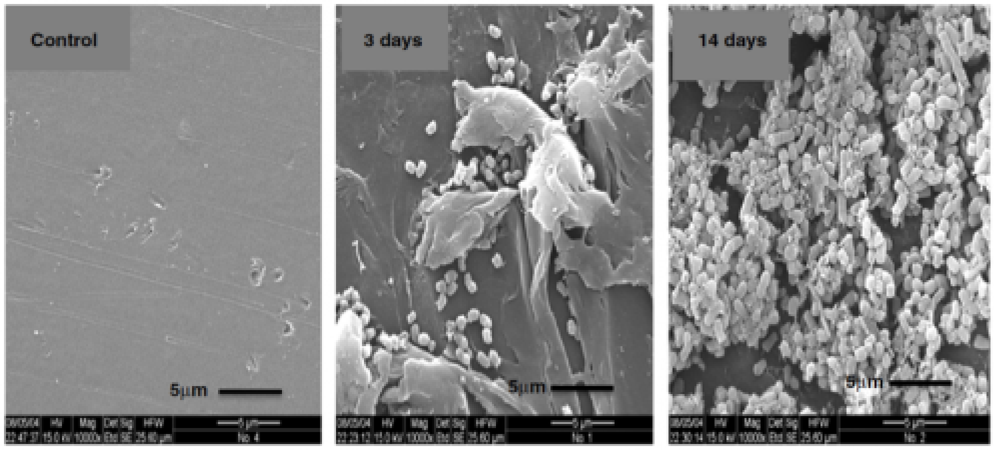

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Fig. 2. Scanning electron microscopy of biofilm formed by Rhodococcus ruber C208 on the surface of UV photo-oxidised polyethylene. Initiation of biodegradation was detected after 3 days. UV irradiated but not inoculated served as control (from Sivan 2011).

The microbial biofilm provokes serious physical and chemical deterioration:

<u>-physical deterioration:</u> the formation of the microbial biofilm is associated to the secretion of extracellular polymeric substances (EPS) that reinforce the cohesion of the biofilm and the adhesion to the plastic surface. The EPS enters the pores, microorganisms can then grow inside, thus increasing the pore size and provoking cracks that weakened the physical properties of the plastic (Bonhomme et al., 2003).

<u>-chemical deterioration:</u> the microbial communities that developed on plastic may be highly diverse (Zettler et al. 2013) and the development of a biofilm may **release acid compounds** such as nitrous acid (e.g. Nitrosomonas spp.), nitric acid (e.g. Nitrobacter spp.) or sulphuric acid (e.g. Thiobacillus spp.) by chemolithotrophic bacteria. Organic acids such as oxalic, citric, fumaric, gluconic, glutaric, glyoxalic, oxalic and oxaloacetic acids may also be released by chemoorganotrophic communities. The pH inside the pores is then modified, resulting in a progressive degradation that changes the microstructure of the plastic matrix.

Tests	Norms	Characteristics		Estimating			
		Difficulty	Reality	AB[a]	BD[b]	BF[c]	A[d]
Out-door exposure		+	++++	X	X		
UV exposure	ISO 4582	+	++	X			
Suntest	ISO 4892 series	+	++	X			
Accelerated weathering chamber		++	+++	X			
Differential scanning calorimetry		++	+	X			
Thermogravimetric analysis		++	+	X			
Pyrolysis		++	+	X			
Microorganisms surface colonisation	ISO 846 ISO 11266 NF X41-513 NF X41-514 ASTM G22-76 ASTM G21-70	+++	+++		X	X	

	ASTM G21-90						
Weight loss	ISO 14852 ISO 14855 NF EN ISO 13432	+	+	X	X	X	
Significant enzymes in batch		++	++		X	X	
Clear zone test		+++	+++		X	X	
Respirometry	OECD series, ISO 14852, ISO 14855 ASTM D 5209	++	++				X

[a] Abiotic degradation.
[b] Biodeterioration.
[c] Biofragmentation.
[d] Assimilation.

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Analytical techniques	Norms	Characteristics		Estimating			
		Cost	Difficulty	AB	BD	BF	A
Morphological							
Yellowness	ASTM D 1925	+	+	X			
Photonic microscopy		++	++	X	X		
Electronic microscopy		++++	++++	X	X		
Polarization microscopy		+++	++	X	X		
Rheological							
Tensile	ISO 527-3	++	+	X	X	X	
X-ray diffraction		++++	+++	X	X	X	
Differential scanning calorimetry		++++	++	X	X	X	
Thermogravimetric analysis		++++	++	X	X	X	
Gravimetric		+	+	X	X	X	
Spectroscopic							
Fluorescence		++	++	X	X	X	
UV–visible		+	+	X	X	X	
FTIR		++	++	X	X	X	
RMN		++++	++	X	X	X	
Mass spectrometry		++++	+++	X	X	X	
Chromatographic							
Gel permeation chromatography		+++	++	X	X	X	
Hight performance Liquid chromatography		+++	++	X	X	X	
Gas phase chromatography		+++	++	X	X	X	

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Table 1: Bio-degradability tests related to different steps of plastic degradation (left) and analytical techniques for bio-degradability estimation (right) (from Lucas et al. 2008).

Bio-fragmentation

The fragmentation of plastic polymers into oligo- and monomers can be of various origins, i.e. mechanical, UV radiation, thermal, chemical and/or biological. Here we focused on the biological aspect, but other reviews are providing more details about the abiotic fragmentation (see for example Singh and Sharma, 2008).

Plastic polymers are molecules with high molecular weight that cannot cross the cell wall. Microorganisms secrete extracellular enzymes (**exoenzymes)** that can catalyze reactions principally at the boundaries of the plastic polymer. They can perform many chemical reactions, but they generally need imbalance of electric charge to perform lysis. The main limit of bio-fragmentation is the stability of the plastic polymers, which are constituted by a long chain of carbons and hydrogens that contains very balanced charges. To destabilize the local electric charge, bacteria that can break down plastics usually contain enzymes called **oxygenases**, which can add oxygen to a long carbon chain. For instance, mono-oxygenases and di-oxygenases incorporate, respectively, one and two oxygen atoms, forming alcohol or peroxyl groups that are less recalcitrant for biodegradation. Other transformations are then catalysed by lipases and esterases after the formation of carboxylic groups, or by endopeptidases for amide groups (Lugauskas et al. 2003).

Some of the **well-known microbes,** which have the capacity to degrade plastic polymers into their respective simple monomeric, are shown in Table 2 (from Ghosh et al. 2013). This table shows 15 bacterial genera which have the capacity to degrade various types of plastics. Among them, Pseudomonas is dominant. It can degrade polythene, PVC, PHB, poly(3-hydroxybutyrate-co-3-mercaptopropionate), and poly(3-droxypropionate). Bacillus brevis can degrade only polycaprolactone while Streptomyces can degrade PHB, poly(3-hydoxybutyarate-co-3-hydroxyvalerate), and starch or polyester. Ochrobactrum TD is also able to degrade PVC. Majority of the strains that are able to degrade PHB belong to different taxa such as Gram-positive and Gram-negative bacteria, Streptomyces, and fungi (Mergaert and Swings 1996). It has been reported that 39 bacterial strains of the classes Firmicutes and Proteobacteria can degrade PHB, PCL, and PBS, but not PLA (Suyama et al. 1998). Other bacterial species identified having the properties of degrading plastics were Bacillus sp., Staphylococcus sp., Streptococcus sp., Diplococcus sp., Micrococcus sp., Pseudomonas sp., and Moraxella sp. (Kathiresan 2003).

For more details on the biodegradation of most prominent synthetic plastics (PEs, PP, PS and PVC), see Raddadi et al. in the present CIESM Workshop Monograph n°46.

Plastic	Microorganism	Reference
Polyethylene	*Brevibacillus borstelensis*	Hadad et al. (2005)
	Rhodococcus rubber	Sivan et al. (2006); Gilan et al. (2004)
	Pseudomonas chlororaphis	Zheng et al. (2005)
	Comamonas acidovorans TB-35	Akutsu et al. (1998)
Polyvinyl chloride	*Pseudomonas putida* AJ	Anthony et al. (2004)
	Ochrobactrum TD *Pseudomonas fluorescens* B – 22	Mogil'nitskii et al. (1987)
BTA copolyester	*Thermomonospora fusca*	Kleeberg et al. (1998)
Some biodegradable/natural plastics and their degrading microorganisms		
Poly(3-hydroxybutyrate-co-3-mercaptopropionate)	*Schlegelella thermodepolymerans*	Elbanna et al. (2004)
Poly(3-hydroxybutyrate)	*Pseudomonas lemoignei*	Jendrossek et al. (1995)
Poly(3-hydroxybutyrate-co-3-mercaptopropionate)	*Pseudomonas indica* K2	Elbanna et al. (2004)
Poly(3-hydroxybutyrate), poly(3-hydroxybutyrate-co-3-hydroxyvalerate)	*Streptomyces* sp. SNG9	Mabrouk and Sabry (2001)
Poly(3-hydroxybutyrate-co-3-hydroxypropionate)	*Ralstonia pikettii* T1 *Acidovorax* sp. TP4	Wang et al. (2002)
Poly (3-hydroxybutyrate), poly(3-hydroxypropionate), poly(4-hydroxybutyrate), polyethylene succinate, polyethylene adipate	*Alcaligenes faecalis* *Pseudomonas stutzeri* *Comamonas acidovorans*	Kasuya et al. (1999)
Poly(3-hydroxybutyrate)	*Alcaligenes faecalis* *Schlegelella thermodepolymerans*	Kita et al. (1997)
	Caenibacterium thermophilum	Romen et al. (2004)
Poly(3-hydroxybutyrate-co-3-hydroxyvalerate)	*Clostridium botulinum* *Clostridium acetobutylicum*	Abou-Zeid et al. (2001)
Polycaprolactone	*Clostridium botulinum* *Clostridium acetobutylicum*	Abou-Zeid et al. (2001)
	Amycolatopsis sp.	
	Bacillus brevis	
Polymer blends and its degrading microorganisms		
Starch/polyester	*Streptomyces*	Lee et al. (1991)

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Table 2: List of microbial strains and the types of plastic which they degrade (from Ghosh et al. 2013).

Assimilation and mineralisation

The formation of monomer does not guaranty their assimilation by microorganisms. They have to use specific carriers to cross the cell wall and/or cytoplasmic membrane. Some monomers may stay in the surrounding of microbial cells without being assimilated. Inside cells, the plastic monomers are oxidized through catabolic pathways to produce energy, cell structure and new biomass. Depending on the microbial abilities to grow in aerobic or anaerobic conditions, there exist three essential on the microbial abilities to grow in aerobic or anaerobic conditions, there exist three essential catabolic pathways to produce the energy to maintain cellular activity, structure and reproduction: aerobic respiration, anaerobic respiration and fermentation. The assimilation refers to the integration of atoms inside microbial cells, but the degradation of the monomers may not be complete. The assimilation result in numerous secondary metabolites that can be transported outside the microorganism that do not have the metabolic capability to transform it or that do not need to metabolize or store it. The secondary metabolites excreted may be used by another cell that can perform further degradation, or can stay further in the pool of non-assimilable compounds. The mineralization refers to the complete degradation of primary and secondary metabolites that resulted in the excretion of completely oxidized metabolites (CO_2, N_2, CH_4, H_2O).

Concluding remarks and perspectives

For the last 30 years, scientists are trying to develop some alternative ways where they can use microbes to degrade these long chain synthetic polymers into their respective monomers. Until now, very few evidences are available where scientists were able to develop some alternative ways to enhance the mode of degradation and make it faster.

Biodegradability tests are necessary to estimate the environmental impact of plastic materials and to find solutions to avoid the disturbing accumulation of polymers. The augmentation of derived biodegradability tests has conducted to confused interpretations about biodegradation mechanisms. To compensate for this problem, it is necessary to explain the different steps involved in biodegradation (i.e. bio-deterioration, bio-fragmentation, assimilation and mineralization). In addition, each biodegradation stage should be associated with the adapted estimation technique.

Better knowledge on the different steps of plastic degradation by microorganisms may also help for improving biodegradation. Several factors may be explored for a better biodegradation of polymer plastics (i) by using surface active agents or inducing the microorganism to produce surfactant to allow better attachment of microorganisms on the polymer surface, (ii) by blending the polymers with biodegradable synthetic polymers such as polylactic acid or polycaprolactum (iii) by the pretreatment of the plastics which includes thermal, UV, high energy radiation and chemical treatment, (iv) by culturing those microorganisms that can efficiently degrade the plastics (bio-addition) and (v) by addition of nutrients that may be limiting in the environment (bio-stimulation).

The biodegradation of synthetic plastics is a complex phenomenon. Nature-like experiments are difficult to realize in laboratory due to the great number of parameters occurring during the biogeochemical recycling. Actually, all these parameters cannot be entirely reproduced and controlled in vitro. To date, most of the knowledge on the microbial ability to degrade synthetic plastics is based on few bacteria able to grow on culture media, that represent <0.1% of the total bacteria. Hence, the great natural source of high diversity of microorganisms is not fully exploited. By using -omics technologies (genomic, transcriptomic, proteomic, metabolomics), it is now possible to discover new non-culturable microorganisms involved in plastic colonization and degradation, and explore the new properties of microorganisms that arise from the interplay of genes, proteins, other macromolecules, small molecules, and the environment.

Low cost, efficient technology, eco-friendly treatments capable of reducing and even eliminating plastics, are of great environmental interest. Among biological agents, microbial enzymes are one of the most powerful tools for the biodegradation of plastics. There is a huge demand in exploring these microbes which can grow in different conditions and, under specific stress conditions, may be directed to grow and use the plastic carbon polymers as energy source.

References cited

Bonhomme S., Cuer A., Delort A.M., Lemaire J., Sancelme M. and G. Scott. 2003. Environmental biodegradation of polyethylene. Polymer Degradation and Stability, 81: 441-452.

Carpenter E.J. and K.L. Smith. 1972. Plastics on the Sargasso Sea surface. Science, 175: 1240-1241.

Colton J.B., Burns B.R. and F.D. Knapp. 1974. Plastic particles in surface waters of the Northwestern Atlantic. Science, 185: 491-497.

Ghiglione J.F., Conan P. and M. Pujo-Pay (2009) Diversity of total and active free-living vs. particle-attached bacteria in the euphotic zone of the NW Mediterranean Sea. FEMS Microbiology Letters, 299: 9-21.

Ghosh S.K., Pal S. and S. Ray. 2013. Study of microbes having potentiality for biodegradation of plastics. Environmental Science and Pollution Research 20:4339-4355.

Helbling C., Abanilla M., Lee L. and V.M. Karbhari. 2006. Issues of variability and durability under synergistic exposure conditions related to advanced polymer composites in civil infrastructure. Composites Part A: Applied Science and Manufacturing, 37: 1102-1110.

Ipekoglu B., Böke H. and O. Cizer. 2007. Assessment of material use in relation to climate in historical buildings. Building and Environment, 42: 970-978.

Kathiresan K. 2003. Diversity and effectiveness of tropical mangrove soil microflora on the degradation of polythene carry bags. Revista de Biologia Tropical, 51:629-634.

Kister G., Cassanas G., Bergounhon M., Hoarau D. and M.Vert. 2000. Structural characterization and hydrolytic degradation of solid copolymers of D, L-lactide-co-e-caprolactone by Raman spectroscopy. Polymer, 41: 925-932.

Law K.L., Morét-Ferguson S., Maximenko N., Proskurowski G., Peacock E.E., Hafner J. and C.M. Reddy. 2010. Plastic accumulation in the North Atlantic subtropical gyre. Science, 329: 1185-1188.

Lucas N., Bieniame C., Belloy C., Queneudec M., Silvestre F. and J.E. Nava-Saucedo. 2008. Polymer biodegradation: mechanisms and estimation techniques. Chemosphere, 73: 429-442.

Lugauskas A., Levinskait L. and D. Peciulyte. 2003. Micromycetes as deterioration agents of polymeric materials. International Biodeterioration and Biodegradation, 52: 233-242.

Mergaert J. and J. Swings. 1996. Biodiversity of microorganisms that degrade bacterial and synthetic polyesters. Journal of Industrial Microbiology, 17:463-469.

Proikakis C.S., Mamouzelous N.J., Tarantili P.A. and A.G. Andreapoulos. 2006. Swelling and hydrolytic degradation of poly(D, L-lactic acid) in aqueous solution. Polymer Degradation and Stability, 91: 614-619.

Shimao M. 2001. Biodegradation of plastics. Current Opinion in Biotechnology, 12: 242–247.

Simon M., Grossart H.P., Schweitzer B., and H. Ploug. 2002. Microbial ecology of organic aggregates in aquatic ecosystems. Aquatic Microbial Ecology, 28: 175-211.

Singh B. and N. Sharma. 2008. Mechanistic implications of plastic degradation. Polymer degradation and stability, 93: 561-584.

Sivan A. 2011. New perspectives in plastic biodegradation. Current Opinion in Biotechnology, 22: 422-426.

Suyama T., Tokiwa Y., Oichanpagdee P., Kanagawa T., Kamagata Y. 1998. Phylogenetic affiliation of soil bacteria that degrade aliphatic polyesters available commercially as biodegradable plastics. Applied and Environmental Microbiology, 64: 5008-5011.

Zettler E.R., Mincer T.J. and L.A. Amaral-Zettler. 2013. Life in the ''plastisphere'': microbial communities on plastic marine debris. Environmental Science and Technology, 47: 7137-7146.

Link to the paper: http://www.sfecologie.org/regards/2014/12/26/r63-plastiques-en-mer-dussud-et-ghiglione/ (http://www.sfecologie.org/regards/2014/12/26/r63-plastiques-en-mer-dussud-et-ghiglione/)