Crotonic Acid Production by Pyrolysis and Vapor Fractionation of Mixed Microbial Culture-Based Poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate)

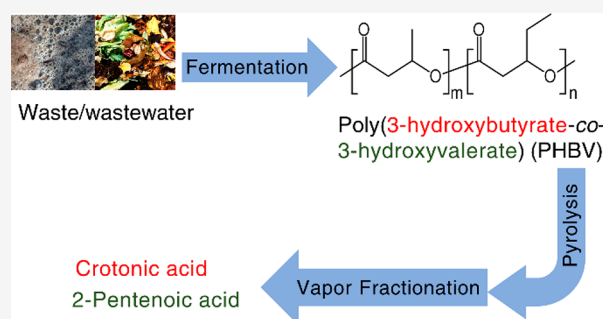Vahideh Elhami, Mark A. Hempenius, and Boelo Schuur*

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ABSTRACT: Crotonic acid (CA) and *trans*-2-pentenoic acid (2-PA) can be obtained from renewable resources by pyrolysis of the bio-based poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV) copolymer. In this study, direct pyrolysis of the PHBV-enriched biomass into CA and 2-PA was studied under an inert atmosphere and N_2 carrier gas flow, aiming at obtaining high acid yields from mixed microbial cultures (MMCs). The highest yields of $80 \pm 2\%$ for CA and $67 \pm 1\%$ for 2-PA were obtained at conditions of 240 °C, 1 h, and 0.15 L/min nitrogen flow rate, corresponding to a mean hot vapor residence time of 20 s. A similar acid yield was achieved when the pyrolysis was performed under reduced pressure (150 mbar) instead of using nitrogen gas. The combined pyrolysis of extracted PHBV at 220 °C and 90 min with vapor fractionation by distillation resulted in yields of 81 and 92% for CA and 2-PA, respectively.

Waste/wastewater → Fermentation → Poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV) → Pyrolysis → Vapor Fractionation → Crotonic acid / 2-Pentenoic acid
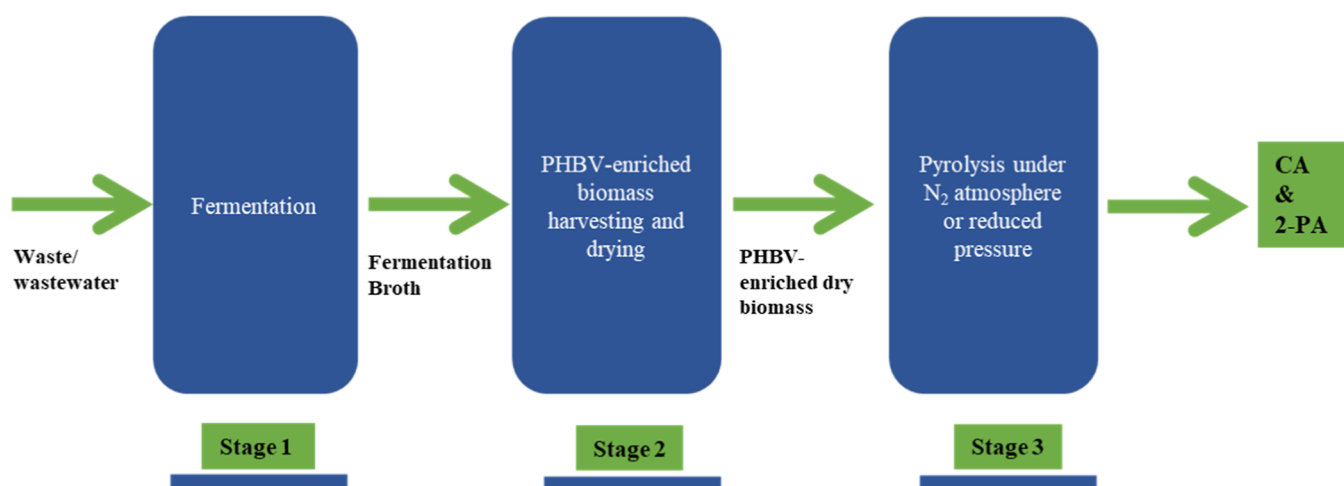
INTRODUCTION

Polyhydroxyalkanoates (PHAs) are a class of biodegradable and renewable polymers which can be produced by various bacteria as an energy reserve, ensuring the long-term survival of the bacteria during nutrient-scarce conditions.[1,2] These bio-based polymers are considered as green polymers of the future due to their potential to replace conventional polymers.[3] It has been shown that the bacteria can yield high-quality PHAs using pure substrates and sterile conditions, but under these expensive growth conditions, the polymers are not economically competitive with the conventional polymers.[4] Alternatively, it is possible to apply an open mixed microbial culture (MMC) to produce low-cost PHAs. MMC enables the use of inexpensive feedstocks such as waste/wastewater under non-sterile conditions.[5−10] This approach involves anaerobic fermentation of waste/wastewater to produce volatile fatty acids (VFAs), followed by aerobic conversion of VFAs into PHAs. During the fermentation process in the MMC, VFAs with an odd number of carbons will produce the poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV) copolymer with varying hydroxyvalerate (HV) ratios and those with an even number of carbons will yield poly(3-hydroxybutyrate) (PHB).[3] Polymers with a low HV content (<10% HV) are hard and brittle, resembling unplasticized polyvinyl chloride (PVC), mid-range HV content (between 10 and 25%) polymers maintain a good balance of toughness and resemble polypropylene (PP), while high-HV content (25−40%) polymers are soft and tough having a polyethylene (PE)-like feel.[11]

In most cases, PHBV is the main biopolymer spontaneously produced by fermentation of VFA-enriched waste/wastewater. Naturally, the composition of waste/wastewater varies from day to day, which can affect the quality of the polymer. For instance, the monomer composition of the co-polymer might vary per batch and consequently the thermal and physical properties of the polymer differ, which can limit the application window of the bio-based polymer. Alternatively, it is still possible to valorize the bio-polymers of low quality by depolymerizing them to produce other value-added chemicals such as 2-alkenoic acids. 2-Butenoic acid/crotonic acid (CA) is the main product of PHB decomposition.[12] CA is used in textile, cosmetic, painting, and coating applications and as a building block in the synthesis of co-polymers, for example, by copolymerization with vinyl acetate.[13] Regardless of its wide range of applications, the current production pathway of CA through a petrochemical route is neither renewable nor straightforward. The 2021 selling price in Europe was about 15.4 USD per kg, while the selling price of CA produced via thermal degradation of the bio-based PHB obtained from a pure culture was estimated to be 7.80−11.05 USD per kg in 2014.[12] With such high market prices, it is worthwhile to

Figure 1. Schematic overview of CA/2-PA production from MMC-based PHBV.

further investigate the efficient production of CA from biobased PHB/PHBV. For example, by replacing a pure culture with an open MMC and applying waste/wastewater as a feedstock, the production cost of CA can be reduced, and sustainable valorization of waste may be realized.[14] Over the last few decades, the thermal degradation mechanism of PHBV/PHB has been investigated in several studies using thermogravimetric analysis (TGA) and pyrolysis gas chromatography−mass spectroscopy (Py GC−MS), in which different reaction paths were considered.[15−17] Generally, it is concluded that a random beta elimination followed by an unzipping beta elimination at the crotonyl chain end is the dominant reaction path.[15−18] First, a random beta elimination occurs in which the PHB/PHBV undergoes chain scission. With beta elimination, the β-hydrogen linked to the ester oxygen is nucleophilically attacked by the ester carbonyl group. In this way, two new chain ends are formed, one having a carboxyl chain end and the other a crotonyl chain end. Finally, if the molecule with the crotonyl chain end undergoes a beta elimination at the ester group neighboring the crotonyl chain end, CA or 2-pentenoic acid (2-PA) is formed depending on which monomer [hydroxybutyrate (HB) or HV] is present at the chain end. The yield of CA produced through pyrolysis of PHB/PHB-enriched biomass at 310 °C is reported to be about 63%, which is 30% higher than the yield obtained by the conventional petroleum-based route.[12,19,20] Moreover, the biobased CA yield can be increased to about 80% using a catalyst[19] or pretreatment technique.[20] Solvent-based pyrolysis of the commercial PHB can increase the CA yield even further.[21,22] Thermal decomposition of the PHB in cyclohexane solvent at 210 °C and 5 h, followed by solvent separation by distillation, resulted in 89% CA yield with a purity of 91%.[22] Ionic-liquid-based media was also examined as a green solvent and catalyst to obtain CA from PHB.[21] About 97% yield of CA was obtained by applying an ionic liquid, namely [EMIM][AcO], as a solvent and catalyst at 140 °C and 90 min. Based on these PHB-pyrolysis results, showing promising potential to obtain CA in scales beyond analytical, we decided to study PHBV pyrolysis from MMC-based PHBV to obtain a mixture of CA and 2-PA, originating from HB and HV repeating units, respectively. Recently, Parodi et al.[23] developed a thermolysis method to directly pyrolyze PHB/PHB-enriched biomass and obtain relatively pure CA. By means of this approach, it was possible to produce CA with a purity of 92% from pyrolysis of

a PHB-enriched biomass containing 30 wt % PHB on a dry basis. Since both catalytic and non-catalyzed thermal approaches have shown highly interesting results for both pure PHB and PHBV on an analytical scale, it was decided to study direct pyrolysis of PHBV co-polymers and examine the impact of process conditions to aid further process design. Moreover, PHBV is the main co-polymer usually obtained from waste digestion in MMC. Thus, it is worth investigating the possibility of producing relatively pure CA from MMC-PHBV which might provide an economic approach due to the low cost of polymer synthesis. Figure 1 shows the schematic overview of the process to produce CA through MMC-based PHBV depolymerization. To the best of our knowledge, the production of CA from MMC-based PHBV using a lab-scale pyrolysis setup has not been studied yet, and such a study can aid process design because aspects like thermal degradation as well as the optimum mean residence time of the hot vapor phase in the oven need to be understood on the lab scale before aiming at further development. As PHBV is the main polymer which can be naturally produced by fermentation of VFA-enriched waste/wastewater, it is worthwhile to investigate the possibility of obtaining relatively pure CA from PHBV depolymerization.

In the present study, we developed a lab-scale pyrolysis setup to study CA production from the decomposition of MMC-based PHBV/PHBV-enriched biomass. The thermal degradation experiments were performed under an inert atmosphere using either N_2 gas or vacuum. The effect of the vapor phase residence time on the acid production yield was studied by varying the flow rate of the N_2 gas. As 2-PA is the main byproduct in the PHBV pyrolysis, a Vigreux was applied to upgrade the purity of the CA by distillation and to study the possibility of in situ separation of CA and 2-PA from the vapor phase.

■ MATERIALS AND METHODS

Chemicals. CA (≥98%), *trans*-2-pentenoic acid (≥98%), 2-methyl tetrahydrofuran [2-MTHF, (≥99%)], and *n*-hexane (≥95%) were purchased from Sigma Aldrich. Acetone (>99%, extra pure) was supplied by Thermo Fischer Scientific. Chemicals were used as received.

MMC-Based PHBV-/PHBV-Enriched Biomass Preparation. The dry PHBV-enriched biomass containing 30 wt % PHBV (35 wt % HV) on a dry basis was obtained from Paques

Biomaterials, which was produced from municipal food waste.[24] For the experiments in which pure PHBV was used, the polymer was extracted using 2-MTHF and characterized according to the procedure described in our previous work.[25] Briefly, a certain quantity of the biomass was added to the 2-MTHF solvent to reach a concentration of 5% [g/mL], followed by heating the mixture at 80 °C for 1 h. Afterward, *n*-heptane was used as an antisolvent to precipitate the polymer. Finally, the collected polymer was dried in a vacuum oven at 50 °C for 24 h.

The water content of the biomass was measured by drying it in an oven at 105 °C for an hour. The difference in the mass of the biomass before and after drying was used to calculate the water content.

Pyrolysis of PHBV and PHBV-Enriched Biomass. A custom-built oven pyrolyzer setup was applied to pyrolyze the MMC-based PHBV/PHBV-enriched biomass. As depicted in the Figure 2, the oven pyrolyzer setup consisted of a
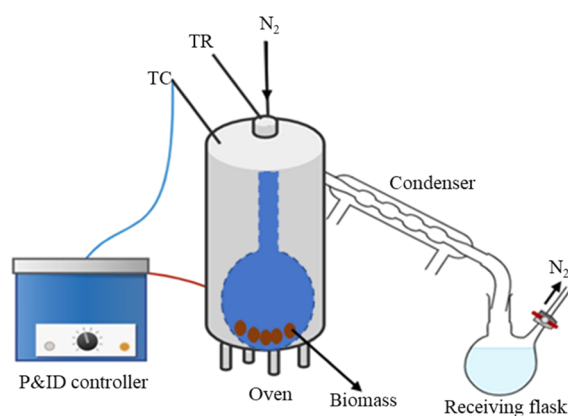
Figure 2. Schematic view of the custom-built oven pyrolyzer setup to produce CA from MMC-based PHBV-/PHBV-enriched biomass; TC is a Pt-100 sensor used to control the temperature of the oven, and TR is the thermometer used to measure the temperature of the round-bottom flask.

temperature-controlled oven, a round-bottom flask loaded with the PHBV/PHBV-enriched biomass and placed inside the oven, a thermocouple to monitor the round-bottom flask temperature with an accuracy of ± 1 °C, a condenser at -5 °C to condense the vapor phase, and a receiving flask to collect the products. The setup was connected to either a vacuum pump or a N_2 supply, depending on the experimental procedure.

First, the PHBV and PHBV-enriched biomass were analyzed by TGA to determine the degradation temperature of the polymer and to set the oven at the required decomposition temperature. Then, the pyrolysis experiment was started by heating the PHBV/PHBV-enriched biomass to the set temperature which took 20 min, after which the oven was kept at this temperature for 1 h. Various nitrogen gas flows (0.05 to 0.2 L/min) were applied, or reduced pressure (either 50 or 150 mbar). After the 1 h of pyrolysis, the condenser was flushed with acetone to collect the pyrolyzates that had crystallized there. Pyrolyzates refer to all the materials which condensed in the condenser and were collected there. It includes CA, 2-PA, and other byproducts. Acetone was removed from the samples by overnight atmospheric evaporation, followed by final evaporation in a vacuum oven at maximum vacuum for 2 h at room temperature.

Subsequently, the pyrolyzates were analyzed by various analysis techniques to determine the yield and purity of the produced acids. Each experiment was duplicated.

Integrated Pyrolysis−Distillation Approach for PHBV Decomposition. In the next step, the goal was to not only obtain relatively pure CA from MMC-based PHBV but also retrieve 2-PA as a side product. Thus, a vacuum-insulated Vigreux distillation column was placed between the oven pyrolyzer and the condenser. To limit the byproduct formation due to reactivity between the formed CA and 2-PA, the experiments were performed at a reduced pressure of 50 mbar, and the main fraction of CA was collected after it passed the condenser, while the majority of 2-PA remained at the bottom of the heated flask.

Analysis. *High-Performance Liquid Chromatography.* The concentrations of CA and 2-PA were measured using a high-performance liquid chromatography (HPLC) system [Agilent Hi-Plex H column (300 × 7.7 mm) with a refractive index detector on an Agilent 1200 series HPLC system; mobile phase, 5 mM H_2SO_4 solution; column temperature, 65 °C at a flow rate of 0.6 mL/min].

Due to the overlapping of the peaks of the acids with their side products, symmetrical peaks were fitted with a Gaussian function using OriginPro 2019b software [see the electronic Supporting Information (ESI) for more details]. Afterward, the fitted peaks were integrated to quantify the acids. The acid yield was defined as eq 1

$$\text{acid yield (wt \%)} = \frac{\text{amount of collected acid (g)}}{\text{theoretical amount of the acid (g)}} \times 100 \tag{1}$$

where the amount of the collected acid was determined by HPLC and the theoretical amount of the acid was defined based on the HB content in PHBV for CA and the amount of HV repeat units for 2-PA. Thus, a 100% CA yield means that the total quantity of HB units present in the PHBV is completely converted into CA.

Gas Chromatography−Mass Spectroscopy. GC−MS analyses were performed to determine the main side products from the PHBV-/PHBV-enriched biomass pyrolysis. The pyrolyzate samples were dissolved in acetone with concentrations of less than 500 ppm and analyzed using a GC-FID/MS (GC—7890A, MS—5975C Agilent Technologies system) equipped with an Agilent HP-5MS HP19091S-433 capillary column (60 m, ID: 0.25 mm, film thickness: 0.25 μm). The column was packed with 5% phenyl methylpolysiloxane. Helium was used as a carrier gas with a constant flow rate of 1.95 mL/min. The oven temperature was programed from 45 °C (4 min) to 280 °C at a heating rate of 3 °C/min and held at 280 °C for 20 min. The injector and the column to MS interface were maintained at a constant temperature range of 250 and 280 °C, respectively. A sample of 1 μL was injected into the GC. The mass spectrometer was operated in the electron ionization mode, and ions were scanned in a m/z range from 15 to 500.

Refinery GC. Refinery GC was used to identify the non-condensed gases. A sample was taken from a N_2 stream in the outlet of the condenser and injected into a Varian 450-RGA. Details of the instrument were previously provided elsewhere.[26]

Thermogravimetric Analysis. TGA was employed to measure the degradation temperature of the polymers using

Table 1. Characteristics of PHBV-Enriched Biomass and Extracted PHBV Used in the Pyrolysis Experiments

| material	PHBV content (%)	T_{deg} (°C)	HB/HV	MW (kDa)	PDI	water content (%)
PHBV-enriched biomass	30	240	65/35	146	3.5	4 ± 1
extracted PHBV	>99	245	65/35	122	4.9	<1

a TGA-550 instrument operated under nitrogen flow. Samples were heated from room temperature to 400 °C at a rate of 10 °C/min under a nitrogen atmosphere.

Karl-Fischer Titration. Karl-Fischer titration (KFT) was used to analyze the water content of the pyrolyzate mixture using a Metrohm 787 KFTitrino. HYDRANAL Composite 5 was titrated from a 20 mL buret filled with a mixture of methanol and dichloromethane in a volume ratio of 3 to 1. The samples were analyzed in triplicate with a relative error of less than 1%.

■ RESULTS AND DISCUSSION

Pyrolysis of the PHBV-Enriched Biomass. The characteristics of the PHBV and PHBV-enriched biomass are given in Table 1. The polymer content of the biomass, the composition (repeat unit ratio) of the polymer, average molecular weight (MW), and polydispersity index (PDI) are taken from our previous work as the same batch of the biomass was used for pyrolysis experiments.[25] The composition, polymer content of the biomass, and water content of the polymer and biomass were taken into account in the calculation of the yield in each experiment. The PHBV and PHBV-enriched biomass are known to be hygroscopic. Therefore, their water content was measured beforehand, resulting in about 4 ± 1 and <1% for the biomass and extracted PHBV, respectively. To apply a proper temperature range for the pyrolysis of the polymer, the degradation temperature (T_{deg}) of the biomass and the extracted PHBV was determined by TGA in which the inflection point of the curve was considered as T_{deg}. As shown in Table 1, the T_{deg} of the polymer in PHBV-enriched biomass is 240 °C; thus, the experiments of direct pyrolysis of the biomass were performed at 240 °C. The T_{deg} of extracted PHBV increased by 5 °C, possibly due to the purification of the polymer during the extraction, as it is known that metal ions present in the biomass can accelerate thermal degradation of the polymer[17] and perhaps other molecules in the biomass.

The heating time of the polymer in the setup and the residence time of the produced thermally sensitive monomers in the vapor phase in the hot setup are crucial parameters to be controlled in order to achieve complete degradation of the polymer and maximize monomer yields. To establish an estimate for the heating time for the polymer pyrolysis, a preliminary experiment was carried out using about 5 g of biomass at 240 °C and 1 h of heating. Afterward, the residual biomass was analyzed by TGA to determine whether or not a significant fraction of the polymer remained in the biomass after pyrolysis. In Figure 3, two TGA experiments are shown, one of biomass before pyrolysis and one of the residue remaining after pyrolysis. In the temperature range of 180 to 280 °C, the line for the biomass before pyrolysis shows a distinct reduction in weight, occurring in the temperature range where PHBV degrades. The mass reduction of the residue of biomass after pyrolysis is not as pronounced as that of the biomass before pyrolysis. Most likely, this indicates that the polymer content of the residual biomass is not significant anymore and from that, it can be concluded that 1 h of pyrolysis at 240 °C sufficed to pyrolyze the vast majority of the
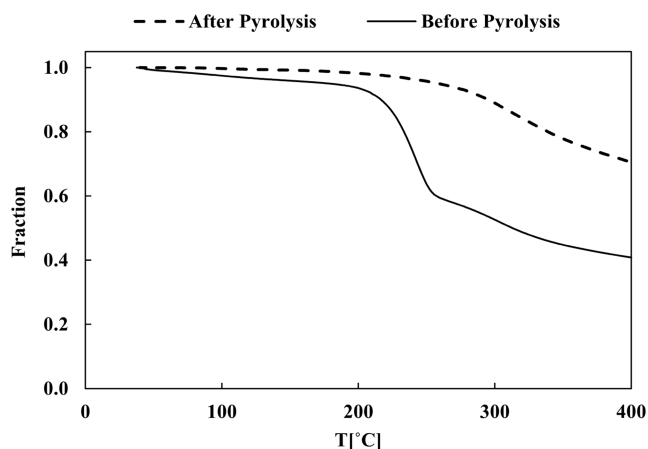
Figure 3. TGA graph displaying the weight fraction remaining of the biomass before and after pyrolysis at 240 °C for 1 h under a nitrogen flow rate of 0.1 L/min.

polymers in the biomass. Thus, the contact time and temperature for pyrolysis were kept constant at 1 h and 240 °C in all experiments.

To study the influence of the residence time of the vapor phase in the hot zone on the acid yield, pyrolysis of the PHBV-enriched biomass and extracted PHBV was carried out under either a N_2 stream or under vacuum, and the obtained results were compared. Applying nitrogen as a carrier gas allows for varying the residence time of the vapor phase both in the hot pyrolyzer and in the condenser. CA and 2-PA are unsaturated acids which are not thermally stable. At long residence times in the hot zone, these monomers can undergo decomposition, isomerization, and polymerization.[27] Therefore, the influence of the hot vapor residence time was experimentally examined by varying the flow rate of the nitrogen carrier gas and assuming that the produced vapor phase has the same flow rate. Figure 4 represents the yield obtained under various pyrolysis conditions. As can be clearly seen from the graph, the acid yield is lowest when there is neither N_2 carrier gas nor reduced pressure to pull out the vapor phase. The yield increased by about 15% at a N_2 flow rate of 0.05 L/min as compared to the experiment where no carrier gas was used. Increasing the flow to 0.15 L/min resulted in an 80 ± 2 and 67 ± 1% yield of CA and 2-PA, respectively. A further increase in the nitrogen flow rate did not significantly enhance the pyrolysis yield. Following the method of Parodi et al.,[23] a few pyrolysis experiments were also performed under reduced pressures of 150 mbar and 50 mbar. As shown in Figure 4, at 150 mbar similar yields were obtained in comparison with applying nitrogen gas at flow rates of 0.15 and 0.2 L/min. However, the acid yields were low at 50 mbar, which may be because they are not condensing in the condenser, for which two explanations may be given. First, it might be due to the short residence time of the vapor phase in both the hot zone and the condenser. Second, due to the pumping out of vapor molecules by the pump as it maintains the setpoint pressure of 50 mbar. Due to the continuous removal of acids from the system in the vapor phase, the system may not reach the
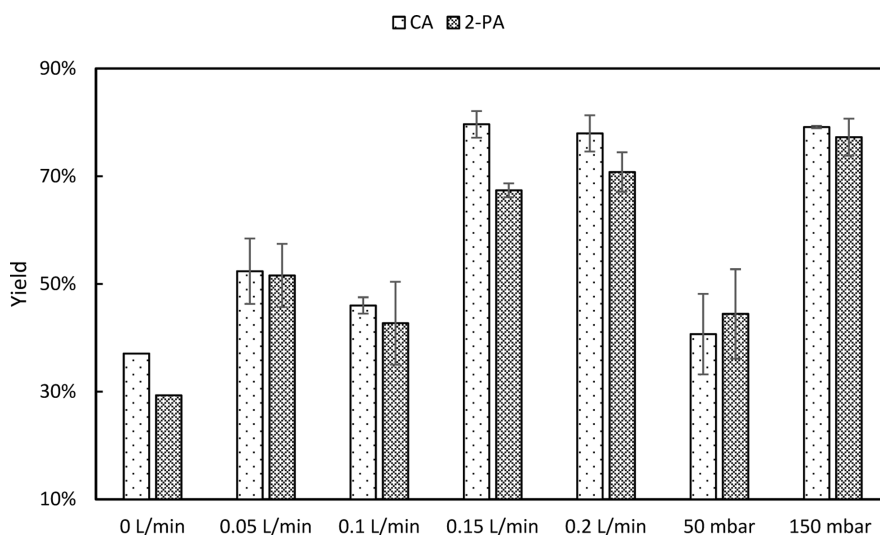
Figure 4. CA and 2-PA yields obtained by direct pyrolysis of the PHBV-enriched biomass at various operation conditions.

dewpoint.[28] Also in the experiments which were conducted using N_2 flow, for situations where the rate of pyrolysis is relatively low compared to the flow of N_2, the mol fractions of the acids also become low, which may result in portions of the acids that do not condense due to the vapor pressure of the acids (in thermodynamic equilibrium equated in eq 2), not reaching the dewpoint.[28]

$$y_i P = \gamma_i x_i P^{sat,i} \qquad (2)$$

When the kinetic effect is the major cause, this could be countered by adding a longer condenser, but when nitrogen flows are applied, some of the vapors do not condense due to the dewpoint not being reached, and then longer condensers do not help. As a check to calculate whether this would be a significant amount of CA not condensing, the dewpoint was calculated (see Supporting Information) as a function of the vapor fraction CA in N_2, and it was determined that at -5 °C the dewpoint corresponds to a mol fraction CA of 5.2×10^{-5} and at 0.2 L/min N_2 flow, this corresponds to a loss of only 0.085% of the CA. This calculation shows that any significant effect must be due to kinetics, as the equilibrium would dictate that most CA would condense and crystallize.

The highest CA yield achieved in this work is higher than what has been published for direct pyrolysis of PHB-enriched biomass (about 60%)[12,23] and comparable to the yield obtained by either catalytic thermal degradation (83%)[19] or by thermal decomposition of pretreated PHB (80%).[20] Although differences in yield may be explained by different characteristics of the polymer and biomass used in this work, the distinct pyrolysis setup and experimental procedure used here also contribute to the high monomer yields. Especially considering the significant impact that process conditions have on the yields, as can be seen in Figure 4, optimization of the procedure is essential. As a further example, Mamat et al.[12] performed pyrolysis experiments at a high temperature of 310 °C, which can accelerate the isomerization and polymerization of CA, resulting in low CA production yields. As the reported CA yields from direct pyrolysis of the biomass[12,23] are similar to the ones we obtained under less favorable conditions (hot vapor residence time of 61 s corresponding to N_2 flow rate of 0.05 L/min), the configuration of the pyrolysis setup and the residence time of the vapor phase in the hot oven are indeed

major parameters determining the differences in yield obtained in the literature and in this work.

When comparing the polymer used in this study with other studies, the most distinctive properties of the polymer in this study are its high HV content and low average MW, obtained by MMC, using food waste as a feedstock. It has been found that the HV content in PHBV polymers does not affect the T_{deg} of the polymer.[29] The glass transition temperature,[30] crystallinity, and melting point[29] of the polymer, however, are lowered by increasing the HV content, and these thermal characteristics may have an impact on the pyrolysis process. Therefore, a generalized conclusion on the impact of the HV content on the thermal decomposition of PHBV polymers cannot be presented yet.

To gain more information regarding the possible reasons for the different yields obtained from various experiments, the composition of the pyrolyzates was investigated, followed by a mass balance closure over the biomass during pyrolysis. Pyrolyzate refers to all the materials condensed in the condenser and collected there. It includes CA, 2-PA, and other side products. Table 2 represents the composition of the pyrolyzate mixture obtained at various pyrolysis conditions, which illustrates that the pyrolysis at different nitrogen flow rates results in mixtures with similar acid content. The mixture

Table 2. Summary of the Composition of Pyrolyzate Mixtures Obtained by Direct Pyrolysis of PHBV-Enriched Biomass at Various Operation Conditions

		composition (wt %)			
exp.	RT[a] (s)	CA	2-PA	water	other condensed byproducts
N_2-0 L/min		50	21	<1	28
N_2-0.05 L/min	61	53 ± 1	27 ± 1	<1	19 ± 0.3
N_2-0.1 L/min	30	52 ± 8	25 ± 2	<1	22 ± 6
N_2-0.15 L/min	20	52 ± 1	24 ± 1	<1	24 ± 1
N_2-0.2 L/min	15	54 ± 5	26 ± 2	<1	19 ± 8
Vacuum-50 mbar		36 ± 1	21 ± 0.3	<1	41 ± 1
Vacuum-150 mbar		45 ± 2	24 ± 0.1	<1	31 ± 2

^{a}RT is the mean residence time of the vapor phase in the hot zone of the oven-pyrolyzer.

from the experiment at 50 mbar has the lowest acids content and the largest amount of condensed side products. The samples were analyzed by GC-FID/MS to identify the side products present in the pyrolyzate mixture. According to GC-FID/MS, isocrotonic acid, 3-butenoic acid, 3-pentenoic acid, 4-pentenoic acid, crotonoamide, and 2,2,6,6-tetramethyl-4-piperidone are the main side products in all the experiments, regardless of the pyrolysis conditions. To compare the quantity of the aforementioned side products in each experiment, their corresponding peak areas were normalized to the area of the 2-PA peak in each sample. It was found that the operation conditions do not have a significant impact on the pyrolyzate composition in terms of the dominant side product masses. Regarding water content in the pyrolyzate mixture which originated from the moisture content of the biomass, it is <1% based on Karl-Fischer analysis which is lower than the water content of the starting biomass ($4 \pm 1\%$). It can be explained by the evaporation of water while removing acetone from the pyrolyzate mixture using a vacuum oven at room temperature. Furthermore, it is also expected that a fraction of the water in the vapor phase leaves the condenser without condensing during pyrolysis (same reasoning as for the acid yields being limited by a fraction of the acids not condensing).

The mass balance closure over the biomass pyrolysis was performed to compare the performance of the various pyrolysis conditions in terms of the overall conversion. As can be seen from Figure 5, the char formation is high (~62%) at low
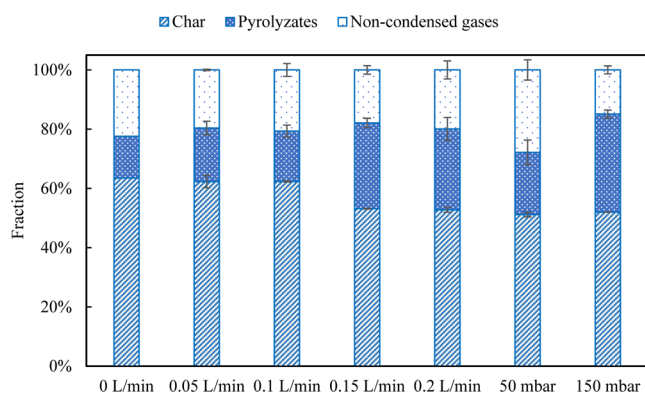
Figure 5. Mass balance closure over the PHBV-enriched biomass pyrolyzed at various operation conditions. Integrated pyrolysis of PHBV and distillation of pyrolyzates under vacuum conditions.

nitrogen flow rates due to the long residence of the vapor in the hot zone. The vapor phase contains mainly CA and 2-PA, which are not thermally stable and can undergo isomerization and dimerization at elevated temperatures during prolonged residence times.[27] Moreover, it was observed that a brown layer forms on the walls of the glassware at low nitrogen flow rates, which can be explained by possible dimerization of the acids. The char formation is around 53% in the experiments under reduced pressures of 50 and 150 mbar, and nitrogen flow rate of 0.15 and 0.2 L/min which is about 10% less than other experiments. The largest quantity of pyrolyzate mixture collected under N_2 flow was around 28% on the basis of the starting biomass amount, using flow rates of 0.15 and 0.2 L/min, while the highest pyrolyzate mass obtained under vacuum (150 mbar) was around 33%. Considering that the amount of polymer in the biomass was estimated at approximately 30 wt % (discussion on results in Figure 3), this yield is excellent. In

all experiments with various pyrolysis conditions, a fraction of the small molecules derived from the PHBV-enriched biomass did not condense in the condenser at −5 °C. Therefore, a gas sample was taken to be analyzed with refinery-GC to identify the non-condensed gases. Since the setup was an open system with a continuous flow of nitrogen, it was not possible to precisely quantify the non-condensed gas phase. According to GC, the vapor phase at a nitrogen flow rate of 0.1 L/min contained ethylene, methane, propylene, 1-butene, 1,3-butadiene, and 1-butyne.

To directly produce the acids with high purity from pyrolysis of the PHBV, a vacuum-isolated Vigreux column was placed in the outlet of the oven pyrolyzer to in situ separate CA and 2-PA in the vapor phase by distillation. A vacuum-isolated Vigreux column prevents the crystallization of CA within the column because it losses much less heat to the surroundings compared to unisolated Vigreux columns. This approach was already mentioned by Parodi et al.[23] Nonetheless, these authors used PHB/PHB-enriched biomass to obtain CA, and not the co-polymer PHBV. We aimed to produce CA and 2-PA with the highest possible purity from a MMC-PHBV, as the PHBV is the dominant polymer usually produced in MMC, using VFA-rich waste streams as feedstock. Therefore, preliminary integrated pyrolysis of PHBV and distillation experiments were performed to examine the fractionation of the vapor phase, containing mainly CA and 2-PA. The experiments were carried out at 50 mbar (instead of using a nitrogen flow). In the first experiment, the temperature was set at 170 °C following the method of Parodi et al.[23] However, there was no vapor flow entering the column after an 1 h. Thus, the temperature was gradually increased until a vapor flow was observed, which occurred at 220 °C. The experiment was repeated, and the temperature was set at 220 °C from the starting point. After 90 min at 220 °C, the flow of vapor was diminished. Thus, the experiment was stopped and samples were taken to be analyzed by HPLC. The yields of CA and 2-PA were 81 and 92%, respectively. The overall mass balance closure indicated that 85% of the PHBV was converted into the CA and 2-PA, including both top and bottom products. About 6% can be accounted for in the side products in the bottom stream, and 6% is converted into non-condensed gas products. 3% was remained as a char in the round-bottom flask. Moreover, a small fraction can also be considered to be lost during handling and collecting the products. As shown in Figure 6, the top product of the distillation column is enriched with CA (76 wt %), while the bottom product contained 2-PA in 51 wt %, meaning that it is possible to separate these acids by distillation and produce them with relatively high purity from MMC-based PHBV. In the current setup, only a limited number of distillation stages was available, and further distillation studies are recommended for follow-up work to reach higher purities.

■ CONCLUSIONS

In this work, the direct pyrolysis of the PHBV-enriched biomass produced by an open MMC using food waste as feedstock was studied in a custom-made oven pyrolyzer, operated either under reduced pressure conditions or a nitrogen atmosphere. Applying nitrogen as a carrier gas enabled to manipulate the mean residence time of the hot vapor phase and experimentally optimized thermal depolymerization of the PHBV toward CA and 2-PA. At low nitrogen flow rates <0.1 L/min, the acid yield is lower and the char
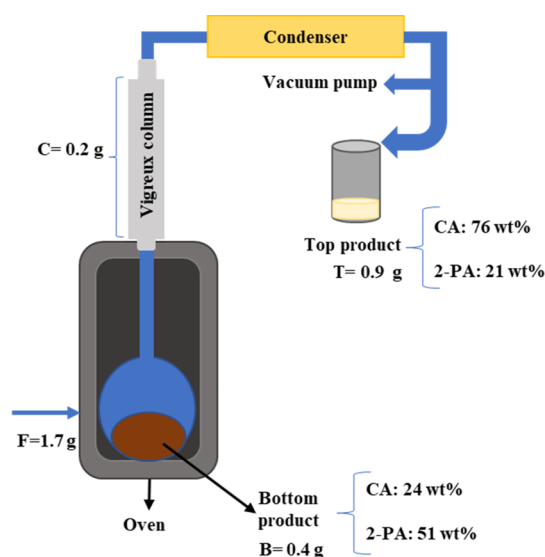
Figure 6. Pyrolysis of PHBV at 220 °C, 50 mbar, and 90 min with in situ fractionation of the vapor phase by distillation. F, C, T, and B represent the mass of PHBV used for pyrolysis, the mass of the acids that remained in the column, and the total mass of the top and bottom products, respectively.

formation is higher than with experiments performed at N_2 flow rates of 0.15 and 0.2 L/min. According to the mass balance closure over the biomass, the non-condensed vapor phase is almost constant in all the experiments performed under nitrogen gas. It indicates that regardless of the operational conditions, a part of the biomass forms non-condensable gases at 240 °C. The GC analysis of the gas phase in the outlet of the condenser indeed confirmed the formation of non-condensable gases such as ethylene, methane, propylene, 1-butene, 1,3-butadiene, and 1-butyne. The maximum acid yields of 80 ± 2 and 67 ± 1% for CA and 2-PA were achieved at a nitrogen flow rate of 0.15 L/min, 240 °C and 1 h. By replacing the nitrogen gas with a vacuum of 150 mbar, similar acid yields were obtained. The highest obtained CA yield is comparable with the results achieved by catalyzed pyrolysis and by pyrolysis of pretreated PHB/PHB-enriched biomass. However, due to the nature of the PHBV, 2-PA is also formed, requiring an appropriate downstream separation technique to yield pure CA. Therefore, the suitability of distillation was investigated by integrated pyrolysis-distillation experiments. The integrated pyrolysis-distillation approach indeed allowed to directly fractionate the vapor phase, originating from pyrolysis of the extracted PHBV. The product at the top of the column was mainly CA (76%), indicating that distillation is a viable technique for separating CA and 2-PA and thereby producing relatively pure CA and 2-PA from MMC-based PHBV. It is expected that the purity of CA can be increased even further by using a proper distillation column with more equilibrium stages.

ASSOCIATED CONTENT

Ⓢ Supporting Information
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.iecr.2c03791.

> GC spectrum of the pyrolyzate mixture; HPLC spectrum of the pyrolyzate mixture; Gaussian fit of CA and 2-PA HPLC peaks; dew point calculation for the

acid mixture in the vapor phase, originating from pyrolysis (PDF)

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V.E.—Development of the experimental methodology, investigation, data analysis, and writing the original draft. M.A.H.—Supervision and reviewing and editing of the manuscript. B.S.—Conceptualization of the project, supervision, formal analysis, editing and reviewing of the manuscript, and funding acquisition.

Notes
The authors declare no competing financial interest.

ABBREVIATIONS

PHAs	polyhydroxyalkanoates
PHB	poly(3-hydroxybutyrate)
PHV	poly(3-hydroxyvalerate)
PHBV	poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate)
HB	hydroxy butyrate
HV	hydroxy valerate
CA	crotonic acid
2-PA	2-pentenoic acid
MW	molecular weight
MMC	mixed microbial culture
VFAs	volatile fatty acids
GC−MS	gas chromatography
TGA	thermal gravimetric analysis
GPC	gel permeation chromatography
HPLC	high-pressure liquid chromatography
THF	tetrahydrofuran
PDI	polydispersity index
2-MTHF	2-methyltetrahydrofuran
Py GC−MS	pyrolysis gas chromatography−mass spectroscopy
KFT	Karl-Fischer titration
PE	polyethylene
PP	polypropylene
PVC	polyvinyl chloride

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