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PHB into PHB: Recycling of polyhydroxybutyrate by a tandem “thermolytic distillation-microbial fermentation” process

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1 **PHB into PHB: recycling of polyhydroxybutyrate by a tandem “thermolytic**  
2 **distillation-microbial fermentation” process**

3

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25 **Abstract**

26 The recycle of polyhydroxybutyrate (PHB) *via* depolymerization-polymerization is here  
27 proposed and assessed for the first time. Effective depolymerization of PHB to crotonic  
28 acid through a thermolytic distillation at mild conditions (170 °C and 150 mbar), and  
29 without the need of any catalyst, gives a crotonic acid-enriched condensate (94% yield  
30 and 98% selectivity towards trans-crotonic acid) that was used without any further  
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  
34 approach, with a 55% overall yield. A preliminary evaluation of the energy  
35 consumption of various depolymerization approaches indicated that the thermolytic  
36 distillation consumes 20-25% less energy than previously reported methods; the energy  
37 requirement for producing crotonic acid from PHB<sub>waste</sub> with 50% moisture has a still  
38 acceptable energy demand (4 MJ/kg<sub>crotonic acid</sub>), comparable to the energy required for  
39 obtaining fermentable sugars (4.4 MJ/kg<sub>sugar</sub>) to be used for feeding bacteria that  
40 accumulate PHB.

41

42 **Keywords:** polyhydroxybutyrate; crotonic acid; thermolysis; chemical recycling;

43 *Cupriavidus necator*

44

45

46 **1. Introduction**

47 Polyhydroxyalkanoates (PHAs) are bio-based polyesters produced by a variety of  
48 organisms able to accumulate PHA granules inside the cells as energy and carbon  
49 storage. Thanks to their biodegradability under aerobic and anaerobic conditions,  
50 PHAs could meet the demand for biobased plastics from renewable resources.  
51 Importantly, PHAs could be applied for functional products expected to be  
52 degraded (e.g. seed or herbicide containing capsules) or in conventional  
53 applications where degradation is not required (e.g. bags, packaging). In this last  
54 case, their biodegradability contributes to avoiding pollution when the plastic  
55 product (or a piece of it) accidentally leaks into the environment.  
56 In the cradle-to-grave approach initially considered for conventional PHAs  
57 applications, its end-of-life management relies on biological strategies like  
58 composting or anaerobic digestion. The former (i.e. with aerobic pathways)  
59 degrade PHAs directly into CO<sub>2</sub> whereas the latter (i.e. with anaerobic pathways)  
60 degrade PHAs into volatile fatty acids (VFA) and subsequently into CH<sub>4</sub>-rich  
61 biogas. In both mineralisation strategies, CO<sub>2</sub> can be reused for example by  
62 cyanobacteria to directly produce PHAs (Reddy et al., 2003) or to grow plants  
63 that can be used in fermentation for renewed PHAs production (Lamberti et al.,  
64 2020), while VFA and CH<sub>4</sub> produced in the anaerobic pathway can be recycled  
65 back into PHAs by mixed microbial cultures (MMC) or type II methanotrophic  
66 (methane-utilizing) bacteria (Myung et al., 2015). However, such PHA-  
67 regeneration options could result in: *i*) a too slow or prolonged overall process for  
68 the plastic market (Yates and Barlow, 2013) and *ii*) energy dispendious since all

69 the efforts for producing the PHAs monomers vanished when mineralisation is  
70 applied.

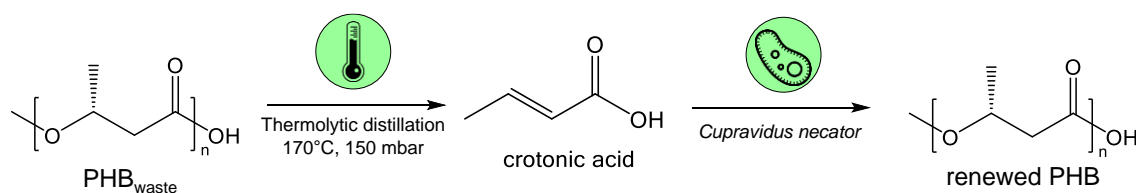
71 Hence, two more straightforward cradle-to-cradle strategies could be adopted for  
72 the end-of-life management of waste PHAs oriented to the synthesis of renewed  
73 PHAs avoiding monomers mineralisation and thus material and energy leakages:  
74 *i)* exploiting (thermo)chemistry to de-polymerize PHAs and chemistry to re-  
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 chemo-  
77 catalysed synthesis of PHA produces polymers with a much lower molecular  
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)  
82 provide intermediate products that can be used as carbon source; 2) achieve the  
83 production of effluents with constant and targeted features, to feed the PHA  
84 production process with non-variable feedstock thus obtaining well-defined and  
85 reproducible products and productivities; 3) avoid the formation of specific  
86 inhibitors of microbial growth such as furans, phenolic compounds, and ketones  
87 (Basaglia et al., 2021).

88 The resulting intermediates of a thermal/chemical depolymerization of the  
89 homopolymer polyhydroxybutyrate (PHB) are propene (Clark et al., 2016; Li and  
90 Strathmann, 2019; Torri et al., 2017), *trans*-2-butenoic acid (crotonic acid, CA)  
91 (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;

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



135 **Figure 1.** Proposed tandem “thermolytic distillation-microbial fermentation” process  
 136 for producing PHB from PHB.

137

138 **2. Materials and methods**



139 *2.1 Chemicals*

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).

144

145 *2.2 Thermolytic distillation to produce CA*

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 
$$Yield_{CA} (w/w_{PHB}\%) = Yield_{distillates} (w/w_{PHB}\%) * Yield_{CA-distillates}(w/w_{distillates}\%) (Eq.$$

159

2)

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<sub>PHB</sub>%)  
163 (Mamat et al., 2014):

164

$$165 \quad Recovery_{CA} (\%) = Yield_{CA} (w/w\%) / Yield_{CA-theoretical} (w/w_{PHB}\%) \quad (Eq. 3)$$

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  $\epsilon$ , 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

$$174 \quad Energy \ economy \ coefficient \ (\epsilon, \ ^\circ C^{-1} \times min^{-1}) = Yield_{CA} (w/w\%) / T (^\circ C) \times t (min)$$

175 *(Eq. 4)*

176

177 where  $t$  is the depolymerization time and  $T$  is the reaction temperature.

178

$$179 \quad Environmental \ factor \ (E, \ a.u.)$$

$$180 \quad = [0.1 * (solvent/PHB) + (catalyst/PHB) + (others/PHB)] / Yield_{CA} (w/w\%) \quad (Eq. 5)$$

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<sub>CA</sub>) of  
186 various depolymerization processes was done by considering the thermal energy  
187 (TE) for heating PHB to the temperatures at which the depolymerization  
188 occurred, and the electrical energy (EE) for pumping down the vapours during  
189 the depolymerization under vacuum (see ESI for further detail).

190 The preliminary evaluation of the energy requirements (expressed as MJ/kg<sub>CA</sub>)  
191 for treating various PHB<sub>waste</sub> through thermolytic distillation was done by  
192 considering the i) TE for drying PHB<sub>waste</sub> to almost 0% moisture, ii) TE for  
193 heating dried PHB<sub>waste</sub> to 170°C for achieving depolymerization of PHB<sub>waste</sub>  
194 chains, and iii) electrical energy (EE) for pumping down the vapours during  
195 thermolytic distillation under vacuum (see Figure 2S and calculations in ESI for  
196 further details).

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  
203 amended with glucose (4.45 g L<sup>-1</sup>) which was used as a carbon source during the  
204 batch balance growth (first phase of cell growth). Thereafter, once glucose and  
205 NH<sub>4</sub><sup>+</sup> were consumed, the second phase (accumulation of PHB) was started by  
206 feeding a concentrated water solution of CA contained in the distilled fraction  
207 (100 g L<sup>-1</sup> of CA) based on a pO<sub>2</sub>-stat strategy. A control condition (0.6 L of  
208 working volume) was also experimented by using commercial crotonic acid

209 (CA<sub>C</sub>) to verify whether other C<sub>4</sub>-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  
220 was performed according to the literature (Parodi et al., 2021b), while the  
221 identification of the depolymerization by-products was done by comparing their  
222 mass spectra with the NIST spectra database, and by comparison with the  
223 fragmentation reported in the literature (Abbondanzi et al., 2017; Torri et al.,  
224 2014).

225 For determining the molecular weight of the produced PHB, microbial biomass  
226 (2-3 g) was collected at the end of the fermentation, freeze-dried, and extracted in  
227 duplicate with dimethyl carbonate (DMC, 20 mL) for 2 h at 90°C (Samorì et al.,  
228 2015). Thereafter, the mixture was filtered with a 0.45 µm porosity Teflon filter,  
229 the solvent mixture evaporated and the PHB film dried at 60°C under vacuum  
230 overnight. The molecular weight (M<sub>w</sub>) and the polydispersity index (PDI) of the  
231 extracted PHB were analysed by gel permeation chromatography (GPC) by using

232 chloroform/1,1,1,3,3,3-hexafluoro-2-propanol as previously reported (Ferri et al.,  
233 2020).

234

### 235 **3. Results and discussion**

236 *3.1 Evaluation of the greenness of PHB-depolymerization approaches to produce*

237 *CA*

238 The thermal degradation of PHAs is known to occur through  $\beta$ -elimination  
239 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-  
241 alkenoic acids, alkenyl-terminated dimers and trimers, and crotonamide (in the  
242 case of PHB inclusions inside bacterial cells) have been reported as minor  
243 products (Li and Strathmann, 2019), whose formation can be drastically reduced  
244 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).

246 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  
249 maximum decomposition temperature of PHB (290°C) (Abdelwahab et al., 2012),  
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  $C_4$  compounds. The obtained distilled  
254 fraction was mainly composed of CA (98%) and minor amounts of cis-crotonic  
255 acid (0.1%) and dimer of PHB (0.2%), in line with the best results reported in the

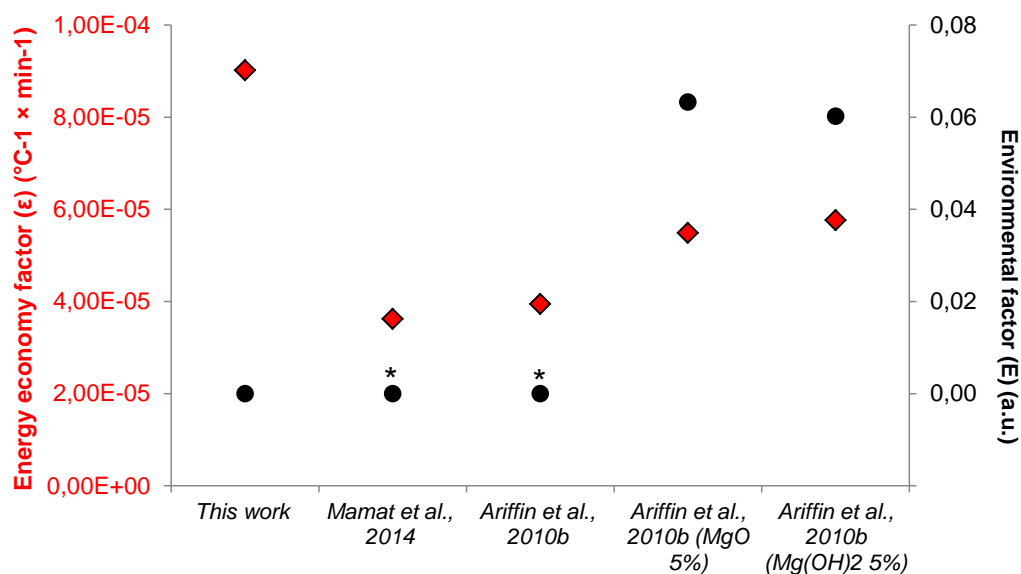
256 literature (Ariffin et al., 2010b; Mamat et al., 2014; see Table 1S in ESI). To  
257 evaluate the depolymerization results currently described (Ariffin et al., 2010b;  
258 Mamat et al., 2014; Parodi et al. 2021) as a function of the depolymerization  
259 variables (temperature, time, and catalyst)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

271 The best processes would tend to present low values of E factor (low mass inputs,  
272 thus low consumption of solvents and catalysts) and high  $\epsilon$  values (low energy  
273 consumption, thus short reaction time and mild reaction temperature). When the  
274 depolymerization of PHB was conducted without catalysts (Ariffin et al., 2010b;  
275 Mamat et al., 2014; Parodi et al., 2021b), the E factor was equal to 0, differently  
276 from what occurred for of the processes that made use of them (5% of MgO or  
277 Mg(OH)<sub>2</sub>, Ariffin et al., 2010b). However, it is worth mentioning that the low  
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  
281 chromatography); therefore the E factor in these cases must be higher than 0  
282 (Figure 2), due to the contribution of other mass inputs (e.g. solvents) not strictly  
283 used in the depolymerization phase but necessary in the purification step. On the  
284 other hand, the high purity of the distilled fraction obtained through the  
285 thermolytic distillation here adopted (98%) is equal to the purity of commercial  
286 CA, guaranteeing its exploitation without any further purification.

287 A preliminary evaluation of the energy consumption ( $\text{MJ}/\text{kg}_{\text{CA}}$ ) 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  
291 pumping down the vapours during the depolymerization under vacuum (Figure  
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  $\epsilon$  value (Figure 2).

295



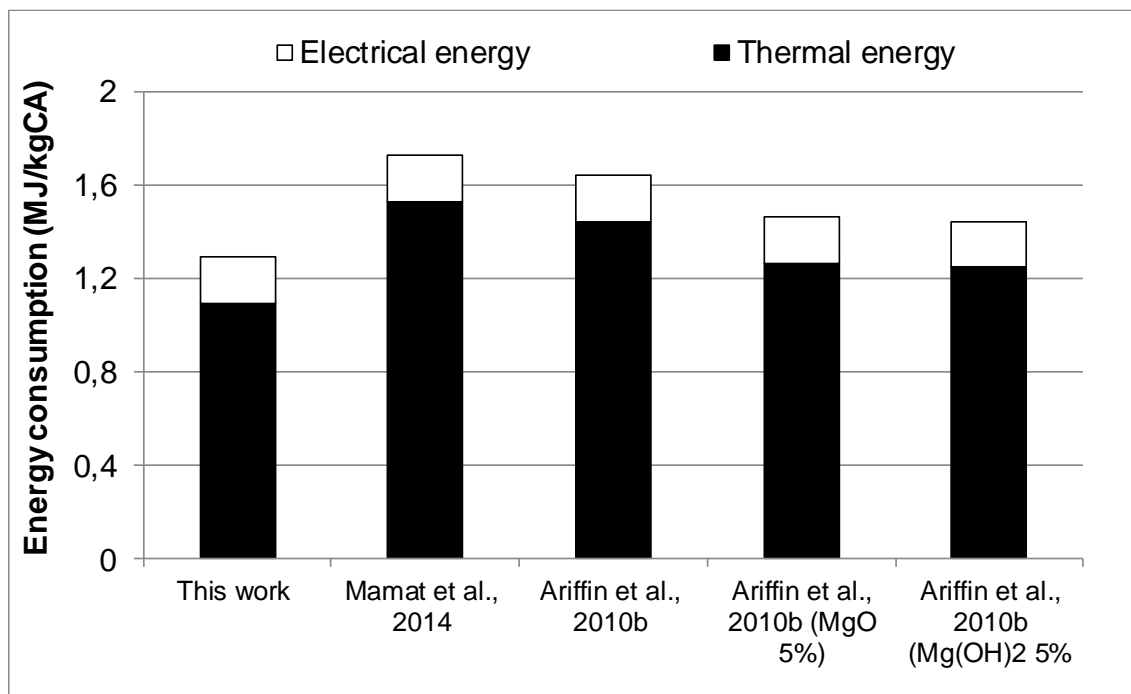
\* solvents will be necessary to purify CA before the use

296

297 **Figure 2.** Energy economy factor ( $\epsilon$ ,  $\blacklozenge$ ) and Environmental factor (E,  $\bullet$ ) for the

298 most significant thermal depolymerizations of PHB reported in the literature.

299



300

301 **Figure 3.** Energy required to produce CA from PHB through the most significant

302 thermal depolymerizations reported in the literature.



303

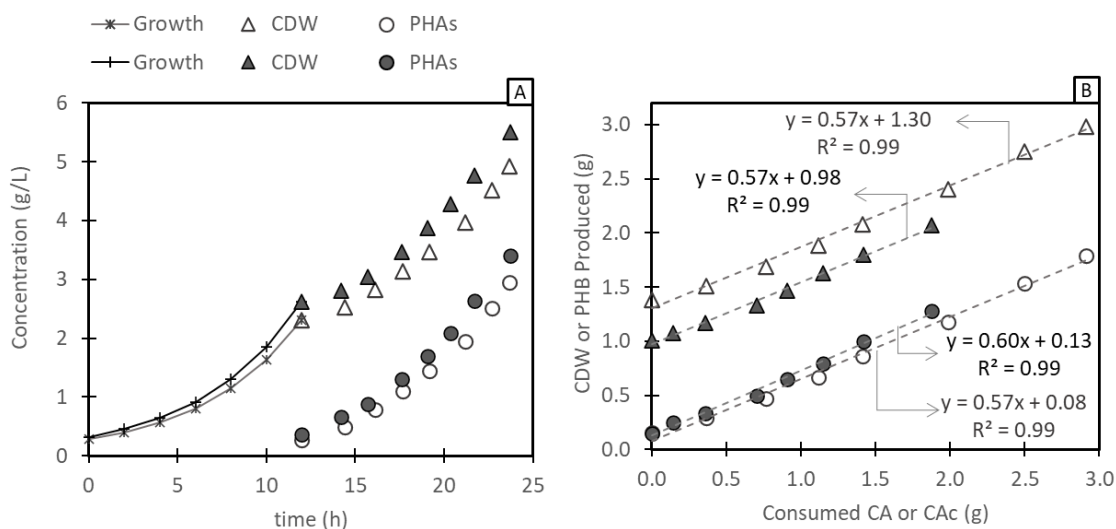
304 To the best of our knowledge, the production of CA through pyrolysis of PHB i)  
305 at temperatures below 200°C, ii) without any catalyst addition, iii) with an overall  
306 yield of 92% and a purity of 98% is peculiar and unique of the thermolytic  
307 distillation approach, representing clear 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<sub>C</sub>) 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).

323



324

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

330

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

340 The PHB yields here obtained with CA and CA<sub>C</sub> (0.60 g<sub>PHB</sub> g<sub>CA</sub><sup>-1</sup> and 0.57 g<sub>PHB</sub>  
341 g<sub>CAc</sub><sup>-1</sup>, respectively, Figure 4b) were similar to what achieved with Na<sup>+</sup> 3-HB  
342 (0.57 g<sub>PHB</sub> g<sub>3-HB</sub><sup>-1</sup>, Flanagan et al., 2016), or to those obtained with volatile fatty  
343 acids from cheese whey (0.54 g<sub>PHAs</sub> g<sub>VFA</sub><sup>-1</sup>, Domingos et al., 2018), and higher  
344 than the yield obtained by using CA and CH<sub>4</sub> as co-substrates (0.45 g<sub>PHA</sub> g<sub>substrate</sub><sup>-1</sup>  
345 Flanagan et al., 2016). The specific PHB production rate (0.08 h<sup>-1</sup>) was similar to  
346 the one calculated from the concentration trend previously reported for a mixed  
347 culture fed with Na<sup>+</sup> 3-HB (0.06-0.1 h<sup>-1</sup>) (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<sup>+</sup> 3-HB) derived from the alkaline hydrolysis of PHB would  
352 require the consumption of inorganic acid (HCl or H<sub>2</sub>SO<sub>4</sub>) during the PHB  
353 production by fermentation: in fact, the microbial consumption of  
354 hydroxybutyrate leads to pH rise since Na<sup>+</sup> gets free.

355 PHB produced by *C. necator* fed with CA or CA<sub>C</sub> 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 *C. necator* after the  
361 fermentation of CA<sub>C</sub> (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

364 PHB. Overall, even if the fermentation could be optimised (e.g. by testing pH-stat  
365 feeding strategy), the results here obtained in the fermentation experiments confirmed  
366 the feasibility of using CA (directly from the thermolytic distillation of PHB, without  
367 any further purification) as substrate, achieving yields and PHB characteristics which  
368 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  
373 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  
375 recycling process capable of by-passing the presence of impurities or small  
376 variations in polymer characteristics seems to be highly promising. According to  
377 the yields here reported ( $0.92 \text{ g}_{\text{CA}}/\text{g}_{\text{PHB}}$  and  $0.60 \text{ g}_{\text{PHB}}/\text{g}_{\text{CA}}$  for the thermolytic  
378 distillation and the microbial conversion processes, respectively), the tandem  
379 thermochemical-biological recycling of  $\text{PHB}_{\text{waste}}$  allows at producing about 0.55  
380  $\text{kg}_{\text{PHB}}/\text{kg}_{\text{PHB}_{\text{waste}}}$  that corresponds to a resource-saving of at least 1  
381  $\text{kg}_{\text{substrate}}/\text{kg}_{\text{PHB}_{\text{waste}}}$ , depending on the carbon source used to feed bacteria and  
382 the PHB yield of such biological process (Domingos et al., 2018; Flanagan et al.,  
383 2016; Myung et al., 2014). Of course, the entire tandem process here proposed is  
384 undoubtedly more energy-demanding than other expected end-of-life (EoL)  
385 scenarios of the  $\text{PHB}_{\text{waste}}$  if managed within a differentiated waste collection  
386 framework (e.g. composting or anaerobic digestion processes together with food  
387 waste). In composting,  $\text{PHB}_{\text{waste}}$  would be partially or completely oxidized to  $\text{CO}_2$

388 and H<sub>2</sub>O, together with the production of compost, whereas anaerobic digestion  
389 would convert PHB<sub>waste</sub> into CH<sub>4</sub>-containing biogas, with a maximum theoretical  
390 yield of 0.42 kgCH<sub>4</sub>/kgPHB<sub>waste</sub> that corresponds to 23.1 MJ of thermal energy  
391 per kg of PHB<sub>waste</sub>. However, in the framework of the PHB circular loop, the  
392 proposed strategy could result in significant energy savings.

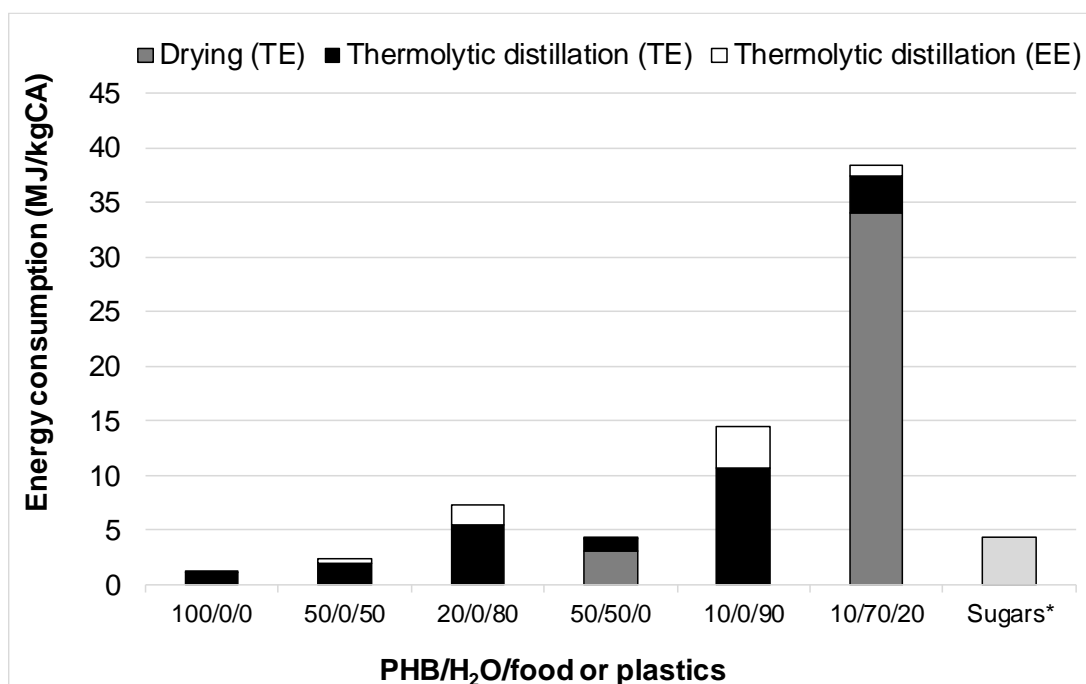
393 Even though a detailed life cycle analysis is beyond the scope of this work, a  
394 preliminary evaluation of the energy required to produce 1 kg of CA from  
395 different PHB sources (PHB<sub>waste</sub>) was performed. Therefore, various PHB<sub>waste</sub>-  
396 scenarios were considered, each including different percentages of PHB, water  
397 and other residues (waste food or other plastics), namely (PHB%/H<sub>2</sub>O%/Other%)  
398 as follows: i) dried and clean PHB<sub>waste</sub> collected through an *ad-hoc* waste  
399 collection and not mixed with other materials (100/0/0); ii) dried PHB<sub>waste</sub> mixed  
400 with other plastics (50/0/50 and 20/0/80) from a plastic waste collection; iii) wet  
401 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  
411 al., 2003) as the contribution of agricultural practice, grain transport and grinding

412 (3.8 MJ/kg<sub>sugar</sub>) together with the contribution of grain cooking and liquefaction  
413 (0.6 MJ/kg<sub>sugar</sub>). The treatment of relatively uncontaminated and dried PHB<sub>waste</sub>  
414 (i.e. 100/0/0 and 50/0/50) requires a limited amount of energy (< 3 MJ/kg<sub>CA</sub>,  
415 Figure 5). If the thermolytic distillation treatment of PHB<sub>waste</sub> with 50% moisture  
416 or of dried PHB<sub>waste</sub> but contaminated with plastic materials (20/0/80) has a still  
417 acceptable energy demand (4 and 7 MJ/kg<sub>CA</sub>, respectively), the production of CA  
418 from PHB<sub>waste</sub> 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<sub>CA</sub>, respectively),  
420 since it needs 3-10 times more energy than that required for obtaining  
421 fermentable sugars (4.4 MJ/kg<sub>sugar</sub>). 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<sub>waste</sub> 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  
 441 **Figure 5.** Energy required to produce CA from various PHB<sub>waste</sub>, or to produce  
 442 fermentable sugars, both usable as substrates by *C. necator* to produce PHB.

443

#### 444 **4. Conclusions**

445 The thermolytic distillation of PHB at low temperature and without any catalyst gives  
 446 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  
 449 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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