



**HAL**  
open science

## Biodegradability under marine conditions of bio-based and petroleum-based polymers as substitutes of conventional microparticles

Jingguang Cheng, Boris Eyheraguibel, Justine Jacquin, Mireille Pujo-Pay, Pascal Conan, Valérie Barbe, Julia Hoypierres, Gaëlle Deligey, Alexandra Ter Halle, Stéphane Bruzaud, et al.

### ► To cite this version:

Jingguang Cheng, Boris Eyheraguibel, Justine Jacquin, Mireille Pujo-Pay, Pascal Conan, et al.. Biodegradability under marine conditions of bio-based and petroleum-based polymers as substitutes of conventional microparticles. *Polymer Degradation and Stability*, 2022, 206, pp.110159. 10.1016/j.polymdegradstab.2022.110159 . hal-03844496

**HAL Id: hal-03844496**

**<https://hal.science/hal-03844496v1>**

Submitted on 27 Oct 2023

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **Title: Biodegradability under marine conditions of bio-based and petroleum-**  
2 **based polymers as substitutes of conventional microbeads**

3

4 **Authors:** Jinguang Cheng<sup>a,1</sup>, Boris Eyheraguibel<sup>b,1</sup>, Justine Jacquin<sup>a</sup>, Mireille Pujo-Pay<sup>a</sup>,  
5 Pascal Conan<sup>a</sup>, Valérie Barbe<sup>c</sup>, Julia Hoypierres<sup>d</sup>, Gaëlle Deligey<sup>d</sup>, Alexandra Ter Halle<sup>e</sup>,  
6 Stéphane Bruzaud<sup>d</sup>, Jean-François Ghiglione<sup>a</sup> & Anne-Leila Meistertzheim<sup>f\*</sup>

7

8 **Affiliations:**

9 <sup>a</sup> CNRS, Sorbonne Universités, UMR 7621, Laboratoire d'Océanographie Microbienne,  
10 Observatoire Océanologique de Banyuls, France

11 <sup>b</sup> CNRS, Univ. Clermont Auvergne, UMR 6296, Institut de Chimie de Clermont-Ferrand,  
12 France

13 <sup>c</sup> Génomique Métabolique, Genoscope, Institut François Jacob, CEA, CNRS, Univ Evry,  
14 Université Paris-Saclay, 91057 Evry, France

15 <sup>d</sup> UMR CNRS 6027, Institut de Recherche Dupuy de Lôme (IRDL), Univ. Bretagne Sud,  
16 Lorient-Pontivy, France

17 <sup>e</sup> Laboratoire des IMRCP, Université de Toulouse, CNRS UMR 5623, Université Paul Sabatier,  
18 118 route de Narbonne 31062 Toulouse Cedex 9, France

19 <sup>f</sup> SAS Plastic@Sea, Observatoire Océanologique de Banyuls, France

20

21 **Author information:**

22 *(<sup>1</sup>) Jinguang Cheng and Boris Eyheraguibel contributed equally to this work and shared co-*  
23 *first authorship*

24 *(\*) Corresponding author:*

25 *Jean-François Ghiglione, Laboratoire d'Océanographie Microbienne, 1 Avenue Fabre, F-*  
26 *66650 Banyuls sur mer, Email : ghiglione@obs-banyuls.fr*

27

28 **Abstract:**

29 Plastic microbeads have been produced at very large scales since several decades for cosmetics  
30 and personal care products, a large amount of which being directly transported in the oceans.  
31 To better understand their biodegradability in marine environment and evaluate their possible  
32 replacement by biodegradable polymers, seven polymer types were studied including three  
33 conventional petroleum-based polymers (PE, PMMA and PCL), two bio-based polymers (PLA  
34 and PHBV) and two natural products (rice seeds and apricot kernel). We used several  
35 innovative approaches by both the experimental design and the set of multidisciplinary  
36 techniques to follow the successive steps of biodegradation together with abiotic degradation  
37 under seawater conditions. Congruent signs of biodegradability were observed by oxygen  
38 consumption, weight loss, and modification of molecular weight, <sup>1</sup>H NMR and mass  
39 spectrometry profiles. We found that microbeads made of PHBV, PCL, rice and in a lesser  
40 extent apricot were biodegradable under our conditions, while not for microbeads made of PE,  
41 PMMA and PLA. Taking into consideration of industrial application, PHBV could be the ideal  
42 material to replace the conventional microbeads, since the material is resistant to hydrolysis,  
43 but susceptible to microbial degradation, as shown by the <sup>1</sup>H NMR results. This study provides  
44 the first time arguments for the use of biodegradable substitutes to replace conventional  
45 microbeads to support the recent legislative rules aiming to reduce the pollution by primary  
46 microplastics in the oceans.

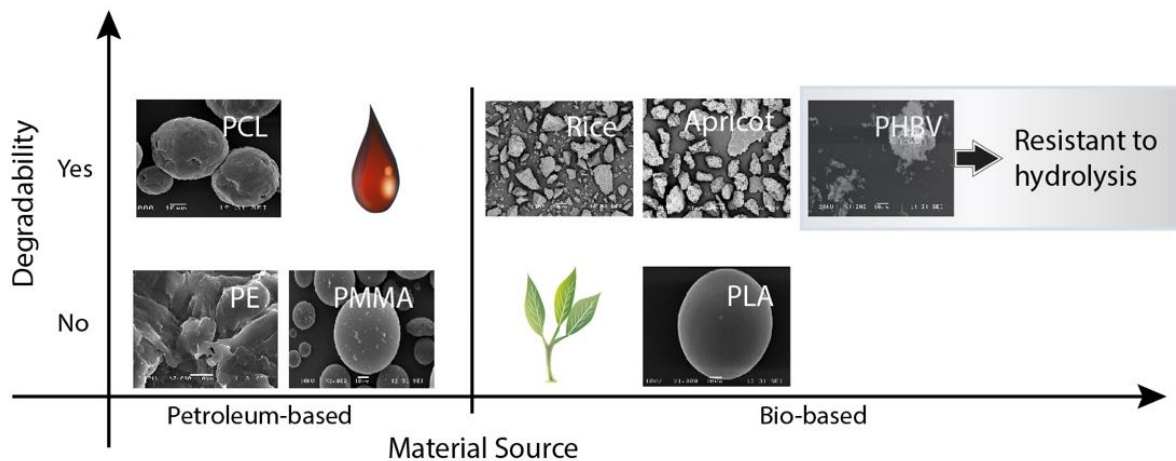
47

48 **Keywords:** microbeads, biosourced and biodegradable polymer, cosmetics, plastic pollution,  
49 microbial ecotoxicology

50 **Highlights:**

- 51 • The biodegradation of 7 polymers in the marine environment was investigated to  
52 challenge their application as potential substitutes of the conventional microbeads used  
53 in cosmetics and personal care products.
- 54 • Innovative 2-stepwise experiments were designed to mimic realistic marine conditions.
- 55 • The four biodegradation steps were characterized, including biodeterioration,  
56 biofragmentation, bioassimilation and mineralization.
- 57 • The biosourced PHBV is proposed as the best biodegradable polymer tested to replace  
58 the conventional microbeads.

59  
60 **Graphical abstract:**



61

## 62 **1. Introduction**

63 Accumulation of plastics in the marine environment has been observed in ocean gyres, on  
64 beaches and in sediments worldwide [1]. The major source of plastic at sea arises from  
65 mismanaged plastic waste and from riverine inputs [2]. Beside entanglement and ingestion of  
66 macro debris by large vertebrates (birds, turtles etc.) [3], adverse effects on marine organisms  
67 were particularly due to microplastics with proven transfer along the food chain [4]. Within  
68 microplastics, distinction has been made between small microplastics (25  $\mu\text{m}$  –1 mm) that can  
69 be as abundant as large microplastics (1–5 mm) in the oceans [5]. Another distinction has been  
70 made between “primary microplastics” purposefully manufactured to be of millimeter size (e.g.  
71 microbeads and plastic pellets) and “secondary microplastic” that result from breaking down of  
72 larger plastic pieces due to UV light, waves or abrasion [6].

73 Microbeads are primary microplastics commercially available in particle sizes ranging from 10  
74  $\mu\text{m}$  to 1 mm, which are used in hundreds of products, often as abrasive scrubbers, including  
75 face washes, body washes, cosmetics, and cleaning supplies [7]. After use, microbeads included  
76 in personal care products are poured down the drain and cannot be collected for recycling.  
77 Wastewater treatment plant is generally not a sufficient solution to prevent microbeads  
78 pollution [8]. Microbeads from personal care and cosmetics products could be a significant  
79 source of microplastics pollution in the ocean [8–11]. It has been estimated that about 8 trillion  
80 microbeads were emitted into aquatic habitats per day from the United States [7].

81 As an alternative way to relieve the problem, it has been also advised to replace the conventional  
82 microplastic, generally made of polyethylene (PE) or polymethyl methacrylate (PMMA) by

83 'biodegradable' microparticles. Biodegradable plastics can be petroleum-based polymers such  
84 as polycaprolactone (PCL), or bio-based polymers (so-called 'biosourced') such as poly(D-  
85 lactide) (PLA) and polyhydroxyalcanoates (PHAs). Other natural compounds such as rice seeds  
86 and apricot kernels have already been used as exfoliating material by some manufacturers and  
87 are considered as bio-based polymers [12].

88 Most of scientific studies on plastic biodegradation were conducted under controlled laboratory  
89 conditions by using soil or compost, but very few studies investigated the aquatic media and  
90 especially the marine environment [13–15]. By now, few standard methods could be applied to  
91 assess the biodegradability of plastics under marine conditions, but several scientists underlined  
92 the drawbacks and biases of these methods [16–18]. Several authors underlined the need of  
93 convergent results from combined approaches, including proof of mineralization by  
94 microorganisms together with tests of chemical structure alteration in order to conclude on the  
95 biodegradability of a polymer [19,20]. Another concern is the necessity to mimic biological,  
96 physical and chemical conditions of the marine environment during the tests.

97 The aim of this study was to test the biodegradability under marine conditions of bio-based  
98 (PLA, PHBV, apricot kernels and rice seeds) and petroleum-based polymers (PCL), as  
99 substitutes of conventional microbeads (PE, PMMA) for cosmetics. We used microbeads of 50  
100 to 250  $\mu\text{m}$  diameter, which were commercially available or laboratory-made by solvent  
101 emulsion-evaporation technique. Biodegradability tests were performed in a two-steps protocol  
102 including a first 2-months incubation of each polymer type with natural seawater communities,  
103 followed by a transfer of the plastic together with its natural microbial biofilm to a marine

104 minimum medium with no other carbon source than the plastic for another 2-months. We  
105 hypothesized that bio-sourced polymers may have a greater biodegradation capability  
106 compared to petroleum-based polymers under marine conditions. Here, we combined different  
107 methodological approaches including weight loss, continuous biological oxygen consumption  
108 (optical fiber luminescent sensor), changes in molecular weight (high temperature size  
109 exclusion chromatography), size and surface properties (laser diffraction particle size analyzer  
110 and scanning electron microscopy), carbonyl index (Fourier transform infrared spectroscopy)  
111 and release of oligomers ( $^1\text{H}$  nuclear magnetic resonance spectroscopy and Liquid  
112 Chromatography-High Resolution Mass Spectrometry).

113

## 114 **2. Materials and Methods:**

### 115 *2.1. Production of petroleum-based and bio-based microbeads*

116 The petroleum-based microbeads PE and PMMA were supplied by Good Fellow company  
117 (Lille, France). Natural microparticles were manufactured by cryogrinding of the rice seeds  
118 (Rice Exfoliator 200, Lessonia, Saint Thonan, France) so called “rice” hereafter and apricot  
119 kernels (Apricot Exfoliator 200, Lessonia, Saint Thonan, France) so called “apricot” hereafter.  
120 PE, PMMA, rice and apricot were sieved in order to recover the microparticles for which the  
121 diameter was ranging from 50 to 250  $\mu\text{m}$ .

122 PCL (CAPA 6800, Perstorp Company, Sweden), PLA (7001D, Nature Works, Blair, USA) and  
123 PHBV (ENMAT Y1000P with a HV molar ratio of 3%, Tianan Biological Materials Co. Ltd.,  
124 China) were obtained as pellets and transformed in spherical microbeads by solvent emulsion-

125 evaporation technique (see Method S1 and Table S1 for material preparation of PCL, PLA and  
126 PHBV).

## 127 *2.2. Microparticle characterization*

128 Microparticle surface morphologies were observed at the step of microbeads production by  
129 using a Jeol JSM-6031 scanning electron microscope (SEM). Prior to any observation, the  
130 fracture surfaces were coated with a thin gold layer by means of a Polaron sputtering apparatus.

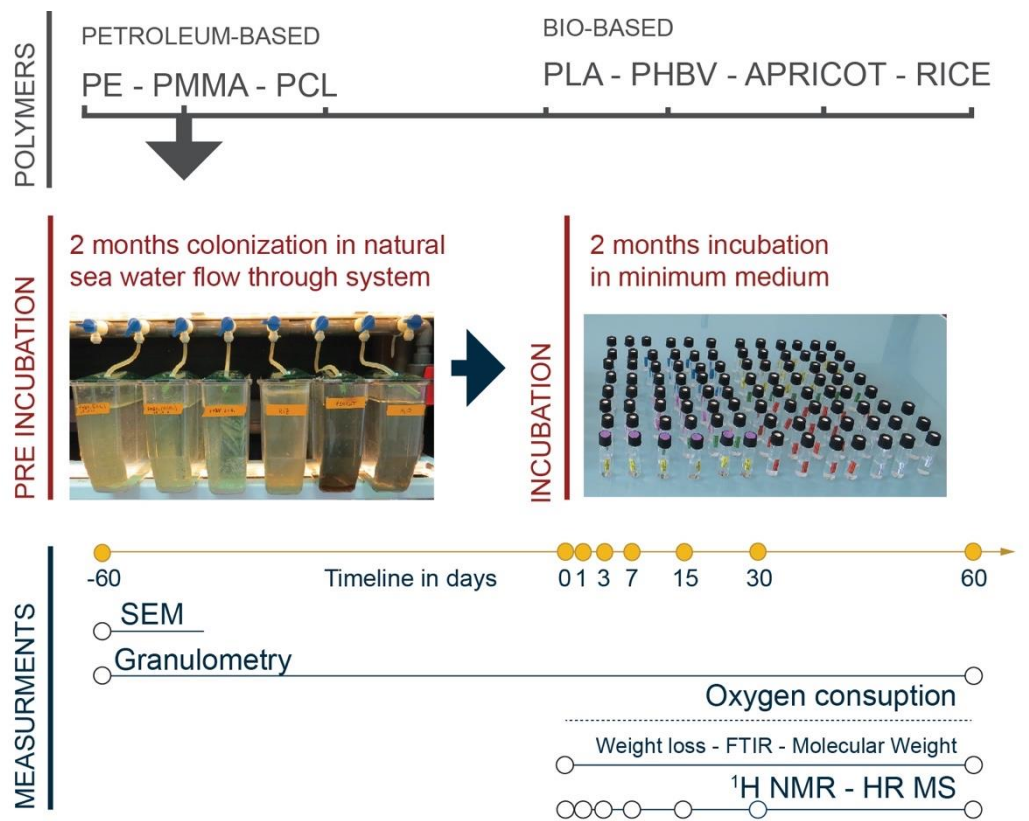
## 131 *2.3. Experimental setup*

132 A two phases stepwise experiment was designed in order to evaluate the biodegradability of the  
133 conventional petroleum-based and bio-based polymers under marine conditions (Fig. 1). The  
134 first step consisted in the formation of a mature biofilm in natural seawater, as previously  
135 described [21,22]. Briefly, each microparticle type (called “microbeads” hereafter) was  
136 incubated for a period of 2-months in 1.8L aquarium (Sodispan, Spain) with direct circulation  
137 to the sea. Seawater was pumped at 14 m depth in the Banyuls Bay close to the SOLA  
138 observatory station of the Laboratoire Arago (SOLA station - NW Mediterranean Sea). A flow  
139 rate of  $50 \text{ ml}\cdot\text{min}^{-1}$  was chosen to ensure a sufficient renewal of natural bacteria (every 30 min)  
140 and the surface outlet water was filtered at  $50 \mu\text{m}$ . Each aquarium contained 12 grams of each  
141 microbead types (PE, PMMA, PCL, PLA, PHBV, apricot and rice) added for a 2-months period.  
142 Throughout the experiment, seawater temperature (between  $18.3^\circ\text{C}$  and  $25.3^\circ\text{C}$ ) and salinity  
143 (38.5) in the aquarium were similar to seawater from the Banyuls bay.

144 Second, about 10 mg of dry microbeads were transferred under sterile conditions to 4.9 mL  
145 closed glass vials (Interchim, Montluçon, France) containing 2 mL of minimum medium (called



146 “MM” hereafter) with microbeads as sole carbon source (see Method S2 for MM composition).  
 147 Vials were incubated in the dark at 18°C under agitation at 110 rpm (orbital agitator) for a period  
 148 of 2 months (called “biotic condition” hereafter). In addition, controls were incubated and  
 149 sampled in the same way and consisted in triplicate vials containing 2 mL of MM with  
 150 microbeads of the same composition but previously sterilized overnight in 70% ethanol and  
 151 evaporation under UV sterile hood (called “abiotic condition” hereafter). A total of 130 vials  
 152 were needed to follow the different parameters detailed below in triplicate samples taken after  
 153 0,1, 3, 7, 15, 30 and 60 days of incubation (Fig. 1).



154  
 155 **Fig. 1 Experimental design.** Schematic presentation of the two phases stepwise experiment of  
 156 (1) a 2-months incubation in natural seawater flow through system (flow rate 50 mL.min<sup>-1</sup>) for  
 157 petroleum-based (PE, PMMA, PCL) and bio-based microbeads (PLA, PHBV, apricot, rice)  
 158 followed by (2) a transfer into 4.9 mL glass vials containing 2 mL minimum medium with  
 159 microbeads as sole carbon source for another 2-months. Lines indicate when measurements  
 160 were possible for respective assays and dots indicate each measurement done during the course  
 161 of the experiment, dashed line indicates daily oxygen measurement.

162

#### 163 2.4. Continuous oxygen measurement and biodegradation

164 During the second step of the experiment (Fig. 1), triplicate vials with each microbead type  
165 were equipped with an optical fiber luminescent oxygen sensor (Presens, SP-PSt3-YAU) using  
166 the instrument of Stand-alone Fiber Optic Oxygen Meter (Fibox 4, Germany). Oxygen sensors  
167 were placed in the liquid phase to obtain the concentration of dissolved oxygen. Oxygen  
168 concentration was manually measured daily in the abiotic (PE and PHBV) and biotic conditions  
169 in minimum medium and oxygen was converted from mole concentration to total oxygen in  
170 vial with the Henry's law (oxygen equilibrium confirmed between the two phases) and ideal  
171 gas law. In the case of PHBV, vials were opened under a sterile laminar flow hood after 15 days  
172 to ensure that oxygen was not limiting for bacterial growth and closed again. In this case,  
173 oxygen concentration was always maintained  $> 15 \mu\text{mol.L}^{-1}$  and the re-opening for 10 minutes  
174 was enough to return to initial values (around  $230 \mu\text{mol.L}^{-1}$ ). For all polymer types, biological  
175 oxygen demand (BOD) was computed from the difference of oxygen concentration. The  
176 consumption of oxygen in abiotic condition served as a negative control. The percentage of  
177 biodegradation was expressed as the ratio of the oxygen consumption over the theoretical  
178 amount of consumed oxygen for complete degradation (ThOD), as previously described [23].

#### 179 2.5. Size change

180 The size distribution of the microparticles were measured at the beginning (*D-60*) and at the  
181 end of the experiment (*D60*) (Fig. 1) with a laser diffraction particle size analyzer (Malvern  
182 Mastersizer 2000 model with a Scirocco 2000 module) through granulometry analyses. The

183 particles were analyzed according to a model of spherical particles of polystyrene and according  
184 to the Fraunhofer diffraction theory [24]. Each measurement was an average of 15 scans and  
185 each sample was analyzed, thus providing the number, volume and length of the particles.

#### 186 *2.6. Weight loss*

187 Microbeads weight was measured on *D0* and *D60* of the incubation in MM in triplicates (Fig.  
188 1). The pellet was washed twice with distilled water to remove salts with 1 min centrifugation  
189 at 13000 rpm, then lyophilized and immediately weighed in analytical balance with the  
190 precision of 0.1 mg (ENTRIS1241-1S, Sartorius).

#### 191 *2.7. Molecular weight*

192 Molecular weight was determined for each polymer before colonization and at the end of the  
193 incubation in MM (Fig 1). High temperature size exclusion chromatography (HT-SEC)  
194 analyses were performed using a Viscotek system (Malvern Instruments) equipped with a  
195 combination of three columns (Polefin 300 mm × 8 mm I. D. from Polymer Standards Service,  
196 porosity of 1000 Å, 100 000 Å and 1 000 000 Å). Samples were dissolved in the mobile phase  
197 (tetrahydrofuran) with a concentration of 5 mg mL<sup>-1</sup> and 200 µL of sample solutions were  
198 injected and eluted in tetrahydrofuran using a flow rate of 1 mL min<sup>-1</sup> at 25 °C. The same  
199 procedure was applied to polyethylene samples using 1,2,4-trichlorobenzene at 150 °C to  
200 dissolve the sample and as mobile phase. The mobile phase was stabilized with 2,6-di(tert-  
201 butyl)-4-methylphenol (200 mg L<sup>-1</sup>). Online detection was performed with a differential  
202 refractive index detector, a multichrom right angle light scattering detector, and a viscometer  
203 detector. A universal calibration curve, obtained with polystyrene standards (Polymer Standards

204 Service, Mainz, Germany) in the range of 0.5–7.106 kg mol<sup>-1</sup>, was used to calculate the molar  
205 mass distribution of the samples. OmniSEC software version 5.12 (Malvern Panalytical, UK)  
206 was used for the calculations. The typical evaluated accuracy for this technique is close to 10%.

### 207 2.8. *Fourier Transform Infrared (FTIR) spectroscopy*

208 Fourier Transform Infrared (FTIR) analyses were performed on microbeads on *D0* and *D60* of  
209 the incubation in MM in biotic condition (Fig. 1). After collection, the supernatant was removed  
210 and samples were dried at 60° in an aerated oven until constant weight to eliminate trace of  
211 water. FTIR measurements were carried out using a Nicolet™ 380 FT-IR (ThermoFisher  
212 Scientific) in ATR (Attenuated Total Reflectance) mode. For each sample, the total amount of  
213 microbeads (10 mg or less) was compressed against a diamond crystal with a constant force.  
214 FTIR spectra were collected using 16 scans from 4000 cm<sup>-1</sup> to 400 cm<sup>-1</sup>, with a resolution of 4  
215 cm<sup>-1</sup>. To assess the variation between spectra, the characteristic absorption bands were realized  
216 using Omnic Spectra software (Thermo Fisher Scientific). For each polymer, manual assessment  
217 of absorption band was done and compared to the literature. The calculations for carbonyl and  
218 crystallinity index are detailed for each polymer in the supporting material (Table S2-8).

### 219 2.9. *Characterization of the degradation products by <sup>1</sup>H NMR spectroscopy*

220 <sup>1</sup>H NMR spectroscopy analysis were carried out to assess the release of degradation products  
221 (oligomers) from microbeads as sole carbon source for the biofilms incubated in minimum  
222 medium for 0, 1, 3, 7, 15, 30 and 60 days in abiotic and biotic condition (Fig. 1). For each point,  
223 samples (microbeads + liquid media) were transferred to 2 mL Eppendorf tube and centrifuged  
224 to remove the microbeads. Supernatants (540 µl) were supplemented with 60 µL of a 2 mM

225 solution of TSPd<sub>4</sub> (sodium tetra deuterated tri methylsilyl propionate, Eurisotop) in deuterated  
226 water (D<sub>2</sub>O, Eurisotop). D<sub>2</sub>O signal was used for locking and shimming while TSPd<sub>4</sub>  
227 constituted a reference for chemical shifts (0 ppm) and quantification. <sup>1</sup>H NMR spectra were  
228 recorded on a Bruker Avance 500 MHz spectrometer, equipped with a 5 mm inverse-triple  
229 tuned (TXI) <sup>1</sup>H/<sup>13</sup>C/<sup>15</sup>N with z-gradient coil probe (Bruker Biospin Wissenbourg, France), with  
230 5 mm-diameter tubes containing 600 µl of sample. 128 scans were collected (90° pulse, 3.24 s  
231 acquisition time, 4.0 s relaxation delay, 4789.272 Hz SW, 65536 data points). Water signal was  
232 eliminated by pre-saturation. An exponential filter was applied before Fourier transformation,  
233 and a baseline correction was performed on spectra before integration. Under these conditions,  
234 the limit of quantification is in the range of 0.01 mM. The relative quantity of oligomers was  
235 expressed using the TSPd<sub>4</sub> as the reference.

#### 236 *2.10. Liquid Chromatography- High Resolution Mass Spectrometry*

237 When detected by NMR spectroscopy, the degradation products were then identified by High  
238 Resolution Mass Spectrometry. The extracts were analyzed using Electrospray Ionization (ESI)  
239 with a Q-Exactive Orbitrap™ mass spectrometer (Thermo Scientific) providing a mass  
240 accuracy lower than 3 ppm and an ultra-high resolution over 100 000. Briefly, 5 µl of  
241 supernatant from extracted samples were analyzed using positive and negative ion mode. The  
242 ion source and the capillary heater were set to operate at 3.2 kV and 320 °C. Nitrogen gas was  
243 used for nebulizing and as the damping and collision gas in the mass analyzer.

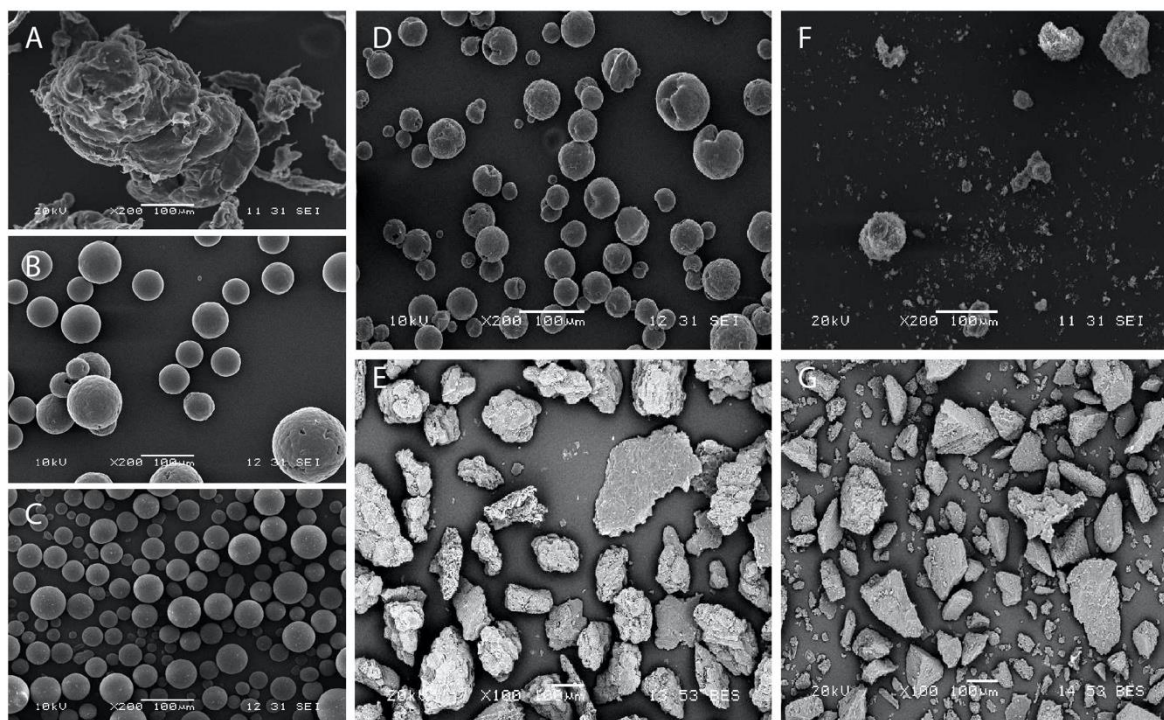
244

### 245 **3. Results**

246 *3.1. Temporal changes of microparticle sizes*

247 The SEM images of the raw particles showed significantly different size, sphericity and  
248 roughness properties depending on how the material was obtained (Fig. 2). The production of  
249 microbeads by cryo-grinding increased the roughness and the size distribution of particle  
250 compared to the solvent emulsion-evaporation technique. Size distribution estimated by  
251 granulometry for PLA, PCL and PHBV microparticles was centered approximately 100  $\mu\text{m}$   
252 (median diameter between 88 and 126  $\mu\text{m}$  depending on the types of bioplastics) (Table 1). The  
253 PE microparticles were the largest with a median diameter around 280  $\mu\text{m}$ . On the other hand,  
254 those made of PMMA were the smallest since their median diameter is around 60  $\mu\text{m}$ . Natural  
255 microparticles made from rice or apricot had much more heterogeneous dimensions over a wide  
256 range of sizes confirming SEM observations. The median diameter for these microparticles was  
257 estimated to be around 120 and 160  $\mu\text{m}$ , respectively.

258 After 2-months of incubation in natural seawater followed by 2-months incubation in minimum  
259 medium, sample aggregation was observed for most samples except for PHBV. No change of  
260 the size distribution was observed for the PMMA. For PLA and PE, values of the median  
261 diameter were similar. For rice, median diameter was higher after 4 months, with no smaller  
262 particles. For PCL, apricot and PHBV (Fig. S1), decreases of the median diameters were  
263 observed with the decrease of bigger particles.



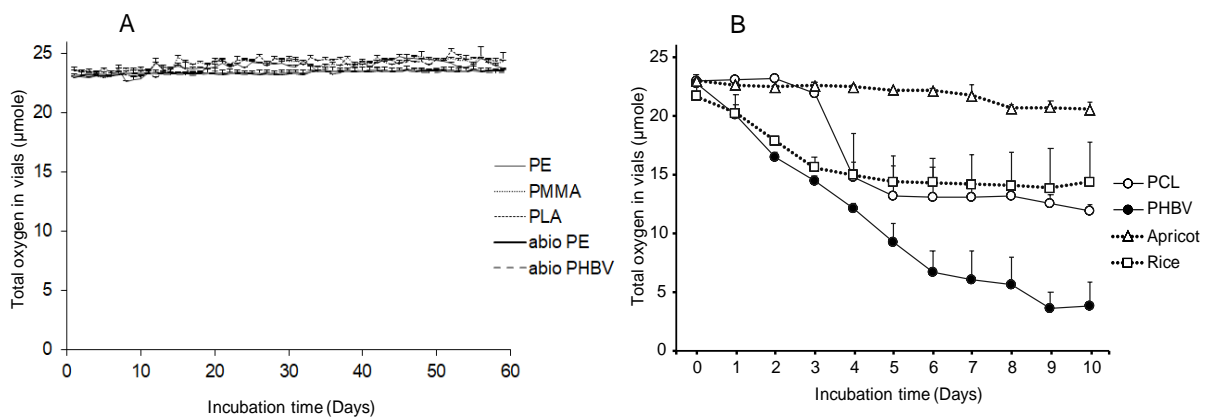
264  
 265 **Fig. 2** Scanning electron microscopy for the microbeads made of seven polymer types: (A) PE,  
 266 (B) PLA, (C) PMMA, (D) PCL, (E) ground apricot kernels, (F) PHBV, (G) ground rice particles.  
 267 The scale bar in all panels are 100  $\mu$ m.

268

269 *3.2. Temporal dynamic of oxygen consumption*

270 The dissolved oxygen was recorded daily for the first 15 days for the triplicates. No oxygen  
 271 consumption was observed for abiotic control, as illustrated by PE and PHBV abiotic conditions  
 272 in Fig. 3A. In biotic condition, the continuous evolution of oxygen consumption by bacteria in  
 273 minimum medium with each polymer as sole carbon source showed different patterns according  
 274 to the polymer types. Polymers including PE, PMMA and PLA did not show any sign of oxygen  
 275 consumption. On the contrary, a drastic decrease of 19  $\mu$ mole was observed for PHBV during  
 276 the first 10 days, PCL, Rice and Apricot also showed significant oxygen consumption of 11.1,  
 277 7.3, and 2.4  $\mu$ mole for the same period, respectively (Fig. 3B). The biological oxygen demand  
 278 was then calculated for the first 10 days, before the first opening of the vials to avoid oxygen

279 limitation for PHBV in biotic conditions. being aware of the possibility of oxygen limitation  
 280 for PHBV. The percentages of biodegradation were  $4.1 \pm 0.4\%$  (average and standard deviation),  
 281  $1.0 \pm 0.1\%$ ,  $3.8 \pm 1.9\%$  and  $0.5 \pm 0.2\%$  for PHBV, PCL, rice and apricot, respectively. It has to be  
 282 noted that vials with rice became visually very turbid, which may affect the transmission of  
 283 luminescence and then underestimate the oxygen consumption and resulting percentage of  
 284 biodegradation. The two different oxygen consumption patterns have clearly classified the  
 285 materials into two groups: the degradable and non-degradable ones (Fig. 3).  
 286



287  
 288 **Fig. 3** Oxygen consumption (in  $\mu\text{mole}$ ) after incubation in minimum medium with microbeads  
 289 made of the seven material types under abiotic (abio PE and abio PHBV) and biotic (with their  
 290 respective biofilm on PE, PMMA, PLA – left ; PCL, PHBV, apricot, rice - right) conditions  
 291 (with standard deviation).



292 **Table 1.** Synthesis of the microbeads composition and characterization before colonization and after biodegradation assessment.

Type	Polymer	Monomer	Median diameter (µm)		Biodegradation rate % (S.D.)	Weight loss %	Molecular weight				Carbonyl */ Crystallinity index		<sup>1</sup> H NMR Signal	Mass Id		
			D-60	D60			D0-D10	D0-D60	D0	Mn	D60	Mw/Mn			D0	D60
Conventional	Low Density Polyethylene (PE)		278	282	n.d.	0%	D0	5200	D60	5700	8.9	9.1	0*	0*	n.d./ n.d.	n.a.
	Poly(methyl methacrylate) (PMMA)		57	56	n.d.	0% (25.5%)	D0	125 000	D60	118 000	2.6	3.0	n.a.	n.a.	n.d./ n.d.	n.a.
	Polycaprolactone (PCL)		88	61	1.0% (0.1%)	17.8% (7.2%)	D0	33 000	D60	23 000	1.7	2.0	7.57* /0.38	4.24* /0.53	n.d./ ++	Caprolactone (and its dimer and trimer)
	Poly(lactic acid) (PLA)		115	119	n.d.	-0.3% (18.6%)	D0	66 270	D60	66 250	1.7	1.7	n.a.	n.a.	n.d./ n.d.	n.a.
Biosourced	Poly (3-hydroxybutyrate-co-3-valerate) (3 mol % HV) (PHBV)		126	106	4.1% (0.4%)	17.0% (6.1%)	D0	34 0000	D60	n.a.	n.a.	n.a.	0.74	0.83	+/ n.d.	3-hydroxybutyric acid 3-hydroxyvaleric acid 3-hydroxybutyrate dimer 3-hydroxybutyrate-3-hydroxyvalerate dimer 3-hydroxyvalerate dimer (and its Heterotrimers)
Natural polymer	Crushed Apricot kernel (APRICOT)		158	120	0.5% (0.2%)	5.9% (8.8%)	D0	n.a.	D60	n.a.	n.a.	n.a.	n.a.	n.a.	n.d./ ++	derivative compounds from lignocellulosis and oil constituents (carbohydrates, lignin and fatty acids)
	Crushed rice seed (RICE)		120	277	3.8% (1.9%)	80.1% (4.8%)	D0	n.a.	D60	n.a.	n.a.	n.a.	n.a.	n.a.	n.d./ ++	derivative compounds from protein and starch constituents (carbohydrates, amino acids)

293 D-60, D0, D10 and D60, please referring to the Figure 1; n.a. non-applicable; n.d. not detected in the condition; + level of signals

294 *3.3. Weight loss*

295 Weight loss measurement on *D0* and *D60* of the incubation in MM separated the polymers in 2  
296 groups similar to those obtained by oxygen measurement (Table 1). No weight loss was found  
297 on polymer for PE, PMMA and PLA. Apricot showed  $5.9\pm 8.8\%$  of weight loss, when PCL,  
298 PHBV showing about  $17.8\pm 7.2\%$  and  $17.0\pm 6.1\%$ . Rice had the highest weight loss with  
299  $80.1\pm 4.8\%$ .

300 *3.4. Changes in the molecular weight*

301 Significant signs of degradation were only observed for PCL that showed a decrease by 30%  
302 of average molecular weight ( $M_n$ ) (from 33 000 to 23 000  $\text{g mol}^{-1}$ ) and an increase of their  
303 polydispersity index (1.7 to 2.0) that indicates a higher molecular weight dispersion (Table 1).  
304 No significant change was found for PE, PMMA and PLA microbeads. Measurement of the  
305 molecular weight was not applicable for the, PHBV, rice and apricot.

306 *3.5. Modification of the chemical properties*

307 The composition of microbeads incubated under biotic conditions was controlled by FTIR after  
308 2 months of incubation in MM. No biodegradability could be found for PE, PMMA and PLA  
309 (Fig. S2-4). Moreover, the absence of characteristic carbonyl band at  $1720\text{ cm}^{-1}$  indicate that  
310 PE microbeads did not undergo photodegradation.

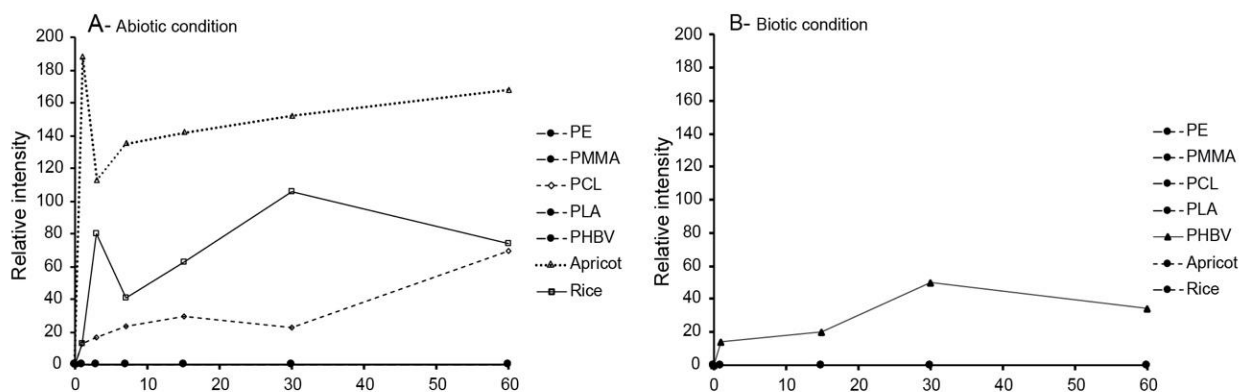
311 The FTIR spectra of PCL microbeads showed a decrease of carbonyl index and a concomitant  
312 increase of crystallinity index (Table1, Fig. S5). Such pattern is typical of ester bonds cleavage  
313 in amorphous region of the polymer chains and confirms the reduction of the molecular weight  
314 of the polymer. Rice microbeads presented a spectrum characteristic of starch with specific

315 monosaccharides and polysaccharides signals (Fig. S6). The strong modification of the spectra  
316 over time revealed a transformation of starch constituent amylopectin, amylose into simple  
317 monomer of glucose (Fig. S7). The FTIR analysis of apricot microbeads presented a typical  
318 spectrum of lignocellulosic material. The spectra of these sample did not change over time, thus  
319 indicating a low transformation of the material as observed by weight loss measurements (Fig.  
320 S8). An increase of the crystallinity index was observed for PHBV samples, suggesting the  
321 possible cleavage of ester bonds in amorphous region of the polymer chains (Table 1, Fig. S9).

### 322 *3.6. Products release during the biodegradation*

323 Under abiotic conditions,  $^1\text{H}$  NMR signal was detected only in the supernatant of PCL, rice and  
324 apricot samples incubated in minimum media while no signal was recorded for the other  
325 materials (Fig. 4A). This indicate the release of small molecular weight compounds from these  
326 polymer matrixes in the aqueous medium when no biological process occurs. The extraction of  
327 oligomers quickly started after incubation. The integration of the total NMR signal clearly  
328 showed a gradual release of oligomers during the 60 days of incubation (Fig. 4A). By  
329 comparison with the literature data, the signal attribution confirms the identification of short  
330 chains of PCL oligomers and typical signal of carbohydrates such as glucose was observed for  
331 the oligomers extracted from rice and apricot samples (Fig. S10 and Table S9). It is worth to  
332 notice that oligomers from PCL, rice and apricot were not detected under biotic conditions,  
333 suggesting an assimilation concomitant to their production. On the contrary, the PHBV  
334 microbeads did not release any measurable oligomers under abiotic conditions but a signal was  
335 recorded in presence of microorganisms, thus confirming the biotic degradation of polymers

336 (Fig. 4B). The release of oligomers (3-hydroxyvalerate, 3-hydroxybutyrate repeating unit)  
 337 started slowly after one day of incubation with microorganisms and increased up to 30 days.  
 338 The signal then decreased until the end of experiment which is typical of microbial consumption.



339 **Fig. 4** Production of oligomers over 60 days of incubation in minimum medium with  
 340 microbeads made of the seven material types (PE, PMMA, PCL, PLA, PHBV, apricot, rice)  
 341 under (A) abiotic (without biofilm) and (B) biotic (with biofilm) conditions. The results  
 342 represent the total integration of <sup>1</sup>H NMR spectra expressed in relative intensity of TSPD<sub>4</sub>  
 343 internal standard.  
 344

345

### 346 3.7. LC Orbitrap TM Mass Spectrometry

347 The oligomers detected in the supernatant were analyzed by mass spectrometry to identify their  
 348 molecular composition. Analysis of PCL, rice and apricot supernatants confirmed the presence  
 349 of small molecular weight compounds corresponding to the abiotic degradation of the polymer  
 350 matrix into various size of oligomers (Fig. S11 and Table S10). Under abiotic conditions, the  
 351 ester hydrolysis of PCL generated caprolactone oligomers that were identified as monomer,  
 352 dimer and trimer of polycaproic acid oligomers. As well, the mass spectra obtained from rice  
 353 and apricot supernatant provided a complex profile of low molecular weight compounds.  
 354 Carbohydrates and amino acids were detected in rice samples while degradation products from  
 355 lignin and fatty acids were identified in apricot samples, resulting from the transformation of

356 the raw material by abiotic processes. Finally, the PHBV microbeads incubated with  
357 microorganisms released various dimer/trimer units of hydroxy-butyrate and hydroxy-valerate.  
358 In our conditions, PHBV microbeads were more sensitive to enzymatic cleavage than to abiotic  
359 degradation processes.

360

## 361 **4. Discussion**

### 362 *4.1. An original multidisciplinary approach for the estimation of polymer biodegradability in* 363 *the marine environment*

364 Standard test procedures for estimating polymer biodegradability are commonly based on  
365 measurements of biological O<sub>2</sub> demand or CO<sub>2</sub> consumption, which are giving indication on  
366 the complete biomineralization of the material by microorganisms [17]. In this study, we  
367 combined the standard test together with other methodologies in order (i) to confirm the  
368 standard test results based on biomineralization processes and (ii) to give more insights on the  
369 other steps involved in biodegradation that include also the biodeterioration, biofragmentation  
370 and the bioassimilation of the polymers [25]. Physico-chemical and structural modification of  
371 the microbeads and evidence of biodeterioration were assessed by granulometry, gravimetry  
372 and FTIR spectroscopy and size exclusion chromatography, <sup>1</sup>H NMR and high-resolution mass  
373 spectrometry were performed to identify degradation products (monomers and oligomers) in  
374 biotic and abiotic conditions and then give information on the biofragmentation. Finally, <sup>1</sup>H  
375 NMR analysis also showed the bioassimilation of the polymers. To our knowledge, this is the  
376 first time that molecular analysis was used to identify the biofragmentation products (i.e.

377 oligomers) and their bioassimilation in polymer degradation by complex natural microbial  
378 communities. Here, we propose that NMR spectroscopy and mass spectrometry provide new  
379 and highly informative results on the molecular fragments of the polymers that can be generated  
380 during its biodegradation. Moreover, it brings a complementary information on direct  
381 assimilation of polymers by microorganisms, that are missing parameter in the classic  
382 methodological approaches which focus either on the growth of microorganisms or on the loss  
383 of material properties, thus addressing the biodeterioration with indirect processes.

384 While there is no harmonized indication today for the preparation of the microbial inoculum to  
385 test its polymer biodegradation capabilities, convergent views indicated that incubation with  
386 pre-selected isolated strains, as it is specified for regional standard tests [26,27], do not mimic  
387 the large diversity of microorganisms living on plastics in the natural conditions, the so-called  
388 'plastisphere' [28,29]. Complex natural marine inocula were adopted by two international  
389 standards [30,31], where it is unfortunately indicated that the inocula could be stored for a  
390 month at 4 °C, which drastically increase the possibility of a strong modification of the plastic-  
391 degrading community [32]. Another novelty of our study is to propose an original two-steps  
392 protocol to test the polymer biodegradability by a natural mature biofilm. The first step of  
393 formation of the mature biofilm was performed by incubating the polymer types in natural  
394 seawater [21]. The second step is the biodegradability test, where the polymers with its mature  
395 biofilm were transferred into a minimal medium with no other carbon source and energy. This  
396 procedure is classically used to test the capability of microorganisms to degrade various organic

397 compounds or pollutants [33,34], but this is the first time that the complex biofilm community  
398 has been used for polymer degradation under marine condition.

399 Finally, another originality of our study is that no other feasibility study exists ranging from the  
400 bio-based and biodegradable microparticle design to the evaluation of its fate at sea. Most of  
401 the biodegradation study so far were using commercially plastics, mainly in the form of film  
402 [17,15] and to a less extent in powder [35,36], rarely in the form of microbeads. The successful  
403 use of cryogrinding and solvent emulsion-evaporation techniques showed that such approaches  
404 may be useful to conduct further studies on other polymer types or for the production of  
405 biodegradable substitutes to replace conventional microbeads [37–39].

#### 406 *4.2. Successive steps of biodegradability by complex marine bacterial communities for bio-* 407 *based and petroleum-based polymers*

408 Our multidisciplinary approach provides the first evidence of the successive steps involved in  
409 polymer biodegradation in seawater. These steps have been theoretically proposed by several  
410 authors [18], but never evidenced before by a complementary set of techniques on the same  
411 samples and by involving a complex mature biofilm grown under natural seawater conditions.  
412 Here, we have highlighted a group of polymers including PCL, PHBV, apricot and rice showing  
413 congruent signs of biodegradation, and another group made of PE, PMMA and PLA that gave  
414 no sign of biodegradation under our conditions. We prove here that PCL, PHBV, rice and  
415 apricot microbeads undergo a succession of physico-chemical transformation (as revealed by  
416 FTIR) that lead to the biofragmentation of the material (weight loss, molecular weight) resulting  
417 in the release of short length oligomers (mass spectrometry, <sup>1</sup>H NMR). Then, these

418 hydrosoluble compounds can be readily assimilated by the microorganisms ( $^1\text{H}$  NMR) and  
419 support the respiratory activity of the biofilm (BOD). This sequential process does not occur  
420 for PE, PMMA and PLA microbeads, as no or slight oxidation can be initiated in our  
421 experimental time frame.

422 Our results are congruent with other studies describing PHBV and PCL as biodegradable  
423 polymers under natural conditions in soil [40,41]. Other studies found no sign of biodegradation  
424 for PLA in freshwater [42]. PCL films were also found to present less biodegradability potential  
425 in freshwater than under marine condition, probably due to very different bacterial communities  
426 inhabiting these ecosystems [43].

427 Interestingly, we found that one of the bio-based polymers (PLA) did not fall into the group of  
428 biodegradable polymers and contrariwise, one of the petroleum-based polymers (PCL) showed  
429 clear signs of biodegradation. These counterintuitive results underline again that bio-based  
430 polymers does not necessary mean environmental degradable polymers, and inversely  
431 petroleum-based polymer does not necessary mean non-biodegradable products, as already  
432 mentioned elsewhere [16].

### 433 *4.3. A set of congruent results for each polymer type*

434 *4.3.1. Biodegradation of PCL.* We found that the weight loss for PCL was 17.8% under our  
435 experimental conditions. Besides, *Lu et al.* [44] found a weight loss around 18% for 2 month  
436 period in natural seawater environment. Oxygen consumption confirmed the presence of  
437 biodegradation, while an unusual behavior was observed for PCL, with a steep decrease of  
438 oxygen concentration after 3 days (Fig. 3), which could be attributed to the modification of



439 bacteria community and/or the availability of the dissolved oligomers. The release of oligomers  
440 in abiotic condition proved here that a slight hydrolysis of PCL occurred in seawater without  
441 any other biological attack, which did not be realized by some other tests [42]. There was no  
442 signal captured by  $^1\text{H}$  NMR for PCL in biotic condition, indicating the rapid utilization of the  
443 oligomers released by abiotic processes and the presence of bioassimilation activity, which is  
444 consistent with other evidence of biotic degradation of PCL in seawater by others [43, 45,46].  
445 Finally, the increase of crystallinity index from the FTIR results together with the reduction of  
446 molecular weight confirmed the degradability of PCL in seawater.

447 *4.3.2. Biodegradability of PHBV.* In our conditions, PHBV showed a weight loss of 17% (2  
448 month and a biodegradability of 4.1% (10 days). The weight loss was also reported for PHBV  
449 with different degree of degradation. For instances, a study performed in natural seawater from  
450 coastal France showed relatively lower value, with the 30% weight loss for a period of 6-months  
451 for plastic film[35], the observed differences could result from the material size and shape.  
452 During this study,  $^1\text{H}$  NMR assay showed oligomer release only in biotic but not in abiotic  
453 condition, thus making evidence of an efficient biofragmentation of PHBV by the marine  
454 bacteria which was under or beyond their capability to assimilate the large amount of oligomer  
455 produced. Thus, our research showed the stability of PHBV by the analysis of the released of  
456 the oligomers ( $^1\text{H}$  NMR). Abiotic degradation has not been observed under other seawater  
457 conditions within 6 months incubation at 25°C by weight loss assay [47]. In addition, negligible  
458 abiotic degradation (weight loss) was also found after 21 days under composting conditions at  
459 60 °C [48]. Therefore, together with our results, PHBV could be described as quite resistant to

460 moisture or abiotic attack. We also found an increase of crystallinity index that showed the  
461 preference of the biofilm to attack amorphous region of the polymer, further proving previous  
462 described results [49–51].

463 *4.3.3. Biodegradation of apricot and rice.* It is noteworthy that it was the first study which  
464 performed the degradation test on the two natural materials in the marine environment. Both  
465 the results of oxygen consumption and <sup>1</sup>H NMR confirmed its degradability in marine  
466 conditions. Under abiotic condition, carbohydrates such as glucose was gradually released from  
467 rice and apricot, and the released soluble carbohydrate fraction was readily assimilated by the  
468 microorganisms. Previous studies on rice straw also showed a high biodegradation rate in the  
469 soil and freshwater environments [52,53].

470 *4.3.4. Other polymers.* No sign of biodegradation was detected by our set of complementary  
471 techniques for PE, PMMA and PLA. Beside their chemical structures and properties (carbon-  
472 carbon bonds and/or crystallinity), the environmental conditions (relatively low temperature,  
473 salinity, low nutrients and others) may also be a cause of limited degradation in seawater [54,55].  
474 The lack of obvious oxidation of these polymers limit their fragmentation, the production of  
475 intermediate products (< 1500 Da) and thus their assimilation by microbes in marine  
476 environment [18,56,57].

477 Our multidisciplinary techniques gave congruent results for the polymer biodegradability,  
478 except for the aggregation problem from granulometry results probably due to the formation of  
479 extracellular polymeric substances after the bacterial colonization [58]. It has been noticed that  
480 PCL, rice and apricot could gradually release the oligomers, thus the weight loss measurement

481 of the three polymers could be overestimated. With the complementary techniques, such as  
482 oxygen consumption and  $^1\text{H}$  NMR results, etc. the three materials showed biodegradable in the  
483 marine environment.

484

## 485 **5. Conclusions**

486 An increasing number of countries have implemented laws to restrict the production of  
487 microbeads over the world. We present here that, out of the seven materials, microbeads made  
488 of PHBV could be good candidate for substitution of conventional microplastics classically  
489 made of PE or PMMA, since the material is quite resistant to hydrolysis and susceptible to  
490 microbial degradation, as proved from this study.

491 Our study presented several novelties regarding its experimental design but also by the various  
492 and complementary technologies used. We advise here that not only respirometry tests  
493 classically used in standard tests, but rather a multidisciplinary approach reflecting the 4 steps  
494 of biodegradation should be considered for further studies dealing with the biodegradability of  
495 polymers in the environment. It includes other banned single-used plastics such as plastic bags,  
496 straws, disposable dishes, cotton swabs (such as Q-tips) and coffee stirrers.

497 We also underlined that clear distinction should be made with bio-based polymers that may not  
498 be biodegradable (such as PLA), when less sustainable substitute materials showed clear signs  
499 of biodegradation at sea (such as the petroleum-based PCL). Finally, further studies are needed  
500 to evaluate the toxicity of the polymers and their degradation products, as well as the release of  
501 additives generally supplemented by manufacturers in the microplastic composition.

502 ***Declaration of Competing Interest***

503 The authors declare that they have no known competing financial interests or personal  
504 relationships that could have appeared to influence the work reported in this paper.

505

506 ***Acknowledgements.*** *This project was founded by the Ministry of ecological and solidarity*  
507 *transition of the French republic (project MICROBEADS). This work was also supported by*  
508 *two European project JRA-ASSEMBLE+, and AtlantEco. This work is part of the PhD thesis*  
509 *of Jingguang Cheng supported by the China Scholarship Council (CSC; NO, 201706330086).*  
510 *We are grateful to Guigui PA, VF, JS, JP for insightful comments on the manuscript.*

511

512 ***Appendix A. Supplementary data:***

513 Supplementary methods including polymer preparation with solvent emulsion-evaporation  
514 method, minimum medium composition and detailed information on molecular weight and <sup>1</sup>H  
515 NMR measurements; supplementary results including spectra and spectra descriptions for FTIR  
516 results on the seven polymers, for <sup>1</sup>H NMR spectra and ESI-MS mass spectra of PCL, rice,  
517 apricot and PHBV.

518

519 **References:**

- 520 [1] D.K.A. Barnes, F. Galgani, R.C. Thompson, M. Barlaz, Accumulation and fragmentation  
521 of plastic debris in global environments, *Philosophical Transactions of the Royal Society*  
522 *B: Biological Sciences*. 364 (2009) 1985–1998.
- 523 [2] L. Weiss, W. Ludwig, S. Heussner, M. Canals, J.F. Ghiglione, C. Estournel, M. Constant,  
524 P. Kerhervé (2021) The missing ocean plastic sink: gone with the rivers. *Science*. 73 (2021)  
525 107-111.
- 526 [3] J.G.B. Derraik, The pollution of the marine environment by plastic debris : a review,  
527 *Marine Pollution Bulletin*. 44 (2002) 842–852.
- 528 [4] B. Harry, E. Cecilia, Origins & biological accumulation of MPs in fur seals from  
529 Macquarie Island, *Royal Swedish Academy of Sciences*. (2003) 380–384.
- 530 [5] M. Poulain, M.J. Mercier, L. Brach, M. Martignac, C. Routaboul, E. Perez, M.C. Desjean,  
531 A. Ter Halle, Small Microplastics As a Main Contributor to Plastic Mass Balance in the  
532 North Atlantic Subtropical Gyre, *Environmental Science and Technology*. 53 (2019)  
533 1157–1164.
- 534 [6] D. Xanthos, T.R. Walker, International policies to reduce plastic marine pollution from  
535 single-use plastics (plastic bags and microbeads): A review, *Marine Pollution Bulletin*.  
536 118 (2017) 17–26.
- 537 [7] C.M. Rochman, S.M. Kross, J.B. Armstrong, M.T. Bogan, E.S. Darling, S.J. Green, A.R.  
538 Smyth, D. Veríssimo, Scientific Evidence Supports a Ban on Microbeads, *Environmental*  
539 *Science and Technology*. 49 (2015) 10759–10761.
- 540 [8] L.S. Fendall, M.A. Sewell, Contributing to marine pollution by washing your face:  
541 Microplastics in facial cleansers, *Marine Pollution Bulletin*. 58 (2009) 1225–1228.
- 542 [9] P. Bhattacharya, A review on the impacts of microplastic beads used in cosmetics, *Acta*  
543 *Biomedica Scientia*. 3 (2016) 47–52.
- 544 [10] P.K. Cheung, L. Fok, Characterisation of plastic microbeads in facial scrubs and their  
545 estimated emissions in Mainland China, *Water Research*. 122 (2017) 53–61.
- 546 [11] N. Ding, D. An, X. Yin, Y. Sun, Detection and evaluation of microbeads and other  
547 microplastics in wastewater treatment plant samples, *Environmental Science and*  
548 *Pollution Research*. 27 (2020) 15878–15887.
- 549 [12] D. Wardrop, C. Bott, C. Criddle, R. Hale, J. McDevitt, M. Morse, C.M. Rochman,  
550 Technical Review of Microbeads Microplastics in the Chesapeake Bay, STAC Publication  
551 16-002. (2016) 1–21.
- 552 [13] J.P. Eubeler, S. Zok, M. Bernhard, T.P. Knepper, Environmental biodegradation of  
553 synthetic polymers I. Test methodologies and procedures, *TrAC Trends in Analytical*  
554 *Chemistry*. 28 (2009) 1057–1072.
- 555 [14] S.M. Emadian, T.T. Onay, B. Demirel, Biodegradation of bioplastics in natural  
556 environments, *Waste Management*. 59 (2017) 526–536.
- 557 [15] L.S. Dilkes-Hoffman, P.A. Lant, B. Laycock, S. Pratt, The rate of biodegradation of PHA  
558 bioplastics in the marine environment: A meta-study, *Marine Pollution Bulletin*. 142  
559 (2019) 15–24.

- 560 [16] A. Krzan, S. Hemjinda, S. Miertus, A. Corti, E. Chiellini, Standardization and certification  
561 in the area of environmentally degradable plastics, *Polymer Degradation and Stability*. 91  
562 (2006) 2819–2833.
- 563 [17] J.P. Harrison, C. Boardman, K. O’Callaghan, A.M. Delort, J. Song, Biodegradability  
564 standards for carrier bags and plastic films in aquatic environments: A critical review,  
565 *Royal Society Open Science*. 5 (2018).
- 566 [18] J. Jacquin, J. Cheng, C. Odobel, C. Pandin, P. Conan, M. Pujo-Pay, V. Barbe, A.L.  
567 Meistertzheim, J.F. Ghiglione, Microbial ecotoxicology of marine plastic debris: A review  
568 on colonization and biodegradation by the “plastisphere,” *Frontiers in Microbiology*. 10  
569 (2019) 1–16.
- 570 [19] N. Lucas, C. Bienaime, C. Belloy, M. Queneudec, F. Silvestre, J.E. Nava-Saucedo,  
571 *Polymer biodegradation: Mechanisms and estimation techniques - A review*,  
572 *Chemosphere*. 73 (2008) 429–442.
- 573 [20] A.A. Shah, F. Hasan, A. Hameed, S. Ahmed, Biological degradation of plastics: A  
574 comprehensive review, *Biotechnology Advances*. 26 (2008) 246–265.
- 575 [21] C. Dussud, C. Hudec, M. George, P. Fabre, P. Higgs, S. Bruzaud, A.-M. Delort, B.  
576 Eyheraguibel, A.-L. Meistertzheim, J. Jacquin, J. Cheng, N. Callac, C. Odobel, S.  
577 Rabouille, J.-F. Ghiglione, Colonization of Non-biodegradable and Biodegradable  
578 Plastics by Marine Microorganisms, *Front. Microbiol.* 9 (2018).
- 579 [22] J. Cheng, J. Jacquin, P. Conan, M. Pujo-Pay, V. Barbe, M. George, P. Fabre, S. Bruzaud,  
580 A. Ter Halle, A.-L. Meistertzheim, J.-F. Ghiglione, Relative Influence of Plastic Debris  
581 Size and Shape, Chemical Composition and Phytoplankton-Bacteria Interactions in  
582 Driving Seawater Plastisphere Abundance, Diversity and Activity, *Front. Microbiol.* 11  
583 (2021) 610231.
- 584 [23] K. Kasuya, K. Takagi, S. Ishiwatari, Y. Yoshida, Y. Doi, Biodegradabilities of various  
585 aliphatic polyesters in natural waters, *Polymer Degradation and Stability*. 59 (1998) 327–  
586 332.
- 587 [24] E.D. Hirtleman, Modeling of multiple scattering effects in Fraunhofer diffraction particle  
588 size analysis, Springer Boston, 1988.
- 589 [25] C. Dussud, J.-F. Ghiglione, Bacterial degradation of synthetic plastics, In *CIESM*  
590 *Workshop Monogr.* 46 (2014) 49–54.
- 591 [26] W. Guo, J. Tao, C. Yang, Q. Zhao, C. Song, S. Wang, The rapid evaluation of material  
592 biodegradability using an improved ISO 14852 method with a microbial community,  
593 *Polymer Testing*. 29 (2010) 832–839.
- 594 [27] ASTM D6691-09, 2009 ASTM D6691-09 Standard test method for determining aerobic  
595 biodegradation of plastic materials in the marine environment by a defined microbial  
596 consortium or natural sea water inoculum, 2009.
- 597 [28] E.R. Zettler, T.J. Mincer, L.A. Amaral-Zettler, Life in the “plastisphere”: Microbial  
598 communities on plastic marine debris, *Environmental Science and Technology*. 47 (2013)  
599 7137–7146.
- 600 [29] C. Dussud, A.L. Meistertzheim, P. Conan, M. Pujo-Pay, M. George, P. Fabre, J. Coudane,  
601 P. Higgs, A. Elineau, M.L. Pedrotti, G. Gorsky, J.F. Ghiglione, Evidence of niche

- 602 partitioning among bacteria living on plastics, organic particles and surrounding seawaters,  
603 Environmental Pollution. 236 (2018) 807–816.
- 604 [30] ISO 18830, Determination of aerobic biodegradation of non- coating plastic materials in a  
605 seawater/sandy sediment interface., 2016.
- 606 [31] ISO 19679, Plastics. Determination of aerobic biodegradation of non-floating plastic  
607 materials in a seawater/sediment interface. Method by analysis of evolved carbon dioxide,  
608 2016.
- 609 [32] B. Stenberg, M. Johansson, M. Pell, K. Dahl-Svensson, J. Stenstroem, L. Torstensson,  
610 Microbial biomass and activities in soil as affected by frozen and cold storage, Soil  
611 Biology and Biochemistry. 30 (1998) 393–402.
- 612 [33] B. De Wilde, Biodegradation testing protocols, ACS Symposium Series. 1114 (2012) 33–  
613 43.
- 614 [34] J.P. Rodríguez, S. Achleitner, M. Möderl, W. Rauch, Č. Maksimović, N. McIntyre, M.A.  
615 Díaz-Granados, M.S. Rodríguez, Sediment and pollutant load modelling using an  
616 integrated urban drainage modelling toolbox: An application of City Drain, Water Science  
617 and Technology. 61 (2010) 2273–2282.
- 618 [35] M. Deroiné, G. César, A. Le Duigou, P. Davies, S. Bruzard, Natural Degradation and  
619 Biodegradation of Poly(3-Hydroxybutyrate-co-3-Hydroxyvalerate) in Liquid and Solid  
620 Marine Environments, Journal of Polymers and the Environment. 23 (2015) 493–505.
- 621 [36] H. Sashiwa, R. Fukuda, T. Okura, S. Sato, A. Nakayama, Microbial degradation behavior  
622 in seawater of polyester blends containing poly(3-hydroxybutyrate-co-3-  
623 hydroxyhexanoate) (PHBHHx), Marine Drugs. 16 (2018) 1–11.
- 624 [37] J. Coombs O'Brien, L. Torrente-Murciano, D. Mattia, J.L. Scott, Continuous Production of  
625 Cellulose Microbeads via Membrane Emulsification, ACS Sustainable Chemistry and  
626 Engineering. 5 (2017) 5931–5939.
- 627 [38] C.A. King, J.L. Shamshina, O. Zavgorodnya, T. Cutfield, L.E. Block, R.D. Rogers, Porous  
628 Chitin Microbeads for More Sustainable Cosmetics †, ACS Sustainable Chemistry and  
629 Engineering. 5 (2017) 11660–11667.
- 630 [39] H.C. Nam, W.H. Park, Aliphatic Polyester-Based Biodegradable Microbeads for  
631 Sustainable Cosmetics, ACS Biomaterials Science and Engineering. 6 (2020) 2440–2449.
- 632 [40] Y. Tokiwa, B.P. Calabria, Biodegradability and biodegradation of polyesters, Journal of  
633 Polymers and the Environment. 15 (2007) 259–267.
- 634 [41] A. Hoshino, H. Sawada, M. Yokota, M. Tsuji, K. Fukuda, M. Kimura, Influence of  
635 weather conditions and soil properties on degradation of biodegradable plastics in soil,  
636 Soil Science and Plant Nutrition. 47 (2001) 35–43.
- 637 [42] A.R. Bagheri, C. Laforsch, A. Greiner, S. Agarwal, Fate of So-Called Biodegradable  
638 Polymers in Seawater and Freshwater, Global Challenges. 1 (2017) 1700048.
- 639 [43] A. Heimowska, M. Morawska, A. Bocho-Janiszewska, Biodegradation of poly( $\epsilon$ -  
640 caprolactone) in natural water environments, Polish Journal of Chemical Technology. 19  
641 (2017) 120–126.
- 642 [44] B. Lu, G.X. Wang, D. Huang, Z.L. Ren, X.W. Wang, P.L. Wang, Z.C. Zhen, W. Zhang,  
643 J.H. Ji, Comparison of PCL degradation in different aquatic environments: Effects of  
644 bacteria and inorganic salts, Polymer Degradation and Stability. 150 (2018) 133–139.

- 645 [45] K. Leja, G. Lewandowicz, Polymer biodegradation and biodegradable polymers - A  
646 review, *Polish Journal of Environmental Studies*. 19 (2010) 255–266.
- 647 [46] M. Suzuki, Y. Tachibana, K. Oba, R. Takizawa, K. ichi Kasuya, Microbial degradation of  
648 poly( $\epsilon$ -caprolactone) in a coastal environment, *Polymer Degradation and Stability*. 149  
649 (2018) 1–8.
- 650 [47] M. Deroiné, A. Le Duigou, Y.M. Corre, P.Y. Le Gac, P. Davies, G. César, S. Bruzard,  
651 Seawater accelerated ageing of poly(3-hydroxybutyrate-co-3-hydroxyvalerate), *Polymer*  
652 *Degradation and Stability*. 105 (2014) 237–247.
- 653 [48] C. Eldsäter, A.C. Albertsson, S. Karlsson, Impact of degradation mechanisms on poly(3-  
654 hydroxybutyrate-co-3-hydroxyvalerate) during composting, *Acta Polymerica*. 48 (1997)  
655 478–483.
- 656 [49] A. Spyros, R. Kimmich, B.H. Briese, D. Jendrossek, 1H NMR imaging study of enzymatic  
657 degradation in poly(3-hydroxybutyrate) and poly(3-hydroxybutyrate-co-3-  
658 hydroxyvalerate). Evidence for preferential degradation of the amorphous phase by PHB  
659 depolymerase B from *Pseudomonas lemoignei*, *Macromolecules*. 30 (1997) 8218–8225.
- 660 [50] M. Hakkarainen, Aliphatic polyesters: abiotic and biotic degradation and degradation  
661 products, in: *Degradable Aliphatic Polyesters*, Springer, Berlin, Heidelberg, 2002: pp.  
662 113–138.
- 663 [51] K. Iggui, N. Le Moigne, M. Kaci, S. Cambe, J.R. Degorce-Dumas, A. Bergeret, A  
664 biodegradation study of poly(3-hydroxybutyrate-co-3-hydroxyvalerate)/organoclay  
665 nanocomposites in various environmental conditions, *Polymer Degradation and Stability*.  
666 119 (2015) 77–86.
- 667 [52] P. Sain, Decomposition of wild rice (*Zizania aquatica*) straw in two natural lakes of  
668 northwestern Ontario., *Canadian Journal of Botany*. 62 (1984) 1352–1356.
- 669 [53] F. Bilo, S. Pandini, L. Sartore, L.E. Depero, G. Gargiulo, A. Bonassi, S. Federici, E.  
670 Bontempi, A sustainable bioplastic obtained from rice straw, *Journal of Cleaner*  
671 *Production*. 200 (2018) 357–368.
- 672 [54] M. Agarwal, K.W. Koelling, J.C. Jeffrey, Characterization of the degradation of polylactic  
673 acid polymer in a solid substrate environment, *Biotechnology Progress*. (1998) 517–526.
- 674 [55] K. Min, J.D. Cuiffi, R.T. Mathers, Ranking environmental degradation trends of plastic  
675 marine debris based on physical properties and molecular structure, *Nature*  
676 *Communications*. 11 (2020).
- 677 [56] A.L. Andrady, Microplastics in the marine environment, *Marine Pollution Bulletin*. 62  
678 (2011) 1596–1605.
- 679 [57] B. Eyheraguibel, M. Traikia, S. Fontanella, M. Sancelme, S. Bonhomme, D. Fromageot,  
680 J. Lemaire, G. Lauranson, J. Lacoste, A.M. Delort, Characterization of oxidized oligomers  
681 from polyethylene films by mass spectrometry and NMR spectroscopy before and after  
682 biodegradation by a *Rhodococcus rhodochrous* strain, *Chemosphere*. 184 (2017) 366–374.
- 683 [58] X. Wang, N. Bolan, D.C.W. Tsang, B. Sarkar, L. Bradney, Y. Li, A review of  
684 microplastics aggregation in aquatic environment: Influence factors, analytical methods,  
685 and environmental implications, *Journal of Hazardous Materials*. 402 (2021) 123496.