



UNIVERSITÀ DI PARMA

ARCHIVIO DELLA RICERCA

University of Parma Research Repository

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

This is the peer reviewed version of the following article:

Original

PHB into PHB: Recycling of polyhydroxybutyrate by a tandem “thermolytic distillation-microbial fermentation” process / Samori, C.; Martinez, G. A.; Bertin, L.; Pagliano, G.; Parodi, A.; Torri, C.; Galletti, P.. - In: RESOURCES, CONSERVATION AND RECYCLING. - ISSN 0921-3449. - 178:(2022), pp. 106082.1-106082.7. [10.1016/j.resconrec.2021.106082]

Availability:

This version is available at: 11381/3006604 since:

Publisher:

Published

DOI:10.1016/j.resconrec.2021.106082

Terms of use:

Anyone can freely access the full text of works made available as "Open Access". Works made available

Publisher copyright

note finali coverpage

(Article begins on next page)

This is the final peer-reviewed accepted manuscript of:

Chiara Samorì, Gonzalo Agustin Martinez, Lorenzo Bertin, Giorgia Pagliano, Adriano Parodi, Cristian Torri, Paola Galletti, 2022. "PHB into PHB: Recycling of polyhydroxybutyrate by a tandem "thermolytic distillation-microbial fermentation" process". Resources, Conservation and Recycling, 178, 106082

The final published version is available online at:
<https://doi.org/10.1016/j.resconrec.2021.106082>

Terms of use:

Some rights reserved. The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. For all terms of use and more information see the publisher's website.

This item was downloaded from IRIS Università di Bologna (<https://cris.unibo.it/>)

When citing, please refer to the published version.

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

3

4 *Chiara Samorì,^{*,a,b} Gonzalo Agustin Martinez, ^{*,c} Lorenzo Bertin,^c Giorgia Pagliano,^a*
5 *Adriano Parodi,^a Cristian Torri,^{a,b} Paola Galletti^{a,b}*

6

7 ^a Dipartimento di Chimica "Giacomo Ciamician", University of Bologna, via
8 Sant'Alberto 163, Ravenna, Italy

9 ^b CIRI-FRAME, University of Bologna, via Sant'Alberto 163, Ravenna, Italy

10 ^c Dipartimento di Ingegneria Civile, Chimica, Ambientale e dei Materiali, University of
11 Bologna, viale Risorgimento 2, Bologna, Italy

12

13 **Corresponding Author**

14 * Chiara Samorì, email address: chiara.samori3@unibo.it

15 * Gonzalo Agustin Martinez, email address: gonzalo.martinez3@unibo.it

16

17

18

19

20

21

22

23

24

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_{waste} with 50% moisture has a still
38 acceptable energy demand (4 MJ/kg_{crotonic acid}), comparable to the energy required for
39 obtaining fermentable sugars (4.4 MJ/kg_{sugar}) 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 CO₂ whereas the latter (i.e. with anaerobic pathways)
60 degrade PHAs into volatile fatty acids (VFA) and subsequently into CH₄-rich
61 biogas. In both mineralisation strategies, CO₂ 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 CH₄ 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.

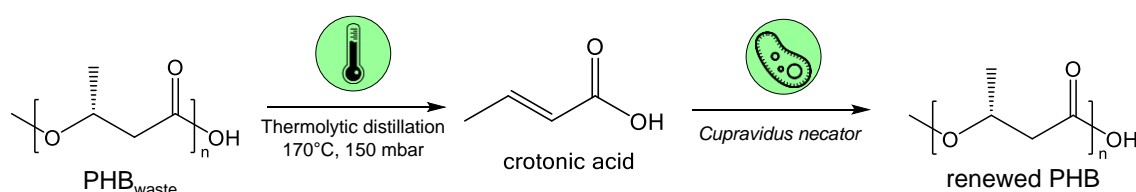
71 Hence, two more straightforward cradle-to-cradle strategies could be adopted for
72 the end-of-life management of waste PHAs oriented to the synthesis of renewed
73 PHAs avoiding monomers mineralisation and thus material and energy leakages:
74 *i)* exploiting (thermo)chemistry to de-polymerize PHAs 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



134

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

185 The preliminary evaluation of the energy consumption (expressed as MJ/kg_{CA}) 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_{CA})
191 for treating various PHB_{waste} through thermolytic distillation was done by
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}
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⁻¹) which was used as a carbon source during the
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
207 (100 g L⁻¹ of CA) based on a pO₂-stat strategy. A control condition (0.6 L of
208 working volume) was also experimented by using commercial crotonic acid

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
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_w) 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 β -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

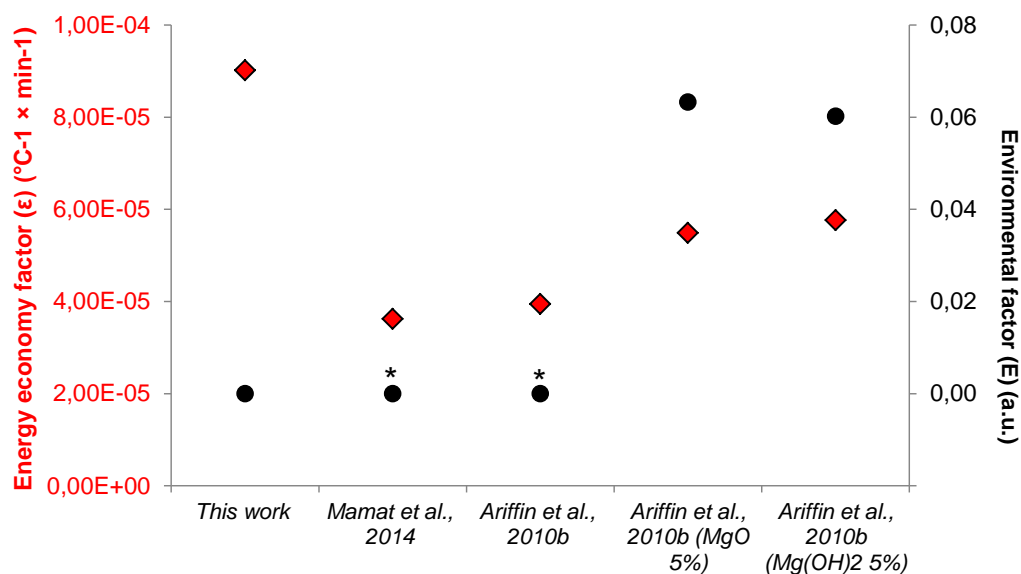
256 literature (Ariffin et al., 2010b; Mamat et al., 2014; see Table 1S in ESI). To
257 evaluate the depolymerization results currently described (Ariffin et al., 2010b;
258 Mamat et al., 2014; Parodi et al. 2021) as a function of the depolymerization
259 variables (temperature, time, and catalyst) and 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 (ϵ , 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 ϵ 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)₂, 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 ϵ value (Figure 2).

295



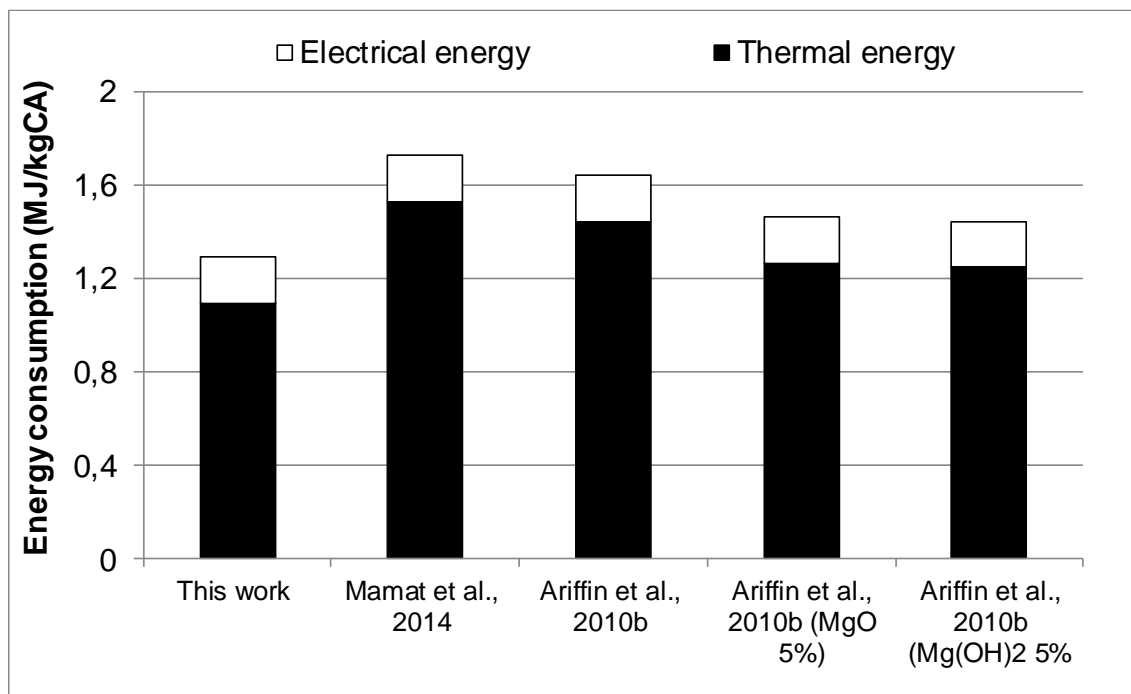
* solvents will be necessary to purify CA before the use

296

297 **Figure 2.** Energy economy factor (ϵ , \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

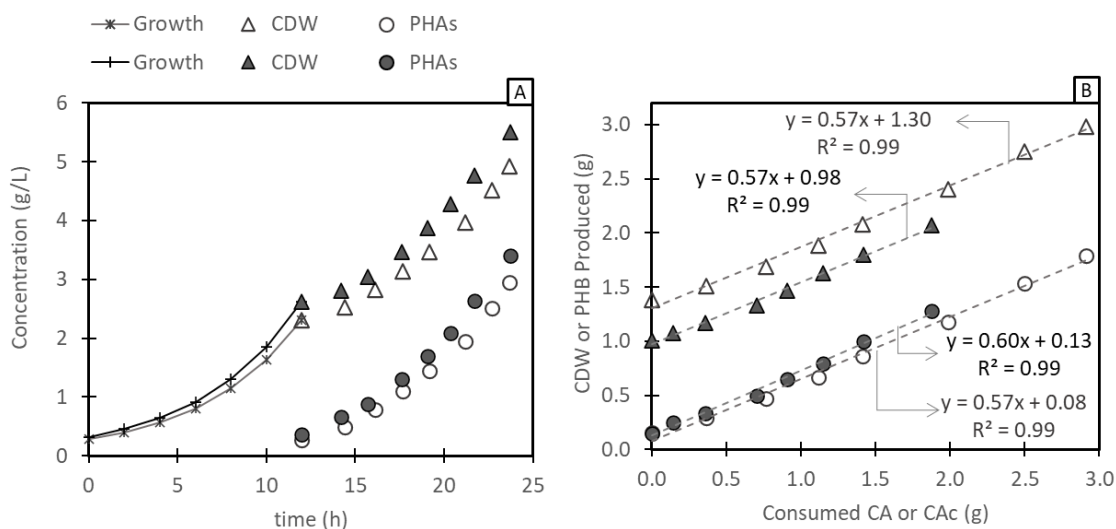
304 To the best of our knowledge, the production of CA through pyrolysis of PHB i)
305 at temperatures below 200°C, ii) without any catalyst addition, iii) with an overall
306 yield of 92% and a purity of 98% is peculiar and unique of the thermolytic
307 distillation approach, representing clear progress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

323



324

325 **Figure 4.** PHB production from CA (filled symbols) and CA_c (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_c) towards bacteria was avoided by keeping
 332 the concentration almost zero all along with the accumulation phase through a
 333 pO₂-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⁻¹ up to 5 g L⁻¹, 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_c at the end of the
 339 accumulation phase was 63%.

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_{CA_C}⁻¹, respectively, Figure 4b) were similar to what achieved with Na⁺ 3-HB
342 (0.57 g_{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 CH₄ 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 H₂SO₄) 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 *C. necator* 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

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 CO_2

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

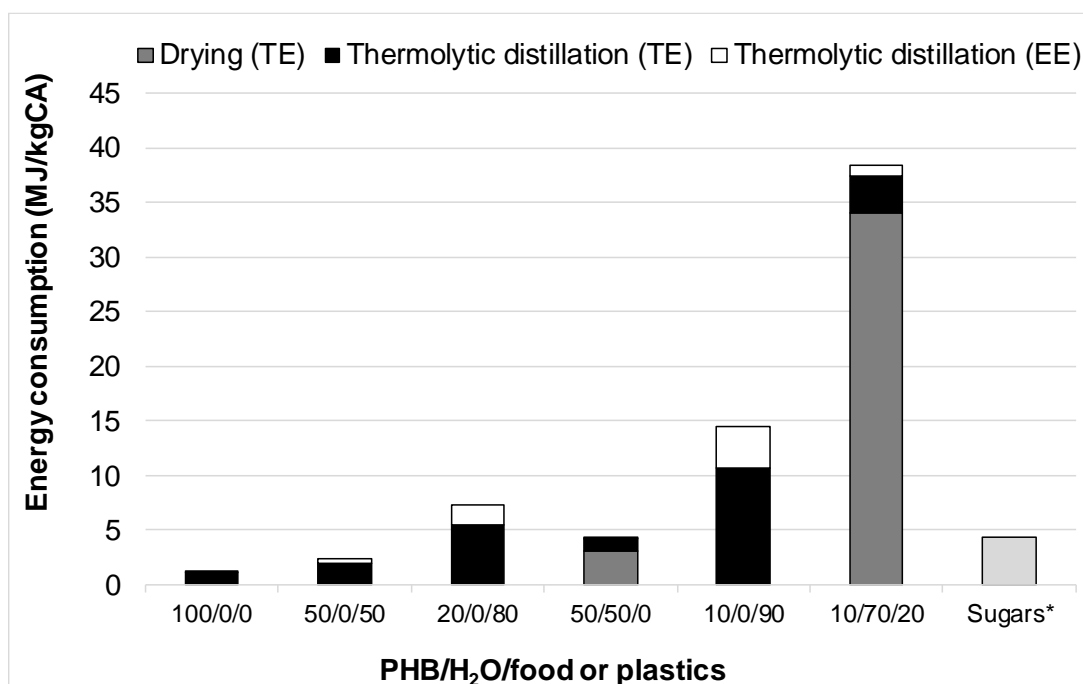
393 Even though a detailed life cycle analysis is beyond the scope of this work, a
394 preliminary evaluation of the energy required to produce 1 kg of CA from
395 different PHB sources (PHB_{waste}) was performed. Therefore, various PHB_{waste}-
396 scenarios were considered, each including different percentages of PHB, water
397 and other residues (waste food or other plastics), namely (PHB%/H₂O%/Other%)
398 as follows: i) dried and clean PHB_{waste} collected through an *ad-hoc* waste
399 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
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_{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
 441 **Figure 5.** Energy required to produce CA from various PHB_{waste}, 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.

Acknowledgement

The study was conducted within the project “B-PLAS DEMO” funded by EIT-CLIMATE KIC. We thank Regione Emilia-Romagna (Bando “Alte competenze per la ricerca e il trasferimento tecnologico” – POR FSE 2014/2020, Obiettivo tematico 10) for PhD funding.

References

- Abbondanzi, F., Biscaro, G., Carvalho, G., Favaro, L., Lemos, P., Paglione, M., Samorì, C., Torri, C., 2017. Fast method for the determination of short-chain-length polyhydroxyalkanoates (scl-PHAs) in bacterial samples by In Vial-Thermolysis (IVT). *N. Biotechnol.* 39. <https://doi.org/10.1016/j.nbt.2017.05.012>
- Abdelwahab, M.A., Flynn, A., Chiou, B.-S., Imam, S., Orts, W., Chiellini, E., 2012. Thermal, mechanical and morphological characterization of plasticized PLA–PHB blends. *Polym. Degrad. Stab.* 97, 1822–1828. <https://doi.org/https://doi.org/10.1016/j.polymdegradstab.2012.05.036>
- Ariffin, H., Nishida, H., Hassan, M.A., Shirai, Y., 2010a. Chemical recycling of polyhydroxyalkanoates as a method towards sustainable development. *Biotechnol. J.* 5, 484–492. <https://doi.org/10.1002/biot.200900293>
- Ariffin, H., Nishida, H., Shirai, Y., Hassan, M.A., 2010b. Highly selective transformation of poly[(R)-3-hydroxybutyric acid] into trans-crotonic acid by catalytic thermal degradation. *Polym. Degrad. Stab.* 95, 1375–1381. <https://doi.org/10.1016/j.polymdegradstab.2010.01.018>
- Barnard, E., Rubio Arias, J.J., Thielemans, W., 2021. Chemolytic depolymerisation of PET: a review. *Green Chem.* <https://doi.org/10.1039/d1gc00887k>

- Basaglia, M., Favaro, L., Torri, C., Casella, S., 2021. Is pyrolysis bio-oil prone to microbial conversion into added-value products? *Renew. Energy* 163, 783–791. <https://doi.org/10.1016/j.renene.2020.08.010>
- Brown, R., 2007. Hybrid Thermochemical / Biological Processing. *Appl. Biochem. Biotechnol.* 136–140, 947.
- Clark, J.M., Pilath, H.M., Mittal, A., Michener, W.E., Robichaud, D.J., Johnson, D.K., 2016. Direct Production of Propene from the Thermolysis of Poly(β -hydroxybutyrate) (PHB). An Experimental and DFT Investigation. *J. Phys. Chem. A* 120, 332–345. <https://doi.org/10.1021/acs.jpca.5b09246>
- David, G., Vannini, M., Sisti, L., Marchese, P., Celli, A., Gontard, N., Angellier-Coussy, H., 2020. Eco-Conversion of Two Winery Lignocellulosic Wastes into Fillers for Biocomposites: Vine Shoots and Wine Pomaces. *Polym.* . <https://doi.org/10.3390/polym12071530>
- Domingos, J.M.B., Puccio, S., Martinez, G.A., Amaral, N., Reis, M.A.M., Bandini, S., Fava, F., Bertin, L., 2018. Cheese whey integrated valorisation: Production, concentration and exploitation of carboxylic acids for the production of polyhydroxyalkanoates by a fed-batch culture. *Chem. Eng. J.* 336, 47–53. <https://doi.org/https://doi.org/10.1016/j.cej.2017.11.024>
- Fernández-Dacosta, C., Posada, J.A., Ramirez, A., 2016. Techno-economic and carbon footprint assessment of methyl crotonate and methyl acrylate production from wastewater-based polyhydroxybutyrate (PHB). *J. Clean. Prod.* 137, 942–952. <https://doi.org/10.1016/j.jclepro.2016.07.152>
- Flanagan, J.C.A., Myung, J., Criddle, C.S., Waymouth, R.M., 2016. Poly(hydroxyalkanoate)s from Waste Biomass: A Combined Chemical–Biological

- Approach. *ChemistrySelect* 1, 2327–2331. <https://doi.org/10.1002/slct.201600592>
- Gorke, J.T., Okrasa, K., Louwagie, A., Kazlauskas, R.J., Srienc, F., 2007. Enzymatic synthesis of poly(hydroxyalkanoates) in ionic liquids. *J. Biotechnol.* 132, 306–313. <https://doi.org/10.1016/j.jbiotec.2007.04.001>
- Goto, M., 2009. Chemical recycling of plastics using sub- and supercritical fluids. *J. Supercrit. Fluids* 47, 500–507. <https://doi.org/https://doi.org/10.1016/j.supflu.2008.10.011>
- Kang, S., Yu, J., 2014. One-pot production of hydrocarbon oil from poly(3-hydroxybutyrate). *RSC Adv.* 4, 14320–14327. <https://doi.org/10.1039/c4ra00892h>
- Kopinke, F.D., Remmler, M., Mackenzie, K., 1996. Thermal decomposition of biodegradable polyesters - I: Poly(β -hydroxybutyric acid). *Polym. Degrad. Stab.* 52, 25–38. [https://doi.org/10.1016/0141-3910\(95\)00221-9](https://doi.org/10.1016/0141-3910(95)00221-9)
- Lamberti, F.M., Román-Ramírez, L.A., Wood, J., 2020. Recycling of Bioplastics: Routes and Benefits. *J. Polym. Environ.* 28, 2551–2571. <https://doi.org/10.1007/s10924-020-01795-8>
- Li, Y., Strathmann, T.J., 2019. Kinetics and mechanism for hydrothermal conversion of polyhydroxybutyrate (PHB) for wastewater valorization. *Green Chem.* 21, 5586–5597. <https://doi.org/10.1039/c9gc02507c>
- Mamat, M.R.Z., Ariffin, H., Hassan, M.A., Mohd Zahari, M.A.K., 2014. Bio-based production of crotonic acid by pyrolysis of poly(3-hydroxybutyrate) inclusions. *J. Clean. Prod.* 83, 463–472. <https://doi.org/10.1016/j.jclepro.2014.07.064>
- Morikawa, H., Marchessault, R.H., 1981. Pyrolysis of bacterial polyalkanoates. *Can. J. Chem.* 59, 2306–2313. <https://doi.org/10.1139/v81-334>
- Mullen, C.A., Boateng, A.A., Schweitzer, D., Sparks, K., Snell, K.D., 2014. Mild

pyrolysis of P3HB/switchgrass blends for the production of bio-oil enriched with crotonic acid. *J. Anal. Appl. Pyrolysis* 107, 40–45.

<https://doi.org/10.1016/j.jaap.2014.01.022>

Myung, J., Strong, N.I., Galega, W.M., Sundstrom, E.R., Flanagan, J.C.A., Woo, S.G., Waymouth, R.M., Criddle, C.S., 2014. Disassembly and reassembly of polyhydroxyalkanoates: Recycling through abiotic depolymerization and biotic repolymerization. *Bioresour. Technol.* 170, 167–174.

<https://doi.org/10.1016/j.biortech.2014.07.105>

Myung, J., Strong, N.I., Galega, W.M., Sundstrom, E.R., Flanagan, J.C.A., Woo, S.G., Waymouth, R.M., Criddle, C.S., Basaglia, M., Favaro, L., Torri, C., Casella, S., Reddy, C.S.K., Ghai, R., Rashmi, Kalia, V.C., Wendlandt, K.D., Jechorek, M., Helm, J., Stottmeister, U., Gorke, J.T., Okrasa, K., Louwagie, A., Kazlauskas, R.J., Srien, F., Before, C., Shen, Y., Jarboe, L., Brown, R., Wen, Z., Flanagan, J.C.A., Myung, J., Criddle, C.S., Waymouth, R.M., 2007. Producing poly-3-hydroxybutyrate with a high molecular mass from methane. *Bioresour. Technol.* 86, 127–133. [https://doi.org/10.1016/S0960-8524\(02\)00212-2](https://doi.org/10.1016/S0960-8524(02)00212-2)

Nishida, H., Ariffin, H., Shirai, Y., Hass, M., 2010. Precise Depolymerization of Poly(3-hydroxybutyrate) by Pyrolysis. *Biopolymers*. <https://doi.org/10.5772/10270>

Parodi, A., Ambrosio, M.D., Mazzocchetti, L., Martinez, G.A., Samorì, C., Torri, C., Galletti, P., 2021a. Chemical Recycling of Polyhydroxybutyrate (PHB) into Bio-Based Solvents and Their Use in a Circular PHB Extraction. *ACS Appl. Biomater.* 9, 12575. <https://doi.org/10.1021/acssuschemeng.1c03299>

Parodi, A., Jorea, A., Fagnoni, M., Ravelli, D., Samorì, C., Torri, C., Galletti, P., 2021b. Bio-based crotonic acid from polyhydroxybutyrate: synthesis and photocatalyzed

- hydroacylation. *Green Chem.* 23, 3420–3427. <https://doi.org/10.1039/d1gc00421b>
- Reddy, C.S.K., Ghai, R., Rashmi, Kalia, V.C., 2003. Polyhydroxyalkanoates: An overview. *Bioresour. Technol.* 87, 137–146. [https://doi.org/10.1016/S0960-8524\(02\)00212-2](https://doi.org/10.1016/S0960-8524(02)00212-2)
- Samorì, C., Basaglia, M., Casella, S., Favaro, L., Galletti, P., Giorgini, L., Marchi, D., Mazzocchetti, L., Torri, C., Tagliavini, E., 2015. Dimethyl carbonate and switchable anionic surfactants: Two effective tools for the extraction of polyhydroxyalkanoates from microbial biomass. *Green Chem.* 17. <https://doi.org/10.1039/c4gc01821d>
- Samorì, C., Kiwan, A., Torri, C., Conti, R., Galletti, P., Tagliavini, E., 2019. Polyhydroxyalkanoates and Crotonic Acid from Anaerobically Digested Sewage Sludge. *ACS Sustain. Chem. Eng.* 7. <https://doi.org/10.1021/acssuschemeng.8b06615>
- Shen, Y., Jarboe, L., Brown, R., Wen, Z., 2015. A thermochemical-biochemical hybrid processing of lignocellulosic biomass for producing fuels and chemicals. *Biotechnol. Adv.* 33, 1799–1813. <https://doi.org/10.1016/j.biotechadv.2015.10.006>
- Song, X., Liu, F., Wang, H., Wang, C., Yu, S., Liu, S., 2018. Methanolysis of microbial polyester poly(3-hydroxybutyrate) catalyzed by Brønsted-Lewis acidic ionic liquids as a new method towards sustainable development. *Polym. Degrad. Stab.* 147, 215–221. <https://doi.org/10.1016/j.polymdegradstab.2017.12.009>
- Spekreijse, J., Le Nôtre, J., Sanders, J.P.M., Scott, E.L., 2015. Conversion of polyhydroxybutyrate (PHB) to methyl crotonate for the production of biobased monomers. *J. Appl. Polym. Sci.* 132, 1–8. <https://doi.org/10.1002/app.42462>
- Torri, C., Cordiani, H., Samorì, C., Favaro, L., Fabbri, D., 2014. Fast procedure for the

analysis of poly(hydroxyalkanoates) in bacterial cells by off-line pyrolysis/gas-chromatography with flame ionization detector. *J. Chromatogr. A* 1359.

<https://doi.org/10.1016/j.chroma.2014.07.008>

Torri, C., Weme, T.D.O., Samorì, C., Kiwan, A., Brilman, D.W.F., 2017. Renewable alkenes from the hydrothermal treatment of polyhydroxyalkanoates-containing sludge. *Environ. Sci. Technol.* 51. <https://doi.org/10.1021/acs.est.7b03927>

Vermeer, C.M., Rossi, E., Tamis, J., Jonkers, H.M., Kleerebezem, R., 2021. From waste to self-healing concrete: A proof-of-concept of a new application for polyhydroxyalkanoate. *Resour. Conserv. Recycl.* 164, 105206.

<https://doi.org/10.1016/j.resconrec.2020.105206>

Yang, X., Odelius, K., Hakkarainen, M., 2014. Microwave-assisted reaction in green solvents recycles PHB to functional chemicals. *ACS Sustain. Chem. Eng.* 2, 2198–2203. <https://doi.org/10.1021/sc500397h>

Yates, M.R., Barlow, C.Y., 2013. Life cycle assessments of biodegradable, commercial biopolymers - A critical review. *Resour. Conserv. Recycl.* 78, 54–66.

<https://doi.org/10.1016/j.resconrec.2013.06.010>

