Degradation of recalcitrant polyurethane and xenobiotic additives by a selected landfill microbial community and its biodegradative potential revealed by proximity ligation-based metagenomic analysis

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Abstract

Polyurethanes (PU) are the sixth more produced plastics with around 19-million tons/year, but since they are not recyclable they are burnt or landfilled, generating ecological damage. To elucidate the mechanisms that landfill microbial communities perform to attack recalcitrant PU plastic, we studied the BP8 community selected by its capability to grow in a water PU dispersion (WPUD) that contains a polyether-polyurethane-acrylate (PE-PU-A) copolymer and xenobiotic additives (N-methyl 2-pyrrolidone, isopropanol and glycol ethers), and performed a proximity ligation-based metagenomic analysis for revealing the community structure and potential biodegradative capacity. Additives were consumed early whereas the copolymer was cleaved throughout the 25-days incubation. BP8 metagenomic deconvolution reconstructed five genomes, three of them from novel species. Genes encoding enzymes for additives biodegradation were predicted. The chemical and physical analysis of the biodegradation process, and the identified biodegradation products show that BP8 cleaves esters, aromatic urethanes, C-C and ether groups by hydrolytic and oxidative mechanisms. The metagenomic analysis allowed to predicting comprehensive metabolic pathways and enzymes that explain the observed PU biodegradation. This is the first study revealing the metabolic potential of a landfill microbial community that thrives within a WPUD system and shows potential for bioremediation of polyurethane- and xenobiotic additives-contaminated sites.

Introduction

Plastic pollution represents a pervasive anthropogenic threat for the survival of natural ecosystems. Worldwide, plastics have become so abundant that they have been proposed as geological markers for the Anthropocene era [1]. In 2017, 348 million tons of plastics were manufactured [2] and their production keeps increasing. Polyurethanes (PU) are versatile plastics produced as thermoplastics, thermosets, coatings, adhesives, sealants and elastomers that are incorporated into our daily life in building insulation, refrigerators and freezers, furniture and bedding, footwear, automotive, coatings, adhesives, and others. PU has been ranked as the sixth most used polymer worldwide with a production of 18 million tons in 2016 [3,4]. The extensive utilization of PU generates wastes that are mainly disposed in municipal landfills where, because of its structural complexity will remain as polymeric structures for decades, or are burnt generating toxic substances that negatively impact human health and ecosystems [3]. Furthermore, some PU such as polyether (PE)-PU are more recalcitrant than others, and additionally, some polyurethane-based liquid formulations contain additives that include secondary alcohols and glycol ethers that function as solvents or coalescing agents. Glycol ethers enter the environment in substantial quantities, are toxic for many microbial species [5-7] and represent a potential hazard for human health [8].

Over the last three decades, several research groups have isolated microorganisms with capacity to attack PU [9-15] and degrade xenobiotic additives [7, 9, 16, 17], and the abilities from several fungal and bacterial communities have been assessed in compost, soil, or liquid cultures [18-21] and in different activated sludges [22-25]. However, PU biodegradation is still a challenge for environmental and biological disciplines and little is known about structure or potential degradative enzymatic

68 pathways of microbial communities capable of PU biodegradation. Metagenomics
69 provides access to the structure and genetic potential of microbial communities, helping
70 to understand the ecophysiological relationships governing the dynamics of their
71 populations in the environment. Recently, a new approach has been developed that
72 allows the reconstruction of individual genomes of microbial species using physical
73 interactions between sequences within cells [26]. This approach involves Hi-C proximity
74 ligation and yields direct evidence of sequences co-occurrence within a genome, which is
75 used for *de novo* assembly, identification of complete and novel genomes [27] and for
76 testing functional and phylogenetic hypotheses, surpassing other methods for clustering
77 contigs by taxonomic origins [28-30].

78 To characterize the biodegradation process of the recalcitrant plastic PE-PU by
79 microbial communities, we adopted the commercial water PU dispersion PolyLack®
80 (Sayer Lack, México) that contains a proprietary aromatic polyether-polyurethane-
81 acrylate (PE-PU-A) copolymer and the xenobiotic additives N-methyl 2-pyrrolidone
82 (NMP), isopropanol (IP) 2-butoxyethanol (2-BE), dipropyleneglycol butyl ether (DPGB),
83 and dipropyleneglycol methyl ether (DPGM). In this work, we provide comprehensive
84 chemical and physical evidences for the capacity of a selected landfill microbial
85 community to degrade an aromatic PE-PU-A copolymer and the aforementioned
86 xenobiotic additives, and analyze its structure and phenotypic potential by applying the
87 Hi-C proximity ligation technology. Based on these analyses, we identified a novel
88 microbial landscape that can deal with PE-PU-A and xenobiotics additives degradation
89 and proposed the putative metabolic pathways and genes that can account for these
90 capabilities. This is one of the few studies that combine physical and chemical analyses
91 with metagenomics to elucidate possible metabolic pathways involved in xenobiotics

92 biodegradation, and the first metagenomic analysis of a polyurethane-degrading enriched
93 landfill community. Understanding these pathways will help to design environmental
94 biotechnological strategies that contribute to mitigate plastics and xenobiotics pollution
95 and to achieve a better environmental quality.

96 **Materials and Methods**

97 **Microbiological techniques**

98 The BP8 community, studied in this work, was selected by inoculating deteriorated pieces
99 of PU foam collected at El Bordo Poniente municipal landfill, as previously described [21],
100 into a minimal medium (MM) [10] containing PolyLack® (0.3% v/v), as the sole carbon
101 source (MM-PolyLack). PolyLack® (Sayer Lack, Prod. Num. UB-0810, México) contains a
102 proprietary aromatic PE-PU-A copolymer (≤30% w/v), and the additives NMP (≤6% v/v),
103 2-BE (≤5% v/v), IP (≤3% v/v), DPGB (≤2% v/v), DPGM (≤1% v/v), and silica (≤3% w/v)
104 [31]. BP8 growth was quantified by dry weight. For that, flasks with MM-PolyLack (25 ml)
105 were inoculated with fresh cells (3 mg/ml) harvested from pre-cultures grown in MM-
106 PolyLack for 48 h at 37ºC, 220 rpm. At different incubation times, cells of one flask were
107 harvested, washed three times with phosphate buffer (50 mM, pH 7) and dried to
108 constant weight. Emulsification index (EI_{24}) and cell surface hydrophobicity (CSH) were
109 determined as described [32]. To observe cell-copolymer interactions, cells were fixed
110 with 3% (v/v) glutaraldehyde in phosphate buffer (100 mM, pH 7.4), at 4°C overnight,
111 washed three times, dehydrated with serial dilutions of ethanol, coated with gold and
112 analyzed in a JEOL JSM-5900-LV electron microscope.

113 **Analytical techniques**

114 Nuclear magnetic resonance spectra from dried PolyLack® dissolved in C_5D_5N (30 mg/ml)
115 were recorded at 298 K in a Bruker Avance 400 NMR (Billerica, MA, USA) at 400 MHz

116 (^{1}H). For most of the analytical techniques, cell-free supernatants (CFS) were obtained by
117 centrifugation at 17 211 x *g* for 10 min, filtered through Whatman grade 41 paper, and
118 dried at 37ºC for 5 days. Carbon content was determined in a Perkin Elmer Elemental
119 Analyzer (2400 CHN/O, Series II, Shelton, CT., USA). For gas chromatography coupled
120 to mass spectrometry (GC-MS) analysis, 25 ml CFS were extracted in 6 ml LC-18
121 cartridges (Supelco) at a flow rate of 2 ml/min, eluted with 2 ml chloroform:methanol (1:1,
122 v/v) and concentrated to 0.5 ml. Samples were injected in an Agilent GC system (7890B,
123 Santa Clara, CA, USA) using two 5%-phenyl-methylpolysiloxane columns (15 m x 250
124 μm x 0.25 μm). Oven was heated from 50ºC to 300ºC at 20ºC/min, Helium was used as
125 carrier gas at a flow rate of 1 ml/min. The injector temperature was 300ºC. For the
126 quantification of additives, pure compounds (Sigma-Aldrich Chemicals ≥98% purity) were
127 used for standard curves. Identification of biodegradation products was performed in an
128 Agilent Quadrupole Mass Analyzer (5977A MSD, Santa Clara, CA, USA) with electronic
129 ionization energy of 1459 EMV and the mass range scanned at 30-550 amu. Scan rate
130 was 2.8 spec/s. Data acquisition was performed with the Enhanced MassHunter software
131 system. Compounds were identified based on mass spectra compared to the NIST
132 database (2002 Library). Fourier transform infrared spectroscopy (FTIR) analyses were
133 performed in a Perkin Elmer spectrometer (Spectrum 400, Waltham, MA, USA) in
134 attenuated total reflection mode; 64 scans with a resolution of 4 cm^{-1} were averaged in
135 the range of 500-4000 cm^{-1}, processed and analyzed (Spectrum v6.3.5.0176 software).
136 Derivative thermogravimetric analyses (DTG) were performed in a Perkin Elmer
137 Thermogravimetric Analyzer (TGA 4000, Waltham, MA, USA) on 25 mg of dried CFS
138 samples heated 30-500ºC at a rate of 20ºC/min, under a N_2 atmosphere analyzing 10 mg
139 of dry CFS. Differential Scanning Calorimetry (DSC) was performed analyzing 10 mg of

140 dry CFS in a Q2000 (TA Instrument, New Castle, DE, USA) at a rate of 10°C/min, under
141 a nitrogen flow of 50 ml/min, at a 20-600ºC range. Gel Permeation Chromatography was
142 performed in a Waters 2695 Alliance Separation Module GPC (Milford, MA, USA) at 30°C
143 in tetrahydrofuran, using a universal column and a flow rate of 0.3 ml/min in CFS. All the
144 analyses were performed at least in three replicates. Controls were non-inoculated MM-
145 PolyLack supernatants similarly processed.

146 **HI-C proximity ligation based metagenomic analysis**

147 BP8 community cells cultured for 5 days in 50 ml of MM-PolyLack were harvested and
148 washed three times with phosphate buffer. Cells were resuspended in 20 ml TBS buffer
149 with 1% (v/v) formaldehyde (J.T. Baker) (crosslinker) and incubated 30 min with periodic
150 mixing. The crosslinker was quenched with glycine (0.2 g) (Bio-Rad) for 20 min,
151 thereafter cells were centrifuged, lyophilized and frozen at -20°C. For DNA extraction, cell
152 pellets (100 µl) were resuspended in 500 µl of TBS buffer containing 1% (v/v) Triton-X
153 100 and protease inhibitors [27]. DNA was digested with *Sau*3AI and *Mlu*CI and
154 biotinylated with DNA Polymerase I Klenow fragment (New England Biolabs) followed by
155 ligation reactions incubated for 4 h and then overnight at 70°C to reverse crosslinking.
156 The Hi-C DNA library was constructed by using the HyperPrep Kit (KAPA Biosystems,
157 Wilmington, MA, USA). A shotgun library was also prepared from DNA extracted from
158 non-crosslinked cells using Nextera DNA Sample Preparation Kit (Illumina). The two
159 libraries were paired-end sequenced using NextSeq 500 Illumina platform (Illumina, San
160 Diego, CA, USA). *De novo* metagenome draft assemblies from the raw reads were made
161 using the metaSPAdes assembler [33]. Hi-C reads were then aligned to the contigs
162 obtained from the shotgun library using the Burrows-Wheeler Alignment tool [34]
163 requiring exact read matching. The ProxiMeta algorithm was used to cluster the contigs

164 of the draft metagenome assembly into individual genomes [27]. Additionally, we
165 performed a community taxonomic profiling from shotgun reads using MetaPhlAn tool
166 [35]. Genome completeness, contamination, and other genomic characteristics were
167 evaluated using CheckM pipeline [36]. Phylogenetic analysis was performed using the
168 single copy molecular markers, DNA gyrase subunit A and ribosomal proteins L3 and S5,
169 selected from each deconvoluted genome and compared to homologous sequences from
170 GenBank. Alignments were cured with Gblocks tool
171 (http://phylogeny.lirmm.fr/phylo_cgi/one_task.cgi?task_type=gblocks) and WAG plus G
172 evolutionary models were selected using Smart Model Selection tool [37]. Finally,
173 phylogeny was inferred with the graphical interface of SeaView [38] using the Maximum
174 Likelihood method. To compare genetic relatedness, Average Nucleotide Identity (ANI)
175 between the genomes and the closest phylogenetic neighbors was calculated [39]. Open
176 reading frames were identified using MetaGeneMark [40]. KO assignments (KEGG
177 Orthology) and KEGG pathways reconstruction were performed with GhostKOALA server
178 and KEGG Mapper tool, respectively [41]. All the xenobiotic degradation pathways were
179 manually curated to only report those pathways in which most of the enzymes were
180 encoded in the BP8 metagenome.

181 **Data availability**

182 Genomes described in this manuscript were deposited to GenBank under Bioproject
183 Accession number: PRJNA488119.

184 **Results**

185 **Growth and interactions of BP8 cells with PolyLack®**

186 The BP8 community cultivated in MM-PolyLack for 25 days exhibited a biphasic growth
187 with a first phase, from 0-13 days, presenting a growth rate (2-4 days) of 0.008 h^{-1} and a

188 second phase, from 13-25 days, with a growth rate (13-20 days) of 0.005 h^{-1}. Biomass
189 increased from 0.32 to 2.9 mg/ml and consumed 50.3% of the carbon from the medium at
190 25 days (Figure 1a). EI_{24} initial value was 70%, it decreased to 24% at 20 days and
191 increased again to 70%. CSH started at 62% and decreased to 25% at the first growth
192 phase, thereafter it increased to 42% and remained constant until 20 days to increase to
193 67% at the end of the second phase (Figure 1b). SEM analysis at 10 days of cultivation
194 revealed multiple-sized (0.5-1.5 μm) rod-shaped cells aggregated and attached to
195 copolymer particles (Figure 1c). The changes in CSH and EI_{24}, reflect the complex cell-
196 substrate interactions involved in promoting substrate bioaccessibility and mineralization,
197 as has been observed in bacteria degrading other xenobiotics [42, 43].

198 **Chemical and physical changes in PolyLack® components generated by the BP8**
199 **community**

200 To characterize the biodegradative activity of the BP8 community on the PolyLack®
201 components, we performed different analytical techniques. GC-MS analysis of the CFS
202 revealed that BP8 metabolized the xenobiotic additives, NMP and IP at the first day of
203 cultivation, 2-BE at the fourth day and DPGM and DPGB were metabolized 85 and 73%
204 respectively at the first day, and remained constant until the end of the experiment
205 (Figure 2). Since the PE-PU-A copolymer structure is unknown, we proposed a
206 hypothetical structure (Figure S1), based on ^{1}H-NMR, the manufacturer's technical sheet
207 and in the most frequently used chemicals for the synthesis of this copolymer [44-46].
208 Since the first day of cultivation, complex and diverse chemical compounds such as
209 aromatics, nitrogen-containing, ethers, esters, aliphatics, alcohols and organic acids,
210 derived from the copolymer breakdown were observed. During the first 3 days (log
211 phase) the degradation products were low abundant, at 10 days (intermediate lag phase)

212 accumulation occurred, and during the second log phase their abundance decreased.
213 Notably, isocyanates (2,4-toluene diisocyanate (TDI) and methylene diphenyl
214 diisocyanate (MDI)) derivatives were aromatic amines observed maximal at the beginning
215 and diminished throughout the cultivation period (Figure 2, S2), suggesting that
216 metabolization of the urethane groups is being achieved. FTIR of CFS revealed changes
217 in PE-PU-A functional groups. The signal intensity of the C=O stretch from urethane and
218 acrylate carbonyl groups (1 730 cm^{-1}) increased at 5 days and lately decreased,
219 suggesting hydrolysis and subsequent catabolism of urethanes and acrylates. The signal
220 for aromatic groups C=C stretch (1 600 cm^{-1}) considerably decreased at 20 days, while
221 the signal for aromatic C-C stretch (1 380 cm^{-1}) showed variable intensities at different
222 days, and a new C-C signal for aromatics (1 415 cm^{-1}) appeared at 20 days, indicating
223 the cleavage of the aromatic rings. The urethane N-H bending plus C-N stretch signal (1
224 530 cm^{-1}) slightly decreased at 15 days and increased at the end of the cultivation time,
225 whereas urethane C-N stretching band (1 231 cm^{-1}) significantly increased, indicating
226 urethane attack. Signals associated with urethane C-O-C stretch (1 086 cm^{-1}, 1 049 cm^{-1})
227 and C-O-C symmetric stretch (977 cm^{-1}) decreased during the cultivation period,
228 indicating microbial activity on the ether groups. The signal for the acrylate's vinyl group
229 C=C-H out of plane (850 cm^{-1}) decreased at 20 days, indicating the cleavage of the
230 acrylate component. Also, the aliphatic chain signals (704 and 520 cm^{-1}) decreased
231 during the cultivation period (Figure 3a). DTG thermograms exhibited four stages of
232 thermal decomposition corresponding to the functional groups of the copolymer. Stages II
233 and IV, for urethane and ether groups respectively, reduced their masses at early
234 cultivation times, while stage III, for esters, steadily kept reducing its mass during the
235 whole experimental period. Interestingly, stage I, which accounts for low molecular weight

236 compounds, in this case biodegradation products, showed a fluctuating behavior that
237 increased at 10 days, and decreased afterwards (Figure 3b). DSC analysis of the
238 copolymer showed multiple thermal transitions revealing complex microstructures: the
239 glass transition temperature (Tg: 50.2ºC) reflects the proportion of soft and hard
240 segments; the three melting temperatures (Tm-I: 70ºC, Tm-II: 210.6ºC, Tm-III: 398.1ºC)
241 are associated with the hard segments of the polymer and the crystallization temperature
242 (Tc: 459.6ºC) is attributed to heat-directed crystallization of copolymer chains [47, 48]
243 (Figure 3c). BP8 biodegradative activity caused Tg decrease (46.2ºC), changes in Tms,
244 and strong decrease in Tc area, indicating that BP8 disrupts both, the soft and the hard
245 segments (associated with urethane groups) (Figure 3, Table S1). GPC analysis showed
246 that the number-average molecular weight of the copolymer decreased 35.6% and the
247 PDI increased to values higher than 2, at 25 days of cultivation with BP8 (Table S2). All
248 these results indicate that the degradative activity of the BP8 community generates
249 changes in the soft and hard segments of the copolymer microstructure resulting from the
250 attack to the different functional groups, including the more recalcitrant ether and
251 urethane groups.

252 **Community structure and metagenomic deconvolution of the BP8 community**

253 Analysis of the BP8 community taxonomic profile with MetaPhlAn, by using 17 282 414
254 reads, detected five bacterial orders (abundance), *Rhodobacterales* (83%), *Rhizobiales*
255 (8.9%), *Burkholderiales* (6.8%), *Actinomycetales* (0.83%), *Sphingobacteriales* (0.08%),
256 and one viral order *Caudovirales* (0.33%). Bacteria included 16 genera, being the most
257 abundant *Paracoccus* (83.9%) and *Ochrobactrum* (8.7%) (Figure S3). *De novo* assembly
258 of the shotgun metagenome sequences generated 3 072 contigs with a total length of 17
259 618 521 bp. Alignment of Hi-C reads to this assembly allowed the deconvolution of five

genome clusters, three near complete drafts (>95%), and two substantially complete drafts (89 and 71%) [36] (Table S3). The phylogenetic analysis showed well-supported clades within *Paracoccus*, *Chryseobacterium*, *Parapedobacter*, a member of the *Microbacteriaceae* family, and *Ochrobactrum intermedium* (Figure S4). The deconvoluted genomes of *Paracoccus* sp. BP8 and *O. intermedium* BP8.5 showed low novelty scores and high ANI values compared to their closest phylogenetic relatives, while *Chryseobacterium* sp. BP8.2, *Parapedobacter* sp. BP8.3 and the *Microbacteriaceae* bacterium BP8.4 showed high novelty scores and low ANI values (<95%) indicating they are new species. GC content and genomes' sizes were similar to the closest relatives except for the *O. intermedium* BP8.5 genome size, probably because of the low genome completeness (Table S3, S4).

Analysis of the xenobiotic metabolism encoded in the BP8 metagenome

In all the genomes, except in *O. intermedium* BP8.5, the genes and proteins assigned were in the range reported for the phylogenetically related members (Table S3, S4). Reconstruction of the metabolic pathways encoded in the BP8 metagenome was performed with 18 386 ORFs from which 8 637 were annotated into KEGG Orthology groups and the rest was not assigned to any orthologous functional category. Analysis of the BP8 xenobiotic metabolism identified 215 sequences encoding 59 unique proteins participating in pathways for benzoate (ko00362), fluorobenzoate (ko00364), aminobenzoate (ko00627), chlorocyclohexane and chlorobenzene (ko00361), and n-alkanes (ko00071) degradation. The most relevant enzymes are listed in Table 1. The genes for benzoate metabolism include all the enzymes for benzoate and 4-methoxybenzoate activation as well as 4-methoxybenzoate monooxygenase, a O-demethylating enzyme that transforms methoxybenzoate to hydroxybenzoate, and for

284 their subsequent transformation to β-ketoadipate (first 18 EC numbers in Table 1). Two
285 genes encoding carboxymethylene butanolidase that cleavages the ring of cyclic ester
286 dienelactone to produce maleylacetate, acting on the fluorobenzoate and
287 chlorocyclohexane and chlorobenzene metabolisms, were identified. Genes encoding
288 enzymes for the aminobenzoate pathway, such as 4-hydroxybenzoate decarboxylase
289 that participates in the transformation of phenol into hydroxybenzoate, amidase that
290 transforms benzamide into benzoate, and benzoyl phosphate phospohydrolase that
291 converts benzoyl phosphate into benzoate, were identified. All the genes encoding
292 enzymes needed for chlorocyclohexane and chlorobenzene degradation, the specific 2,4-
293 dichlorophenol 6-monooxygenase, the enzymes that transform 4-chlorophenol to cis-
294 acetylacrylate (EC1.13.11.1, EC5.5.1.1 and EC3.1.1.45), and the 2-haloacid
295 dehalogenase, which eliminates halogens from alkanes, were found. Likewise, genes
296 encoding enzymes for n-alkanes degradation (Table 1 Alkanes metabolism), as well as
297 all the enzymes for beta-oxidation were also detected.

298 **BP8 community phenotypic potential to biodegrade the xenobiotic additives**
299 **present in PolyLack®**

300 *NMP degradation.* Genes encoding putative proteins for NMP degradation, with
301 significant similarity (>40%) to the enzymes of *Alicycliphilus denitrificans* BQ1 [52] were
302 identified in several BP8 genomes (Table 1). However, only in *Paracoccus* sp. BP8 a
303 gene cluster (RQP05666.1-RQP05671.1) comparable to the BQ1 *nmp* cluster was
304 identified. *Isopropanol degradation.* Genes encoding proteins with significant similarity
305 to NAD^+-dependent secondary ADH with capability to oxidize IP to acetone were
306 identified in the BP8 metagenome [49], but not the genes encoding the enzymes for the
307 oxidative transformation of acetone. However, the three genes encoding acetone

308 carboxylase, that transforms acetone into acetoacetate, were identified, as well as the
309 enzymes that convert acetoacetate into acetoacetyl-CoA and this to acetyl-CoA are also
310 encoded in the BP8 metagenome (Figure 4a, Table 1). *Glycol ethers degradation.* In
311 the BP8 metagenome, homologous genes to PEG-degrading ADHs and ALDHs [50, 51],
312 and diverse enzymes that could attack the ether bonds, such as glycolate oxidase
313 (RQP04511.1, RQP04512.1, RQP04513.1, RQP11464.1, RQP19624.1, RQP19625.1,
314 RQP16322.1, RQP16256.1), dye decoloring peroxidase (RQP04907.1, RQP09154.1)
315 and superoxide dismutase (RQP04715.1, RQP13424.1, RQP09887.1, RQP11889.1,
316 RQP18047.1, RQP18034.1, RQP09190.1, RQP20377.1), as well as genes encoding
317 enzymes involved in glutathione metabolism, which have been proposed to participate in
318 PEG metabolism [53] were identified (Figure 4b, Table 1).

319 **BP8 community phenotypic potential to degrade polyurethane**

320 Genes encoding PU-esterases verified for PU degradation [54-57] and confirmed
321 carbamate-hydrolyzing enzymes *i.e.* arylamidase A [58], amidase [59], urethanase [60,
322 61], and carbaryl hydrolase [62], were searched by standalone BLASTP analyses. Six
323 and five sequences with similarity to PU-esterases and carbamate hydrolases were
324 retrieved from the BP8 metagenome, respectively (Table 2). We also identified genes
325 encoding ureases (EC3.5.1.5), suggested to act on PU degradation [63], in
326 *Parapedobacter* sp. BP8 (RQP19536.1, RQP19537.1 RQP19538.1) and *O. intermedium*
327 BP8.5 (RQP17756.1, RQP17448.1, RQP17449.1, RQP17450.1) genomes.

328 **Discussion**

329 To elucidate the mechanisms that landfill microbial communities perform to degrade the
330 recalcitrant PE-PU plastic, here we studied the degradative activity of the BP8 microbial
331 community that was selected because of its capability to grow in PolyLack®, a WPUD that

332 contains a proprietary PE-PU-A copolymer and several xenobiotic additives (NMP, IP, 2-
333 BE, DPGB and DPGM). Chemical and physical analyses demonstrated that BP8
334 consumes the additives and breaks the copolymer whereas Hi-C based metagenomic
335 analysis allowed us to unveil the phenotypic potential to degrade PU and xenobiotics of
336 five deconvoluted genomes from the community. The diauxic growth of BP8 observed
337 during 25 days of cultivation in MM-PolyLack suggested that two different metabolic
338 processes were involved in degrading the components of the WPUD. We hypothesized
339 that the additives were consumed during the first phase whereas the copolymer was
340 broken during the second one. However, the biomass increment and the carbon
341 decrease observed in the first growth phase (Figure 1a) resulted not only from additive
342 consumption, but also from the copolymer breakdown (Figures 2, 3, S2, Tables S1, S2).
343 These observations indicate that the diauxic growth is the result of simultaneous
344 degradation of additives and copolymer and that microbial enrichment could have
345 selected a more effective PU-degrading community that accounts for the second
346 exponential growth phase. Further studies to demonstrate this possibility are being
347 undertaken.

348 Exploring the BP8 metagenome, genes encoding enzymes presumably involved in
349 the degradation of the PolyLack® additives were identified in several of the deconvoluted
350 genomes. Genes for NMP degradation, similar to the ones reported for *A. denitrificans*
351 BQ1 [52] were identified in the *Paracoccus* sp. BP8 genome. *Paracoccus* strains able to
352 utilize NMP as carbon source have been reported [64], but the genes sustaining this
353 capability have not been described. IP biodegradation occurs by oxidative pathways in *P.*
354 *denitrificans* GH3 and *Gordonia* sp. TY-5. In these strains, IP is transformed by NAD^+-
355 dependent secondary ADH into acetone that is oxidized by a specific monooxygenase to

356 produce methyl acetate, which is transformed to acetic acid and methanol [49, 65].
357 However, the enzymes for metabolizing acetone by these reactions are not encoded in
358 the BP8 metagenome. Instead, genes encoding enzymes for acetone carboxylation, to
359 produce acetoacetate (acetone carboxylase), and for its subsequent transformation to
360 acetoacetyl-CoA by 3-oxoacid-CoA transferase and thereafter to acetyl-CoA by acetyl-
361 CoA C-acetyltransferase [66] were identified (Figure 4a, Table 1). The possibility that IP
362 degradation occurs by transformation to acetyl-CoA, via acetone in BP8 is supported by
363 the observation that in the *Paracoccus* sp. BP8 genome, a gene encoding an ADH
364 (RQP05888.1), homologous to the *Gordonia* sp. TY-5 *adh*2, and genes encoding the
365 acetone carboxylase subunits (RQP05866.1, RQP05867.1, RQP05889.1) are
366 contiguously located. Adjacent to these genes, a sequence encoding a sigma-54-
367 dependent transcriptional regulator (RQP05868.1) was observed, suggesting an operon-
368 like organization. This presumptive IP degradative operon has not been described in any
369 other bacteria. Degradation of 2-BE, DPGM and DPGB, the glycol ethers present in
370 PolyLack®, has not been reported in bacteria. Degradation pathways for PEG and PPG
371 reported in *Sphingomonads* species and *Microbacterium* (formerly *Corynebacterium*) sp.
372 No. 7 [5, 67, 68-70] show similar reactions where the glycols' hydroxyl terminal groups
373 are sequentially oxidized by specific ADHs and ALDHs to produce aldehydes, and
374 thereafter carboxylic acids [50,51], suggesting a widespread strategy for glycol ethers
375 metabolism in prokaryotes. Nevertheless, few enzymes involved in scission of ether
376 bonds, present in these compounds, have been identified in bacteria. A glycolic acid
377 oxidase [71] and a glycolic acid dehydrogenase [72] have been reported acting on PEG,
378 although several other enzymes such as superoxide dismutase, monooxygenase, ether
379 hydrolase, carbon-oxygen lyase, peroxidase and laccase have been suggested [5].

380 Homolog genes for specific ADHs and ALDHs were identified in the *Paracoccus* sp. BP8
381 genome (Table 1). Therefore, we hypothesize that 2-BE can be oxidized to 2-
382 butoxyacetic acid, DPGM to 2-methoxypropionic acid, which has been reported as a
383 metabolite in the degradation of DPGM by rats [73], and DPGB to 2-butoxypropionic acid
384 (Figure 4b). In *Paracoccus* sp. BP8, and in other genomes of the BP8 community, genes
385 encoding glycolate oxidase, dye decoloring peroxidase, 4-methoxybenzoate
386 monooxygenase and unspecific monooxygenase, which could account for the ether
387 scission of the aforementioned carboxylic acids, were identified (Table 1). The cleavage
388 of the carboxylates produced by ALDHs would generate the metabolizable intermediaries
389 glyoxylate, butyraldehyde, propylene glycol and formaldehyde (Figure 4b). Glyoxylate can
390 be funneled to the glyoxylate metabolism, butyraldehyde to the butanoate metabolism,
391 propylene glycol to the pyruvate metabolism, by lactaldehyde and lactate
392 dehydrogenases as suggested in *P. yeei* TT13 [74], and formaldehyde can be channeled
393 to the formate metabolism where glutathione-dependent enzymes could oxidize it to
394 formate and thereafter to CO_2 (Figure 4b, Table 1). All the enzymes for the aforesaid
395 metabolic pathways are encoded in the BP8 metagenome. Additionally, in PEG
396 metabolism, long chains of PEG-carboxylate can be processed by acyl-CoA synthetase
397 and glutathione-S transferase forming glutathione-conjugates [53]. Although these
398 reactions would not be needed for glycol ethers catabolism, they could be required for the
399 degradation of long polypropylene glycol moieties that are part of the PE-PU-A copolymer
400 (Figure S1).

401 By using different analytical techniques, we demonstrate that the BP8 community
402 attacks the main functional groups of the PE-PU-A copolymer; from the more
403 enzymatically susceptible ester bonds, present in acrylate and carbamate, to the more

404 recalcitrant C-C from aliphatics and aromatics, C-N from urethane, and C-O-C from ether
405 bonds of polypropylene glycol (Figures S1, 2, 3). The changes in the chemical and
406 physical properties of the polymer when incubated with BP8, and the generation of
407 diverse degradation products, some of them potential metabolic intermediates in the
408 degradation process, are evidences of the BP8's degradative capability, which is
409 sustained by the diverse xenobiotic degrading enzymes encoded in its metagenome
410 (Table 1). Some of the biodegradation products (Figure 2) seem to be the result of
411 oxidative reactions on C-C bonds flanking TDI, MDI or the acrylates' styrene ring (Figure
412 3, S1), generating aromatic compounds containing hydroxyl, aldehydes or organic acids.
413 Additionally, the copolymer aromatic compounds could be destabilized by
414 monooxygenases, which introduces hydroxyl groups to the aromatic rings, and by
415 dioxygenases that catalyzes reductive dihydroxylation, generating central intermediates
416 that can be cleaved by dearomatizing dioxygenases producing carboxylic acids [75, 76].
417 The enzymes for the complete benzoate metabolism are encoded in the BP8
418 metagenome and could account for PE-PU-A aromatic rings catabolism (Table 1).
419 Aliphatic chains from acrylates and polypropylene glycols can be metabolized by alkane
420 1-monooxygenases, that activate aliphatic chains by terminal or subterminal oxidations
421 and by the activities of ADH and ALDH, generating compounds that can be channeled by
422 beta-oxidation into the fatty acids metabolism (Table 1). If terminal oxidations are
423 introduced, primary alcohols are generated and transformed into aldehydes, carboxylic
424 acids and acyl-CoA. If subterminal oxidations of aliphatic chains occur, secondary
425 alcohols are formed, which upon breakdown, will produce ketones and thereafter esters,
426 which are hydrolyzed to alcohol and organic acids [77, 78]. Many different esters
427 compounds were identified in the BP8's degradation products, suggesting that

428 subterminal oxidation of alkanes could be an important route in PU metabolism (Figures
429 2, 3, S1). The cleavage of ester bonds by PU-esterases would produce alcohols and
430 organic acids, and the cleavage of urethane groups by carbamate-hydrolases would
431 produce nitrogen-containing compounds and aromatic isocyanate derivatives. As we
432 detected these degradation products by GC-MS analysis (Table 1, 2, Figure 2),
433 hydrolysis of ester and urethane bonds are accomplished during PE-PU-A degradation by
434 BP8. The identification of several PU-esterases and carbamate hydrolases encoded in
435 most of the BP8 genomes support this conclusion (Table 2).

436 The metabolic reactions proposed for the degradation of the additives and the PE-
437 PU-A copolymer present in PolyLack® by the BP8 community are based on the
438 phenotypic potential encoded in its metagenome. The use of proximity ligation Hi-C
439 technology allowed to define, with high confidence, what genes belong to each of the
440 different species of BP8 (Table 1). In this community, xenobiotic degradation is a niche
441 dominated by *Paracoccus* sp. BP8 and *Ochrobactrum intermedium* BP8.5, in whose
442 genomes, key enzymes for different steps of biodegradation are widely represented
443 (Table 1), which must be the reason for their preponderance in the BP8 community. In
444 addition, Microbacteriaceae bacterium BP8.4 genome encodes enzymes for the
445 metabolism of aromatic compounds suggesting that metacleavage ring excision and
446 muconate lactone formation might be functional. On the other hand, *Chryseobacterium*
447 sp. BP8.2 and *Parapedobacter* sp. BP8.3 genomes, harbor genes encoding
448 complementary metabolic activities for alkanes oxidation, such as hydrolysis and
449 oxidation of linear intermediates. The finding of such a diverse genetic repertoire in the
450 BP8 metagenome suggests a remarkable metabolic versatility, with strong hydrolytic and
451 oxidative capabilities that can play significant roles in the degradation of diverse

452 environmental contaminants. The abundance and distribution of these catabolic enzymes
453 among the different members of the BP8 community, suggest syntrophic mechanisms
454 driving community behavior. However, incomplete genome reconstruction in the
455 deconvolution analysis, resulting in potential pathway gaps in certain genomes, cannot be
456 ruled out, nor can the collapsing of multiple strains into a single cluster. On the other
457 hand, although *Paracoccus* and *Ochrobactrum* are predominant in the BP8 community by
458 far, we cannot discard that specific enzymatic activities encoded in genomes of little
459 abundant species can be crucial for the successful performance of BP8.

460 The present work provides deep understanding of the microbial ecology of a
461 selected landfill microbial community capable of PU and xenobiotics degradation by
462 revealing its composition and its outstanding phenotypic potential observed in the
463 catalytic capabilities that its members could display to cleave different recalcitrant
464 functional groups. Altogether, these features place BP8 community as a quite promising
465 source for developing environmental biotechnology strategies contributing to mitigate
466 anthropogenic plastics and xenobiotics pollution for achieving better environmental
467 quality. Moreover, further exploration of individual species of the community will allow the
468 manipulation of novel catabolic capabilities in order to improve biodegradative
469 technological processes.

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Conflict of interest statement

IL, MP, and SS are employees and shareholders of Phase Genomics, a company commercializing proximity ligation technology.

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Table 1. Distribution of genes encoding relevant proteins involved in xenobiotics degradation in the BP8 metagenome.

Activity	K Number	EC	Name	*Paracoccus* sp. BP8	*Chryseobacterium* sp. BP8.2	*Parapedobacter* sp. BP8.3	Microbacteriaceae bacterium BP8.4	*O. intermedium* BP8.5
Benzoate and related compounds metabolism								
Benzoate/toluate 1,2-dioxygenase	K05549 K05550 K05784	1.14.12.10 1.18.1.-	*benA-xylX* *benB-xylY* *benC-xylZ*	1 1 1	-	-	-	-
Dihydroxyclohexadiene carboxylate dehydrogenase	K05783	1.3.1.25	*benD-xylL*	1	-	-	-	-
p-Hydroxybenzoate 3-monooxygenase	K00481	1.14.13.2	*pobA*	1	-	-	-	1
Catechol 1,2-dioxygenase	K03381	1.13.11.1	*catA*	1	-	-	-	-
Catechol 2,3-dioxygenase	K07104	1.13.11.2	*catE*	1	-	-	1	1
Protocatechuate 3,4-dioxygenase	K00448 K00449	1.13.11.3	*pcaG-pcaH*	1 1	- -	- -	- -	1 1
Muconate cycloisomerase	K01856	5.5.1.1	*catB*	1	-	-	1	-
3-Carboxy-cis,cis-muconate cycloisomerase	K01857	5.5.1.2	*pcaB*	1	-	-	-	1
Muconolactone isomerase	K01856	5.3.3.4	*catC*	1	-	-	-	-
4-Carboxymuconolactone decarboxylase	K01607	4.1.1.44	*pcaC*	2	-	-	-	1
Enol-lactone hydrolase	K01055	3.1.1.24	*pcaD*	3	-	-	-	1
β-ketoadipate:succyinyl-CoA transferase,	K01031 K01032	2.8.3.6	*pcaI-pcaJ*	1 1	- -	- -	- -	2 1
β-ketoadipyl-CoA thiolase	K00632	2.3.1.16	*pcaF*	-	1	1	1	1
2-Oxopent-4-enoate hydratase (benzoate)	K02554	4.2.1.80	*mhpD*	-	-	-	1	-
4-Hydroxy 2-oxovalerate aldolase	K01666	4.1.3.39	*mhpE*	-	-	-	2	-
Acetaldehyde dehydrogenase	K04073	1.2.1.10	*mhpF*	-	-	-	2	-
4-Methoxybenzoate monooxygenase (O-	K22553	1.14.99.15	CYP199A2	1	-	-	-	-

demethylating)								
Carboxymethylene butanolidase	K01061	3.1.1.45	*clcD*	-	-	-	1	1
4-Hydroxybenzoate decarboxylase	K03186	4.1.1.61	*ubiX*	2	-	1	-	1
Amidase	K01426	3.5.1.4	*amiE*	1	-	-	1	1
Benzoyl phosphate phosphohydrolase	K01512	3.6.1.7	*acyP*	-	-	1	1	-
2,4-Dichlorophenol 6-monooxygenase	K10676	1.14.13.20	*tfdB*	1	-	-	-	-
2-Haloacid dehalogenase	K01560	3.8.1.2		2	-	-	-	1
Alkanes metabolism								
Alkane 1-monooxygenase	K00496	1.14.15.3	*alkB1_B2 alkM*	2	-	-	-	-
Ferredoxin NAD reductase component	K00529	1.18.1.3	*hcaD*	2	-	-	-	1
Unspecific monooxygenase	K00493	1.14.14.1		2	-	-	-	1
Long-chain-alkane monooxygenase	K20938	1.14.14.28	*LadA*	-	-	-	1	-
Alcohol dehydrogenase propanol preferring	K13953	1.1.1.1	*adhP*	2	-	1	-	1
Aldehyde dehydrogenase (NAD^+ dependent)	K00128	1.2.1.3	ALDH	6	2	-	-	3
Aldehyde dehydrogenase (NADP dependent)	K14519	1.2.1.4	*aldH*	-	1	-	-	-
Lipocalin family protein	K03098	-	*Blc*	-	1	1	-	1
Long chain fatty acid transport protein	K06076	-	*fadL*	1	-	-	-	-
N-methyl 2-pyrrolidone metabolism								
N-methylhydantoin amidohydrolase	K01473	3.5.2.14	*nmpA*	5	-	-	-	1
	K01474		*nmpB*	5	-	-	1	-
Aminoacid oxidase	-	-	*nmpC*	3	-	-	2	1
Succinate-semialdehyde dehydrogenase	K00135	1.2.1.16	*nmpF*	7	-	1	2	1
Isopropanol metabolism								
[a]Alcohol dehydrogenase propanol preferring	K13953	1.1.1.-	*adh1*	1	-	-	-	-

Alcohol dehydrogenase	K18369	1.1.1.-	*adh2*	2	-	-	-	-
[b]Aldehyde dehydrogenase	K00138	1.2.1.-	*adh3*	1	-	-	1	-
Acetone carboxylase	K10854 K10855 K10856	6.4.1.6	*acxB* *acxA* *acxC*	1 1 1	-	-	-	1
3-Oxoacid-CoA transferase	K01028 K01029	2.8.3.5		1 1	1 1	1 1	1 1	- -
Acetoacetate-CoA ligase	K01907	6.2.1.16	*acsA*	-	-	-	-	1
Acetyl-CoA C-acetyltransferase	K00626	2.3.1.9	*atoB*	10	1	1	3	5
Glycol ethers and polypropylene glycols metabolism								
[c]Alcohol dehydrogenase,	.	1.1.1.-	*pegdh*	3	-	-	-	3
[d]Aldehyde dehydrogenase	-	1.2.1.3	*pegC*	3	-	-	1	-
Glycolate oxidase	K00104 K11472 K11473	1.1.3.15	*glcD* *glcE* *glcF*	1 1 1	- - -	- - -	- - 1	2 1 1
Superoxide dismutase	K04564 K04565	1.15.1.1	SOD2 SOD1	- 1	1 1	3 -	1 -	1 -
Dye decoloring peroxidase	K15733	1.11.1.19	*DyP*	1	-	-	1	-
Glutathione S-transferase	K00799	2.5.1.18	*gst*	11	-	-	-	8
Acyl Co-A synthetase	K01897	6.2.1.3	*ACSL*	2	3	2	2	1
S-(hydroxymethyl) glutathione dehydrogenase	K00121	1.1.1.284	*frmA*	3	-	-	-	2
S-formylglutathione hydrolase	K01070	3.1.2.12	*fghA*	1	-	-	-	1

[a] *Adh1* was identified by BLAST analysis using the *adh1* sequence (Acc. num. BAD03962.1) reported for *Gordonia* sp. TY-5 [49] as query (Query cover ≥ 99%; E-value ≤ 4E-42; Identity 34%). The gene accession number in the BP8 metagenome is RQP06405.1.

[b] *Adh3* genes were identified by BLAST analysis using the *adh3* sequence (Acc. num. BAD03965.1) reported for *Gordonia* sp. TY-5 [49] as query (Query cover ≥ 97%; E-value ≤ 1E-97; Identity ≥ 38.8%). The gene accession numbers in the BP8 metagenome are RQP06404.1 and RQP13157.1. These genes were classified as aldehyde dehydrogenases by KEGG, similarly as described in [49].

[c] *Pegdh* genes were identified by BLAST analysis using the polyethylene glycol dehydrogenase sequence (*pegdh*) from *Sphingophyxis terrae* (Acc. num. BAB61732) [50] as query (Query cover ≥ 97%; E-value ≤ 2.0E-122; Identity ≥ 38.5%). The gene accession numbers in the BP8 metagenome are RQP05609.1, RQP06903.1, RQP07092.1, RQP19606.1, RQP18819.1, RQP20974.1.

[d] *Pegc* genes were identified by BLAST using polyethylene glycol aldehyde dehydrogenase sequence from *Sphingophyxis macrogoltabida* (Acc. num. BAF98449.1) [50] as query (Query cover ≥ 98%; E-value ≤ 1.0E-80; Identity ≥ 38%; Similarity ≥ 56%). The gene accession numbers in the BP8 metagenome are RQP06197.1, RQP04172.1, RQP06015.1, RQP13157.1. Only RQP06197.1 was identified as K00128 by KEGG.

Table 2. Esterases and carbamate hydrolyzing enzymes encoded in the BP8 metagenome.

Enzyme Query Organism (Accession number)	E.C. num.	Amino acids in the query	Hit in the BP8 metagenome	E value/ [a]Identity/ Similarity	Amino acids in the hit	Reference
Polyurethane esterase *Delftia acidovorans* (BAA76305)	3.1.1.6	548	*Parapedobacter* sp. BP8.3 (RQP17780.1)	1.0E-07/ 32%/ 48%	640	[53]
Polyurethanase esterase A *Pseudomonas chlororaphis* (AAD22743)	3.1.1.-	617	*Paracoccus* sp. BP8 (RQP07762.1)	4.0E-16/ 34%/ 50%	783	[54]
			Paracoccus sp. BP8 (RQP06646.1)	2.0E-14/ 33%/ 46%	980	
			Paracoccus sp. BP8 (RQP04598.1)	7.0E-12/ 30%/ 43%	854	
			Paracoccus sp. BP8 (RQP06839.1)	5.0E-12/ 32%/ 43%	612	
Esterase CE_Ubrb uncultured bacterium (SIP63154)	3.1.1.-	295	*Microbacteriaceae* bacterium BP8.4 (RQP12977.1)	8.0E-05/ 35%/ 48%	309	[56]
Arylamidase A *Paracoccus huijuniae* (AEX92978)	3.4.11.2	465	*Paracoccus* sp. BP8 (RQP04489.1)	6.0E-25/ 41%/ 52%	471	[57]
Amidase *Ochrobactrum* sp. TCC-2 (ANB41810)	3.5.1.4	474	*Microbacteriaceae* bacterium BP8.4 (RQP11486.1)	2.0E-47/ 35%/49%	475	[58]
			O. intermedium BP8.5 (RQP19215.1)	6.0E-24/ 39%/50%	326	
Urethanase *Lysinibacillus fusiformis* (KU353448)	3.5.1.4	472	*Microbacteriaceae* bacterium BP8.4 (RQP12064.1)	1.0E-60/ 32%/ 48%	499	[60]
Carbaryl hydrolase *cahA* *Arthrobacter* sp. RC100 (BAC15598)	3.6.3.5	506	*Paracoccus* sp. BP8 (RQP06118.1)	1.0E-15/ 35%/ 48%	326	[61]

[a]Only genes with identity ≥ 30% are presented.

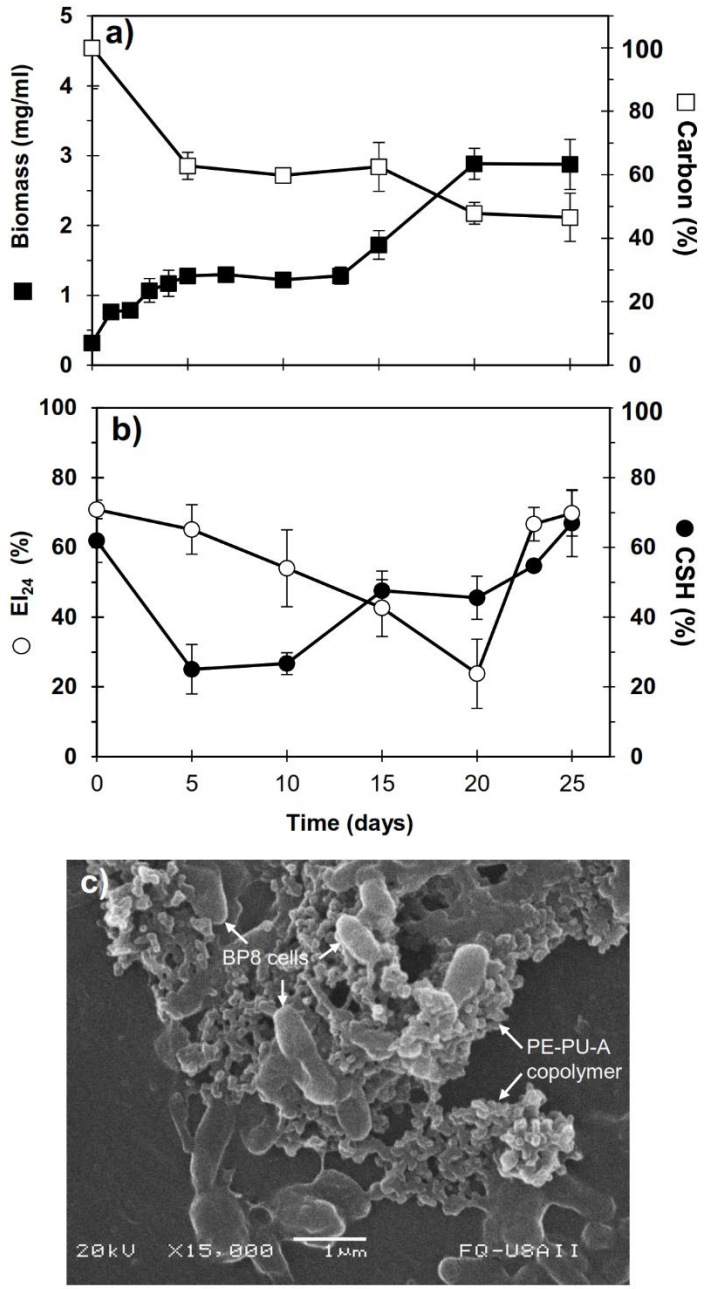

Figure 1. Characteristics of the BP8 community growing in MM-PolyLack. a) Growth and carbon consumption, **b)** emulsification index (EI_{24}) and cell surface hydrophobicity (CSH) at different cultivation times; **c)** SEM micrograph of BP8 cells attached to the PE-PU-A copolymer at 10 days of cultivation. Bars represent standard deviation. n=3.

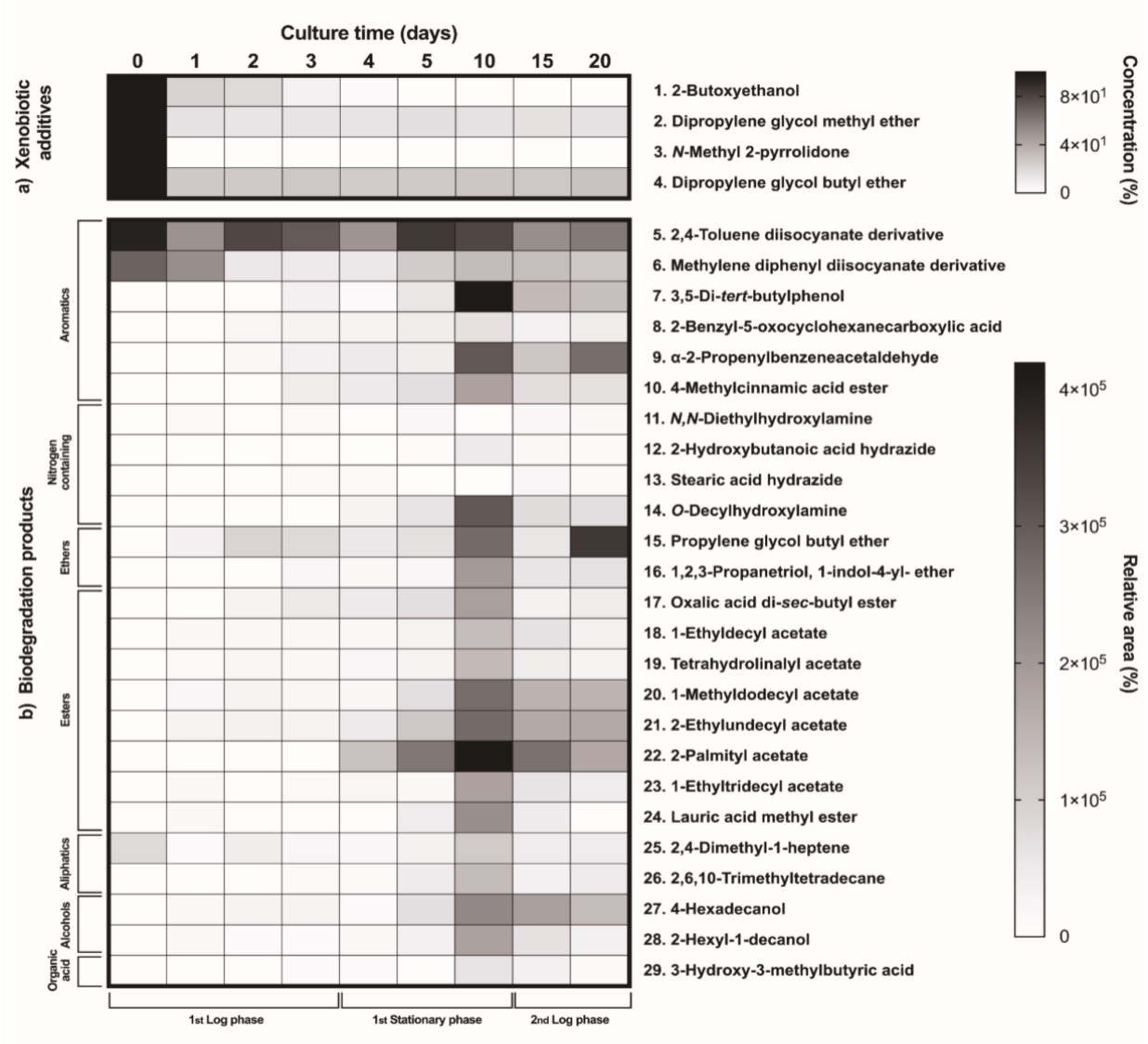

Figure 2. Xenobiotic additives consumed (a) and PE-PU-A biodegradation products generated (b) by the BP8 community. Cell-free supernatants were extracted at differents culture times with chloroform:methanol and analyzed by GC-MS. **a)** Additives were quantified using standard curves for each compound and **b)** biodegradation products by analyzing their relative areas in independent chromatograms. n=3. Compounds with mass spectra similarity values over 700 were considered the same compounds of the Library hits. The numbers in the compounds correspond to signals in the chromatograms of Figure S2.

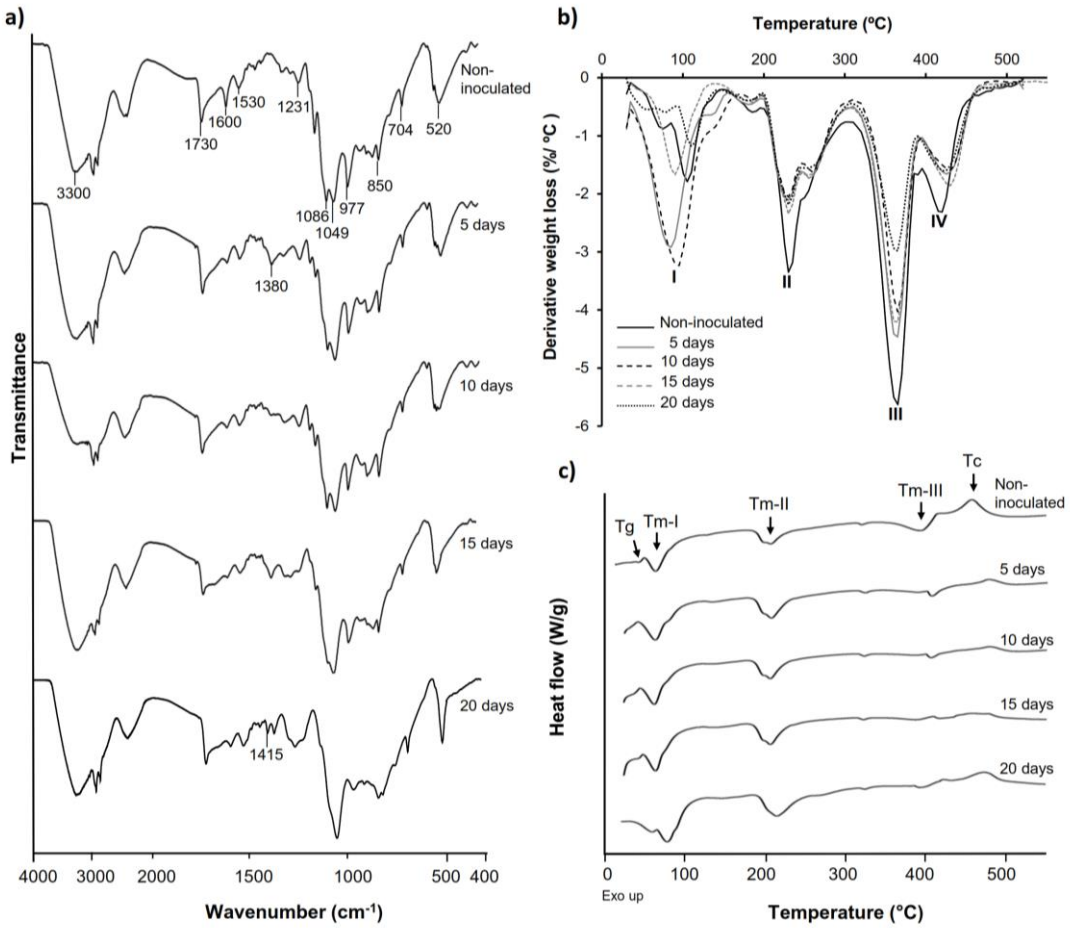

Figure 3. Physical and chemical analyses of the aromatic PE-PU-A copolymer after incubation with the BP8 community. a) FTIR spectra. **b)** DTG analysis. Thermal degradation stages correspond to the following functional groups: I. Low molecular weight compounds, II. Urethane, III. Ester, IV. Ether; **c)** DSC analysis. Glass transition temperature (Tg) represents the relative amount of soft and hard segments; melting temperatures, Tm-I, Tm-II and Tm-III are associated with hard domains, and crystallization temperature (Tc) represents heat-directed crystallization of copolymer chains**.**

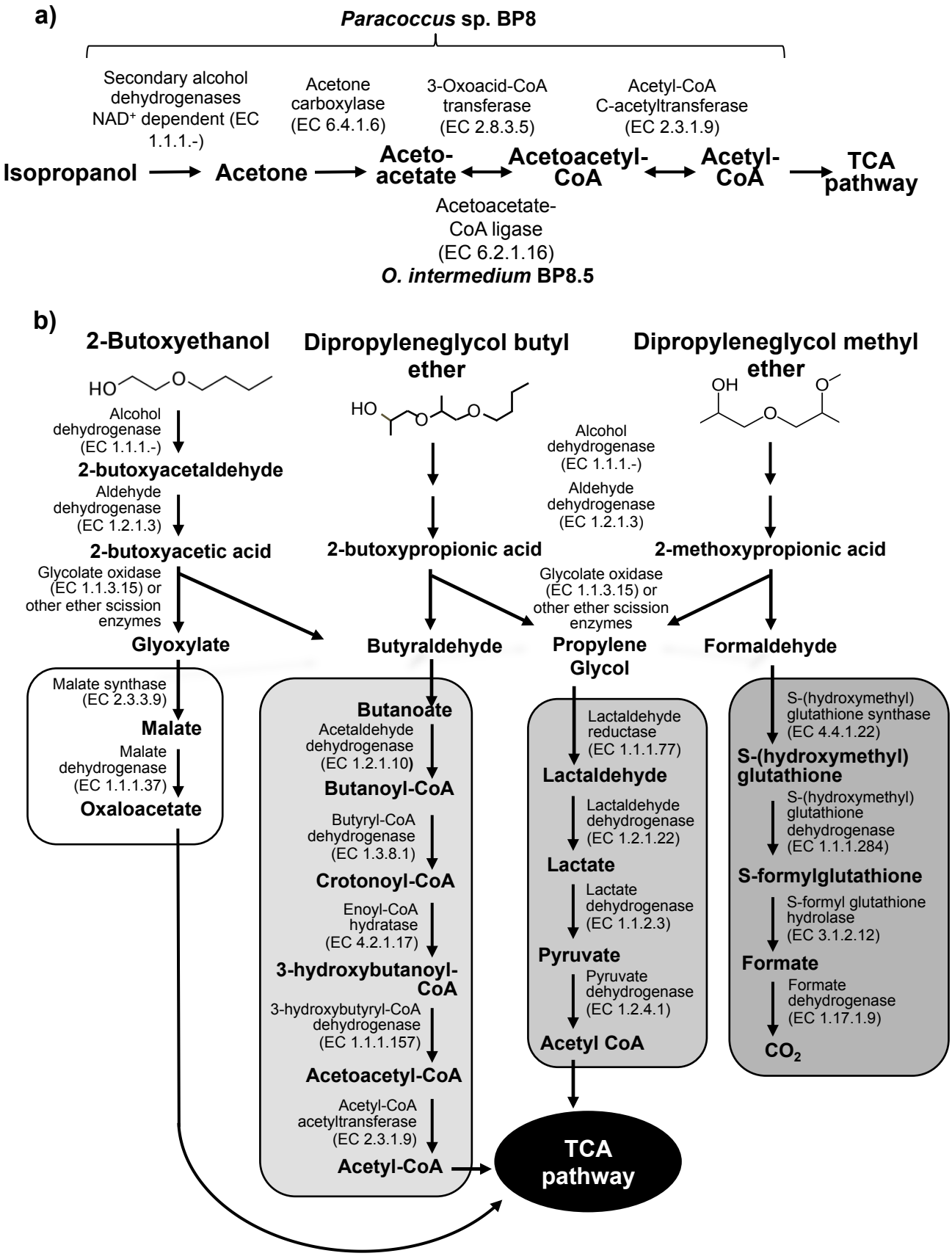
a)
Paracoccus sp. BP8
Secondary alcohol dehydrogenases NAD+ dependent (EC 1.1.1.-)
Acetone carboxylase (EC 6.4.1.6)
3-Oxoacid-CoA transferase (EC 2.8.3.5)
Acetyl-CoA C-acetyltransferase (EC 2.3.1.9)
Isopropanol
Acetone
Aceto-acetate
Acetoacetyl-CoA
Acetyl-CoA
TCA pathway
Acetoacetate-CoA ligase (EC 6.2.1.16)
O. intermedium BP8.5
b)
2-Butoxyethanol
Dipropyleneglycol butyl ether
Dipropyleneglycol methyl ether
Alcohol dehydrogenase (EC 1.1.1.-)
2-butoxyacetaldehyde
Aldehyde dehydrogenase (EC 1.2.1.3)
2-butoxyacetic acid
2-butoxypropionic acid
2-methoxypropionic acid
Glycolate oxidase (EC 1.1.3.15) or other ether scission enzymes
Glyoxylate
Butyraldehyde
Propylene Glycol
Formaldehyde
Malate synthase (EC 2.3.3.9)
Malate
Malate dehydrogenase (EC 1.1.1.37)
Oxaloacetate
Butanoate
Acetaldehyde dehydrogenase (EC 1.2.1.10)
Butanoyl-CoA
Butyryl-CoA dehydrogenase (EC 1.3.8.1)
Crotonoyl-CoA
Enoyl-CoA hydratase (EC 4.2.1.17)
3-hydroxybutanoyl-CoA
3-hydroxybutyryl-CoA dehydrogenase (EC 1.1.1.157)
Acetoacetyl-CoA
Acetyl-CoA acetyltransferase (EC 2.3.1.9)
Acetyl-CoA
Lactaldehyde reductase (EC 1.1.1.77)
Lactaldehyde
Lactaldehyde dehydrogenase (EC 1.2.1.22)
Lactate
Lactate dehydrogenase (EC 1.1.2.3)
Pyruvate
Pyruvate dehydrogenase (EC 1.2.4.1)
Acetyl CoA
S-(hydroxymethyl) glutathione synthase (EC 4.4.1.22)
S-(hydroxymethyl) glutathione
S-(hydroxymethyl) glutathione dehydrogenase (EC 1.1.1.284)
S-formylglutathione
S-formyl glutathione hydrolase (EC 3.1.2.12)
Formate
Formate dehydrogenase (EC 1.17.1.9)
CO2
TCA pathway

Figure 4. Potential degradation pathways for isopropanol (a) and glycol eters (b) encoded in the BP8 metagenome. a) *Paracoccus* sp. BP8 genome encodes ADH enzymes that can oxidize IP to acetone, but no genes encoding enzymes for acetone metabolism were found. Instead, the genes encoding the three subunits of acetone carboxylase that reductively transforms acetone to acetoacetate were found. Acetoacetate can be transformed to acetoacetyl-CoA by 3-oxoacid-CoA transferase activity, present in *Paracoccus* sp. BP8 or by acetoacetate-CoA ligase, present in *O. intermedium* BP8.5. Acetoacetyl CoA is transformed by acetyl-CoA C-acetyltransferase to acetyl CoA, that enters the TCA pathway encoded in the BP8 metagenome (See Table 1). **b)** Degradation of 2-BE could be carried out by subsequent oxidations of the hydroxy terminal group by PEG-DH and PEG-ALDH, followed by scission of the ether bond by glycolate oxidase or other ether scission enzymes to produce glyoxylate and butyraldehyde [5, 67]. Glyoxylate would be funneled to the glyoxylate metabolism (white rectangle) and butyraldehyde to the butanoate metabolism (light gray rectangle). DPGB and DPGM can also be degraded by initial oxidation of the hydroxy terminal groups and further be ether-cleaved by ether scission enzymes. The products of these processes would be butyraldehyde an propylene glycol from DPGB and propylene glycol and formaldehyde from DPGM. Propylene glycol can be funneled to the pyruvate metabolism (medium gray rectangle) and formaldehyde can be transformed by the methane metabolism (dark gray rectangle), Genes encoding homologs for PEG-DH and PEG-ALDH (*pegdh* and *pegc*) from *Sphyngophyxis terrae* and *S. macrogoltabida*, the three subunits of glycolate oxidase (*glcD*, *glcE*, *glcF*) and other possible ether scission enzymes were identified in *Paracoccus* sp. BP8 (See Table 1). Pathways for glyoxylate, butanoate, pyruvate and methane metabolisms as well as the TCA pathway were fully reconstructed from the BP8 metagenome based on KEGG annotated genes, using KEGG Mapper.