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

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Abstract

The recycle of polyhydroxybutyrate (PHB) *via* depolymerization-polymerization is here 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 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 purification as a substrate for producing renewed PHB with a culture of *Cupriavidus necator.* The yield of PHB (0.6 g PHB per g of crotonic acid), final PHB content (63%), 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 consumption of various depolymerization approaches indicated that the thermolytic distillation consumes 20-25% less energy than previously reported methods; the energy 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 obtaining fermentable sugars (4.4 MJ/kg_{sugar}) to be used for feeding bacteria that accumulate PHB.

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

1. Introduction

Polyhydroxyalkanoates (PHAs) are bio-based polyesters produced by a variety of organisms able to accumulate PHA granules inside the cells as energy and carbon storage. Thanks to their biodegradability under aerobic and anaerobic conditions, PHAs could meet the demand for biobased plastics from renewable resources. Importantly, PHAs could be applied for functional products expected to be degraded (e.g. seed or herbicide containing capsules) or in conventional applications where degradation is not required (e.g. bags, packaging). In this last case, their biodegradability contributes to avoiding pollution when the plastic product (or a piece of it) accidentally leaks into the environment.

In the cradle-to-grave approach initially considered for conventional PHAs applications, its end-of-life management relies on biological strategies like composting or anaerobic digestion. The former (i.e. with aerobic pathways) degrade PHAs directly into CO_2 whereas the latter (i.e. with anaerobic pathways) degrade PHAs into volatile fatty acids (VFA) and subsequently into CH_4-rich biogas. In both mineralisation strategies, CO_2 can be reused for example by cyanobacteria to directly produce PHAs (Reddy et al., 2003) or to grow plants that can be used in fermentation for renewed PHAs production (Lamberti et al., 2020), while VFA and CH_4 produced in the anaerobic pathway can be recycled back into PHAs by mixed microbial cultures (MMC) or type II methanotrophic (methane-utilizing) bacteria (Myung et al., 2015). However, such PHA-regeneration options could result in: *i)* a too slow or prolonged overall process for the plastic market (Yates and Barlow, 2013) and *ii*) energy dispendious since all

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

Hence, two more straightforward cradle-to-cradle strategies could be adopted for the end-of-life management of waste PHAs oriented to the synthesis of renewed PHAs avoiding monomers mineralisation and thus material and energy leakages: *i)* exploiting (thermo)chemistry to de-polymerize PHAs and chemistry to re-polymerize it or *ii)* exploiting (thermo)chemistry to depolymerize PHAs and biology to re-polymerize it (Brown, 2007; Shen et al., 2015). Since the chemo-catalysed synthesis of PHA produces polymers with a much lower molecular weight than what is achievable through microbial processes (Gorke et al., 2007), the second strategy that avoids downgrading PHAs mechanical properties seems more suitable for a circular economy scenario. Considering that the biological 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 production of effluents with constant and targeted features, to feed the PHA production process with non-variable feedstock thus obtaining well-defined and reproducible products and productivities; 3) avoid the formation of specific inhibitors of microbial growth such as furans, phenolic compounds, and ketones (Basaglia et al., 2021).

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; 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 al., 2016; Spekreijse et al., 2015), methyl acrylate (Fernández-Dacosta et al., 2016), cyclic and linear oligomers (Morikawa and Marchessault, 1981; Nishida et al., 2010), 3-hydroxybutyric acid (3-HB) (Li and Strathmann, 2019; Yang et al., 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 alkaline hydrolysis of PHB has been demonstrated to be a fermentable substrate for producing PHB through aerobic processes mediated by a mixed microbial consortium (*Comamonas*, *Brachymonas* and *Acinetobacter*, Myung et al., 2014). Recently, we have explored the use of PHB as a polymeric platform for producing fine chemicals and solvents: to this purpose, two PHB monomer derivatives obtainable by alcoholysis and thermolysis of PHB bonds (i.e. methyl 3-hydroxybutanoate and CA, respectively) have been used as building blocks for synthesising bio-based solvents capable to extract PHB from inside the bacteria (Parodi et al., 2021a), and photo-functionalized aldehydes (Parodi et al., 2021b). To this purpose, we have developed an innovative thermal depolymerization process (named “thermolytic distillation”) capable to break PHB or PHB inclusions inside bacterial cells, achieving yields and selectivity of CA never reported before (Parodi et al., 2021b). To extend the potentiality of PHB as a polymeric platform and widen the exploitation of PHB monomers, herein we used CA as an unprecedented building block for synthesising PHB. Specifically, we aimed at integrating our depolymerization protocol (thermolytic distillation) using the resulting monomeric fraction enriched in CA for directly producing PHB, providing the first tandem depolymerization/polymerization cycle of PHB.

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

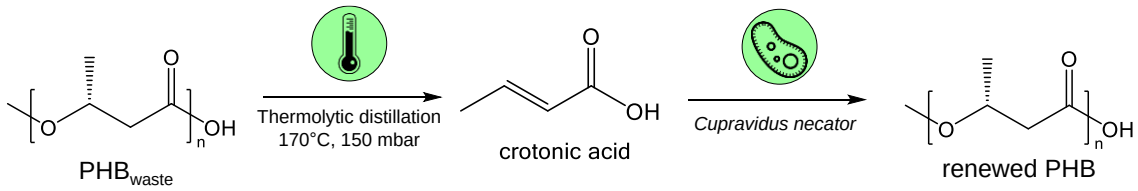

Figure 1. Proposed tandem "thermolytic distillation-microbial fermentation" process for producing PHB from PHB.

2. Materials and methods

2.1 Chemicals

All solvents (dimethyl carbonate, and acetonitrile) and chemicals (trans-crotonic acid, and glucose) used in this study were obtained from Sigma-Aldrich (purities ≥ 98%) and were used without purification. Standard PHB was purchased from Biomer (DE).

2.2 Thermolytic distillation to produce CA

CA was produced from PHB following the procedure reported in the literature (Parodi et al., 2021b) by treating PHB (5 g) at 170°C under reduced pressure (150 mbar). The yield of the distilled fraction was defined as follows (Parodi et al., 2021b):

$$Yield_{distillates}\ (w/w_{PHB}\%) = Distillates\ (g)\ /\ PHB\ (g) * 100 \quad (Eq.\ 1)$$

The yield of CA on a distilled fraction weight basis ($Yield_{CA\text{-}distillates}$, $w/w_{distillates}\%$) was calculated by GC-MS analysis as described below. Consequently, the yield of CA on a PHB weight basis was determined as follows (Parodi et al., 2021b):

$$Yield_{CA}\ (w/w_{PHB}\%) = Yield_{distillates}\ (w/w_{PHB}\%) * Yield_{CA\text{-}distillates}(w/w_{distillates}\%) \quad (Eq.\ 2)$$

The recovery of CA (%) achievable from PHB was calculated as follows, on the assumption that 1 g of PHB will give 1 g of CA ($Yield_{CA\text{-}theoretical}$, w/w_{PHB}%) (Mamat et al., 2014):

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

2.3 Evaluation of green metrics and energy consumption

The comparison between the various depolymerization procedures of PHB reported in the literature was done by applying two coefficients (Energy economy coefficient ε, and Environmental factor E,) proposed for PET depolymerization (Barnard et al., 2021), and here adopted to the specific case of thermal PHB depolymerization:

$$Energy\ economy\ coefficient\ (\varepsilon,\ °C^{-1} \times min^{-1}) = Yield_{CA}\ (w/w\%)\ /\ T\ (°C) \times t\ (min) \quad (Eq.\ 4)$$

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

$$Environmental\ factor\ (E,\ a.u.) = [0.1*(solvent/PHB)+(catalyst/PHB)+(others/PHB)]/\ Yield_{CA}\ (w/w\%) \quad (Eq.\ 5)$$

where the solvent to PHB mass ratio has been multiplied by 0.1 considering that 90% of solvents are typically recycled in industrial processes (Barnard et al., 2021).

The preliminary evaluation of the energy consumption (expressed as MJ/kg_{CA}) of various depolymerization processes was done by considering the thermal energy (TE) for heating PHB to the temperatures at which the depolymerization occurred, and the electrical energy (EE) for pumping down the vapours during the depolymerization under vacuum (see ESI for further detail).

The preliminary evaluation of the energy requirements (expressed as MJ/kg_{CA}) for treating various PHB_{waste} through thermolytic distillation was done by considering the i) TE for drying PHB_{waste} to almost 0% moisture, ii) TE for 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 thermolytic distillation under vacuum (see Figure 2S and calculations in ESI for further details).

2.4 Production of PHB from CA with Cupriavidus necator

The production of PHB was carried out using two bench-scale bioreactors (Infors, Minifors 2, 2L) using *Cupriavidus necator* strain (DSMZ 545) according to a dual-phase process, the procedures and conditions reported in the literature (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 batch balance growth (first phase of cell growth). Thereafter, once glucose and NH_4^+ were consumed, the second phase (accumulation of PHB) was started by feeding a concentrated water solution of CA contained in the distilled fraction (100 g L^{-1} of CA) based on a pO_2-stat strategy. A control condition (0.6 L of working volume) was also experimented by using commercial crotonic acid

(CA_C) to verify whether other C4-byproducts in the collected distilled fraction derived from the thermal distillation of PHB could affect the subsequent PHB accumulation in bacteria. Both experimental conditions were carried out in triplicate and monitored by sampling periodically. After measuring the absorbance, samples were centrifuged (14.000 rpm, 10 min), the liquid fraction was analysed by HPLC-RID (Domingos et al., 2018) for determining residual CA amount and the pellet was freeze-dried, weighted to determine the cell dry weight (CDW) and analysed to determine PHB content (Abbondanzi et al., 2017).

2.5 Analytical procedures

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).

3. Results and discussion

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

The thermal degradation of PHAs is known to occur through β-elimination reactions that randomly break the chains and give dehydrated trans-alkenoic acids (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 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 (Ariffin et al., 2010a, 2010b; Flanagan et al., 2016; Kopinke et al., 1996).

In the present paper, we used a peculiar one-step procedure called "thermolytic distillation" that was developed in our previous paper (Parodi et al. 2021). Although the applied temperature (170°C) was largely below the maximum decomposition temperature of PHB (290°C) (Abdelwahab et al., 2012), it resulted enough for giving a yield of CA of 92% based on PHB input. This finding could be supported by the described weight loss at 180°C found for bacterial biomass containing PHB as inclusions (Kopinke et al., 1996), presumably associated with the formation of volatile C_4 compounds. The obtained distilled fraction was mainly composed of CA (98%) and minor amounts of cis-crotonic acid (0.1%) and dimer of PHB (0.2%), in line with the best results reported in the

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; Mamat et al., 2014; Parodi et al. 2021) as a function of the depolymerization variables (temperature, time, and catalyst) and not only in terms of depolymerization performances, we applied different green chemistry metrics parameters, useful for making a numerical comparison of different depolymerization approaches and determining their relative feasibility (Figure 2): 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 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 evaluation is crucial for the scalability of the technology, assisting in finding the best routes for viable implementation of present and future studies of depolymerization in the field of polymeric material recycling (Barnard et al., 2021).

The best processes would tend to present low values of E factor (low mass inputs, thus low consumption of solvents and catalysts) and high ε values (low energy consumption, thus short reaction time and mild reaction temperature). When the depolymerization of PHB was conducted without catalysts (Ariffin et al., 2010b; Mamat et al., 2014; Parodi et al., 2021b), the E factor was equal to 0, differently 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 selectivity (57-63%, see Table 1S in ESI) of the two literature procedures that did not make use of catalysts hampers the exploitation of such distilled fractions as

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 (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 other hand, the high purity of the distilled fraction obtained through the thermolytic distillation here adopted (98%) is equal to the purity of commercial CA, guaranteeing its exploitation without any further purification.

A preliminary evaluation of the energy consumption (MJ/kg_{CA}) of the depolymerization processes reported in the literature by taking into account the thermal energy (TE) for heating PHB to the temperature at which the depolymerization of PHB chains occurs, and the electrical energy (EE) for pumping down the vapours during the depolymerization under vacuum (Figure 3), highlighted that the process here applied was the least energy-intensive (20-25% lower than the non-catalytic approaches and 12% than the catalytic ones) and the highest in terms of ε value (Figure 2).

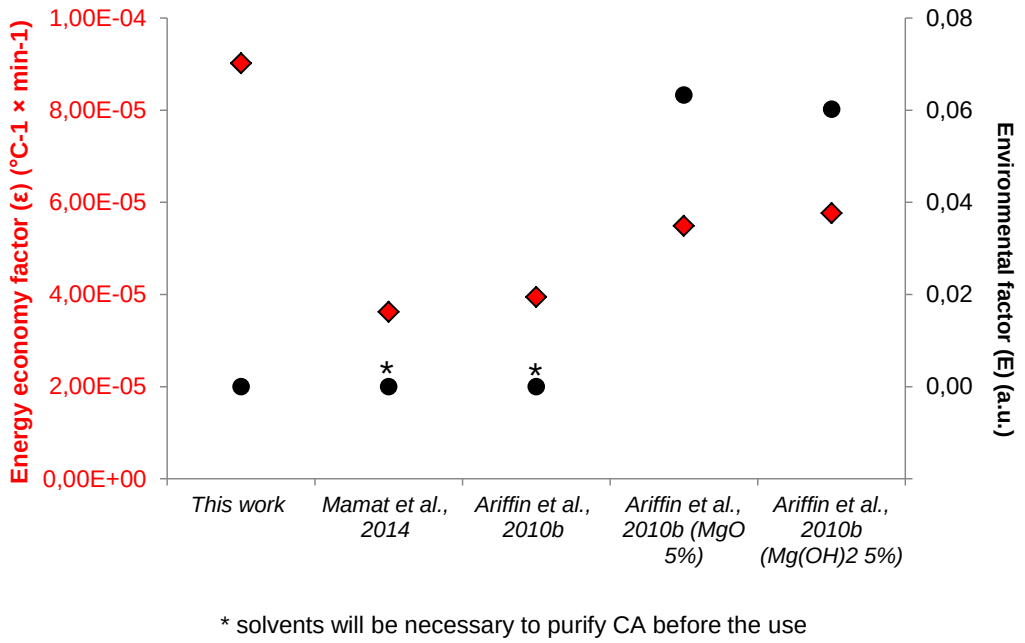

Figure 2. Energy economy factor (ε,◆) and Environmental factor (E,●) for the most significant thermal depolymerizations of PHB reported in the literature.

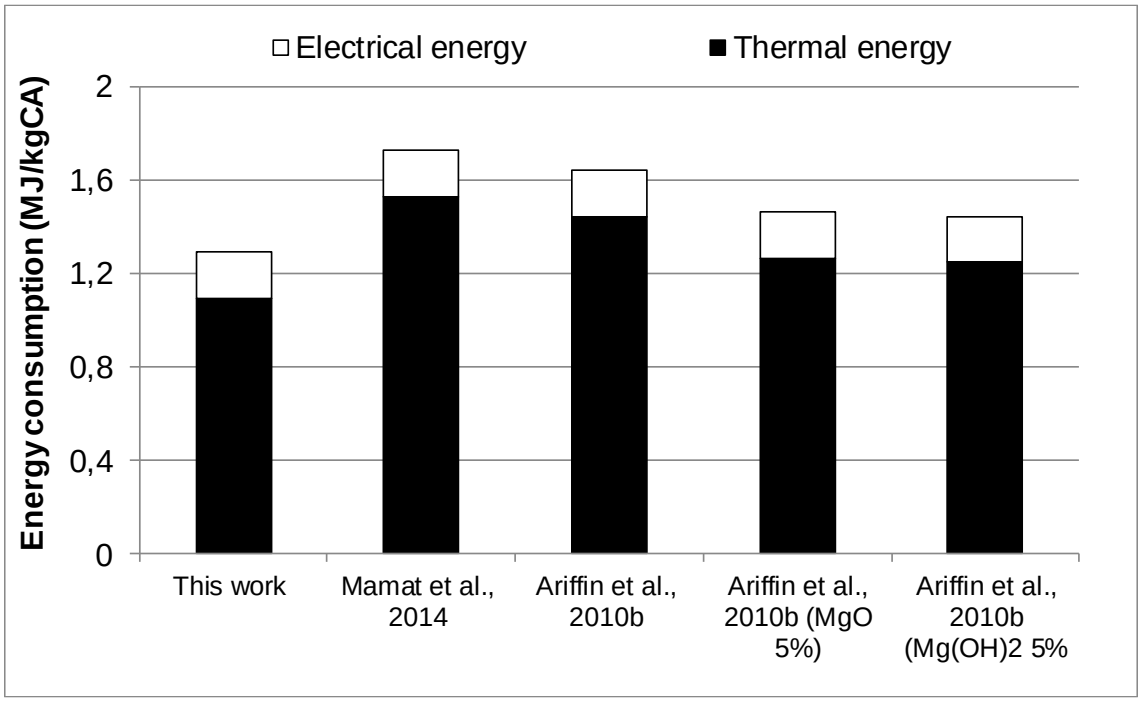

Figure 3. Energy required to produce CA from PHB through the most significant thermal depolymerizations reported in the literature.

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

3.2 Production of PHB from CA with Cupriavidus necator

The entire distilled fraction produced via the thermolytic distillation of PHB without any further purification and commercial CA (CA_C) were used as carbon sources for *C. necator* in the PHB-accumulation phase of a dual-stage fermentation process (Figures 4a and 4b).

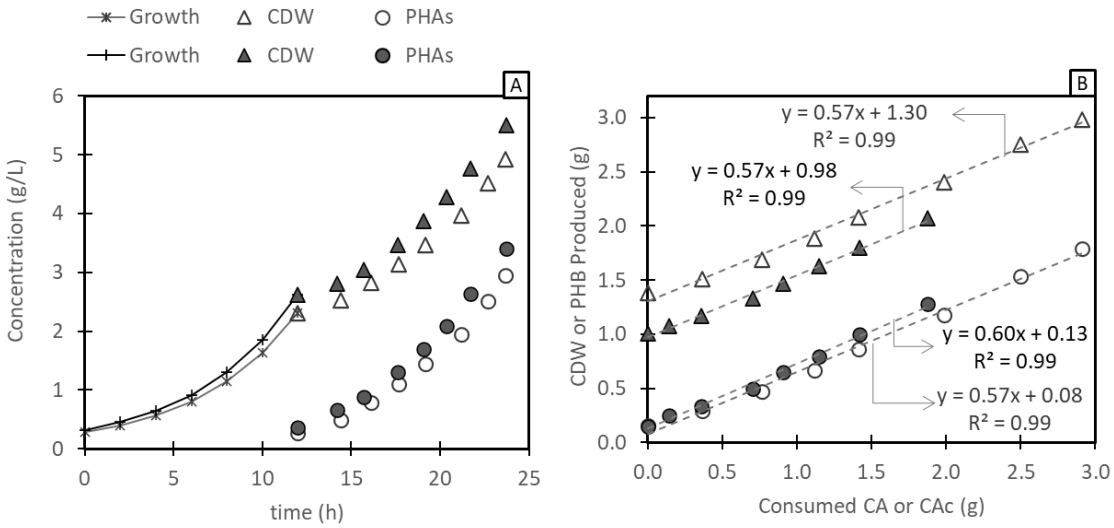

Figure 4. PHB production from CA (filled symbols) and CA_c (empty symbols): a) concentration trends during the growth and accumulation phases (CDW and PHAs); b) yield on the produced CDW and PHAs from the total acids consumed during the accumulation phase. Reported points represent the average of the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

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 pO_2-stat feeding strategy. Concentration profiles were almost identical for both conditions: in fact, both CDW parameters increased by a similar trend, from about 2 g L^{-1} up to 5 g L^{-1}, confirming the equivalency of the two carbon sources used. As expected, during the accumulation phase the cell duplication did not occur, i.e. the non-PHB biomass concentration did not increase. The PHB content in *C. necator* after the fermentation of both CA and CA_C at the end of the accumulation phase was 63%.

The PHB yields here obtained with CA and CA_C (0.60 g_{PHB} g_{CA}^{-1} and 0.57 g_{PHB} g_{CAc}^{-1}, respectively, Figure 4b) were similar to what achieved with Na^+ 3-HB (0.57g_{PHB} $g_{3\text{-}HB}^{-1}$, Flanagan et al., 2016), or to those obtained with volatile fatty acids from cheese whey (0.54 g_{PHAs} g_{VFA}^{-1}, Domingos et al., 2018), and higher than the yield obtained by using CA and CH_4 as co-substrates (0.45 g_{PHA} $g_{substrate}^{-1}$ Flanagan et al., 2016). The specific PHB production rate (0.08 h^{-1}) was similar to the one calculated from the concentration trend previously reported for a mixed culture fed with Na^+ 3-HB (0.06-0.1 h^{-1}) (Myung et al., 2014). All of this confirms the biochemical equivalence of alkenoic acids and hydroxyacids/short-chain carboxylic acids reported in the literature (Eggers and Steinbüchel, 2013; Janssen and Schink, 1993). However, it is worth mentioning that the formation of carboxylates (e.g. Na^+ 3-HB) derived from the alkaline hydrolysis of PHB would require the consumption of inorganic acid (HCl or H_2SO_4) during the PHB production by fermentation: in fact, the microbial consumption of hydroxybutyrate leads to pH rise since Na^+ gets free.

PHB produced by *C. necator* fed with CA or CA_C was finally extracted and characterized. The polymer recovery was in both cases above 90%, and the molecular weight (1.5 and 1.0 MDa, respectively) was in agreement with what is achievable by feeding the same bacterial strain with conventional carbon sources (e.g., glucose, 1.1 MDa, Samorì et al., 2015). The polydispersity index (PDI) here obtained by using CA (2.6) was almost a half than that of the PHB obtained from *C. necator* after the fermentation of CA_C (4.5), and in line with the PDI obtained when glucose is used (2.7, Samorì et al., 2015). Such characteristics of the renewed PHB and the high PHB yield 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.

3.3 Preliminary evaluation of energy requirements for producing CA from various PHB end-of-life scenarios

Introducing waste-PHB into the conventional plastic market disposal scenario is claimed to be a lengthy and complicated procedure (Vermeer et al., 2021). 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 distillation and the microbial conversion processes, respectively), the tandem thermochemical-biological recycling of PHB_{waste} allows at producing about 0.55 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 framework (e.g. composting or anaerobic digestion processes together with food waste). In composting, PHB_{waste} would be partially or completely oxidized to CO_2

and H_2O, together with the production of compost, whereas anaerobic digestion would convert PHB_{waste} into CH_4-containing biogas, with a maximum theoretical yield of 0.42 $kgCH_4/kgPHB_{waste}$ 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 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%/H_2O%/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 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 or 10/70/20) from an organic/food waste collection. This preliminary evaluation focused on CA produced through thermolytic distillation was compared to the energy demand necessary to produce sugars, among the most used feedstock for feeding *C. necator* and giving a similar PHB yield (Choi and Lee, 1997). Therefore, the system boundaries have been chosen to include just the energy consumption associated with the C-source production, and consider the further fermentation stage as the same, independently by the C-source (CA or sugars). The energy requirement for sugar production was calculated from previously reported values for fermentable sugars used in bioethanol distilleries (Jacques et al., 2003) as the contribution of agricultural practice, grain transport and grinding

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

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

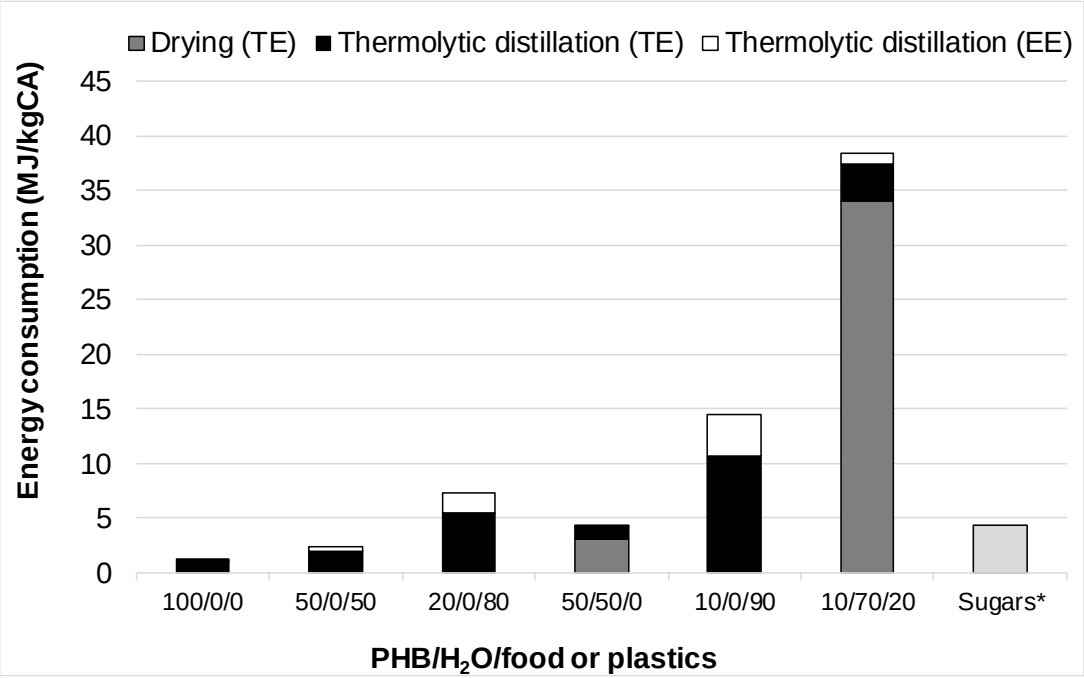

Figure 5. Energy required to produce CA from various PHB_{waste}, or to produce fermentable sugars, both usable as substrates by *C. necator* to produce PHB.

4. Conclusions

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

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

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