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

3

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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, ¹H 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 ¹H 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.

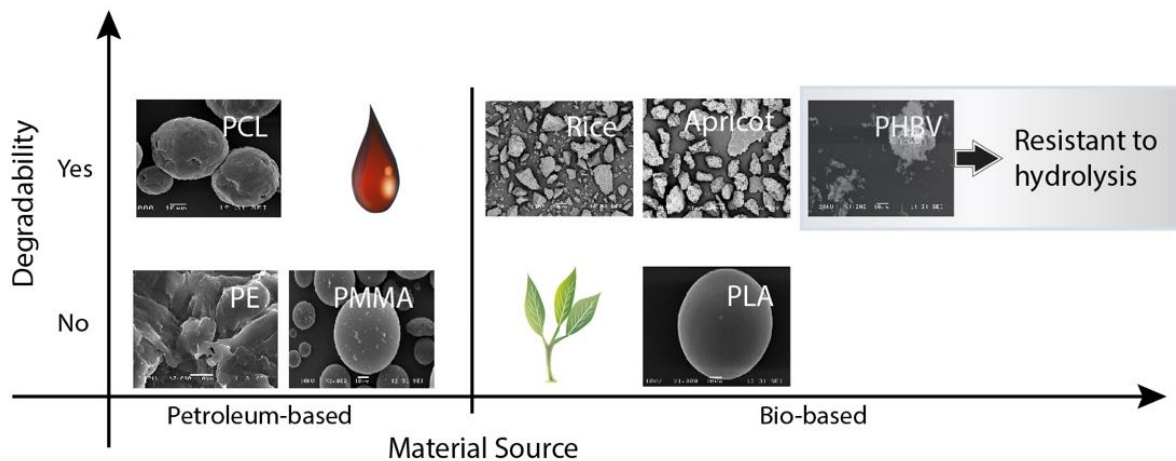
47

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 μm –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 μm 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 μm 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 (^1H 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 μm .

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*

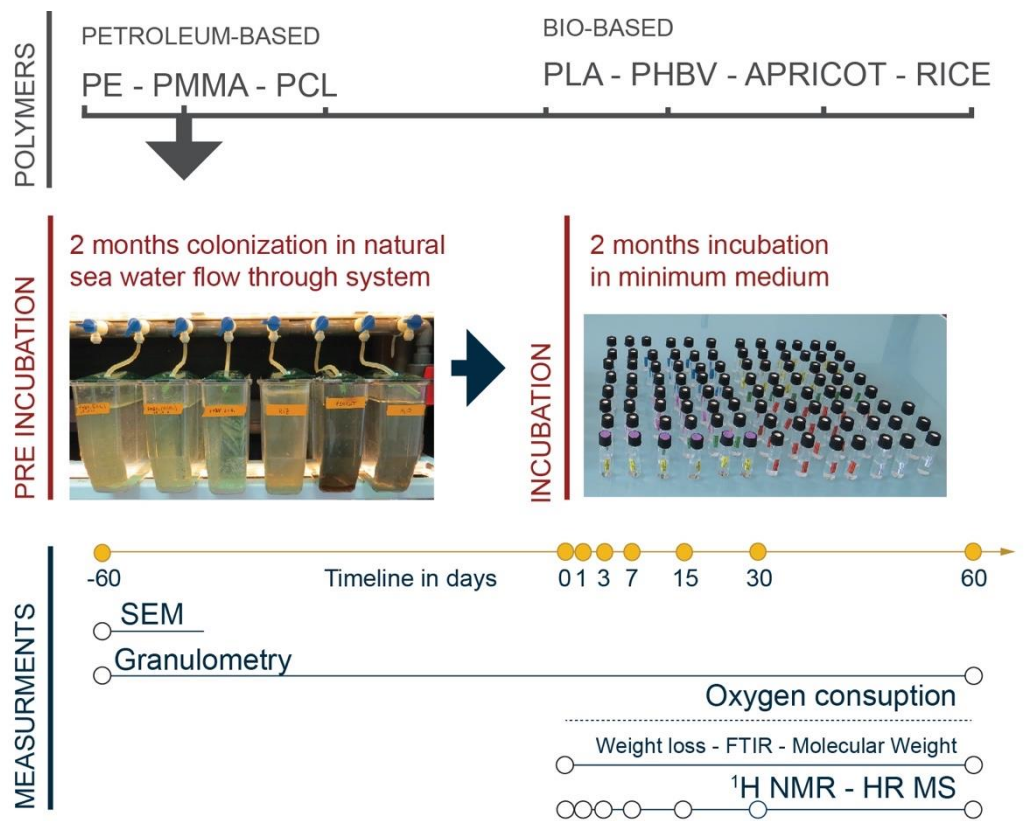
128 Microparticle surface morphologies were observed at the step of microbeads production by
129 using a Jeol JSM-6031 scanning electron microscope (SEM). Prior to any observation, the
130 fracture surfaces were coated with a thin gold layer by means of a Polaron sputtering apparatus.

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 ml.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 µm. 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°C and 25.3°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⁻¹) 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⁻¹ and 200 µL of sample solutions were
198 injected and eluted in tetrahydrofuran using a flow rate of 1 mL min⁻¹ 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⁻¹). 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⁻¹, 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⁻¹ to 400 cm⁻¹, with a resolution of 4
215 cm⁻¹. 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 ¹H NMR spectroscopy*

220 ¹H 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 TSPd₄ (sodium tetra deuterated tri methylsilyl propionate, Eurisotop) in deuterated
226 water (D₂O, Eurisotop). D₂O signal was used for locking and shimming while TSPd₄
227 constituted a reference for chemical shifts (0 ppm) and quantification. ¹H NMR spectra were
228 recorded on a Bruker Avance 500 MHz spectrometer, equipped with a 5 mm inverse-triple
229 tuned (TXI) ¹H/¹³C/¹⁵N 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 TSPd₄ 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.

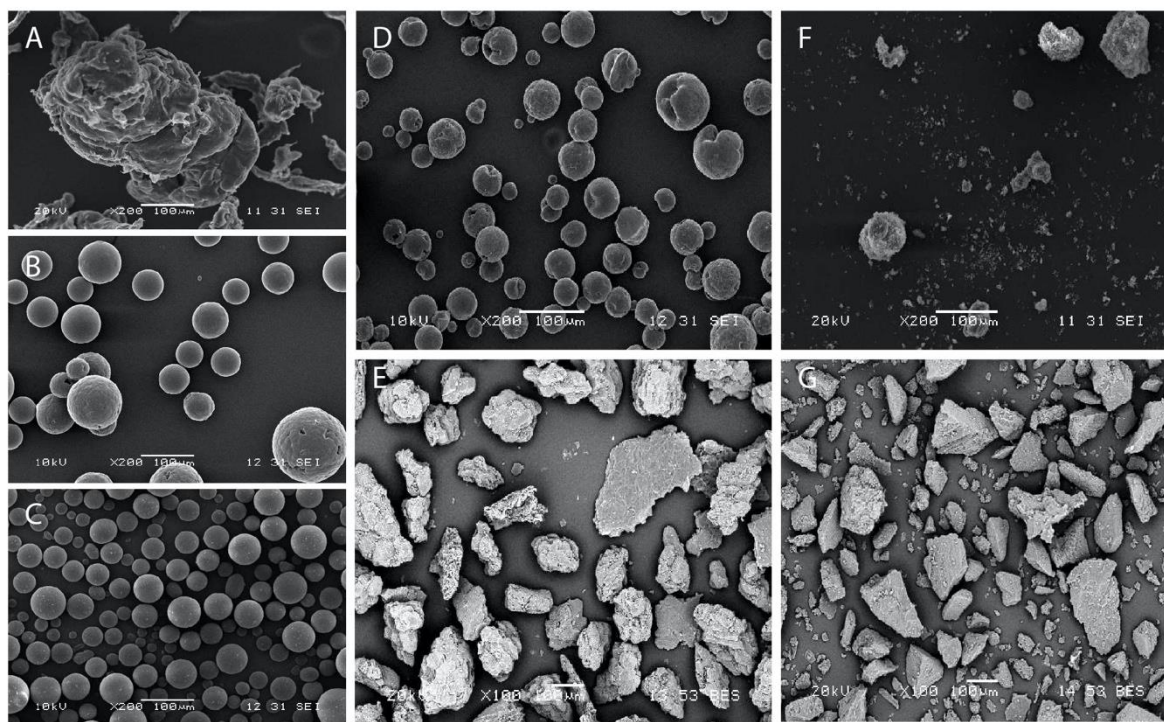
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245 **3. Results**

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 μm
252 (median diameter between 88 and 126 μm depending on the types of bioplastics) (Table 1). The
253 PE microparticles were the largest with a median diameter around 280 μm . On the other hand,
254 those made of PMMA were the smallest since their median diameter is around 60 μm . 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 μm , 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.

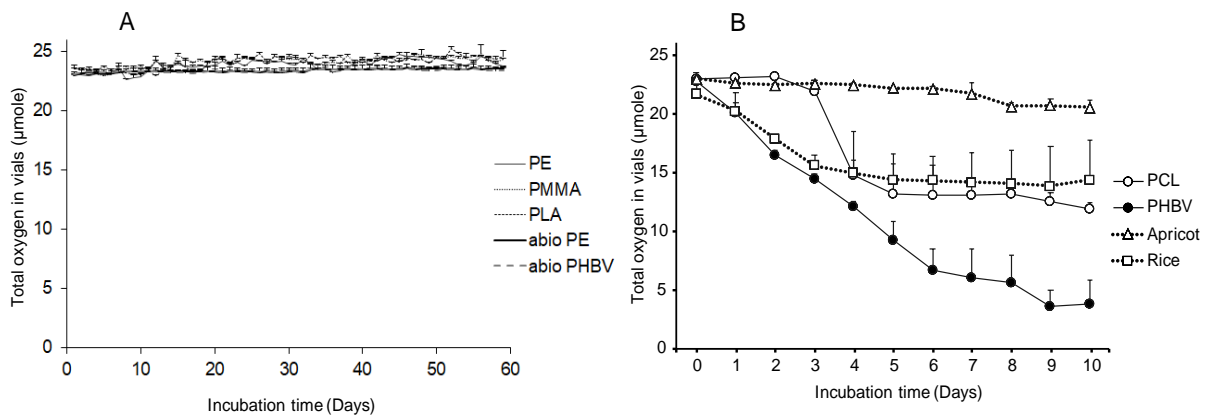


264
 265 **Fig. 2** Scanning electron microscopy for the microbeads made of seven polymer types: (A) PE,
 266 (B) PLA, (C) PMMA, (D) PCL, (E) ground apricot kernels, (F) PHBV, (G) ground rice particles.
 267 The scale bar in all panels are 100 μm .

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 μmole 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 μmole 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 μmole) 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		¹ 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 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 cm^{-1} indicate that
310 PE microbeads did not undergo photodegradation.

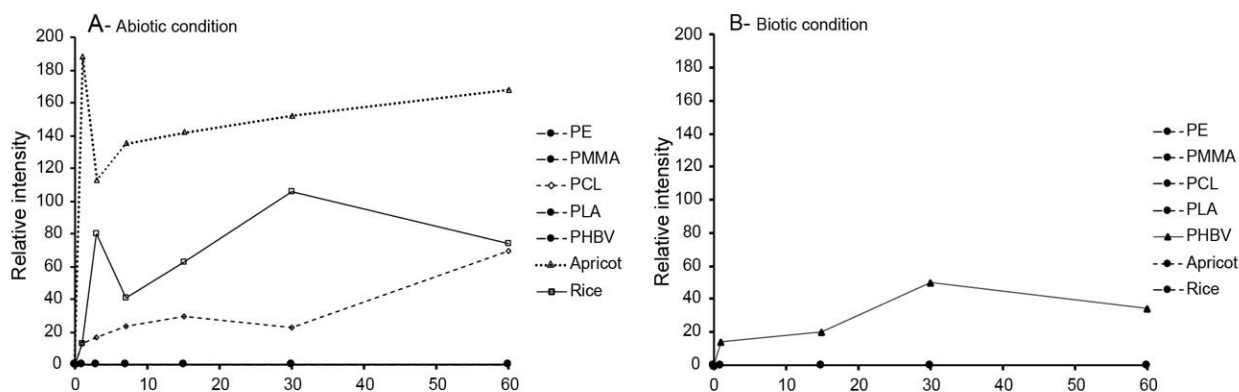
311 The FTIR spectra of PCL microbeads showed a decrease of carbonyl index and a concomitant
312 increase of crystallinity index (Table1, Fig. S5). Such pattern is typical of ester bonds cleavage
313 in amorphous region of the polymer chains and confirms the reduction of the molecular weight
314 of the 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, ^1H 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 ¹H NMR spectra expressed in relative intensity of TSPD₄
 343 internal standard.
 344

345 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 O₂ demand or CO₂ 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, ¹H 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, ¹H
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, ¹H NMR). Then, these

418 hydrosoluble compounds can be readily assimilated by the microorganisms (^1H 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 ^1H 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, ^1H 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 (^1H 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 ¹H 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 ^1H 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
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505

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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 ¹H
515 NMR measurements; supplementary results including spectra and spectra descriptions for FTIR
516 results on the seven polymers, for ¹H NMR spectra and ESI-MS mass spectra of PCL, rice,
517 apricot and PHBV.

518

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