A plant wide simulation of polyhydroxyalkanoate production from wastewater and its conversion to methyl crotonate

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HIGHLIGHTS

- Comprehensive process developed for MC production from wastewater via PHA platform.
- The simulation results were in great agreement with experimental data.
- The origin of the wastewater has great influence on the MC quantity and purity.
- Intracellular PHA reduces the purification steps with little effects on MC purity.

GRAPHICAL ABSTRACT
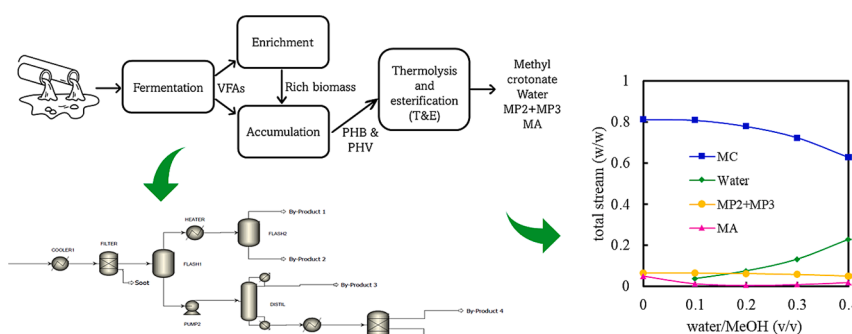
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ABSTRACT

This work simulates the production of methyl crotonate from various industrial wastewaters. In the upstream process, wastewater is fermented into volatile fatty acids which are then converted into polyhydroxyalkanoates (PHA) by means of mixed microbial cultures. In the downstream, PHA undergoes a series of thermolysis and esterification reactions to produce methyl crotonate. The origin of the wastewater was found to have a great influence on the composition of the PHA with the effluent of a candy bar factory producing a high polyhydroxybutyrate/polyhydroxyvalerate ratio of 86/14 in favour of methyl crotonate production. It was observed that the use of intracellular polyhydroxybutyrate, instead of purified, significantly lowers the number of separation steps and yet reduces the methyl crotonate recovery by only 20 %. An operating pressure higher than 18 bar led to more transesterification of polyhydroxybutyrate, producing byproducts instead of methyl crotonate. Finally, a 3 h reaction was found sufficient for completion of polyhydroxybutyrate conversion.

1. Introduction

The transition of chemical industries from fossil resources toward the use of biobased feedstock is becoming a necessity due to the environmental concerns. Top-down initiatives as policy guidelines encourage the researchers to find proper solutions for a smooth transition (Termeer & Metze, 2019). This could be partially achieved by advancing the technologies for chemical production from sustainable feedstocks (Bhatia et al., 2022; Narisetty et al., 2022). Polyhydroxyalkanoates (PHAs) as intracellular storage materials synthesized

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by prokaryotic microbes such as *Loktanella* sp. SM43, *Pseudomonas* sp. B14-6 and *Bacillus* sp. YHY22 have recently attracted great attraction as renewably sourced bioplastics (Lee et al., 2022a; Lee et al., 2021; Lee et al., 2022b). PHAs could be potentially applied in packaging, coatings and disposable products (Reddy et al., 2003), and are increasingly gaining attention for high end applications such as medical implants and drug release carriers (Chen & Patel, 2012). At the end of shelf life, PHAs can be composted and mineralized to CO_2, which is then reused in photosynthesis, closing the carbon cycle (Dietrich et al., 2017). Production of PHA for commercial use started in 1959 (Chanprateep, 2010) and is continuously increasing, having a market size of 80.4 M€ in 2021, and an expected increase of 16.0 M€ in the 2020–2024 period (Amadu et al., 2021). However, its market penetration is limited, with a production capacity of 1.7 % of all bioplastics, while bioplastics capacitate 1 % of the total plastic market (Leal Filho et al., 2021). This is due to the requirement of expensive carbon sources such as pure sugar or lipids as well as the use of pure microbial cultures for PHA production which significantly increases the maintenance and sterilization costs (Chen, 2009; Vandi et al., 2018).

Exploring the feasibility of alternative and cheaper carbon sources such as agricultural waste (Liu et al., 2021), biowaste (Bhatia et al., 2021), biomass (Bhatia et al., 2019) and organic industrial wastewater with a wide range of carbon compounds (Montiel-Corona & Buitrón, 2021; Novelli et al., 2021) is an active field of research. A challenge for the use of agricultural waste is the required pre-treatment step, as obstinate lignocellulosic material is present. Hydrolyzation of this material can have negative effects on costs and the fermentation process. Crude dairy waste also requires a pre-treatment step (e.g., use of acid treatment). Industrial wastewaters are more attractive as they are inexpensive and readily available. Additionally, mixed microbial cultures (MMCs) can take up the wide range of carbon sources present in wastewater allowing for a non-sterile operation (Fang et al., 2021; Li et al., 2021; Morgan-Sagastume et al., 2020; Perez-Zabaleta et al., 2021; White et al., 2017). Production of PHA in lab scale with these conditions has shown to be economically and environmentally more attractive compared to using pure cultures and a single carbon source, with a 30 % decrease in production costs (Estévez-Alonso et al., 2021; Kleerebezem & van Loosdrecht, 2007; Lorini et al., 2021).

Despite the benefits of producing PHAs from industrial wastewater, the selectivity of the process remains low and the product is often a mixture of different PHAs (see supplementary material). The presence of 3-hydroxybutyrate (HB) and 3-hydroxyvalerate (HV) monomers results in a product mixture containing polyhydroxybutyrate (PHB) and polyhydroxyvalerate (PHV) in varying compositions (Estévez-Alonso et al., 2021; Spekreijse et al., 2015). The ratio of PHB/PHV is mainly determined by the type of carbon sources that are present in the industrial wastewater which often fluctuates with time (Tamis et al., 2014b).

Moreover, fractionation costs and product applications of wastewater based PHAs are not yet competitive with those of their petrochemical counterparts such as polyester, as the material is stiff and brittle and the processing temperature is close to that of decomposition (Fernández-Dacosta et al., 2015; Madkour et al., 2013). In addition to the traditional use of PHA as a biopolymer, it could be utilized as a platform to produce building block chemicals. Studies have shown the possibility of pure PHB conversion to methyl crotonate (MC) via thermolysis and subsequent esterification, which can be further processed into methyl acrylate via metathesis (see supplementary material) (Fernández-Dacosta et al., 2016; Schweitzer & Snell, 2015). These products are drop-in chemicals which can be used in multiple chemical industries as base compounds.

A promising process design for PHB conversion to MC is carried out by Fernández-Dacosta et al. using aqueous and intracellular PHB (Fernández-Dacosta et al., 2015; Fernández-Dacosta et al., 2016). Starting with aqueous and intracellular PHB can help to avoid the expensive downstream processing and lowers the carbon footprint compared to processing PHB that is first extracted from the cell.

Lab- and pilot scale experiments have demonstrated the potential of PHA production from wastewater, and a promising design for pure PHB conversion to value added chemicals has also been shown separately. However, an in-depth and complete process design (starting with wastewater and ending with MC) is lacking and wastewater properties and the presence of PHV in the PHA mixture have not been incorporated in the previous studies. Therefore, this study aims to assess the feasibility of such process by developing a plant wide up- and downstream process simulation for the production of MC from wastewater, with PHA mixture as an intermediate platform. The model would be first validated against experimental data and then used to study the influence of the wastewater characteristics as well as various operational conditions on the process performance.

2. Materials and methods

2.1. Experimental

The conversion of PHA to MC and other intermediate compounds through thermolysis and esterification reactions was experimentally studied. Staring materials of 0.6 g of either PHA (including 2 mol % PHV, Mn = 450 kDa and Mw/Mn = 1.3), methyl crotonate (MC, 98 % Sigma-Aldrich), crotonic acid (CA, 98 % Sigma-Aldrich), methyl-3-hydroxybutyric acid (M3HB, 99 % Sigma-Aldrich) were introduced to a reaction vessel. The container consisting of a glass liner with a glass-coated stirring bar was placed in a Parr reactor (Parr multiple reactor system series 5000, 6 X 75 mL, Hastelloy C-276). 10 mL Methanol (99 % Sigma-Aldrich) was added with a syringe while keeping the reactor purged with argon. While keeping the reactor at room temperature, the reactor was flushed and pressurized with nitrogen (Nitrogen 3.0, purity ≥ 99.9 %). Next, heating was applied, causing a pressure build-up to the desired pressure. At T = 200 °C, the reaction time started (t = 0 h). After the desired reaction duration, the reactor was left to cool down to room temperature. The product mixture was then withdrawn and subjected to a Büchner filter to remove any solids. The solution was then passed through a 0.20 μm single use filter unit and subsequently analyzed by High-Performance Liquid Chromatography (HPLC).

2.2. Simulation

For the simulation of wastewater-based MC production with PHA as intermediate platform Aspen Plus V12.1 was used. Fig. 1 visualizes the schematic overview of the proposed design. The up- and downstream process is defined with respect to PHA. The details of each unit operation will be discussed in the following sections.

2.2.1. Upstream process design (PHA production)

The production of PHA from wastewater is carried out in the upstream section. The process starts with the fermentation of wastewater into volatile fatty acids (VFAs) which is then fed to the enrichment and accumulation reactors to produce PHA by means of an MMC. The kinetics for these conversions is introduced by Langmuir-Hinshelwood-Hougen-Watson (LHHW) equation in Aspen. The LHHW rate kinetic includes three main factors: the kinetic factor, the driving force, and the adsorption expression, as shown in Eq. (1).

$$LHHW \ rate = \frac{(kinetic \ factor) \ (driving \ force)}{adsorption \ expression} \qquad (1)$$

For all the upstream reactors, kinetic parameters were extracted from experimental data. The parameters of Monod equation presented in literature (Tamis et al., 2015; Tamis et al., 2014a) were used to calculate the parameters of LHHW equation according to Eq. (2). The kinetic factor in LHHW equation includes a constant k, a temperature variable T^n, an exponent factor with the activation energy E_A and gas constant R (Eq. (3)). The driving force is dependent on an equilibrium constant k_f and substrate concentration $C_{sub}(t)$. The k_f is re-written as the maximum
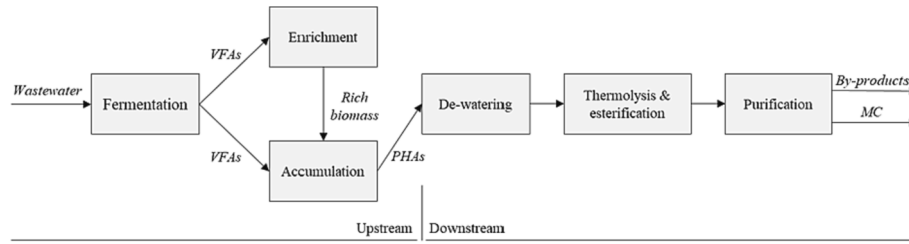
Fig. 1. Schematic overview of the complete simulation of MC production from wastewater. Upstream: wastewater is fermented to volatile fatty acids (VFAs) and converted to PHAs using an enrichment-accumulation approach. Downstream: The PHA stream is de-watered and converted to MC and byproducts using a thermolysis & esterification step, after which the MC is purified.

conversion rate q_{sub}^{max} (Eq. (4)). For the adsorption expression, k_i represents the adsorption coefficient for each substrate, c_i represents the corresponding substrate concentration, γ represents the exponent coefficient for each compound separately, and δ represents the general exponent covering the whole reaction. This is re-written as a summation of the half-velocity constant (K_{sub}) and the concentration of the substrate $C_{sub}(t)$ (Eq. (5)).

$$LHHW \ rate(q_{sub}) = \frac{k*T^n*e^{-\frac{E_A}{R}\left(\frac{1}{T}\right)}\left(k_f[sub]\right)}{\left(\sum k_i(\prod c_i^\gamma)\right)^\delta} = \frac{1*\left(q_{sub}^{max}*C_{sub}(t)\right)}{K_{sub} + C_{sub}(t)}$$
$$= Monod \ rate \qquad (2)$$

$$kinetic \ factor = k*T^n*e^{-\frac{E_A}{R}\left(\frac{1}{T}\right)} = 1 \qquad (3)$$

$$driving \ force = k_f*C_{sub}(t) = q_{sub}^{max}*C_{sub}(t) \qquad (4)$$

$$Adsorption \ expression = \left(\sum k_i\left(\prod c_i^\gamma\right)\right)^\delta = K_{sub} + C_{sub}(t) \qquad (5)$$

As the reaction is set to be irreversible and q_{sub}^{max} and K_{sub} are constants, the rate is determined by the substrate concentration. The kinetic parameters for all the upstream reactors is listed in Table 1. For more details (see supplementary material).

2.2.1.1. Fermentation. Fermentation is the first step in the upstream process, where the sugar-rich wastewater is fermented into VFAs (Fig. 1 - Fermentation). Along with VFAs, solids and ethanol are produced which negatively influence the PHA production (Tamis et al., 2015). Therefore, the fermentation is designed as a granular anaerobic sludge bioreactor (ASBR) with an additional settling step to limit the ethanol production and presence of solids, while delivering similar amounts of VFAs compared to other fermentation techniques (Tamis et al., 2015). Additionally, this approach allows for effective fermentation at pH 4.5,

Table 1
Maximum conversion rate (q_{sub}^{max}) and half-velocity constant (K_{sub}) for each of the substrates in the ASBR, enrichment and accumulation reactor. The constants are obtained using data from experiments (Tamis et al., 2015; Tamis et al., 2014a).

Substrate	$q_{sub}^{max}\left(\frac{kmol}{h*m^3}\right)$	$K_{sub}\left(\frac{kmol}{m^3}\right)$
ASBR		
Glucose	0.043	0.001
Enrichment reactor		
Acetic acid	1.98	8.33E-3
Propionic acid	0.86	6.75E-3
Butyric acid	2.04	5.67E-3
Valeric acid	0.31	4.90E-3
Caproic acid	0.99	4.30E-3
Accumulation reactor		
Acetic acid	0.26	8.33E-3
Propionic acid	0.12	6.75E-3
Butyric acid	0.27	5.67E-3
Valeric acid	0.04	4.90E-3
Caproic acid	0.13	4.30E-3

reducing the amounts of required additional chemicals. Therefore, the kinetic data described by Tamis et al. (Tamis et al., 2015) with a ASBR method is used in the simulation. The additional settling step removes 97 % of the volatile suspended solids (VSS). The produced VFAs include acetic acid (AA), propionic acid (PA), butyric acid (BA), valeric acid (VA) and caproic acid (CA) (Lee et al., 2014).

2.2.1.2. Enrichment and accumulation. Once the produced VFAs leave the fermentation, they enter the next unit operation where they are converted into PHA using MMCs. This conversion can be performed either by an *enrichment accumulation* or a *direct accumulation* method. This process is built based on an *enrichment accumulation* approach where the focus is on maximizing the PHA production through applied optimum selective pressures. In contrast, *direct accumulation* approach primarily focuses on the use of the PHA-storage capacity of the waste activated sludge for PHA production. The *enrichment accumulation* method generally results in higher PHA content than *direct accumulation* with increased content of up to 0.80 g_{PHA}/g_{VSS} compared to 0.52 g_{PHA}/g_{VSS}, respectively (Estévez-Alonso et al., 2021).

In the *enrichment accumulation* technique, half of the produced VFA stream is used as nutrients for the enrichment of PHA producing microorganisms (Fig. 1 - Enrichment). The enrichment follows a feast famine approach, where the VFA stream is added during the feast phase. During the famine phase, no nutrients are added and the stored PHA will be used for biomass maintenance and growth. At the end of the famine phase, only biomass with PHA storing capabilities have survived (rich biomass). Half of the enrichment tank broth with rich biomass is transferred to the accumulation reactor, while the remaining volume of the enrichment tank is re-used for another enrichment reaction. In the accumulation reactor, the rich biomass accumulates the PHA with the second half of the VFA stream as nutrients using a fed-batch approach (Fig. 1 - Accumulation). The accumulation results in an aqueous biomass stream with intracellular PHA (PHB + PHV). The ratio of PHB/PHV is dependent on the type of VFA, as AA, BA and CA result in production of PHB, while PA and VA result in the production of PHV (Lee et al., 2014).

The type of MMC also influences the fermentation, enrichment, and accumulation steps. Two types of MMC systems are used: the ASBR uses an MMC that is capable of acidifying sugar into VFAs, and the enrichment reactor uses an MMC with PHA storing properties. Lab experiments have shown that MMC systems that are dominated by *Plasticicumulans acidivorans* achieve the highest PHA content (Jiang et al., 2011; Johnson et al., 2009). Kinetic data from a pilot plant initiative with *P. acidivorans* is used for calculation of the enrichment and accumulation kinetics. The results are most promising compared to other pilot plant initiatives, resulting in a relative high PHA content of 0.70 ± 0.05 g_{PHA}/g_{VSS} and a high *PHB/PHV* ratio of 84/16 (Estévez-Alonso et al., 2021).

2.2.2. Downstream process design (PHA conversion)
The downstream section is responsible for the conversion of PHA into MC and its isolation from other byproducts. The downstream process starts with thermolysis and esterification (T&E) of PHA (Fig. 1 – Thermolysis & esterification). Intracellular PHA can be used in the

esterification, therefore no extensive purification steps are required beforehand, except for a centrifugation step to remove the excess water (Fig. 1 – De-watering). Methanol is added in excess (13.2 $g_{methanol}/g_{PHA}$) to use in the T&E reactor. The T&E reactor is modelled with mass balances, and kinetic rates are not included in this reactor. The time, temperature, pressure, and concentration of water have influence on the type and quantity of the (by)products. Using experimental yields on these variables, the stoichiometry and mass balances for different operation conditions are established and integrated into the simulation for a sensitivity analysis. A final purification process is implemented to recover and purify the produced MC (Fig. 3 – Purification).

3. Results and discussion

Fig. 2 depicts the developed simulation for both up- and downstream processes. Each unit operation will be discussed in detail in the next sections. The experimental results from the thermolysis and esterification reactions were used to develop the T&E unit in the simulation (see supplementary material).

3.1. PHA production

3.1.1. Model validation

To study the reliability of the model, various waste streams with known VFA compositions are introduced to the process and the subsequent PHB/PHV ratio is determined and compared to the experimental values. A review on pilot plant-scale operations of PHA production with *P. acidivorans* dominated MMC systems presents a variety of VFA compositions from different feedstocks (Estévez-Alonso et al., 2021). A selection of various wastewaters is presented in Table 2, including the concentration of original chemical oxygen demand (COD) in the wastewater ($g_{COD/L}$) and the corresponding produced VFA content (g_{VFA}/g_{COD}). The composition of VFAs is known; therefore, the ASBR step can be cut and the solution of VFAs is submitted as feed stream in the model.

As can be seen in Table 2, a good agreement between the modelled and the experimental PHA compositions can be achieved across different wastewater compositions, demonstrating the reliability of the simulation and the kinetic model. It can also be seen that AA, BA and CA result in production of PHB, while PA and VA result in the production of PHV.

3.2. PHA conversion

3.2.1. T&E of PHB and PHV

The downstream process includes the conversion of PHA to MC and a purification procedure to isolate the produced MC from byproducts. The established PHB/PHV composition (86/14) from the candy bar factory wastewater will be used for downstream processing.

The reaction pathway of PHB and PHV conversion inside the T&E reactor (Fig. 2, T&E) is presented in Fig. 3. The main pathway (orange line) represents the thermolysis of PHB into crotonic acid (CA). Here water is used as catalyst and little amounts are required for the reaction to take place. Therefore, it is not included in the mass balance. With addition of methanol the CA can be esterified into MC. The esterification reaction is reversible as hydrolysis with presence of water. PHB can also be hydrolysed into hydrobutyric acid (HBA) with water or converted to methyl-3-hydroxybutyric acid (M3HB) using methanol in a trans-esterification reaction. HBA and M3HB can also react into one another with esterification and hydrolysis. At higher temperatures, CA can degrade into propylene and CO_2. Degradation of M3HB takes place with an intermediate ring formation and follow-up thermolysis into methyl acetate (MA) and acetaldehyde (Zapata et al., 2007).

The conversion of PHV follows a similar reaction pathway. PHV is converted into pent-2-enoic acid (PA2) and pent-3-enoic acid (PA3) via thermolysis. With presence of methanol, PA2 and PA3 can be esterified to methyl pent-2-enoic acid (MP2) and methyl pent-3-enoic acid (MP3), respectively. PHV can also be hydrolysed into hydroxypentanoic acid (HPA) or *trans*-esterified into methyl-3-hydroxypentanoic acid (M3HP). HPA and M3HP can convert into each other with hydrolysis or esterification. At elevated temperatures, MP2 and MP3 can degrade into 2-butene and 3-butene, respectively. M3HP is assumed to degrade into methyl acetate and propanal.

3.2.2. Purification

To isolate the produced MC from other byproducts a purification process is included after the T&E reaction. It is assumed that the cell material surrounding PHA ends up as sediment during the esterification (Fernández-Dacosta et al., 2016). A filtration step is therefore introduced to remove the sediment (Fig. 2, FILTER). Most of the produced gases such as CO_2 and propylene can be separated via a flash evaporation step with a set vapor fraction of 0.10 at 45 °C (Fig. 2, FLASH1). A
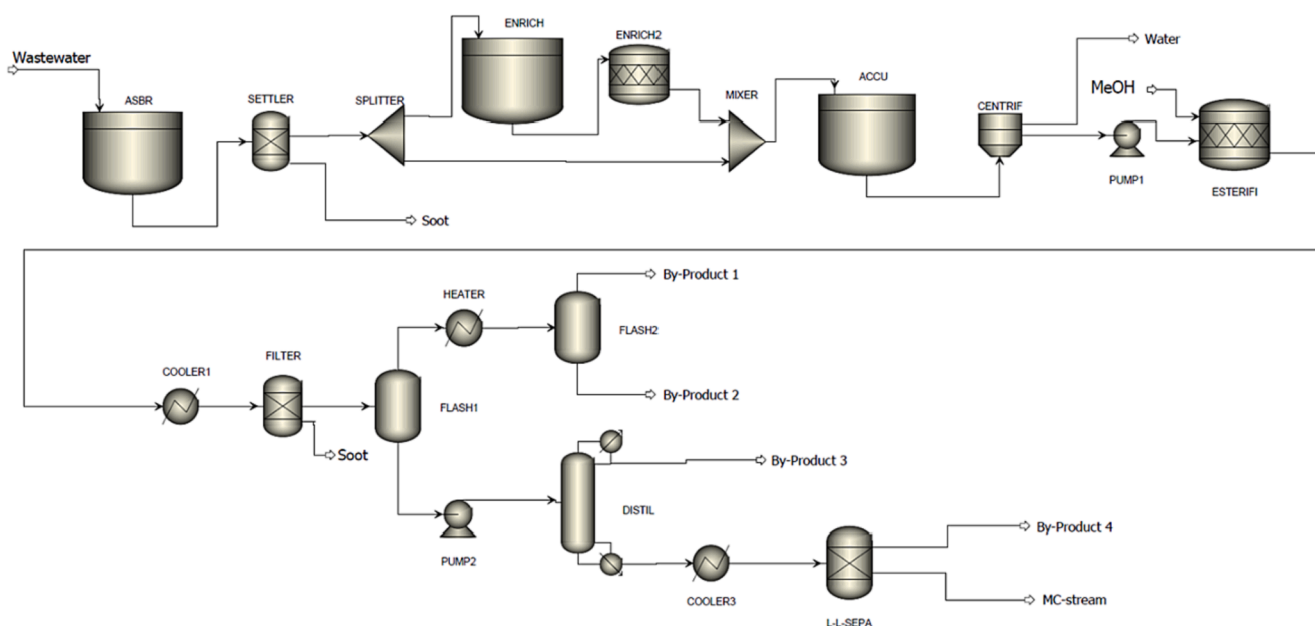

Fig. 2. The developed flow diagram of the process. The required product MC resides in the MC-stream (bottom right), and four side streams are created from the downstream process.
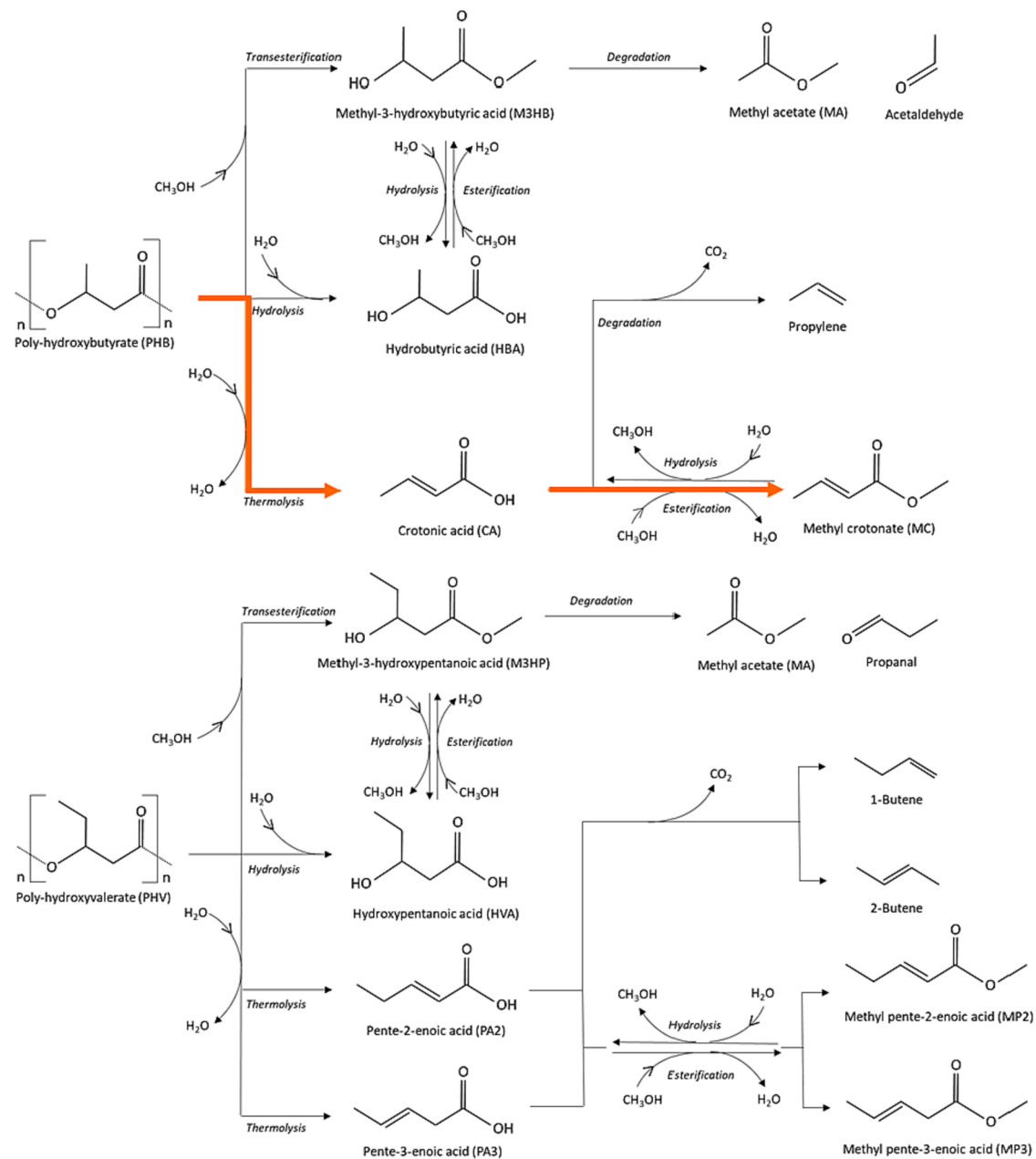
Fig. 3. Pathway of PHB conversion to MC via thermolysis to crotonic acid and follow-up esterification (orange line). A total of 7 products can be created from the conversion of PHB. Likewise, a total of 10 products can be produced from the conversion of PHV. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
The COD concentration, VFA to COD yield, and VFA composition in various fermented wastewaters.

Feedstock	$\frac{g_{COD}}{L}$	$\frac{g_{VFA}}{g_{COD}}$	VFA composition (%)					PHB/PHV		Ref.
			AA	PA	BA	VA	CA	Experimental	Simulation	
Fermented candy bar factory wastewater	7.8	0.64	32	14	33	5	16	84/16	86/14	(Tamis et al., 2014a)
Fermented beet process wastewater	6.95	0.62	28	33	39	0	0	–	75.4/24.6	(Anterrieu et al., 2014)
Fermented paper mill wastewater	6.2	0.72	37	21	29	16	0	75/25	71.3/28.7	(Tamis et al., 2018)
Fermented OFMSW*	16	0.91	21	13	38	12	16	87–93/7–13	87.8/12.2	(Valentino et al., 2019)
Tomato waste concentrate	11.05	0.83	34	23	17	16	10	51–58/42–49	77.9/22.1	(Bengtsson et al., 2017)

* OFMSW: organic fraction of municipal solid waste.

second flash evaporation after de-pressurisation separates the gases from remaining liquids like methanol (MeOH) and water, with a vapor fraction of 0.05 at 20 °C (Fig. 2, FLASH2). The resulting streams are labelled as Side stream 1 (vapor) and Side stream 2 (liquid). The set vapor fractions are determined via an iterative approach, identifying a balance between the portion of the stream that needs to be separated

while making sure the MC recovery remains high-levelled. Next, a distillation column is introduced for the remaining stream (Fig. 2, DISTIL). The pressure of the boiler and condenser is set to 2 bar. The recovery of water and MeOH is determined as 0.01 and 0.99, respectively. MeOH is released as the top product (Side stream 3) and the bottom product containing MC is depressurized and cooled to 20 °C (Fig. 2, COOLER3). Two main phases are present in the remaining liquid stream, based on different polarities. MC, MP2 and MP3 have water-immiscible properties, while the remaining components with an OH group dissolve more easily in water. Therefore, separation based on polarity removes the MC, MP2 and MP3 from the aqueous stream. A temperature of 20 °C is required, as less mixing of the phases will take place. The separation can be executed with chromatography with a polar stationary phase (Fig. 2, L-L-SEPA). The chromatography is modelled as a liquid–liquid separator with pre-determined split factors. For the polar components, a split factor of 0.01 is used while for the non-polar components, a split factor of 0.99 is used. As a result, 99 % of MC is separated from the water, and 1 % of the water is left in the MC stream. The aqueous stream is labelled as Side stream 4 and the remaining liquid with the desired MC is called MC-stream. Table 3 summarizes the composition of all the side streams. MC stream consists of mainly MC and traces of MP, MP3 and MA. As expected, propylene and CO_2 make up most of the side stream 1 which is the gas outlet of the second flash separation. In side stream 2, methanol is recovered from the top of the distillation tower, whereas water exits from the bottom of the distillation tower and is finally recovered in side stream 4.

3.2.3. The influence of operating conditions

The influence of various parameters including the operating pressure, reaction duration and the water concentration during the transesterification reaction on the purity and the quantity of MC is investigated. Experimental yields are used to identify the selectivity of the 7 products from PHB conversion (see supplementary material) as described in Fig. 3. These are copied to the PHV pathway and used as input for the T&E reactor in the simulation, upon which the general purification process is performed. Subsequently, the composition of the final MC stream is determined and analysed. Only component contents of above 0.005 w/w are included in the analysis and the remaining compounds are assumed negligible.

Since in the experiments the MA, acetaldehyde, and propylene production were not measured, only the total amount of degraded M3HB and CA is known (Fig. 3). Therefore, in the simulation, it is assumed that 50 % of the degraded compounds is propylene and 50 % is MA and acetaldehyde.

In the first sensitivity analysis experiment, the water to MeOH ratio was varied between 0 and 0.4 v/v, while the temperature, the pressure and the reaction time were kept constant at 200 °C, 18 bar and 6 h, respectively. As shown in Fig. 4a, a water to MeOH ratio of 0 v/v results in the highest MC concentration of 0.81 w/w. Upon increasing the water concentration to 0.4 v/v, the MC concentration in the MC-stream decreases to 0.63 w/w. This could be due to increased hydrolysis activity in the T&E reactor which leads to lower MC production. Additionally, a larger presence of water for the chromatography step (Fig. 2, L-L-SEPA) results in a larger content of water to remain in the MC stream, due to its pre-determined split factors. Limiting the water content to a near 0 value

is beneficial for the MC purity, however extracting all the water from the PHA-rich biomass stream after the accumulation reaction could be energy intensive due to extensive centrifugation or evaporation.

The influence of operating pressure is tested by changing the pressure between 2 and 35 bar. Other variables are kept constant (T = 200 °C, t = 6 h, water/MeOH = 0 v/v). A pressure of 18 bar results in the highest MC concentration of 0.81 w/w (Fig. 4b). At low and high pressures, the MC concentration decreases to 0.45 and 0.47 w/w, respectively, while MA concentration increases to 0.46 and 0.43 w/w, respectively. More (trans)esterification occur at higher pressures (Spekreijse et al., 2015), increasing the selectivity toward M3HB and M3HP production and subsequent degradation into MA (Fig. 3).

To study the effects of reaction duration, the reaction time was varied between 3 h and 8 h while other variables were kept constant at T = 200 °C, P = 18 bar, and water /MeOH = 0 v/v. It is assumed that PHB and PHV are fully converted after 3 h of reaction, as the sum of MC and CA content does not increase anymore after 3 h (see supplementary material). When the reaction is incomplete, it is uncertain how much PHB is left, as well as how much gaseous compounds are produced via degradation, as both are not measured in the experimental results (see supplementary material). Therefore, a reaction time between 0 and 3 h is not investigated. As can be seen in Fig. 4c, the reaction time after 3 h does not have significant influence on the composition of the MC stream. Although, a slight increase in MA concentration can be seen upon increasing the reaction time. This is mainly due to increased M3HB and M3HP degradation at 200 °C, at longer reaction times.

As described in Fig. 3 MP2 and MP3 are produced from the conversion of PHV with the same reaction pathway as MC production from PHB. Therefore, these compounds have comparable properties, with the main difference being one CH_3 extension at the chain for MP2 and MP3. Separation of MC from MP2 and MP3 based on volatility could be possible, as the boiling point of MC is 119 °C, compared to 135 °C and 136 °C for MP2 and MP3, respectively. However, the elevated temperature could result in additional degradation of the components and would require substantial amounts of energy. Moreover, MA also remains present across all experiments, in varying concentrations. Degradation of M3HB and M3HP results in MA production (Fig. 3). Due to a slightly lower boiling point compared to MeOH (57 vs 65 °C, respectively) most of the MA is distilled away from MC (Fig. 2, DISTIL), however the remaining MA stays present until the final MC stream, due to similar polarity.

The influence of the operating conditions on the recovery of MC in terms of weight of MC per weight of PHA is presented in Fig. 5. As expected, increasing the water concentration has a negative effect on MC recovery due to increased hydrolysis of PHB which is in competition with thermolysis reaction that is responsible for the production of MC. Operating at high pressures (above 18 bar) enhances the transesterification reaction producing MA, which occurs in parallel to thermolysis reaction. The reaction time has limited impact on the MC recovery and therefore no more than 3 h of reaction would be beneficial for MC recovery (Fig. 5c).

4. Conclusions

A coherent plant design for the production of PHB and PHV from

Table 3
The composition of the MC and by product streams (T = 200 °C, P = 18 bar, t = 6 h, and water content = 0.1 v/v).

| MC stream Component	(w/w)	Side stream 1 Component	(w/w)	Side stream 2 Component	(w/w)	Side stream 3 Component	(w/w)	Side stream 4 Component	(w/w)
Water	0.037	CO_2	0.450	Water	0.048	Methanol	0.988	Water	0.801
MC	0.809	Methanol	0.123	CO_2	0.010	MA	0.007	Methanol	0.045
MP3	0.065	Propylene	0.365	Methanol	0.900			M3HP	0.098
MP2	0.065	1-butene	0.016	Propylene	0.007			CA	0.017
MA	0.015	Acetaldehyde	0.040	MA	0.008			HBA	0.016
				Acetaldehyde	0.018			M3HP	0.015
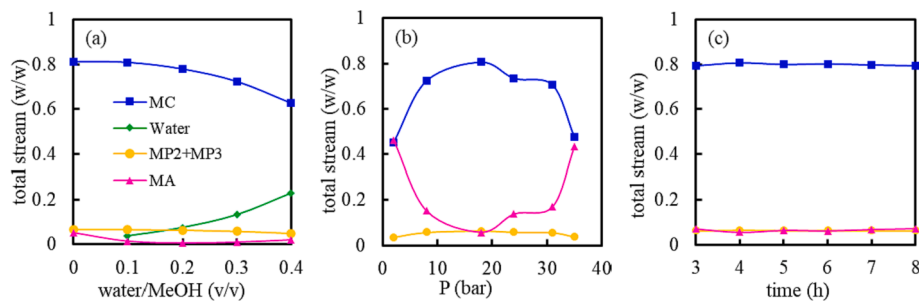
Fig. 4. The influence of the water concentration, pressure, and reaction time on the composition of the MC stream.
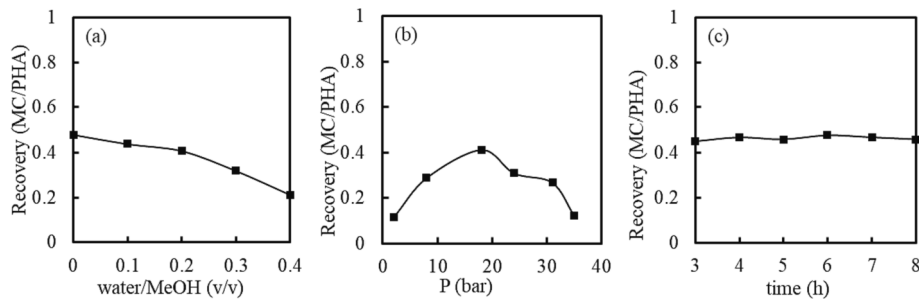

Fig. 5. The influence of the water concentration, pressure, and reaction time on the recovery of MC (the nominal condition is water/MeOH = 0 v/v, P = 18 bar, T = 200 °C and t = 6 h).

wastewater, and subsequent thermolysis and esterification to building block chemicals is developed. The predicted PHB/PHV composition was in agreement with the experimental data across various fermented industrial wastewaters. In the downstream process design, a series of reaction and separation steps were developed to convert the PHA into MC and 16 other byproducts, with MC purities varying between 40 % and 80 %. Amongst the various operating conditions, the water content and operating pressure were found to have substantial influence on the purity and yield of MC.

5. Data availability

Data will be made available on request.

CRediT authorship contribution statement

Joris Koch: Formal analysis, Investigation, Data curation, Software, Writing – original draft. **Elinor Scott:** Resources, Supervision, Writing – review & editing. **Johannes Bitter:** Supervision, Writing – review & editing. **Akbar Asadi Tashvigh:** Conceptualization, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biortech.2022.127994.

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