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1	PHB into PHB: recycling of polyhydroxybutyrate by a tandem "thermolytic
2	distillation-microbial fermentation" process
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25 Abstract

The recycle of polyhydroxybutyrate (PHB) via depolymerization-polymerization is here 26 27 proposed and assessed for the first time. Effective depolymerization of PHB to crotonic acid through a thermolytic distillation at mild conditions (170 °C and 150 mbar), and 28 29 without the need of any catalyst, gives a crotonic acid-enriched condensate (94% yield and 98% selectivity towards trans-crotonic acid) that was used without any further 30 31 purification as a substrate for producing renewed PHB with a culture of *Cupriavidus* 32 necator. The yield of PHB (0.6 g PHB per g of crotonic acid), final PHB content (63%), 33 and PHB-molecular weight (1.5 MDa) confirmed the technical feasibility of this tandem approach, with a 55% overall yield. A preliminary evaluation of the energy 34 35 consumption of various depolymerization approaches indicated that the thermolytic distillation consumes 20-25% less energy than previously reported methods; the energy 36 37 requirement for producing crotonic acid from PHB_{waste} with 50% moisture has a still acceptable energy demand (4 MJ/kg_{crotonic acid}), comparable to the energy required for 38 obtaining fermentable sugars (4.4 MJ/kg_{sugar}) to be used for feeding bacteria that 39 40 accumulate PHB.

41

42 Keywords: polyhydroxybutyrate; crotonic acid; thermolysis; chemical recycling;
43 *Cupriavidus necator*

44

46 **1. Introduction**

47 Polyhydroxyalkanoates (PHAs) are bio-based polyesters produced by a variety of organisms able to accumulate PHA granules inside the cells as energy and carbon 48 storage. Thanks to their biodegradability under aerobic and anaerobic conditions, 49 50 PHAs could meet the demand for biobased plastics from renewable resources. Importantly, PHAs could be applied for functional products expected to be 51 52 degraded (e.g. seed or herbicide containing capsules) or in conventional applications where degradation is not required (e.g. bags, packaging). In this last 53 case, their biodegradability contributes to avoiding pollution when the plastic 54 55 product (or a piece of it) accidentally leaks into the environment. In the cradle-to-grave approach initially considered for conventional PHAs 56 57 applications, its end-of-life management relies on biological strategies like 58 composting or anaerobic digestion. The former (i.e. with aerobic pathways) degrade PHAs directly into CO₂ whereas the latter (i.e. with anaerobic pathways) 59 degrade PHAs into volatile fatty acids (VFA) and subsequently into CH₄-rich 60 61 biogas. In both mineralisation strategies, CO₂ can be reused for example by cyanobacteria to directly produce PHAs (Reddy et al., 2003) or to grow plants 62 63 that can be used in fermentation for renewed PHAs production (Lamberti et al., 2020), while VFA and CH₄ produced in the anaerobic pathway can be recycled 64 65 back into PHAs by mixed microbial cultures (MMC) or type II methanotrophic (methane-utilizing) bacteria (Myung et al., 2015). However, such PHA-66 regeneration options could result in: i) a too slow or prolonged overall process for 67 the plastic market (Yates and Barlow, 2013) and *ii*) energy dispendious since all 68

the efforts for producing the PHAs monomers vanished when mineralisation isapplied.

Hence, two more straightforward cradle-to-cradle strategies could be adopted for 71 the end-of-life management of waste PHAs oriented to the synthesis of renewed 72 73 PHAs avoiding monomers mineralisation and thus material and energy leakages: i) exploiting (thermo)chemistry to de-polymerize PHAs and chemistry to re-74 75 polymerize it or *ii*) exploiting (thermo)chemistry to depolymerize PHAs and 76 biology to re-polymerize it (Brown, 2007; Shen et al., 2015). Since the chemocatalysed synthesis of PHA produces polymers with a much lower molecular 77 78 weight than what is achievable through microbial processes (Gorke et al., 2007), 79 the second strategy that avoids downgrading PHAs mechanical properties seems 80 more suitable for a circular economy scenario. Considering that the biological 81 step is fed with the produced intermediate/s, the thermochemical step should: 1) provide intermediate products that can be used as carbon source; 2) achieve the 82 production of effluents with constant and targeted features, to feed the PHA 83 84 production process with non-variable feedstock thus obtaining well-defined and reproducible products and productivities; 3) avoid the formation of specific 85 inhibitors of microbial growth such as furans, phenolic compounds, and ketones 86 (Basaglia et al., 2021). 87

The resulting intermediates of a thermal/chemical depolymerization of the
homopolymer polyhydroxybutyrate (PHB) are propene (Clark et al., 2016; Li and
Strathmann, 2019; Torri et al., 2017), *trans*-2-butenoic acid (crotonic acid, CA)
(Ariffin et al., 2010b, 2010a; Li and Strathmann, 2019; Mamat et al., 2014;

92 Morikawa and Marchessault, 1981; Mullen et al., 2014; Nishida et al., 2010;

Samorì et al., 2019; Yang et al., 2014), methyl crotonate (Fernández-Dacosta et 93 94 al., 2016; Spekreijse et al., 2015), methyl acrylate (Fernández-Dacosta et al., 2016), cyclic and linear oligomers (Morikawa and Marchessault, 1981; Nishida et 95 al., 2010), 3-hydroxybutyric acid (3-HB) (Li and Strathmann, 2019; Yang et al., 96 97 2014), methyl 3-hydroxybutanoate (Song et al., 2018) and hydrocarbon oil (Kang and Yu, 2014). To the best of our knowledge, only 3-HB obtained through 98 alkaline hydrolysis of PHB has been demonstrated to be a fermentable substrate 99 for producing PHB through aerobic processes mediated by a mixed microbial 100 consortium (Comamonas, Brachymonas and Acinetobacter, Myung et al., 2014). 101 102 Recently, we have explored the use of PHB as a polymeric platform for 103 producing fine chemicals and solvents: to this purpose, two PHB monomer 104 derivatives obtainable by alcoholysis and thermolysis of PHB bonds (i.e. methyl 105 3-hydroxybutanoate and CA, respectively) have been used as building blocks for synthesising bio-based solvents capable to extract PHB from inside the bacteria 106 (Parodi et al., 2021a), and photo-functionalized aldehydes (Parodi et al., 2021b). 107 108 To this purpose, we have developed an innovative thermal depolymerization 109 process (named "thermolytic distillation") capable to break PHB or PHB inclusions inside bacterial cells, achieving yields and selectivity of CA never 110 reported before (Parodi et al., 2021b). To extend the potentiality of PHB as a 111 112 polymeric platform and widen the exploitation of PHB monomers, herein we used CA as an unprecedented building block for synthesising PHB. Specifically, 113 114 we aimed at integrating our depolymerization protocol (thermolytic distillation) using the resulting monomeric fraction enriched in CA for directly producing 115 116 PHB, providing the first tandem depolymerization/polymerization cycle of PHB.

117	The entire concept relies on the use of CA derived from PHB as the key
118	fermentable substrate to obtain renewed PHB through aerobic bacteria. The
119	integrated process here proposed aimed also at unlocking bottlenecks in
120	biopolymers recycling (Yates and Barlow, 2013) by <i>i</i>) exploiting selective
121	depolymerization to obtain CA from all sorts of PHB, including short-chain PHB
122	(partially degraded PHB) and PHB mixed with other organic compounds, thus
123	avoiding cleaning process issues, and <i>ii</i>) exploiting the product of such
124	depolymerization (the CA-enriched condensate) to re-synthesise PHB with the
125	same chemo-physical properties of the virgin PHB (Figure 1). Therefore, this
126	tandem approach could pose the basis for a full circular recycle of PHB (from
127	PHB-waste to renewed PHB), without any downgrading of the polymer itself,
128	thus representing a breakthrough in the field of recycling of biodegradable
129	polymers. In the present paper, we proposed the use of the PHB-
130	depolymerization mixture as it is to feed Cupriavidus necator and accumulate
131	PHB, and we evaluate the feasibility in terms of energy consumption and
132	environmental performances of such an integrated process.
133	



Figure 1. Proposed tandem "thermolytic distillation-microbial fermentation" processfor producing PHB from PHB.

137

138 2. Materials and methods

139 2.1 Che	micals
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140	All solvents (dimethyl carbonate, and acetonitrile) and chemicals (trans-crotonic
141	acid, and glucose) used in this study were obtained from Sigma-Aldrich (purities
142	\geq 98%) and were used without purification. Standard PHB was purchased from
143	Biomer (DE).

145	2.2 Thermo	lytic d	istillation	to prod	luce CA
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146 CA was produced from PHB following the procedure reported in the literature

147 (Parodi et al., 2021b) by treating PHB (5 g) at 170°C under reduced pressure (150

148 mbar). The yield of the distilled fraction was defined as follows (Parodi et al.,

149 2021b):

150

151	$Yield_{distillates} (w/w_{PHB}\%) = Distillates (g) / PHB (g) * 100 (Eq. 1)$
152	
153	The yield of CA on a distilled fraction weight basis (Yield _{CA-distillates} ,
154	$w/w_{distillates}$ %) was calculated by GC-MS analysis as described below.
155	Consequently, the yield of CA on a PHB weight basis was determined as follows
156	(Parodi et al., 2021b):
157	
158	$Yield_{CA} (w/w_{PHB}\%) = Yield_{distillates} (w/w_{PHB}\%) * Yield_{CA-distillates} (w/w_{distillates}\%) (Eq.$

2)

- 159
- 160

161	The recovery of CA (%) achievable from PHB was calculated as follows, on the
162	assumption that 1 g of PHB will give 1 g of CA (Yield _{CA-theoretical} , w/w_{PHB} %)
163	(Mamat et al., 2014):
164	
165	$Recovery_{CA}(\%) = Yield_{CA}(w/w\%) / Yield_{CA-theoretical}(w/w_{PHB}\%)$ (Eq. 3)
166	
167	2.3 Evaluation of green metrics and energy consumption
168	The comparison between the various depolymerization procedures of PHB
169	reported in the literature was done by applying two coefficients (Energy economy
170	coefficient ε , and Environmental factor E ,) proposed for PET depolymerization
171	(Barnard et al., 2021), and here adopted to the specific case of thermal PHB
172	depolymerization:
173	
174	Energy economy coefficient (ε , $^{\circ}C^{-1} \times min^{-1}$) = Yield _{CA} (w/w%) / T ($^{\circ}C$) × t (min)
175	(Eq. 4)
176	
177	where t is the depolymerization time and T is the reaction temperature.
178	
179	Environmental factor (E, a.u.)
180	=[0.1*(solvent/PHB)+(catalyst/PHB)+(others/PHB)]/Yield _{CA} (w/w%) (Eq. 5)
181	
182	where the solvent to PHB mass ratio has been multiplied by 0.1 considering that
183	90% of solvents are typically recycled in industrial processes (Barnard et al.,
184	2021).

185 The preliminary evaluation of the energy consumption (expressed as MJ/kg_{CA}) of various depolymerization processes was done by considering the thermal energy 186 (TE) for heating PHB to the temperatures at which the depolymerization 187 occurred, and the electrical energy (EE) for pumping down the vapours during 188 189 the depolymerization under vacuum (see ESI for further detail). The preliminary evaluation of the energy requirements (expressed as MJ/kg_{CA}) 190 for treating various PHB_{waste} through thermolytic distillation was done by 191 192 considering the i) TE for drying PHB_{waste} to almost 0% moisture, ii) TE for 193 heating dried PHB_{waste} to 170°C for achieving depolymerization of PHB_{waste} chains, and iii) electrical energy (EE) for pumping down the vapours during 194 195 thermolytic distillation under vacuum (see Figure 2S and calculations in ESI for further details). 196

197

198 2.4 Production of PHB from CA with Cupriavidus necator

199 The production of PHB was carried out using two bench-scale bioreactors (Infors, 200 Minifors 2, 2L) using Cupriavidus necator strain (DSMZ 545) according to a 201 dual-phase process, the procedures and conditions reported in the literature 202 (Domingos et al., 2018). Briefly, 0.39 L of culture medium 81 from DSMZ was amended with glucose (4.45 g L^{-1}) which was used as a carbon source during the 203 204 batch balance growth (first phase of cell growth). Thereafter, once glucose and 205 NH₄⁺ were consumed, the second phase (accumulation of PHB) was started by 206 feeding a concentrated water solution of CA contained in the distilled fraction (100 g L⁻¹ of CA) based on a pO₂-stat strategy. A control condition (0.6 L of 207 working volume) was also experimented by using commercial crotonic acid 208

209	(CA _C) to verify whether other C4-byproducts in the collected distilled fraction
210	derived from the thermal distillation of PHB could affect the subsequent PHB
211	accumulation in bacteria. Both experimental conditions were carried out in
212	triplicate and monitored by sampling periodically. After measuring the
213	absorbance, samples were centrifuged (14.000 rpm, 10 min), the liquid fraction
214	was analysed by HPLC-RID (Domingos et al., 2018) for determining residual CA
215	amount and the pellet was freeze-dried, weighted to determine the cell dry weight
216	(CDW) and analysed to determine PHB content (Abbondanzi et al., 2017).
217	
218	2.5 Analytical procedures
219	GC-MS analysis of the chemicals produced by the thermolytic distillation of PHB

Was performed according to the literature (Parodi et al., 2021b), while the
identification of the depolymerization by-products was done by comparing their
mass spectra with the NIST spectra database, and by comparison with the
fragmentation reported in the literature (Abbondanzi et al., 2017; Torri et al.,
2014).

For determining the molecular weight of the produced PHB, microbial biomass
(2-3 g) was collected at the end of the fermentation, freeze-dried, and extracted in
duplicate with dimethyl carbonate (DMC, 20 mL) for 2 h at 90°C (Samorì et al.,
2015). Thereafter, the mixture was filtered with a 0.45 μm porosity Teflon filter,
the solvent mixture evaporated and the PHB film dried at 60°C under vacuum
overnight. The molecular weight (Mw) and the polydispersity index (PDI) of the
extracted PHB were analysed by gel permeation chromatography (GPC) by using

chloroform/1,1,1,3,3,3-hexafluoro-2-propanol as previously reported (Ferri et al.,
2020).

234

235 3. Results and discussion

3.1 Evaluation of the greenness of PHB-depolymerization approaches to produce
CA

238 The thermal degradation of PHAs is known to occur through β -elimination

reactions that randomly break the chains and give dehydrated trans-alkenoic acids

240 (e.g. CA) as the major product. Cis-alkenoic acids (e.g. isocrotonic acid), 3-

alkenoic acids, alkenyl-terminated dimers and trimers, and crotonamide (in the

case of PHB inclusions inside bacterial cells) have been reported as minor

243 products (Li and Strathmann, 2019), whose formation can be drastically reduced

if catalysts like Mg(OH)₂, MgO or CaO are used during the thermal treatment

245 (Ariffin et al., 2010a, 2010b; Flanagan et al., 2016; Kopinke et al., 1996).

In the present paper, we used a peculiar one-step procedure

247 called "thermolytic distillation" that was developed in our previous paper (Parodi 248 et al. 2021). Although the applied temperature (170°C) was largely below the maximum decomposition temperature of PHB (290°C) (Abdelwahab et al., 2012), 249 250 it resulted enough for giving a yield of CA of 92% based on PHB input. This 251 finding could be supported by the described weight loss at 180°C found for 252 bacterial biomass containing PHB as inclusions (Kopinke et al., 1996), presumably 253 associated with the formation of volatile C₄ compounds. The obtained distilled fraction was mainly composed of CA (98%) and minor amounts of cis-crotonic 254 acid (0.1%) and dimer of PHB (0.2%), in line with the best results reported in the 255

256 literature (Ariffin et al., 2010b; Mamat et al., 2014; see Table 1S in ESI). To evaluate the depolymerization results currently described (Ariffin et al., 2010b; 257 Mamat et al., 2014; Parodi et al. 2021) as a function of the depolymerization 258 variables (temperature, time, and catalyst) and not only in terms of 259 260 depolymerization performances, we applied different green chemistry metrics parameters, useful for making a numerical comparison of different 261 262 depolymerization approaches and determining their relative feasibility (Figure 2): 263 the Environmental factor (E, that highlights the effect of materials input that results in waste generation), and the Energy economy coefficient (ε , that relates the yield 264 265 of the main monomer produced from each depolymerization process with the time and temperature required for the reaction) (Barnard et al., 2021). This kind of 266 evaluation is crucial for the scalability of the technology, assisting in finding the 267 best routes for viable implementation of present and future studies of 268 269 depolymerization in the field of polymeric material recycling (Barnard et al., 270 2021).

The best processes would tend to present low values of E factor (low mass inputs, 271 272 thus low consumption of solvents and catalysts) and high ε values (low energy 273 consumption, thus short reaction time and mild reaction temperature). When the depolymerization of PHB was conducted without catalysts (Ariffin et al., 2010b; 274 Mamat et al., 2014; Parodi et al., 2021b), the E factor was equal to 0, differently 275 276 from what occurred for of the processes that made use of them (5% of MgO or Mg(OH)₂, Ariffin et al., 2010b). However, it is worth mentioning that the low 277 278 selectivity (57-63%, see Table 1S in ESI) of the two literature procedures that did 279 not make use of catalysts hampers the exploitation of such distilled fractions as

280 they are, without any further separation to get isolated CA (e.g. through column chromatography); therefore the E factor in these cases must be higher than 0 281 282 (Figure 2), due to the contribution of other mass inputs (e.g. solvents) not strictly used in the depolymerization phase but necessary in the purification step. On the 283 284 other hand, the high purity of the distilled fraction obtained through the thermolytic distillation here adopted (98%) is equal to the purity of commercial 285 286 CA, guaranteeing its exploitation without any further purification. 287 A preliminary evaluation of the energy consumption (MJ/kg_{CA}) of the 288 depolymerization processes reported in the literature by taking into account the 289 thermal energy (TE) for heating PHB to the temperature at which the 290 depolymerization of PHB chains occurs, and the electrical energy (EE) for pumping down the vapours during the depolymerization under vacuum (Figure 291 292 3), highlighted that the process here applied was the least energy-intensive (20-293 25% lower than the non-catalytic approaches and 12% than the catalytic ones) 294 and the highest in terms of ε value (Figure 2).



* solvents will be necessary to purify CA before the use

Figure 2. Energy economy factor (ε , \diamond) and Environmental factor (E, \bullet) for the most significant thermal depolymerizations of PHB reported in the literature.





300

Figure 3. Energy required to produce CA from PHB through the most significant
thermal depolymerizations reported in the literature.

304	To the best of our knowledge, the production of CA through pyrolysis of PHB i)
305	at temperatures below 200°C, ii) without any catalyst addition, iii) with an overall
306	yield of 92% and a purity of 98% is peculiar and unique of the thermolytic
307	distillation approach, representing clear progress beyond the state of the art of
308	PHB depolymerization and posing the best conditions for operating the further
309	biochemical polymerization to PHB, as required for a successful "hybrid
310	(thermo)chemical/biological" tandem process (Brown, 2007; Shen et al., 2015).
311	The high conversion efficiency and selectivity achieved by applying the
312	thermolytic distillation to PHB are similar to the most efficient chemolysis
313	techniques able to depolymerize condensation polymers (e.g. polyethylene
314	terephthalate can be depolymerized in supercritical water, methanol, or ethanol
315	with a monomer yield higher than 98.5%, in 30 min and without any catalyst)
316	(Goto, 2009).

317

318 *3.2 Production of PHB from CA with Cupriavidus necator*

319 The entire distilled fraction produced via the thermolytic distillation of PHB

320 without any further purification and commercial CA (CA_C) were used as carbon

321 sources for *C. necator* in the PHB-accumulation phase of a dual-stage

322 fermentation process (Figures 4a and 4b).



324

Figure 4. PHB production from CA (filled symbols) and CA_c (empty symbols): a)
concentration trends during the growth and accumulation phases (CDW and PHAs); b)
yield on the produced CDW and PHAs from the total acids consumed during the
accumulation phase. Reported points represent the average of the corresponding
triplicates, with standard deviations <20%.

331 The potential inhibition of CA (or CA_C) towards bacteria was avoided by keeping the concentration almost zero all along with the accumulation phase through a 332 333 pO₂-stat feeding strategy. Concentration profiles were almost identical for both conditions: in fact, both CDW parameters increased by a similar trend, from 334 about 2 g L⁻¹ up to 5 g L⁻¹, confirming the equivalency of the two carbon sources 335 used. As expected, during the accumulation phase the cell duplication did not 336 occur, i.e. the non-PHB biomass concentration did not increase. The PHB content 337 in C. necator after the fermentation of both CA and CA_C at the end of the 338 accumulation phase was 63%. 339

340	The PHB yields here obtained with CA and CA _C (0.60 g_{PHB} g_{CA}^{-1} and 0.57 g_{PHB}
341	g_{CAc} ⁻¹ , respectively, Figure 4b) were similar to what achieved with Na ⁺ 3-HB
342	$(0.57g_{PHB} g_{3-HB}^{-1}, Flanagan et al., 2016)$, or to those obtained with volatile fatty
343	acids from cheese whey (0.54 $g_{PHAs} g_{VFA}^{-1}$, Domingos et al., 2018), and higher
344	than the yield obtained by using CA and CH ₄ as co-substrates (0.45 $g_{PHA} g_{substrate}^{-1}$
345	Flanagan et al., 2016). The specific PHB production rate (0.08 h ⁻¹) was similar to
346	the one calculated from the concentration trend previously reported for a mixed
347	culture fed with Na ⁺ 3-HB (0.06-0.1 h^{-1}) (Myung et al., 2014). All of this
348	confirms the biochemical equivalence of alkenoic acids and hydroxyacids/short-
349	chain carboxylic acids reported in the literature (Eggers and Steinbüchel, 2013;
350	Janssen and Schink, 1993). However, it is worth mentioning that the formation of
351	carboxylates (e.g. Na^+ 3-HB) derived from the alkaline hydrolysis of PHB would
352	require the consumption of inorganic acid (HCl or H ₂ SO ₄) during the PHB
353	production by fermentation: in fact, the microbial consumption of
354	hydroxybutyrate leads to pH rise since Na ⁺ gets free.
355	PHB produced by C. necator fed with CA or CA _C was finally extracted and
356	characterized. The polymer recovery was in both cases above 90%, and the molecular
357	weight (1.5 and 1.0 MDa, respectively) was in agreement with what is achievable by
358	feeding the same bacterial strain with conventional carbon sources (e.g., glucose, 1.1
359	MDa, Samorì et al., 2015). The polydispersity index (PDI) here obtained by using CA
360	(2.6) was almost a half than that of the PHB obtained from <i>C. necator</i> after the
361	fermentation of CA_{C} (4.5), and in line with the PDI obtained when glucose is used (2.7,
362	Samorì et al., 2015). Such characteristics of the renewed PHB and the high PHB yield
363	here achieved, highlighted the suitability of CA as a substrate for producing renewed

PHB. Overall, even if the fermentation could be optimised (e.g. by testing pH-stat
feeding strategy), the results here obtained in the fermentation experiments confirmed
the feasibility of using CA (directly from the thermolytic distillation of PHB, without
any further purification) as substrate, achieving yields and PHB characteristics which
are comparable to those obtained when using glucose or volatile fatty acids.

369

370 3.3 Preliminary evaluation of energy requirements for producing CA from

371 various PHB end-of-life scenarios

372 Introducing waste-PHB into the conventional plastic market disposal scenario is

claimed to be a lengthy and complicated procedure (Vermeer et al., 2021).

374 Considering some issues critical for the development of a feasible EoL strategy, a

recycling process capable of by-passing the presence of impurities or small

variations in polymer characteristics seems to be highly promising. According to

the yields here reported (0.92 g_{CA}/g_{PHB} and 0.60 g_{PHB}/g_{CA} for the thermolytic

378 distillation and the microbial conversion processes, respectively), the tandem

thermochemical-biological recycling of PHB_{waste} allows at producing about 0.55

380 kgPHB/kgPHB_{waste} that corresponds to a resource-saving of at least 1

 $kg_{substrate}/kgPHB_{waste}$, depending on the carbon source used to feed bacteria and

the PHB yield of such biological process (Domingos et al., 2018; Flanagan et al.,

2016; Myung et al., 2014). Of course, the entire tandem process here proposed is

undoubtedly more energy-demanding than other expected end-of-life (EoL)

scenarios of the PHB_{waste} if managed within a differentiated waste collection

386 framework (e.g. composting or anaerobic digestion processes together with food

387 waste). In composting, PHB_{waste} would be partially or completely oxidized to CO_2

and H₂O, together with the production of compost, whereas anaerobic digestion
would convert PHB_{waste} into CH₄-containing biogas, with a maximum theoretical
yield of 0.42 kgCH₄/kgPHB_{waste} that corresponds to 23.1 MJ of thermal energy
per kg of PHB_{waste}. However, in the framework of the PHB circular loop, the
proposed strategy could result in significant energy savings.

Even though a detailed life cycle analysis is beyond the scope of this work, a

preliminary evaluation of the energy required to produce 1 kg of CA from

395 different PHB sources (PHB_{waste}) was performed. Therefore, various PHB_{waste}-

scenarios were considered, each including different percentages of PHB, water

and other residues (waste food or other plastics), namely (PHB%/H₂O%/Other%)

as follows: i) dried and clean PHB_{waste} collected through an *ad-hoc* waste

collection and not mixed with other materials (100/0/0); ii) dried PHB_{waste} mixed

400 with other plastics (50/0/50 and 20/0/80) from a plastic waste collection; iii) wet

and clean PHB (50/50/0); iv) PHB mixed with dried or wet food waste (10/0/90

402 or 10/70/20) from an organic/food waste collection. This preliminary evaluation

403 focused on CA produced through thermolytic distillation was compared to the

404 energy demand necessary to produce sugars, among the most used feedstock for

405 feeding *C. necator* and giving a similar PHB yield (Choi and Lee, 1997).

406 Therefore, the system boundaries have been chosen to include just the energy

407 consumption associated with the C-source production, and consider the further

408 fermentation stage as the same, independently by the C-source (CA or sugars).

409 The energy requirement for sugar production was calculated from previously

410 reported values for fermentable sugars used in bioethanol distilleries (Jacques et

al., 2003) as the contribution of agricultural practice, grain transport and grinding

412	(3.8 MJ/kg _{sugar}) together with the contribution of grain cooking and liquefaction
413	(0.6 MJ/kg _{sugar}). The treatment of relatively uncontaminated and dried PHB _{waste}
414	(i.e. 100/0/0 and 50/0/50) requires a limited amount of energy (< 3 MJ/kg _{CA} ,
415	Figure 5). If the thermolytic distillation treatment of PHB_{waste} with 50% moisture
416	or of dried PHB_{waste} but contaminated with plastic materials (20/0/80) has a still
417	acceptable energy demand (4 and 7 MJ/kg _{CA} , respectively), the production of CA
418	from PHB _{waste} mixed with wet organic material like food waste (10/0/90 or
419	10/70/20) is less energetically favourable (14 and 38 MJ/kg _{CA} , respectively),
420	since it needs 3-10 times more energy than that required for obtaining
421	fermentable sugars (4.4 MJ/kg_{sugar}). This preliminary evaluation suggests that the
422	production of PHB from CA through the tandem thermochemical-biological
423	process here proposed could be energetically feasible and comparable to the
424	production of PHB from sugars, especially if CA could be obtained starting from
425	PHB_{waste} with a relatively low level of humidity (< 50%). In principle the process
426	here proposed could be applied to "capture" the value of other kinds of PHAs
427	containing monomers with longer alkyl chains (e.g. polyhydroxybutyrate-valerate
428	or polyhydroxybutyrate-hexanoate): the thermolytic distillation step would
429	benefit from the lower decomposition temperature of longer PHAs and
430	preliminary data on polyhydroxybutyrate-valerate or polyhydroxybutyrate-
431	hexanoate suggest that the monomer ratio of the PHA subjected to the
432	depolymerization procedure would be maintained in the distilled fraction (data
433	not shown); however, the different boiling points of the corresponding alkenoic
434	acids (e.g. 2-pentenoic acid or 2-hexenoic acid) could be exploited to achieve
435	their separation. Additionally, some bacterial strains have been already proven to

- 436 be capable to use these alkenoic acids to produce renewed PHAs (Myung et al.,
- 437 2014). All of this suggests the possibility of recycling PHA mixtures and
- 438 extending the versatility of the proposed integrated approach.
- 439



440

Figure 5. Energy required to produce CA from various PHB_{waste}, or to produce
fermentable sugars, both usable as substrates by *C. necator* to produce PHB.

444 **4. Conclusions**

445 The thermolytic distillation of PHB at low temperature and without any catalyst gives

high yield (92%) and selectivity (98%) in CA, suitable for feeding *C. necator* and

- 447 producing renewed PHB at high yields (60%) and molecular weight (1.5 MDa). The
- 448 proposed thermochemical depolymerization/biological polymerization tandem process
- had an overall yield of 55%, encouraging for a circular EoL management of PHB-waste;
- 450 in fact, the results here obtained suggest that it is possible to recover CA from PHB-
- 451 waste through a technically simple procedure and convert it into renewed high-quality

452	PHB, opening the possibility to a chemical-biological recycling pathway oriented to
453	decrease the amount of virgin feedstock used in PHB synthesis. A preliminary
454	comparative evaluation of the energy demand required to synthesize C-feedstocks
455	fermentable into PHB suggested the convenience of using the proposed tandem
456	recycling approach for producing renewed PHB instead of using virgin C-sources like
457	sugars. This finding demonstrates the versatility of PHB as a polymeric platform, giving
458	building blocks that can be converted again into PHB or other bio-based
459	chemicals/solvents.

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