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Microbes and Persistent Organic Pollutants in the Marine Environment

--Manuscript Draft--

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Abstract:	<p>Marine pollution has increased reaching the entire marine environment, from the surface to the deepest sediment and has become more concerning in the last seventy years. Persistent Organic Pollutants (POPs) are a fraction of ocean waste that includes, among the others, Polycyclic Aromatic Hydrocarbons (PAHs) and plastic polymers. These chemicals have an extremely long half-life, (bio)accumulate and damage the marine flora and fauna and, ultimately, human health. Some organisms have evolved enzymes to attack POPs in the environment and transform them into biomass and CO₂. Several microorganisms degrade many POPs in relatively short time. A wide variety of bacteria has been isolated with different techniques, and key catabolic enzymes used to degrade the most persistent oil hydrocarbon fractions have been identified. For plastic waste there is less evidence of microbial degradation, but a few recent studies are revealing that a biodegradation potential exists for some of the most recalcitrant plastic polymers as well. The scientific community is focusing on microorganisms and their enzymes for POPs uptake and removal from the environment, while searching novel biopolymers (also from microbiological origin) to substitute oil-derived plastics.</p>
Response to Reviewers:	<p>Dear Editor,</p> <p>We appreciate the opportunity to revise and resubmit our paper .</p> <p>We would like to thank the reviewers for the carefull reading of this manuscript. We also greatly appreciate the complimentary comments and suggestions, which help to improve the quality of this manuscript. We have revised the manuscript accordingly. Please find bellow a point-by-point response to the concerns. We hope that you will find our responses satisfactory.</p> <p>Sincerely,</p> <p>The corresponding author Paola Quatrini</p> <p>Rebuttal</p> <p>Reviewer #1: The type of this is review article and this work enhanced our understanding about pollutant in marine environment and Minor revisions need. I read this article carefully. information about plastic pollution in the marine environment is limited and this review can have enhanced our information about this problem. some minor edition need as follow:</p>

	<p>- some information about interaction of fungi and plastics must be added</p> <p>Authors: Thanks for your comments . Several information of the original paper already addressed the role of fungi in plastic degradation. However we agree to strengthen this point and added new information about interaction of fungi and plastics and the fungal key enzymes of the biodegradation pathway. We added various sentences in the paragraphs « The Plastisphere » (lines 158-161) and « Promising microbial Taxa and Enzymes for Plastic Attack » (167-168; 176-178; 188-195; 197-201; 211-220) ; . Having further added information on fungi and plastics, we changed the title « Promising bacterial taxa and enzymes for plastic attack » in « Promising microbial taxa and enzymes for Plastic Attack »). New references and new abbreviations have been added as well.</p> <p>Reviewer #1: - some information about environment assessment of this pollution needed</p> <p>Authors: Thanks for your suggestion. Indeed the text was missing this information, we added a part in the introduction dedicated to environmental assessment needs and methods (lines 68-83). New references have been added as well. (Tickner et al., 2019; Wu et al., 2008; Borah et al., 2020)</p> <p>Reviewer #1- References: This three reference is related to your work and you can use this references</p> <p>Abarian, M., Hassanshahian, M., Esbah, A. Degradation of phenol at high concentrations using immobilization of Pseudomonas putida P53 into sawdust entrapped in sodium-alginate beads. Water Science and Technology. 2018. 79 (7): 1387-1396.</p> <p>Bayat, Z., Hassanshahian, M., Cappello, S. Immobilization of Microbes for Bioremediation of Crude Oil Polluted Environments: A Mini Review. Open Microbiol J. 2015; 9: 48-54.</p> <p>Hassanshahian, M. Amini, N. 2016. Enrichment and identification of naphthalene-degrading bacteria from the Persian Gulf. Marine Pollution Bulletin. 107, 59-65.</p> <p>Ghanavati, H., Emtiazi, G., Hassanshahian, M., 2008. Synergism effects of phenol degrading yeast and ammonia oxidizing bacteria for nitrification in coke wastewater of Esfahan steel company. Waste Manage. Res. 26 (2), 203-208.</p> <p>Hassanshahian, M. 2014. Isolation and characterization of biosurfactant producing bacteria from Persian Gulf (Bushehr provenance). Marine Pollution Bulletin. 86, 361-366</p> <p>Karimi, M, Hassanshahian, M. 2016. Isolation and characterization of phenol degrading yeasts from wastewater in the coking plant of Zarand, Kerman. Brazilian Journal of Microbiology. 47, 18-24.</p> <p>Authors: Thanks for the suggestions. We added the references suggested limited to those related to POPs.</p> <p>Reviewer #2: Dear Author the manuscript submitted is interesting and can be published in this form.</p> <p>Authors: no answers are given to reviewer 2, we only thank him for the revision of our paper.</p>
<p>Additional Information:</p>	
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<p>address, and e-mail address of three potential reviewers who do not pose a conflict of interest. Note that this information will be checked to ensure it is credible. If you do not enter the information here, your manuscript will not be sent out for review and will be returned to you, therefore increasing the time to process your manuscript.</p>	<p>1, Simone Cappello, National Research Council (CNR) – Institute for Biological Resources and Marine Biotechnology, Via S. Raineri, 86 - 98122 Messina (ME)simone.cappello@iamc.cnr.it</p> <p>2, Santina Santisi, National Research Council (CNR) – Institute for Biological Resources and Marine Biotechnology, Via S. Raineri, 86 - 98122 Messina (ME), sanny.santisi@yahoo.it</p> <p>3. Mehdi Hassanshahian, Department of Biology, Faculty of Science, Shahid Bahonar University of Kerman, Kerman, Iran.</p> <p>Email: mshahi@uk.ac.ir</p>
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1 **Microbes and Persistent Organic Pollutants in the Marine Environment**

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14

15 **Keywords**

16 POPs, pollution, PAHs, plastisphere, marine biodegraders, bioplastics.

17 **Abstract**

18 Marine pollution has increased reaching the entire marine environment, from the surface to the deepest sediment
19 and has become more concerning in the last seventy years. Persistent Organic Pollutants (POPs) are a fraction of
20 ocean waste that includes, among the others, Polycyclic Aromatic Hydrocarbons (PAHs) and plastic polymers.
21 These chemicals have an extremely long half-life, (bio)accumulate and damage the marine flora and fauna and,
22 ultimately, human health. Some organisms have evolved enzymes to attack POPs in the environment and
23 transform them into biomass and CO₂. Several microorganisms degrade many POPs in relatively short time. A
24 wide variety of bacteria has been isolated with different techniques, and key catabolic enzymes used to degrade
25 the most persistent oil hydrocarbon fractions have been identified. For plastic waste there is less evidence of
26 microbial degradation, but a few recent studies are revealing that a biodegradation potential exists for some of
27 the most recalcitrant plastic polymers as well. The scientific community is focusing on microorganisms and
28 their enzymes for POPs uptake and removal from the environment, while searching novel biopolymers (also
29 from microbiological origin) to substitute oil-derived plastics.

30

31 **POPs Ocean Contamination**

32 In recent decades, oceanic pollution by anthropogenic litter, mainly Persistent Organic Pollutants (POPs), has
33 been recognized as a serious global environmental concern for its multiple implications on the environment,
34 wildlife and human health (Andrady, 2011). POPs are chemical substances that are retained in the environment,
35 even in the deepest marine sediments up to 10,000 meters of depth (Jamieson et al., 2017), causing the risk of
36 adverse effects to the ecosystem (Islam et al., 2018). Their effects are exacerbated by bioaccumulation through
37 the food web (Matthies et al., 2016) and by the outcomes of climate change (Nadal et al., 2015). Among POPs,
38 Polycyclic Aromatic Hydrocarbons (PAHs) and plastic polymers at sea are of major concern (González-Gaya et
39 al., 2019).

40 PAHs contamination results from activities related to the petrochemical industry, combustion and oil spill
41 accidents (Ghosal et al., 2016). The amount of crude oil that is introduced from anthropogenic or natural sources
42 into the marine environment is estimated to be more than 800 million liters per year (Kleindienst et al., 2015).
43 Due to their complex structure among hydrocarbons, PAHs are highly resistant to degradation and remain
44 persistent in the ecosystem, they have a natural potential for bioaccumulation in food chains they are hazardous
45 environmental pollutants being highly toxic, genotoxic, mutagenic and carcinogenic (Abdel-Shafy & Mansour,
46 2016). PAHs, like other Hydrocarbons (HCs) such as long chain *n*-alkanes (Lo Piccolo et al., 2011), are
47 ultimately degraded by environmental microorganisms that have been recognized as key players in cleanup
48 events (Restrepo- Flórez et al., 2014; Catania et al., 2015, , [Hassanshahian, 2014](#); [Hassanshahian et al., 2016](#)).
49 However, we still need to expand the knowledge on microbial degradation mechanisms and conditions to fully
50 exploit their catabolic potentialities (Ghosal et al., 2016).

51 Since the 1940s, the annual global demand for plastics consistently increased and presently stands at about 245
52 million tons per year, the variety and amount of POPs in the marine environment has consequently increased
53 (Thompson et al., 2009; Cole et al., 2011). Plastic polymers are among the most recalcitrant substances and
54 include Polyethylene (PE), Polypropylene (PP), Polystyrene (PS), Polyethylene Terephthalate (PET) and
55 Polyvinyl Chloride (PVC) (Kale et al., 2015). Plastic is subject to deterioration by biotic and abiotic factors that
56 reduce it into small fragments (microplastics) which are then mixed by waves and distributed to all habitats (Do
57 Sul., et al., 2014). Ingestion of microplastics by the marine biota has probable negative consequences for its
58 health (Browne et al., 2011). Moreover, some of the additives used to modify the properties of plastics are
59 biologically active, potentially affecting development and reproduction (Oehlmann et al., 2009; Savoca et al.,
60 2018). In addition, hydrophobic POPs in seawater are adsorbed onto plastic items (Mato et al., 2001; Teuten et
61 al., 2009) and can accumulate toxins (Andrady, 2011).

62 Microbes tackle plastic polymers with direct and indirect action, by deteriorating the plastic itself or producing
63 bioproducts that influence the polymer structure (Caruso, 2015). Both actions, enzymes and microbial products
64 affecting plastic polymers are to be studied in the nearest future.

65 The use of microbial enzymes in coupled biotic-abiotic technologies for POPs degradation is starting to attract
66 both the engineering and scientific world as a worthwhile and environmental-friendly solution (Jeon et al.,
67 2013).

68 [Due to growing concentration of persistent organic pollutants in the marine environment, combined with an](#)
69 [increasing knowledge of their toxicity, in the last years extensive studies and monitoring programs have been](#)
70 [carried out to determine POPs concentrations in water, sediment, and more recently, in biota; focusing](#)
71 [increasingly on “possible biological effects” to assess the public health risk, environmental and ecological risk.](#)
72 [An ecological and environmental risk assessment approach should be adopted for measuring POPs in the marine](#)
73 [environment; routine monitoring and reporting of abiotic and biotic concentrations currently are the main](#)
74 [methods used for environmental assessment risk \(Jacobs et al., 2016; Tickner et al., 2019\).](#)

75 [The primary objectives of most monitoring surveys consist in the comparisons of spatial changes to identify](#)
76 [sources and “hot spots” containing great contaminant concentrations; comparisons of temporal changes to](#)
77 [detect deterioration or improvement of contaminant concentrations in the environment, or checks on compliance](#)
78 [according to governmental standards and established guidelines alternatives \(Wu et al., 2008\)](#)

79 The environmental assessment of marine POPs require rigorous and expensive chemical analysis; the
80 conventional techniques performed to evaluate usually the presence assessment in the marine environment of
81 POPs are Gas or Liquid Chromatography, Mass Spectrometry and tandem Mass Spectrometry (MS/MS) or
82 analytical techniques such as UV-Vis Spectroscopy and Surface-Enhanced Raman Scattering (Borah et al.,
83 2020).

84

85 **PAHs Biodegradation: Taking Over Persistent Organic Pollutants**

86 Crude oil consists of many fractions, including the highly persistent and toxic (poly)aromatic compounds
87 (Varjani, 2017). PAHs biodegradation by microorganisms has been demonstrated and widely described (Jeon
88 and Madsen 2013; Ghosal et al., 2016), in pure-culture isolates (Oyehan et al., 2017, Djahnit et al., 2019) and in
89 contaminated field sites (Vila et al., 2015; Catania et al., 2016; Dombrowski et al., 2016).

90 Laboratory experiments with pure bacterial cultures showed that low-molecular weight (LMW, two or three
91 rings) PAHs reach complete degradation within 20 to 24 days of incubation (Dandie et al., 2004, Darmawan et
92 al., 2015). High-molecular weight (HMW, more than three rings) PAHs can be degraded up to more than 95%
93 in 16 days (Darmawan et al., 2015).

94 Many bacterial PAH-degrading genera, pathways and key enzymes involved in the biodegradation of PAHs
95 have been characterized (Peng et al., 2008). The aerobic PAHs biodegradation is accomplished by
96 monooxygenase or dioxygenase enzymes that incorporate oxygen atoms into the aromatic ring leading to the
97 formation of intermediates that are ultimately converted to tricarboxylic acid cycle (TCA) cycle intermediates
98 (Ghosal et al, 2016; Gupte et al., 2016). In anaerobic degradation pathways, the demolition of the aromatic ring
99 is based on reductive reactions catalyzed by dehydrogenases, which lead to formation of aromatic central
100 intermediates, which are then dearomatized and channeled to the central metabolism of the cell (Carmona et al.,
101 2009).

102 A high proportion of PAH-degrading bacteria belong to the sphingomonads, mainly to the genera
103 *Sphingomonas*, *Sphingobium* and *Novosphingobium*, (Ghosal et al., 2016). In contaminated marine
104 environments PAH-degraders are either obligate HC-degrading bacteria such as *Cycloclasticus*, that results as
105 the dominant species in contaminated surface sediment, or non-obligate PAHs degraders such as *Halomonas*,
106 *Thalassospira*, and *Lutibacterium* genera (Gutierrez et al., 2015; Catania et al., 2018).

107 Halophilic archaea, fungi and algae can also mediate PAHs degradation and transformation (Sharma et al.,
108 2018), but only with the advancement in genetic, genomic, proteomic and metabolomic studies, bioremediation
109 is going to be a valid alternative for pollution management (Ghosal et al., 2016). Effort has been put in studying
110 the most efficient environment for the bacteria to perform biodegradation: while oxygen concentration was at
111 first considered the limiting factor, new pathways have been studied, using sulfate-reducing, denitrifying,
112 methanogen or metal-ion-reducing bacteria (Nzila, 2018). *In situ* PAHs biodegradation can be achieved by
113 biostimulation (Dell'Anno et al., 2018), or bioaugmentation (by the addition of selected microbes able to
114 degrade the specific organic contaminants) that allow maintaining low costs and low environmental impacts
115 (Dell'Anno et al., 2018).

116

117 **Assessing Plastic Biodegradation**

118 Studies on the biodegradation of plastics and factors that affect their biodegradability are now of great interest
119 for the scientific community. Petroleum-based plastics have generally been considered highly recalcitrant to
120 biodegradation, and data on the biodegradability of synthetic plastics have rarely been reported (Cacciari et al.,
121 1993; Shah et al., 2008; Restrepo-Flórez et al., 2014; Alshehrei, 2017). The evaluation of plastics'
122 biodegradability by microorganisms is mainly based on their chemical structure and physical properties which
123 affect the efficiency of enzymes (Tokiwa et al., 2009), and the environmental conditions that encourage
124 microbial growth (Gu, 2003). Abiotic factors such as UV irradiation, oxygen and temperature play a crucial role
125 in the degradation of PE and PP in the environment (Bonhomme et al., 2003; Jakubowicz, 2003).

126 Biodegradation of plastic polymers consists of three important steps: (1) biodeterioration, which is the
127 modification of mechanical, chemical, and physical properties of the polymer due to the growth of
128 microorganisms on or inside its surface; (2) biofragmentation, which is the conversion of polymers to oligomers
129 and monomers by microbial action and (3) assimilation, where microorganisms are supplied by necessary
130 carbon, energy and nutrient sources from the fragmented polymers and convert plastic carbon to CO₂ and
131 biomass (Lucas et al., 2008).

132 Different methods are used to assess polymer degradation. Biotic degradation and biodeterioration are mainly
133 associated to physical tests (e.g. thermal transitions and tensile changes, tensile strength-elongation at fail and
134 modulus of the polymer via Dynamic Mechanical Analysis), while biofragmentation is revealed by the
135 identification of fragments of lower molecular weight using chromatographic methods; microcracks are revealed
136 via Scanning Electron Microscopes, density with the contact angle, viscosity, and molecular weight distribution
137 via High Temperature Gel Permeation Chromatography. The melting and glass transition temperature is
138 detected via thermogravimetric analyses, and the crystalline and amorphous region with X-diffraction, small-
139 and wide-angle-X-ray-scattering (Restrepo- Flórez et al., 2014; Kale et al., 2015).

140 Biodegradation can also be assessed measuring assimilation by microorganisms or monitoring the development
141 of microbial biomass on plastic surface by microscopic observations, by testing the concentration of Adenosine
142 Triphosphate and Fluorescein Diacetate, and protein analysis in general (Lucas et al., 2008; Kale et al., 2015);
143 The microbiological characterization of the biofilm formed on plastic surface can be carried out using a wide
144 range of cultural and culture-independent techniques (Dussud et al., 2018).

145

146 **The Plastisphere**

147 Any biotic and abiotic surface in the marine/aquatic environment can be colonized by micro- and
148 macroorganisms that can form biofilms (Pinto et al., 2019). As expected, also plastic marine debris (PMD) hosts
149 a diverse microbial community that exploits its hydrophobic surface forming a biofilm recently defined as the
150 "plastisphere" (Zettler et al., 2013; Quero et al., 2017). The plastisphere is a unique consortium that differs from
151 the surface water one, as plastics have a longer half-life than most of other floating substrates (Quero et al.,
152 2017). Variation within plastisphere communities on different polymer types was observed, suggesting that
153 polymer composition could shape the biofilm community structure (Oberbeckmann et al., 2014). This new
154 colonized habitat hosts typical biofilm-forming bacteria and fungi, opportunistic pathogens, cyanobacteria and,
155 interestingly, several hydrocarbon-degrading bacteria (Zettler et al., 2013). Similarly, PMD trapped in the
156 sediment hosts bacterial genera affiliated with HC contaminant mineralization in cold ecosystems, supporting
157 the possibility that these microbes play a role in degrading PMD (Harrison et al. 2014). However, the role of

158 plastic surface colonizers in polymer biodegradation is still unclear, and especially fungal communities still
159 represent an unexplored component of the plastisphere. The knowledges on fungi-plastic interaction
160 mechanisms, especially in marine habitat are limited, although numerous fungal strains was reported for the
161 capability to biodegrade plastic (Shah et al., 2008.; Jacquin et al., 2019).

162

163 **Promising Microbial Taxa and Enzymes for Plastic Attack**

164 Among plastic polymer biodegraders, the most active species are considered fungi and Actinobacteria (Tosin et
165 al., 2012).

166 **Fungi and Actinobacteria** are an ecologically significant group, possessing unique metabolic and physical
167 characteristics and **are considerate of special interest due to their vast metabolic potential and ability to degrade**
168 **plastics and recalcitrant molecules making them good candidates for bioremediation** (Olajuyigbe & Ehiosun,
169 2016; Chen et al., 2016, Rogers et al., 2020). Actinobacteria play a role in several biological processes and are
170 capable of producing pharmaceutically, industrially and clinically important bioactive compounds or enzymes
171 (Ramírez & Calzadía, 2016). They are known to decompose a large number of biomolecules (lignin, cellulose,
172 and hemicellulose) and to metabolize recalcitrant polymers such as long chain *n*-alkanes (Lo Piccolo et al.,
173 2011), xenobiotics (Borozan et al., 2013), pesticides (Alvarez et al., 2017), and rubber (Shivlata & Tulasi,
174 2015). The Actinobacterium *Rhodococcus ruber*, for example, is reported as plastic (Andrady, 2011) and PS
175 (Mor and Sivan, 2008) degrader, producing biofilm which helps to improve degradation (Auta et al., 2017).
176 **Saprophytic fungi are known to biodegrade PE and PS, they belong mainly to the phyla Chytridiomycota,**
177 **Cryptomycota and Ascomycota and dominate fungal degrading communities in aquatic ecosystems (Kettner et**
178 **al., 2017)**

179 The pathways involved in plastic polymer degradation have been tentatively characterized and the key enzymes
180 of the biodegradation pathway resulted to be plant polymer-degrading enzymes such as laccases, cutinases,
181 hydrolases, and other enzymes such as esterase, protease and urease (Pathak, 2017). Polymers with hydrolysable
182 chemical bonds in their backbone such as PET (Webb et al., 2013) and polyurethane (PUR) (Cregut et al., 2013)
183 are more susceptible to biodegradation than PE, PS, PP and PVC (Zheng et al., 2005). Microbial laccases from
184 *Trametes versicolor* and *Rhodococcus*, *Bacillus cereus* have been described as able to strongly reduce the
185 molecular weight of a PE membrane (Singh et al., 2016). Manganese peroxidase from the fungi *Phanerochaete*
186 *chrysosporium*, *Penicillium simplicissimum* and the bacterium *Bacillus cereus* have been reported to be involved
187 in the biodegradation of PE, such as the lignin peroxidases detected in lignocellulose- degrading *Streptomyces*
188 species (Wei & Zimmermann, 2017). **Recently the marine fungus *Zalerion maritimum* has been isolated and his**
189 **ability to degrade polyethylene microplastics in 14 days has been described (Paco et al., 2017). *Aspergillus* spp.**
190 **Species showed good growth in medium supplemented with both low-density polyethylene (LDPE) (Pramila et**
191 **al 2011) and high-density polyethylene (HDPE) (Devi et al. 2015) as unique carbon source. Degradation**
192 **pathway involved in the biodegradation of LDPE comprise hydrophobic proteins and degrading enzymes that**
193 **can attach to the polymer surface (Pramila et al 2011). Other strains of *Aspergillus*, together with *Phanerochaete***
194 **and *Lentinus* species, show significant potential also for biodegradation of PVC plastics (Devi et al.,**
195 **2016).Several microorganisms capable of growing and degrading PUR were isolated and the enzymes related to**
196 **polyurethane biodegradation have been described (Schmidt et al., 2017). *Pseudomonas* spp. degrades PUR using**
197 **proteases, esterases and imbranilases (Loredo-Treviño et al., 2012). Various species of fungi, including,**

198 *Curvularia senegalensis*, *Fusarium solani*, *Aureobasidium pullulans* and *Cladosporium* sp, have been isolated
199 as polyurethane-degrading microbes from environment (Devi et al., 2016). Key enzymes of the biodegradation
200 pathway include an endopolyurethanases that hydrolyze the PUR molecule at random locations, and
201 exoenzymes that remove monomer units from the chain ends (Howard et al., 2002). *Pseudomonas* and *Vibrio*
202 degrade PP by oxidative degradation causing a decrease in viscosity and the formation of carbonyl and carboxyl
203 groups during the biodegradation (Alshehrei, 2017). A number of lipases, esterases, cutinases, carboxylesterases
204 from fungi (*Fusarium oxysporum* and *F. solani*) has been isolated (Nimchua et al., 2007). Key enzymes for PET
205 biodegradation from Actinobacteria (*Thermobifida fusca*) and Firmicutes (*Bacillus licheniformis*, *B. subtilis*)
206 were found to change its crystalline structure (Wei & Zimmermann, 2017).
207 Recently, a newly discovered bacterium, *Ideonella sakaiensis* 201-F6, was shown to exhibit the rare ability to
208 grow on PET as a major carbon and energy source (Son et al., 2019). Central to its PET biodegradation
209 capability is a secreted polyester hydrolase called PETase (PET-digesting enzyme) that shows features common
210 to both cutinases and lipases (Yoshida et al., 2016). This enzyme was also engineered for improved PET
211 degradation capacity (Austin et al., 2018). Several fungal strains are also known to biodegrade PET, such as
212 *Humicola insolens*, *Fusarium* spp., and *Penicillium citrinum* (Ribitsch et al., 2012); PET-colonizing fungal
213 communities that have adapted to plastics as a surface for colonization and degradation, constituted mainly to
214 Ascomycota, Basidiomycota, Chytridiomycota have been identified and characterized (Oberbeckmann et al.,
215 2016). In fungal PET biodegradation, the strategy proposed suggests that fungi adhere to the PET surface and
216 secrete extracellular enzymes PETase (hydrolase) and MHETase. PETase hydrolyze PET to mono-(2-
217 hydroxyethyl) terephthalate (MHET), terephthalic acid (TPA), and bis (2-hydroxyethyl) terephthalate (BHET)
218 (Taniguchi et al., 2019). The MHETase hydrolyzes MHET to TPA and ethylene glycol (EG) (Jacquin et al.,
219 2019). *P. citrinum* hydrolyze PET with a polyesterase that increases PET surface hydrolysis (Liebminger et al.,
220 2007)
221 Weathered polystyrene (PS) films incubated under simulated marine developed convergent biofilm communities
222 enriched with hydrocarbon and xenobiotics degradation genes that efficiently reduced the weight of PS films
223 and decreased the number-average molecular weight of films (Syranidou et al., 2017).
224 These recently obtained results suggest that marine microbial populations carry a plastic biodegradation
225 potential that is still largely unexplored.

226

227 **Future Directions**

228 POPs continue to be produced and spread in the environment despite the worldwide efforts to reduce their
229 impact by recycling, reusing and substituting them with less toxic and less persistent molecules (Nadal et al.,
230 2015).

231 The platisphere has been object to many studies to assess microbial polymer biodegradation at sea, but new and
232 combined monitoring parameters have yet to be defined and confirmed with *in situ* experiments, to define the
233 steps of biofilm formation and bacterial colonization, the impact on global biogeochemical cycles and the spread
234 of invasive species to both the animal and human world (Jacquin et al., 2019).

235 While great efforts are devoted to reducing the huge amount of plastic waste in the environment, the focus of
236 research is to substitute petroleum-based plastics with bio-based plastics and to discover mechanisms that can
237 make plastics degradable by acting on their chemical structure (Iwata, 2015). Additives, such as pro-oxidants

238 and starch, are applied in synthetic materials to modify and make plastics biodegradable (Zheng et al., 2005) and
239 promote microbial colonization (Fontanella et al., 2010). Both routes lead ultimately to plastic removal by
240 biodegradation carried out by microorganisms in the environment.

241 Bioplastics, bio-based plastics synthesized from biomass and renewable resources (Scaffaro et al., 2017), should
242 prevent the disposal of recalcitrant plastic waste in the environment because of their
243 biodeteriorability/biodegradability (Tosin et al., 2012; Accinelli et al., 2012). Bioplastics can be exploited for
244 various applications and are a good alternative to petroleum-based plastics due to their similar properties
245 (Spierling et al., 2018). However, biodegradation rates are strongly dependent on the manufacturing procedure
246 of the materials (Chinaglia et al., 2018). Higher surface roughness and/or the presence of humidity in the
247 polymer matrix and high temperatures can sensibly increase the biodegradation rates (Lo Re et al., 2013).
248 Microorganisms are also able to synthesize biopolymers as intracellular, structural, and extracellular polymers
249 for their function and survival (Sukan et al., 2015). The polyhydroxyalkanoates (PHAs) and exopolysaccharides
250 (EPS) are gaining importance over the other biopolymers (de Jesus Assis et al., 2016) in several applications
251 including drug delivery, food, pharmaceuticals, paper industry, textile printing, cosmetics and agriculture
252 (Vijayendra, 2015).

253 Finally, microorganisms appear the only organisms able to turn plastic polymers into less complex chemical
254 constituents facilitating their mineralization thanks to their enzymes (Kanaly et al., 2010). PAHs-biodegradation
255 pathways, genes, enzymes and molecular mechanisms are the major study-areas selected until this moment:
256 efficiency in high salinity or anaerobic environments and factors enhancing bioremediation like oxygen
257 concentration, pH, temperature, and nutrient availability are to be analyzed (Ghozal et al., 2016). In an attempt
258 to develop an efficient pathway of anaerobic biodegradation, PAHs-degrading bacteria have been used in
259 sulfate-, nitrate-, and metal-ion-reducing conditions as well as methanogenic conditions, that has highlighted the
260 need to develop biosurfactants to ease access to hydrophobic molecules via biochemical and molecular methods
261 (Nzila, 2018). Biosurfactants with different characteristics and efficiency are produced by many microbial taxa:
262 production and effectiveness are to be studied in order to develop eco-friendly *in situ* applications to increase the
263 availability of oil and plastic compounds lessening eco-toxicological risks (Dell'Anno et al., 2018).

264 The development of environmental friendly and sustainable solutions to manage the waste of plastics mixtures
265 in the environment could rely on the use of indigenous consortia tailored for the degradation of plastic polymers
266 in the marine environment (Syranidou et al., 2017) or microorganisms with a set of complementary enzymes
267 either native or engineered using state of the art biotechnologies (Kelwick et al., 2017). Controlled degradation
268 in immobilized conditions can be used for cleaning up environments and wastewaters to perfect survival and
269 performance of bacterial cells (Bayat et al., 2015; Abarian et al., 2019). With modern means of biotechnology,
270 genes involved in pollutant degradation extracted from metagenomic libraries can be inserted into strains with
271 strong fecundity and high adaptability, and overexpressed by plasmids, creating consortia of cooperating
272 bacteria to further enhance biodegradation potential (that can ultimately be coupled with nitrification)
273 (Ghanavati et al., 2008; Liu et al., 2019).

274

275 **Abbreviations**

EPS	Exopolysaccharides
HC	Hydrocarbon

HDPE	High-density polyethylene
HMW	High-Molecular Weight
LDPE	Low density polyethylene
LMW	Low-Molecular Weight
PAH	Polycyclic Aromatic Hydrocarbons
PE	Polyethylene
PET	Polyethylene Terephthalate
PHA	Polyhydroxyalkanoates
PMD	Plastic Marine Debris
POP	Persistent Organic Pollutant
PP	Polypropylene
PS	Polystyrene
PUR	Polyurethane
PVC	Polyvinyl chloride
TCA	Tricarboxylic Acid Cycle

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278 **References**

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