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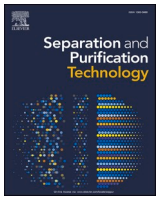

# Extraction of low molecular weight polyhydroxyalkanoates from mixed microbial cultures using bio-based solvents

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ABSTRACT

(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV) obtained from waste/wastewater using a mixed microbial culture (MMC) usually varies in its properties due to daily variation in the waste/wastewater composition applied as feedstock. In the current study, the average molecular weight (MW) of PHBV was purposely reduced from about 1 MDa to about 200 kDa by drying the PHBV-rich biomass at elevated temperature of 120 °C for 18 h to ease extraction and handling. Furthermore, conversion into value-added chemicals such as *trans*-crotonic acid (*trans*-CA) and *trans*-2-pentenoic acid (2-PA) by thermal decomposition (pyrolysis) benefits from the lower MW. For the extraction of low MW PHBV, the use of the bio-based solvents 2-methyltetrahydroxyfuran (2-MTHF) and dihydrolevoglucosenone (cyrene) was studied. The maximum extraction yield of 62 ± 3 % with purity of > 99 % was achieved with 2-MTHF at 80 °C for an hour with high biomass to solvent ratio of 5 % (g/mL). Cyrene-based extractions resulted in the highest yield of 57 ± 2 % with purity of > 99 % at 120 °C in 2 h with 5 % (g/mL) biomass to solvent ratio. The mass balance closure over the extraction process indicated that about 15 % and 10 % of polymer has remained in the residual biomass after extraction by 2-MTHF and cyrene, respectively. The performance of these new solvents to extract polymers with various average MW was compared to the benchmark extractions using chloroform and dimethyl carbonate (DMC). It was found that for the polymers with low average MW the extraction efficiency of the proposed solvents exceeds the benchmark solvents.

## 1. Introduction

Polyhydroxyalkanoates (PHAs) are a class of thermoplastics derived from renewable biomass sources [1]. The variability in the applications of PHAs is diverse, ranging from biodegradable packaging to medical products [2]. PHAs are considered as an attractive alternative to conventional fossil fuel derived polymers due to their biodegradability and biocompatibility, as well as their renewable character, thereby reducing fossil feedstock dependency and its associated environmental impact [3]. Two routes exist for PHAs produced via bacterial fermentation, being either through pure cultures, or via mixed cultures. Sterile conditions are used to grow monocultures in the pure culture-route, which contains a single species of microorganism. The monocultures are fed with substrates like glucose, starch, or vegetable oil to produce PHA rich biomass. Whereas via a mixed culture, more than one type of microorganisms is grown on the same substrate, using volatile fatty acids (VFAs) as ideal precursors for PHA synthesis. This approach is characterized by reduced fermentation costs as it is not required to pretreat the substrate and sterilization is not necessary. These benefits make mixed cultures an attractive route for PHA production, extending the possibility to use cheaper carbon sources such as municipal solid waste and industrial wastewaters [4].

Food waste is currently a global concern due to the rapid growth of urban population and economic development [5]. Although the number of food waste treatment plants has also increased, traditional treatment techniques come with several limitations. Greenhouse gases emission, odor production and leaching are the main issues driven from conventional food waste treatment techniques such as incineration, composting and landfilling [6]. Therefore, valorizing these waste streams by fermentation to produce high-value bio-based platform chemicals

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increasingly gains interest [7,8]. For instance, food waste is a potential feedstock for microorganisms due to large quantity of VFAs production during its anaerobic digestion process [9–11]. In 2012, Paques Biomaterials B.V. established a pilot plant to produce PHA from different waste streams, such as industrial wastewater and food waste. From 2015 to 2021, the pilot plant operated continuously using source separated organics, which are dominated by food and garden waste. This process on source separated organics, including food waste, has the potential to produce a biomass with high PHBV content e.g. up to 77 ± 18 gPHA. $gVSS^{-1}$ (n = 3) in pilot-scale trials [12].

During the fermentation process in the MMC, the ratio between VFAs with an even number of carbons and with an odd number of carbons, will impact the ratio of HB (3-hydroxybutyrate) and HV (3-hydroxyvalerate) monomers in the final PHBV (Poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) copolymer [13]. When a waste stream is used as feedstock, it is challenging to produce PHBV copolymer with a constant quality in large scale due to the daily variation in the composition of the waste stream, for that reason, the value of the polymer is less than when produced from highly controlled fermentation cultures. Alternatively, it is possible to obtain highly valuable chemicals by pyrolysis of PHBV copolymers [14]. Trans-CA and 2-PA are the main pyrolysates in thermal decomposition of PHBV, the estimated market price of tranc-CA justifies the added process cost of the pyrolysis [15]. Trans-CA and its esters have a wide range of applications in textile, paint, coating and as a building block in synthesis of co-polymer with vinyl acetate [14,16]. The esters of 2-PA can be used in flavoring essences. However, its function is currently limited to use as a raw chemical in biomedical and pharmaceutical research purposes [17,18]. Despite their great potential to be employed in various applications, the current market of these unsaturated carboxylic acids is limited as a result of their complex production routes. Thus, providing a relatively straightforward and bio-based pathway is expected to enhance their market. In the current study, we focused on the solvent extraction of MMC-based PHBV with various average MW. The thermal decomposition of the polymer towards 2-alkenoic acids will be studied in the follow up work.

The molecular weight of PHBV copolymers is one of the crucial characteristics determining their solubility. The higher the MW, the lower the solubility of polymer, which leads to lower extraction yields in the solvent extraction method for polymer recovery. Solvent extraction of PHA from biomass has been widely studied [19,20]. It involves dissolution of the polymer in a solvent followed by an appropriate recovery technique, for which mostly precipitation techniques are used to retrieve the polymer in the solid form. Various types of solvents have been reported to extract PHA from a MMC [21]. Among them, dimethyl carbonate (DMC) is gaining more interest as a green solvent [21–24]. Samori et al. [25] reported an extraction yield of 49 % with the purity of 94 % for PHB recovery from a MMC using DMC. Although applying a good solvent such as DMC enhances the extraction yield of the polymer, next to the extraction also the recovery from the solvent should be considered. To retrieve the polymer from the solvent in high purity, an antisolvent must be applied. For solvents such as DMC and chloroform (not green, but often reported as a reference solvent [23,26,27]), it can be challenging to precipitate out the polymers when they have a lower MW due to their high solubility. Overall, the performance of a solvent-antisolvent couple can be affected by the nature of the biomass as well as the characteristics of the polymer.

In this study, three batches of biomass containing PHBV with different MW were produced. The recovery of low molecular weight polymer has been investigated using two biobased solvents, 2-MTHF and cyrene. Cyrene is a new bio-based solvent produced by reduction of levoglucosenone which is a pyrolyzate from fast pyrolysis of cellulose [28]. The application of this new solvent is dramatically growing over the time. It is used as lipase-catalyzed bio-transformations [29], polymerization to of the methacrylic derivative of cyrene [30] and as a solvent for polyethersulfone and poly(vinylidene fluoride) membrane preparation via phase inversion [31]. Cyrene has also been used as a diluent in extractive distillation to purify bio-based levulinic acid [32]. Moreover, it was found that cyrene can be a promising candidate in separation of aromatic/aliphatic systems [33]. To the best of our knowledge, these solvents have never been employed to extract PHA from MMC. Thus, the effect of several operational conditions on PHA recovery from MMC using cyrene and 2-MTHF were investigated. Furthermore, Chloroform and DMC based extraction were performed as benchmark extractions for the comparative assessment of the biobased solvents in terms of extraction yield, polymer purity and characterizations of the extracted polymers. Lastly, the performance of all four solvents were assessed for extraction of PHBV with various average MW from a MMC.

## 2. Material and methods

### 2.1. Chemicals

2-Methyl tetrahydrofuran (2-MTHF, (≥99 %)), chloroform ($CHCl_3$, (≥99.8 %), dimethyl carbonate (DMC, (≥99 %)), *n*-heptane (≥99 %), *n*-hexane (≥95 %), benzoic acid (≥99.5 %), sulfuric acid (95–98 %), polyhydroxybutyrate (PHB (99 %)), methyl(R)-3hydroxyvalerate (≥98 %), chloroform-D (≥99.8 %), Methanol (MeOH, (≥99.9 %)), ethanol (EtOH,(≥99.9 %)), *trans*-crotonic acid (≥98 %), *trans*-2-pentenoic acid (≥98 %), acetone, 1,2,4,5-Tetrachloro-3-nitrobenzene and tetrahydrofuran were purchased from Sigma-Aldrich. Cyrene (≥98.5 %) was kindly provided by the CIRCA Group. The water used was ultrapure (Milli-Q, with a resistance of 18.2 μΩ cm at 25 °C). The chemicals and nutrient composition used for PHA production are described in the latest publication of the Paques Biomaterials pilot plant [12].

### 2.2. PHBV production using mixed microbial culture

Four separate batches of dry biomass were used in this work. Three batches contained PHBV-rich biomass that was produced in a pilot bioreactor system with a liquid volume of ca. 200 L (Biocel Orgawold, Lelystad, The Netherlands) from leachate from organic waste [12]. They were obtained by mechanical dewatering with a centrifuge at 3000*g* and dried in a tray oven for 18 h at 120 °C. Batch 3 was produced in a different 4 $m^3$ bioreactor system from Paques Biomaterials according to the procedure described by Werker et al. [34] and the PHBV biomass was stabilized with acid [35] and dried with a contact type dryer at around 100 °C for 1 min.

### 2.3. Thermal stability of PHBV during drying PHBV-rich biomass

To evaluate the polymer degradation during its drying step, the PHBV-rich biomass (batch 1) was produced as explained in the previous section. After dewatering, the biomass was placed in an oven at 120 °C, followed by sampling over the time. The samples were analyzed by GPC to determine the average MW, the number average (Mn) and polydispersity index (PDI) of PHBV.

To quantify experimental results, a correlation was applied that relates the scission rate and the degree of polymerization (DP). The DP is the average number of structural units per polymer chain [36], defined in Eq. (1) as the number of total monomers (Nm) divided by the total number of polymer chains (Np):

$$DP = \frac{N_m}{N_p} \tag{1}$$

Another expression for DP is presented by Carothers' equation, which expresses the degree of polymerization as function of the extent of reaction [36]:

$$DP = \frac{1}{1-p} \tag{2}$$

where p is the conversion of the reaction. Off course, this relation simplifies the real situation, as eventually this would result in an infinite degree of polymerization, no side reactions are considered, and all monomers end up in a single polymer molecule. Nevertheless, important relations are based on Carothers' equation, and using the definition of Mn, Eq. (2) can be expanded, resulting in a link between DP and Mn:

$$Mn = \frac{M_0}{1-p} = M_0 \times DP \tag{3}$$

In the expression, Mn is the number average molecular weight, and $M_0$ is the molar weight of the repeating unit in the polymer.

Degradation of PHBV is reported to follow random scission in literature [37]. The possibility of breaking each bond was assumed to be the same. The Ekenstam linear relation between time and reciprocal DP has been used to describe thermal degradation of PHBV. The equation of the proposed relationship is given in Eq. (4) [38]:

$$\frac{1}{DP_t} - \frac{1}{DP_0} = kt \tag{4}$$

The rate constant k is defined as the scission rate constant with a unit of $s^{-1}$. Total number of monomers was assumed to stay constant during degradation, so upon breaking of polymer chains, Np increases by the number of the scissions [36,39].

$$N_{P_t} = N_{P_{t-1}} + s \tag{5}$$

Combining Eqs. (1), (4) and (5), the physical meaning of the Ekenstam relation becomes that the number of scissions is proportional to time when random scission dominates (Eq. (6)):

$$\frac{1}{DP_t} - \frac{1}{DP_0} = \frac{N_{P_0}+s}{N_m} - \frac{N_{P_0}}{N_m} = \frac{s}{N_m} \tag{6}$$

### 2.4. Recovery of PHBV from biomass

A schematic view on the recovery process is shown in Fig. 1. In all experiments, first the polymer was extracted from the biomass by solvent extraction. After extraction, the solution was separated from the residual biomass by either centrifugation or filtration. Next, an antisolvent was added to the solution to precipitate the PHBV. The precipitated polymer was separated from the supernatant (the remaining solution) using a centrifuge at 7000 rpm for 10 min.

For selection of solvent extraction conditions to obtain PHBV from biomass, an important consideration is that there is not one generalized method that can be applied for all solvents to compare the performance of these solvents with each other. The optimum conditions vary from solvent to solvent. Therefore, for the biobased solvents investigated in this study, a range of conditions was applied, and for the reference solvents chloroform and DMC, the methods reported by Mongili et al. [23] was used.

Investigations on the extraction conditions with the biobased solvents 2-MTHF and cyrene were executed using biomass from batch 1. The optimization was carried out in two steps. First, the precipitation step was enhanced by varying solvent to antisolvent volume ratio (1–5), time (24–48 h) and temperature (20–4 °C). To consider the possible impact of the impurities associated to the soluble non-polymeric part of biomass on precipitation efficiency, an extraction was carried out at certain condition for all the experiments, followed by precipitation at various conditions. Second, the operation parameters of extraction (time, temperature, and biomass to solvent ratio) were optimized. The temperature was kept constant at 80 °C for 2-MTHF and differed from 80 to 140 °C for cyrene based extraction experiments.

In the solvent extraction experiments, a quantity of 1–6 % (g/mL) of dry biomass was suspended in 5 mL of the selected solvent in a borosilicate glass screw cap tube lined with a polytetrafluoroethylene (PTFE) rubber cap. The solution was heated using a block heater and agitation was carried out with a magnetic stirrer at 8000 rpm. After the chosen extraction time (0.5–3 h), the solution was hot filtered through a predamped Whatman filter paper (8–12 μm retention) to remove the cell debris. Afterwards, the polymer was precipitated by adding a desired amount of an antisolvent to the filtered solution. Finally, the polymer was separated by centrifuge at 7000 rpm for 10 min followed by washing with ethanol and dried in vacuum oven at 50 °C for 24 h.

The mass balance over the extraction process was checked to discover the limiting stages during the extraction. The initial PHBV content of the biomass and remaining quantity of the PHBV in residual biomass and supernatant was determined by gas chromatography (GC). The fraction of the polymer that was lost during the handling was calculated based on mass balance closure over the PHBV for the entire process.

### 2.5. PHBV quantification

GC and thermal gravimetric analysis (TGA) were applied to quantify PHBV. Following the method of Braunegg et al [40], about 10 mg of a sample was treated with 2 mL of acidified methanol with $H_2SO_4$ (3 %v/v), 2 mL of chloroform and an internal standard of 50 μL benzoic acid (2 g benzoic acid in 50 mL methanol). The samples were placed in screw cap borosilicate glass tubes and heated to 100 °C for 4 h. The tubes were vortexed every 30 min for roughly 15 s. After the solution had cooled

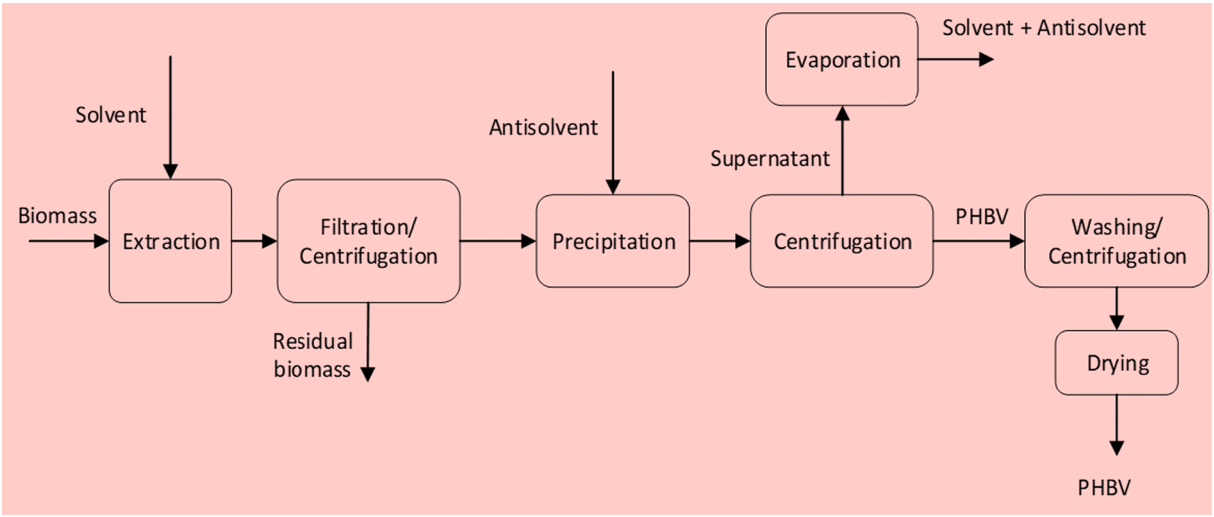


**Fig. 1.** The schematic view of the extraction process to recover PHBV from the biomass.

down, it was transferred to a falcon centrifuge tube followed by adding 2 mL of milli-Q water to remove non-reacted acid. Then, the solution was centrifuged for 5 min at 8000 rpm. Afterwards, the bottom layer of the solution was transferred to a GC vial using a syringe. An amount of 1 μL of the sample was injected in a Thermo Scientific Trace 1300 gas chromatograph equipped with a Flame ionization detector (FID) and an Agilent DB-Wax column (60 m, 0.25 mm, 0.25 μm). The gas carrier was helium at a flow rate of 35 mL/min. The temperature gradient started at 30 °C with a constant heating rate of 5 °C/min until it reached 120 °C and then at a rate of 25 °C/min till it reached 250 °C. The samples were analyzed in triplicate. A calibration curve for PHB and PHBV was produced using pure PHB and methyl(R)-3hydroxyvalerate, respectively.

The fraction of PHB and PHBV in the sample were defined by:

$$x_{PHB} = \frac{m_{PHB,GC}}{m_{Sample}} \tag{7}$$

$$x_{PHV} = 1 - x_{PHB} \tag{8}$$

$$Purity\% = (x_{PHB} + x_{PHBV}) \times 100 \tag{9}$$

where $m_{PHB,GC}$ is the amount of PHB calculated from the GC, $m_{Sample}$ is the amount of extracted material applied for analysis. Eq. (10) was used to determine the recovery yield of the polymer:

$$Extraction\ \ yield\ \ \% = \frac{m_{PHBV,E} \times Purity}{m_{DB} \times x_{PHBV,DB}} \times 100 \tag{10}$$

where $m_{PHBV,E}$ is the total amount of the collected polymer after extraction, $m_{DB}$ is the mass of dry biomass used for extraction and $x_{PHBV,DB}$ is the polymer fraction in the biomass.

### 2.6. PHBV qualification

**Thermal Gravimetric Analysis (TGA)** The degradation temperature of the extracted polymers was measured using TGA-550 instrument operated under nitrogen flow and heated samples from room temperature to 400 °C at a rate of 10 °C/min.

**Gel Permeation Chromatography (GPC)** The sample was dissolved in tetrahydrofuran (THF) with 6 mg/mL concentration and filtered through 0.2 μm disc filters into a vial. In the case of high molecular weight polymers, the samples were prepared in the screw cap vials and heated at 55 °C until about 90 % of the polymer is dissolved. GPC analysis was done at room temperature by injecting 20 μL of the dissolved polymer in an Agilent 1200 series GPC using THF as the eluent at a flowrate of 0.35 mL/min. Polystyrene was used as a calibration standard. The analysis was done in triplicate to determine the analysis error which was less than 0.5 %.

For analysis of the thermal stability of the polymers during drying, the samples were first dissolved in chloroform and diluted in THF. About 10 μL of the sample was injected for analysis by GPC on a Shimadzu Prominence GPC system with THF as the eluent at flow rate of 1 mL/min and operation temperature of 40 °C.

## 3. Results and discussion

### 3.1. Production of PHBV-rich biomass from wastewater

The biomass from batch 1 and 2 was produced in a bioreactor that was operated to produce PHBV-rich biomass from organic waste leachate [12]. After the aerobic digestion process resulting in PHBV-rich biomass, the biomass was collected and dried at high temperature (120 °C) for longer periods of time(18 h) to *in-situ* lower the average MW of PHBV. As can be seen from Table 1, the drying reduced the MW of the sample from about 1.5 MDa, measured using freeze drying, to about 200 kDa.

In order to examine the thermal stability of the PHBV during the biomass drying procedure, samples were taken to analyze the average MW and Mn of the polymer by GPC during the drying at 120 °C. As shown in Fig. 2, the Mn of PHBV drops from about 550 kDa to 77 kDa during 18 h drying at 120 °C. The experimental data were fit with the Ekenstam model to obtain the bond-breaking rate constant, k. By having k, the molecular weight could be predicted at a certain time length. The scission rate was assumed to be constant over the whole process in this model for simplicity and convenience. According to Eq. (4), molar mass measured by GPC was first converted to the degree of polymerization, and then k was obtained by linearly fitting the data points. The k value describes how many bonds per repeated unit were breaking over time. The scission rate constant after the fitting was 9.23 * $10^{-7}$ $min^{-1}$ which is slightly lower than $10^{-6}$ $min^{-1}$ which is reported by Kunioka et al. [37] for PHBV with 45 % HV at 140 °C. The variation can be explained by the difference in the applied temperature and HV content of the polymer which are 120 °C and 22 wt% in the current study.

**Table 1**
Average molecule weight (MW) of polymers from the same production batch with various drying methods.

| Dewatering method | Mn (kDa) | MW (kDa) | PDI |
|---|---|---|---|
| Oven drying at 120 °C | 77.21 | 204.05 | 2.64 |
| Freeze dry | 545.81 | 1465.75 | 2.69 |

The scission rate, however, started with a faster scission and then slow down at later stage. The cause of this phenomena might be the impurities remained inside biomass [41]. Thermal degradation of PHBV was reported to be dominated by β-elimination. Carbon at α-position gave away the electron and caused a bond break [42]. Metal ions, as one of the essential elements during the cultivation of microorganism, were presented in the biomass as the impurities. $Ca^{2+}$, for example, is Lewis acids and acted as the electrophile that was willing to interact with the carbonyl carbon and pulled the electron away to initiate the elimination [43].

The average MW of a polymer has a great impact on its solubility and consequently its solvent extraction efficiency. Therefore, the solvent extraction of this low MW was also optimized in the current study as most of the previously reported works have mainly focused on solvent extraction of PHA with high MW. Moreover, to evaluate the effect of MW on extraction efficiency, different batches of polymer with various MW were synthesized and applied in extraction process using different solvents.

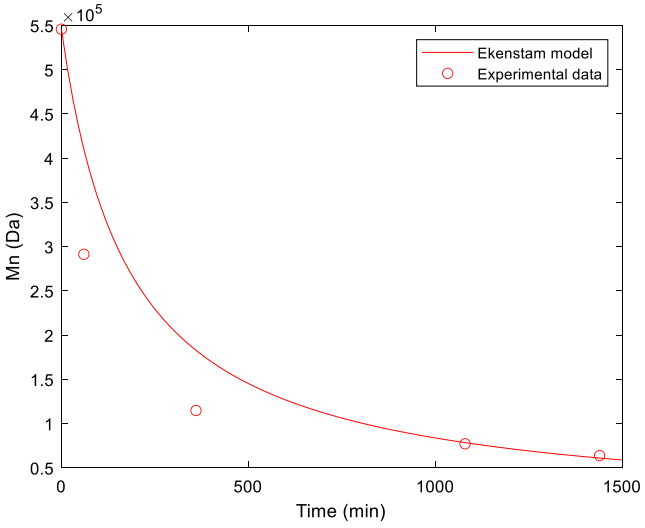


**Fig. 2.** PHBV degradation over the time during drying of PHBV-rich biomass at 120 °C and fitted with Ekenstam model (Eq. (4)) which resulted in k value of 9.23 * $10^{-7}$ $min^{-1}$.

To obtain a polymer sample with a high average MW, the PHBV-rich biomass from batch 3 was produced in a different bioreactor system according to the method of Werker at al. [34] and hardly any degradation occurred due to the acid stabilization and very fast drying conditions (1 min at 100 °C).

### 3.2. Optimization of the extraction with 2-MTHF and cyrene

To the best of our knowledge, 2-MTHF and cyrene have not been used before to recover PHA from biomass. In this study, the capability of these new bio-based solvents to extract PHBV was examined, and the extraction conditions were optimized to achieve the highest yield.

### 3.3. 2-MTHF

Initially, the precipitation step was optimized. By performing an extraction experiment using 2-MTHF at 80 °C, 1 h, and biomass to solvent ratio of 5 % g/mL), followed by precipitation of the polymer at various conditions. Due to the insolubility of the polymer in alkenes and full miscibility of 2-MTHF and the alkenes, *n*-pentane and *n*-heptane were selected as an antisolvent. Moreover, the solubility of the polymer in the solvent reduces by decreasing the temperature. Therefore, the precipitation was examined at room temperature (20 °C) and in the fridge (4 °C). Finally, the ratio of the antisolvent to solvent was varied from 1 to 4. The contact time for all the samples were kept constant at 24 h. as can be seen from the Fig. 3, *n*-heptane performs better than *n*-pentane in precipitation the PHBV dissolved in 2-MTHF. Moreover, the precipitation efficiency increases about 10 % by decreasing the temperature from ambient condition to 4 °C. Regarding the required amount of the antisolvent, the volume ratio of the antisolvent to solvent varied from 1 to 4. As shown in Fig. 3C, the yield gradually rises by increasing the antisolvent to solvent ration from 1 to 3. However, the further increasing the amount of the antisolvent does not result in higher yield. Overall, it was found that *n*-heptane results in high precipitation efficiency at antisolvent to solvent ratio of 3, and temperature of 4 °C within 24 h.

After obtaining the optimum precipitation conditions, the extraction parameters were optimized. One of the critical operation parameters is extraction temperature. The higher the temperature, the higher dissolution capacity of the polymer in the solvent and consequently the higher extraction yield. However, PHBV is a polyester which is not a thermally stable polymer, meaning that at too high temperature, the polymer will thermally degrade and there is an optimum extraction temperature to achieve the highest recovery yield without degrading the polymer. The boiling point of 2-MTHF is 87 °C. In order to operate it at atmospheric pressure, 80 °C was selected for extraction. The thermal stability of the polymer was examined by a preliminary extraction experiment and sampling over the course of time. The samples were analyzed by GPC to measure the average MW and PDI of the extracted polymer. As shown in Fig. 4, the average MW and PDI of the polymer are approximately constant within the extraction. This indicates that the PHBV is stable at extraction conditions and 2-MTHF is capable to

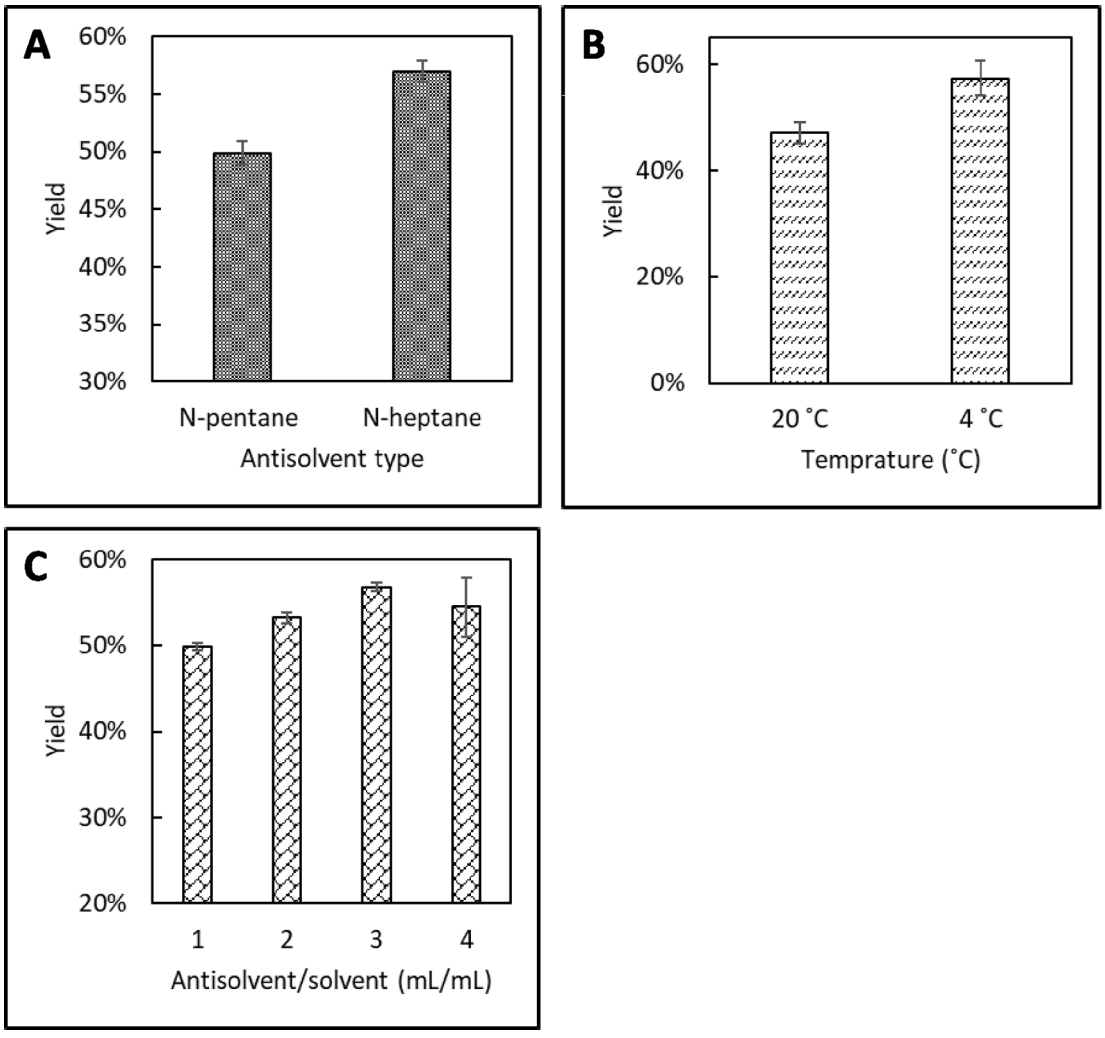


**Fig. 3.** Optimization of the precipitation step for the extraction using 2-MTHF at 80 °C, 1 h, and biomass to solvent ratio of 5 % g/mL).

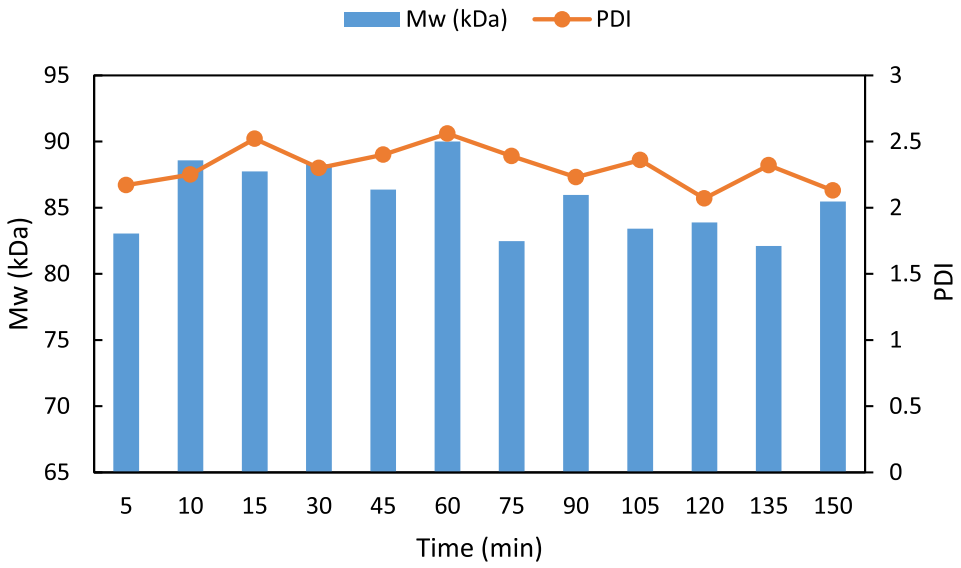


**Fig. 4.** Extraction of PHBV from the biomass (batch 1) using 2-MTHF at 80 °C with the biomass to solvent ratio of 5 % (g/mL) and *n*-heptane as an antisolvent with a volume ratio of 1:3 at 4 °C for 24 h.

dissolve PHBV with average MW of 80 kDa at 80 °C.

Where Fig. 4 displays the molar weight and polydispersity index, the extraction yield as function of time is presented in Fig. 5. As can be clearly seen from Fig. 5, the extraction yield reaches a steady value within the first hour, and while the extraction yield increases from 54 to 62 % during the first hour, it does not enhance beyond 1 h extraction time. The stabilized yield around 60 % is likely due to saturation of the solvent. Therefore, 1 h was considered as an optimum extraction time. In a next series of experiments, the minimum required amount of the solvent was investigated by varying the ratio of biomass to solvent from 1 to 6 % (g/mL). The results are shown in Fig. 6, the yield is improved about 3 % by decreasing the ratio from 5 to 2 %. However, from the economic point of view, gaining only 3 % more polymer by increasing the volume of the solvent with 2.5 factor is not efficient. In the next experiment, the ratio of the biomass to solvent was increased to 6 % (g/mL). As shown in Fig. 5, the recovery yield is slightly decreasing from about 62 to 55 % by rising the ratio from 5 to 6 % (g/mL). Although the reduction in the yield might not be statistically significant, performing the experiments at a ratio higher than 5 % (g/mL) was challenging, because of formation of a sludge when a limited volume of the solvent is

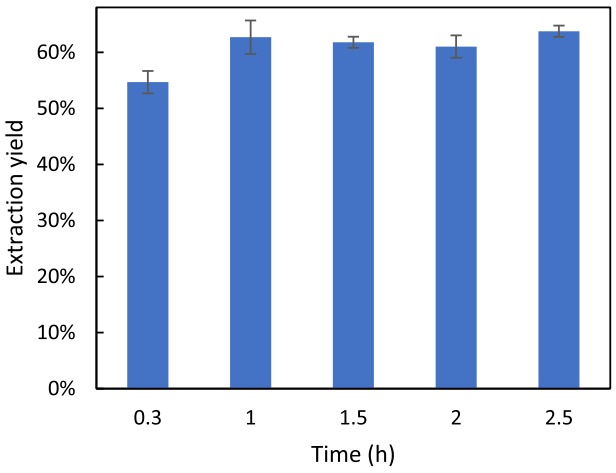


**Fig. 5.** Optimization of the extraction time to recover PHBV from the biomass using 2-MTHF at 80 °C with the biomass to solvent ratio of 5 % (g/mL) and *n*-heptane as an antisolvent with a volume ratio of 1:3 at 4 °C for 24 h.

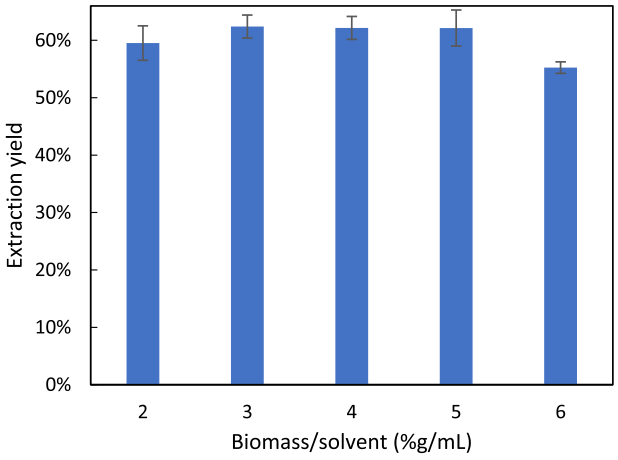


**Fig. 6.** Optimization of the biomass to solvent ratio to recover PHBV from the biomass using 2-MTHF at 80 °C for 1 h with and *n*-heptane as an antisolvent with a volume ratio of 1:3 at 4 °C for 24 h.

applied. Thus, the optimum biomass to solvent ratio with regards to experimental handling was set at 5 % (g/mL). Overall, the maximum yield of 62 ± 3 % was obtained at optimum operation conditions (including both extraction and antisolvent precipitation). Since this yield is far from the theoretical maximum yield of 100 %, the polymer should remain either in the biomass or in the solvent. To gain more information, the mass balance over the complete recovery process was studied to figure out the limiting stages during the recovery.

The schematic view of the process is shown in Fig. 1. The polymer content of each step was measured as explained in Section 2.4. After each extraction experiment, the polymer content of the residual biomass and supernatant were determined by GC. Regarding the extraction process using the biomass from batch 1 with 2-MTHF, it was found that about 15 % of the polymer was left in the supernatant after the precipitation by antisolvent. The large fraction of polymer not precipitating upon addition of the antisolvent can be explained through the relatively low average MW and resulting high solubility of the polymer. For this PHBV it can be concluded that the main limiting stage in the overall process is likely the precipitation. Moreover, about 15 % of the polymer is remained in the residual biomass after extraction at optimum

operation condition. This content did not reduce significantly even though by applying a second extraction with 2-MTHF. The cross flow multistage extraction with 2-MTHF on the residual biomass increased the yield only about 3 %. It confirms that a strong and complex cellular matrix is formed that results in an impenetrable barrier around the polymer in MMC. This formation of the complex cellular matrix was first suggested by Patel et al. [44] and later confirmed by Samori et al [25]. Mass balance closure over the polymer in a single stage extraction indicates that less than 10 % of polymer was lost during the handling. To confirm the mass balance closure of the PHBV, the extraction was repeated without the antisolvent precipitation. The polymer was retrieved by evaporating the solvent leading to 85 ± 7 % recovery yield with the purity of 79 ± 5 % which is in line with the previous method.

### 3.4. Cyrene

For optimization of the precipitation, a preliminary extraction was performed at 180 °C, 15 min, and solvent to antisolvent ratio of 5 %(g/mL) for all the experiments, followed by precipitating the polymer at various conditions. Due to insolubility of the PHBV in water and ethanol, and full miscibility of cyrene with water and ethanol, they were chosen as an antisolvents. The performance of each antisolvent stand alone and a mixture of them were examined. As can be seen from Fig. 7, ethanol is not able to precipitate the majority of the dissolved polymer. On the other hand, water retrieves not only the polymer but also biomass residuals resulting in non-pure brown polymer. However, the mixture of these two acids in the ratio of 1:1 delivers the highest yield. Due to the low average molecule weight (MW) of the polymer, it was not possible to completely precipitate the polymer. Moreover, reducing the temperature resulted in the precipitation of dissolved residuals of the biomass as well. Therefore, all the precipitation experiments were done at room temperature for 24 h. In general, the highest precipitation efficiency was achieved an equal volume ratio of solvent to antisolvent (water: ethanol (1:1 (v:v))) at 20 °C within 24 h.

After optimizing the precipitation step, the extraction parameters were further investigated to obtain the highest extraction yield using cyrene. Fig. 8 represents the extraction yield at different temperature over the course of time. As can be seen from the graph, there is a clear increase in the extraction yield over the time at 80 °C (the lowest temperature), while the initially much higher yield at 140 °C (the highest temeprature applied) clearly drops after>1 h. The time-dependent yield profiles are much less pronounced for the intermediate temperatures of 100 and 120 °C. Overall, the highest yield seems to be obtained at 120 °C. The opposite trends at lowest and highest operational temperatures are the result of two opposing effects that occur simultaneously. An improved solubility of the polymer in cyrene is observed at increasing temperature, while at the higher temperatures a limiting thermal stability of the polymer reduces the yield after prolongued exposure to such high temperatures. When 120 °C was set as an extraction temperature, the recovery yield reached a plateau after 2 h which might be because of the saturation of the solvent. Thereupon, the proper mass ratio of the biomass to solvent was further examined by keeping the mass of the biomass constant at 0.5 g while the amount of the solvents differed to achieve the ratio of 4 to 8 % (g/mL). As can be seen from Fig. 9, there is not a remarkable reduction in the yield by increasing the ratio from 3 to 5 %(g/mL). However, further increase in

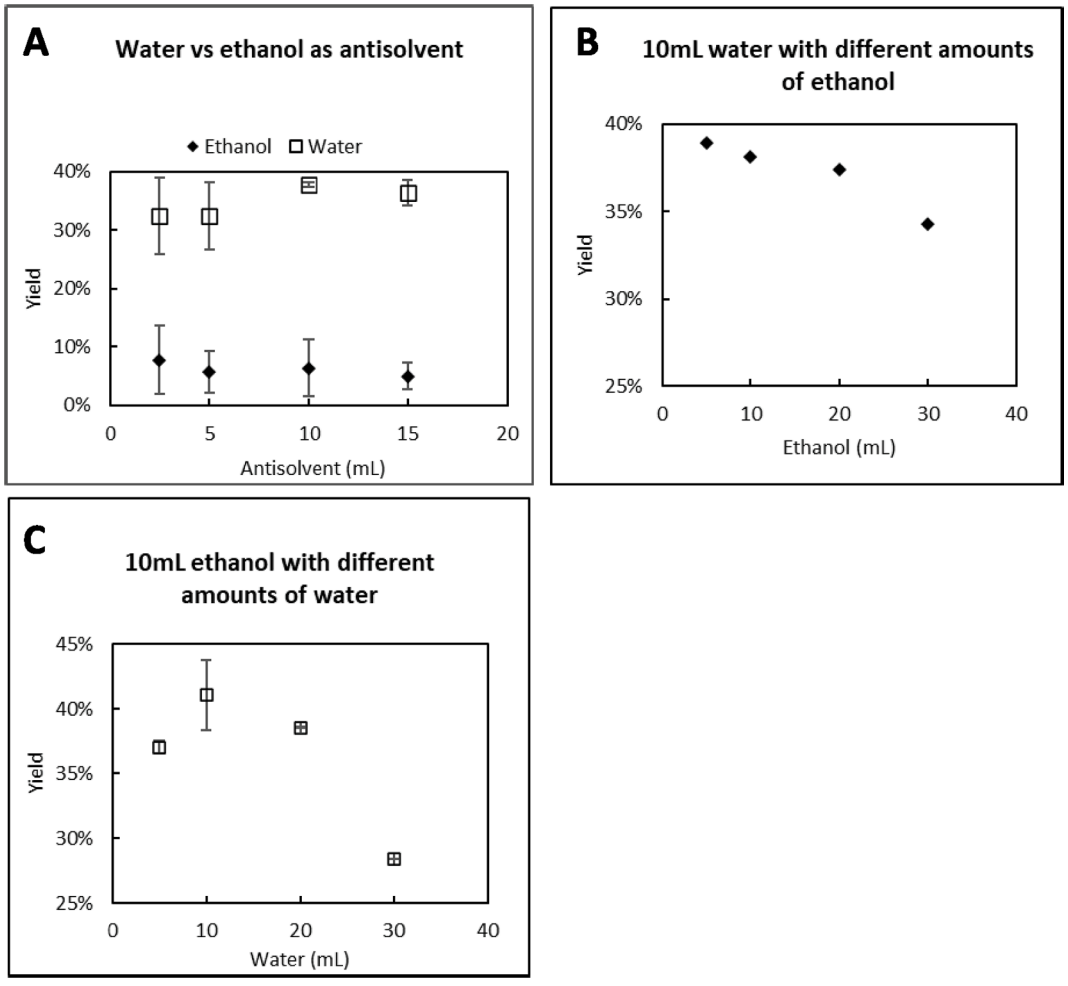


**Fig. 7.** Optimization of the precipitation for cyrene based extraction at 180 °C and 15 min using ethanol and water as an antisolvent AT 20 °C.

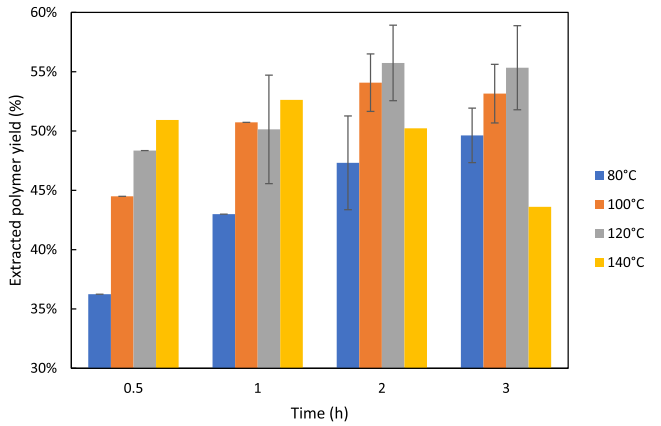


**Fig. 8.** Extraction yield as function of extraction time and temperature. Displayed is the amount of PHBV recovered from the biomass using cyrene with a biomass to solvent ratio of 5 % (g/mL) and in the antisolvent precipitation stage a water: ethanol (1:1(v: v)) as an antisolvent with a volume ratio of 1:1 at 20 °C for 24 h.

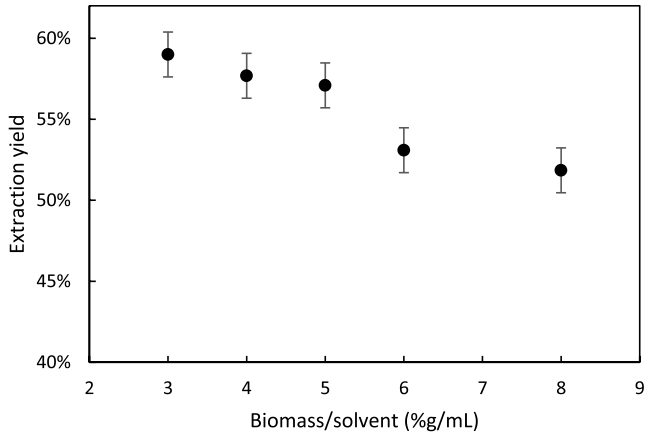


**Fig. 9.** Optimization of biomass to solvent ratio to recover PHBV from the biomass using cyrene at 120 °C for 2 h with water: ethanol (1:1(v: v)) as an antisolvent with a volume ratio of 1:1 at 20 °C for 24 h.

the ratio arose both reduction in the yield and processing problems due to the high concentration of the mixture. It brings to the conclusion that the minimum required amount of solvent is 10 mL which is equal to 5 % (g/mL) of biomass to solvent ratio. Overall, the highest yield of 57 ± 2 % was gained at 120 °C and 2 h.

The analysis of the residual biomass illustrated that about 10 % of the polymer has left in the residual biomass at optimized extraction conditions. The quantification of the polymer by GC requires transesterification reaction in the present of a strong mineral acid, due to the instability of cyrene in acidic condition, the remaining polymer in the supernatant was not measured by GC. However, it is reasonable to assume that a quantity of polymer was lost during handling. The lost polymer is expected to be of similar amount as observed during handling after extraction with 2-MTHF (which was about 7 % loss). Based on this assumption, it is calculated that about 26 % of the polymer did not precipitate from the supernatant.

### 3.5. Comparative assessment of the solvents for extraction of PHBV from mixed microbial culture

The average MW of a polymer has a great impact on its solubility in corresponding solvent and consequently on the maximum capacity of the solvent to extract it. In this study, the average MW of the PHBV is much lower than what has been reported in the literature. Therefore, it was not possible to fairly compare the performance of these new solvents with previously discovered solvents. Furthermore, the capacity of the solvent can be affected by the nature of the biomass as well. For instance, Somori et al. [25] found that using DMC to extract PHBV from a MMC allows 49 % recovery yield. Later, de Souza Reis et al. [24] reported 90 % recovery at the same extraction condition with DMC. They concluded that the difference might be due to applying different wastewater as a feedstock for the microorganism and complex interaction between the PHBV and non-polymeric cell debris. To allow for direct comparison of the yields with the solvents DMC and chloroform and to eliminate the impact of unknown parameters associated to biomass-specific effects on extraction efficiency of each solvent, also with DMC and chloroform extractions were performed. The extraction condition for chloroform and DMC were based on the latest work by Mongili [23]. The results are shown in Table 2. As explained in Section 2.2, the biomass named batch 1 and batch 2 are both produced via the fermentation of the food wastes. They differ only in average MW which is higher for batch 2. On the other hand, Batch 3 was prepared by fermentation of a secondary sludge of the municipal wastewater treatment plant which possesses the highest average MW of kDa. It became clear that by decreasing the average MW of the polymer the extraction yield with chloroform and DMC decreases by cause of the difficulty in the precipitation of highly soluble molecules. An inverse trend was observed for 2-MTHF and cyrene, showing the highest yield for lowest MW PHBV of 62 and 57 %, respectively. However, the solubility of the polymers with high average MW is limited for these solvents. It leads to the conclusion that there is an optimum average MW for every solvent-antisolvent pair to achieve the highest recovery yield. Up to that point the yield is limited by the precipitation using an antisolvent and above that MW, the solubility of the polymer in the solvent is limiting the extraction.

For batch 3 with the highest average MW, the maximum quantity of the polymer was harvested using chloroform with the yield of 74 % and purity of 96 %. Similar results were obtained with DMC, illustrating that indeed DMC has a high potential to replace chlorinated solvents [21,24].

Also, the effect of the extraction procedure on monomer composition of the polymer and its thermal degradation temperature were investigated by GC and TGA, respectively. According to the GC results for the biomass, the mass ratio of the HB to HV monomer before the extraction is 2, 3 and 3 for batch 1, batch 2 and batch 3, respectively. The mass ratio of the monomers for the polymer after extraction by various solvents are presented in Table 2. There is an increase in the monomer ratio for some polymer meaning that the corresponding solvent has possibly dissolved more HB units. Regarding the thermal analysis of the polymer, as can be seen from Table 2, the degradation temperature ($T_{deg.}$) of the polymer with low average MW does not vary using various solvent-antisolvent couple. However, the $T_{deg.}$ for the polymer extracted from batch 3 by 2-MTHF is about 35 °C lower than the polymer extracted by other solvents using the same batch of the biomass. It might be due to the relatively lower average MW of the polymer obtained by 2-MTHF.

### 3.6. Mass balance over the benchmark extraction process

The mass balance closure over the PHBV was applied for the extraction process with reference solvents using the biomass from batch 1. The overall results are displayed in Table 3. Based on the highest fraction of the polymer remaining in the supernatant, the main limiting stage in extraction with the benchmark solvents is the precipitation. Because of the high solubility of the polymer with low average MW, the applied antisolvents are not capable of completely retrieving the polymer. It may be possible to improve the recovery of the polymer from the solvent by applying another antisolvent such as an alkane. The optimization of the precipitation step for the reference solvents was out of this work's scope. The focus of the present work is to investigate the impact of average MW of PHBV on the extraction yield for the investigated solvents and to select a proper solvent-antisolvent couple and extraction

**Table 2**
Solvent extraction of PHBV from mixed microbial culture with chloroform, DMC, 2-MTHF and cyrene at their optimum operation conditions using three biomass batches with different average MW.

| Biomass | solvent | Extraction condition | Precipitation condition | Yield % | Purity % | HB/HV (g/g) | Mw (kDa) | Mn (kDa) | PDI | $T_{deg.}$ (5%) |
|---|---|---|---|---|---|---|---|---|---|---|
| Batch 1 | Chloroform | 2 h, 60 °C, 5 % (g/mL) | *n*-Hexane,1:3 (v: v), 24 h, 4 °C | 32 | >99 | 4 | 100 | 40 | 2.5 | 240 |
| | DMC | 1.5 h, 90 °C, 2.5 % (g/mL) | EtOH,1:3 (v: v), 24 h, 4 °C | 20 | >99 | 4 | 148 | 78 | 1.9 | 240 |
| | 2-MTHF | 1 h, 80 °C, 5 % (g/mL) | *n*-Heptane,1:3 (v: v), 24 h, 4 °C | 63 | >99 | 3 | 74 | 19 | 3.9 | 235 |
| | Cyrene | 2 h, 120 °C, 5 % (g/mL) | Water: EtOH 1:1:1 (v: v), 24 h, 20 °C | 57 | >99 | 3 | 79 | 25 | 3.16 | 240 |
| Batch 2 | Chloroform | 2 h, 60 °C, 5 % (g/mL) | *n*-Hexane,1:3 (v: v), 24 h, 4 °C | 44 | >99 | 4 | 146 | 41 | 3.5 | 245 |
| | DMC | 1.5 h, 90 °C, 2.5 % (g/mL) | EtOH,1:3 (v: v), 24 h, 4 °C | 39 | >99 | 4 | 178 | 81 | 2.2 | 245 |
| | 2-MTHF | 1 h, 80 °C, 5 % (g/mL) | *n*-Heptane,1:3 (v: v), 24 h, 4 °C | 64 | >99 | 4 | 122 | 25 | 4.9 | 240 |
| | Cyrene | 2 h, 120 °C, 5 % (g/mL) | Water: EtOH 1:1:1 (v: v), 24 h, 20 °C | 65 | >99 | 3 | 120 | 28 | 4.29 | 243 |
| Batch 3 | Chloroform | 2 h, 60 °C, 5 % (g/mL) | *n*-Hexane,1:3 (v: v), 24 h, 4 °C | 74 | 96 | 5 | 581 | 298 | 2 | 265 |
| | DMC | 1.5 h, 90 °C, 2.5 % (g/mL) | EtOH,1:3 (v: v), 24 h, 4 °C | 71 | 96 | 4 | 526 | 260 | 2 | 260 |
| | 2-MTHF | 1 h, 80 °C, 5 % (g/mL) | *n*-Heptane,1:3 (v: v), 24 h, 4 °C | 11 | 99 | 3 | 475 | 208 | 2.3 | 230 |
| | Cyrene | 2 h, 120 °C, 5 % (g/mL) | Water: EtOH 1:1:1 (v: v), 24 h, 20 °C | 40 | 98 | 4 | 525 | 238 | 2.2 | 265 |

**Table 3**
The summary of the mass balance closure over PHBV during extraction from batch 1 with chloroform, DMC, 2-MTHF and cyrene at corresponding optimum extraction conditions.

| Extraction solvent | Yield (%) | PHBV in residual biomass (%) | PHBV in supernatant (%) | PHBV lost during handling (%) |
|---|---|---|---|---|
| 2-MTHF | 63 | 15 | 15 | 7 |
| Cyrene | 57 | 10 | 33 | |
| Chloroform | 32 | 6 | 58 | 4 |
| DMC | 20 | 6 | 69 | 5 |

process.

The amount of remaining polymer in residual biomass is much lower for the DMC and chloroform than reported by Samori et al. [25]. They achieved an extraction yield of 49 % with DMC followed by solvent evaporation. The second extraction from residual biomass with DMC improved the yield up to 61 %. The third extraction did not increase the yield any further, meaning that about 39 % of the polymer remained in the residual biomass and is not extractable. Such large amount of polymer not being extractable is most likely due to the complex interaction between polymeric and non-polymeric parts of the microbial cells in MMC. It is noteworthy that the source of biomass and particularly the average MW of the polymer are very different in our study. As already mentioned, a similar behavior for DMC was also found by Souza Reis et al. [24].

### 3.7. Investigation the combined solvent evaporation-antisolvent as precipitation method

2-MTHF showed a great potential to recover the PHBV with low average MW. However, obtaining pure polymer requires using *n*-heptane as an antisolvent with volume ratio of 3. From economic point of view, the lower antisolvent usage is preferred. Therefore, we investigated further on how to reduce the required amount of the antisolvent while still obtaining relatively pure polymer. Combing two precipitation methods, solvent evaporation and applying an antisolvent, is the approach which was taken to lower the antisolvent consumption for retrieving pure polymer. At the optimized condition, 2-MTHF and DMC based extraction experiments were carried out, followed by evaporating a fraction of the solvent using a vacuum oven at 30 °C. Afterwards, antisolvent (3 times the volume of remaining solvent) was added to retrieve the polymer. As shown in Table 4, regarding 2-MTHF based

**Table 4**
Extraction with 2-MTHF (1 h, 80 °C and 5 %(g/mL)) and DMC (1.5 h, 90 °C, 2.5 % (g/mL)) retrieving the polymer by evaporation of a fraction of the solvent, followed by precipitation using the antisolvent.

| Solvent | Evaporated fraction of the solvent (%) | Yield (%) | Purity (%) |
|---|---|---|---|
| 2-MTHF | 0 | 63 | >99 |
| 2-MTHF | 75 | 67 | 96 |
| 2-MTHF | 91 | 67 | 94 |
| 2-MTHF | 100 | 85 ± 7 | 79 ± 5 |
| DMC | 0 | 20 | >99 |
| DMC | 80 | 32 | 98 |
| DMC | 91 | 80 | 98 |
| DMC | 100 | 94 ± 5 | 98 ± 1 |

extractions, the yield is slightly increased from 63 to 67 % by evaporating the majority of the solvent prior to adding antisolvent. Also, the purity dropped from > 99 to 94 % by removing 91 % of the solvent, meaning that it is possible to obtain relatively pure polymer with less antisolvent consumption by removing a large fraction of the solvent beforehand. Recovering the polymer with complete solvent evaporation resulted in 85 ± 7 % extraction yield with the purity of 79 ± 5 %. A picture of the polymer obtained by solvent evaporation and antisolvent precipitation is shown in Fig. 10. Precipitating the polymer with an antisolvent yields into white granules while the product obtained by solvent evaporation has a yellowish cake appearance. Similarly, for DMC based extractions, removing 91 % of the solvent before adding the antisolvent increases the yield from 20 to 80 % with relatively high purity of 98 %. It is in line with the previous conclusion that the precipitation is the main limiting stage to recover low average MW polymer by DMC. Moreover, the precipitation of the polymer by complete evaporation of DMC results in 94 ± 5 % extraction yield and 98 ± 1 % purity.

### 3.8. PHBV extraction from wet biomass

Extraction of PHBV directly from a wet biomass is preferred to reduce the cost associated to the drying of the biomass. Cyrene is fully miscible in water and thereby it is not recommended to apply for polymer extraction from a wet biomass. However, the solubility of 2-MTHF is limited in water. Therefore, the performance of 2-MTHF (at 80 °C, 1 h, 2.5 % (g/mL))to recover the polymer from a wet biomass was compared to DMC (at 90 °C, 1.5 h, 2.5 %(g/mL)). The wet biomass contained 90 wt% water and 43 % PHBV on dry basis. It was found that 2-MTHF is capable to extract PHBV with 73 ± 1 % yield and purity of >

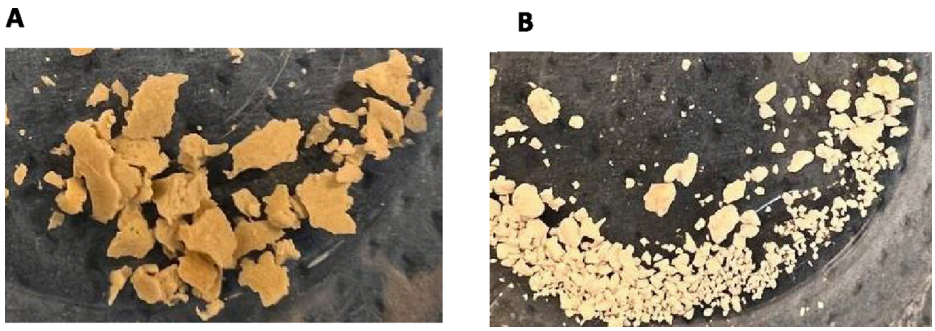


**Fig. 10.** A picture of the polymer extracted by 2-MTHF at 80 °C for 1 h, A) retrieving the polymer by solvent evaporation and B) with *n*-heptane as an antisolvent with a volume ratio of 1:3 at 4 °C for 24 h.

99 % which is a similar performance compare to DMC. Since DMC-based extraction resulted in 66 ± 8 % yield and > 99 % purity. The polymer obtained by 2-MTHF and DMC have an average MW of 535 and 555 kDa, respectively.

### *3.9. Solvent and antisolvent recovery consideration*

The recovery and reusability of the solvent and antisolvent is crucial for an economic extraction process. 2-MTHF is a volatile solvent with a boiling point of 87 °C. The extraction was performed at 80 °C in a closed vial with a sufficient volume to prevent the evaporation of the solvent. After the extraction, the remaining 2-MTHF in the biomass residual can be easily recovered by drying the residuals and condensing the vapor phase. The separation of 2-MTHF and *n*-heptane can be also done by distillation. Regarding cyrene, it is not volatile and has a high boiling point of 227 °C. The remaining solvent in the residual biomass can be obtained by rinsing the residuals with ethanol, followed by ethanol/cyrene separation with distillation. Similarly, the mixture of the antisolvent (ethanol/water) can be also separated from cyrene by distillation, where it is noted that a small part of the cyrene will be in the geminal diol [45].

## 4. Conclusion

In this study, bio-based PHBV-rich biomass was prepared using organic waste leachate as a feedstock. The wet produced biomass was subjected to an elevated temperature of 120 °C for 18 h to simultaneously dry the biomass and reduce the average MW of the polymer. As the reduction of the average MW can enhance the solubility of the polymer and increase the extraction efficiency by solvent extraction. The extraction of the polymer with low average MW was optimized using 2-MTHF and cyrene as a solvent. The maximum extraction yield of 62 ± 3 % with purity of > 99 % was achieved with 2-MTHF at 80 °C for an hour with high biomass to solvent ratio of 5 % (g/mL). Cyrene-based extractions resulted in the highest yield of 57 ± 2 % with purity of > 99 % at 120 °C in 2 h with 5 % (g/mL) biomass to solvent ratio. The mass balance closure over the extraction process indicated that about 15 % and 10 % of polymer has remained in the residual biomass after extraction by 2-MTHF and cyrene, respectively.

Moreover, the performance of these proposed bio-based solvents was compared with chloroform and DMC as a benchmark solvent using different batches of the PHBV-enriched biomass with various average MW. The results indicate that the proposed new solvents are preferred over the benchmarks when the average MW of polymer is low. For example, 2-MTHF resulted in 62 ± 3 % extraction efficiency for PHBV with MW of about 100 kDa while it was only 32 and 20 % for chloroform and DMC, respectively. It can be explained with the difficulties in precipitating out the small polymer molecules from the good solvents due to their high solubility. In fact, the mass balance closure over the extraction process confirmed that the precipitation step is the main limiting stage when a good solvent is used to recover a polymer with relatively low average MW. As about 58 and 69 % of the polymer remained in the supernatant using respectively chloroform and DMC to extract PHBV with the average MW of 100 kDa while it was only 15 % for 2-MTHF based extraction for the same batch of the biomass. Coming to the conclusion that selecting a proper solvent-antisolvent couple to recover pure polymer strongly depends on the average MW of the polymer.

To reduce the antisolvent usage, the solvent evaporation and adding an antisolvent were combined for 2-MTHF based extraction experiment. It was found that by evaporating 91 % of the solvent prior to adding the antisolvent, it is still possible to recover the PHBV with relatively high purity of 94 %. Moreover, applying 2-MTHF for the PHBV extraction from wet biomass which resulted in 73 ± 1 % yield and purity of > 99 % which is a comparable performance to DMC based wet extraction.

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## CRediT authorship contribution statement

**Vahideh Elhami:** Investigation, Methodology, Data analysis, Writing – original draft. **Noor van de Beek:** Investigation, Methodology, Data analysis. **Liangshin Wang:** Investigation, Methodology, Data analysis. **Stephen J. Picken:** Supervision, Writing – review & editing. **Jelmer Tamis:** Investigation, Supervision, Conceptualization, Writing – review & editing. **João A.B. Sousa:** Supervision, Validation, Writing – review & editing, Funding acquisition. **Mark A. Hempenius:** Supervision, Conceptualization, Polymer analysis, Writing – review & editing. **Boelo Schuur:** Supervision, Project administration, Funding acquisition, Writing – review & editing, Methodology.

## Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Jelmer Tamis and João Sousa work for Paques Biomaterials BV, a company working on commercialization of wastewater digesters that produce PHA.

## Data availability

Data will be made available on request.

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## Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.seppur.2022.121773.

## References


[1] E. Bugnicourt, P. Cinelli, A. Lazzeri, V. Alvarez, Polyhydroxyalkanoate (PHA): Review of synthesis, characteristics, processing and potential applications in packaging, eXPRESS Polym. Lett. 8 (11) (2014) 791–808, https://doi.org/10.3144/expresspolymlett.2014.82.

[2] B. Kunasundari, K. Sudesh, Isolation and recovery of microbial polyhydroxyalkanoates, eXPRESS Polym. Lett. 5 (7) (2011) 620–634, https://doi.org/10.3144/expresspolymlett.2011.60.

[3] A. Aramvash, N. Gholami-Banadkuki, F. Moazzeni-Zavareh, S. Hajizadeh-Turchi, An Environmentally Friendly and Efficient Method for Extraction of PHB Biopolymer with Non-Halogenated Solvents, J. Microbiol. Biotechnol. 25 (11) (2015) 1936–1943, https://doi.org/10.4014/jmb.1505.05053.

[4] C. Nielsen, A. Rahman, A.U. Rehman, M.K. Walsh, C.D. Miller, Food waste conversion to microbial polyhydroxyalkanoates, Microb. Biotechnol. 10 (6) (2017) 1338–1352, https://doi.org/10.1111/1751-7915.12776.

[5] L. Zhang, K.-C. Loh, Y. Dai, Y.W. Tong, Acidogenic fermentation of food waste for production of volatile fatty acids: Bacterial community analysis and semi-continuous operation, Waste Manage. (Oxford) 109 (2020) 75–84, https://doi.org/10.1016/j.wasman.2020.04.052.

[6] G. Capson-Tojo, E. Trably, M. Rouez, M. Crest, N. Bernet, J.-P. Steyer, J.-P. Delgenès, R. Escudié, Cardboard proportions and total solids contents as driving factors in dry co-fermentation of food waste, Bioresour. Technol. 248 (2018) 229–237, https://doi.org/10.1016/j.biortech.2017.06.040.

[7] C. Da Ros, V. Conca, A.L. Eusebi, N. Frison, F. Fatone, Sieving of municipal wastewater and recovery of bio-based volatile fatty acids at pilot scale, Water Res. 174 (2020) 115633, https://doi.org/10.1016/j.watres.2020.115633.

[8] M. Ramos-Suarez, Y. Zhang, V. Outram, Current perspectives on acidogenic fermentation to produce volatile fatty acids from waste, Rev. Environ. Sci. Bio/Technol. 20 (2) (2021) 439–478, https://doi.org/10.1007/s11157-021-09566-0.

[9] H. Ma, X. Chen, H. Liu, H. Liu, B. Fu, Improved volatile fatty acids anaerobic production from waste activated sludge by pH regulation: Alkaline or neutral pH? Waste Manage. (Oxford) 48 (2016) 397–403, https://doi.org/10.1016/j.wasman.2015.11.029.

[10] R.J. Jones, R. Fernández-Feito, J. Massanet-Nicolau, R. Dinsdale, A. Guwy, Continuous recovery and enhanced yields of volatile fatty acids from a continually-fed 100 L food waste bioreactor by filtration and electrodialysis, Waste Manage. (Oxford) 122 (2021) 81–88, https://doi.org/10.1016/j.wasman.2020.12.032.

[11] Y. Chen, R. Ruhyadi, J. Huang, W. Yan, G. Wang, N. Shen, W. Hanggoro, A novel strategy for improving volatile fatty acid purity, phosphorus removal efficiency, and fermented sludge dewaterability during waste activated sludge fermentation, Waste Manage. (Oxford) 119 (2021) 195–201, https://doi.org/10.1016/j.wasman.2020.09.044.

[12] M. Mulders, J. Tamis, B. Abbas, J. Sousa, H. Dijkman, R. Rozendal, R. Kleerebezem, Pilot-scale polyhydroxyalkanoate production from organic waste: process characteristics at high pH and high ammonium concentration, J. Environ. Eng. 146 (7) (2020) 04020049, https://doi.org/10.1061/(ASCE)EE.1943-7870.0001719.

[13] X. Wang, G. Carvalho, M.A.M. Reis, A. Oehmen, Metabolic modeling of the substrate competition among multiple VFAs for PHA production by mixed microbial cultures, J. Biotechnol. 280 (2018) 62–69, https://doi.org/10.1016/j.jbiotec.2018.06.342.

[14] A. Parodi, A. Jorea, M. Fagnoni, D. Ravelli, C. Samorì, C. Torri, P. Galletti, Bio-based crotonic acid from polyhydroxybutyrate: synthesis and photocatalyzed hydroacylation, Green Chem. 23 (9) (2021) 3420–3427, https://doi.org/10.1039/D1GC00421B.

[15] M.R.Z. Mamat, H. Ariffin, M.A. Hassan, M.A.K. Mohd Zahari, Bio-based production of crotonic acid by pyrolysis of poly(3-hydroxybutyrate) inclusions, J. Cleaner Prod. 83 (2014) 463–472, https://doi.org/10.1016/j.jclepro.2014.07.064.

[16] J. Blumenstein, J. Albert, R.P. Schulz, C. Kohlpaintner, Crotonaldehyde and crotonic acid, in: Ullmann's Encyclopedia of Industrial Chemistry, pp. 1-20. https://doi.org/10.1002/14356007.a08_083.pub2.

[17] G.J. Raugi, T.C. Liang, J.J. Blum, Effects of pentanoic acid and 4-pentenoic acid on the intracellular fluxes of acetyl coenzyme A in Tetrahymena, J. Biol. Chem. 250 (11) (1975) 4067–4072, https://doi.org/10.1016/S0021-9258(19)41387-2.

[18] N. Ahmad, B. Colak, M.J. Gibbs, D.-W. Zhang, J.E. Gautrot, M. Watkinson, C. R. Becer, S. Krause, Peptide cross-linked poly(2-oxazoline) as a sensor material for the detection of proteases with a quartz crystal microbalance, Biomacromolecules 20 (7) (2019) 2506–2514, https://doi.org/10.1021/acs.biomac.9b00245.

[19] G. Pagliano, P. Galletti, C. Samorì, A. Zaghini, C. Torri, Recovery of polyhydroxyalkanoates from single and mixed microbial cultures: A review, Front. Bioeng. Biotechnol. 9 (54) (2021), https://doi.org/10.3389/fbioe.2021.624021.

[20] M. Koller, Established and advanced approaches for recovery of microbial polyhydroxyalkanoate (PHA) biopolyesters from surrounding microbial biomass, EuroBiotech J. 4 (2020) 113–126, https://doi.org/10.2478/ebtj-2020-0013.

[21] C. Samorì, M. Basaglia, S. Casella, L. Favaro, P. Galletti, L. Giorgini, D. Marchi, L. Mazzocchetti, C. Torri, E. Tagliavini, Dimethyl carbonate and switchable anionic surfactants: two effective tools for the extraction of polyhydroxyalkanoates from microbial biomass, Green Chem. 17 (2) (2015) 1047–1056, https://doi.org/10.1039/C4GC01821D.

[22] S. Righi, F. Baioli, C. Samorì, P. Galletti, E. Tagliavini, C. Stramigioli, A. Tugnoli, P. Fantke, A life cycle assessment of poly-hydroxybutyrate extraction from microbial biomass using dimethyl carbonate, J. Cleaner Prod. 168 (2017) 692–707, https://doi.org/10.1016/j.jclepro.2017.08.227.

[23] B. Mongili, A. Abdel Azim, S. Fraterrigo Garofalo, E. Batuecas, A. Re, S. Bocchini, D. Fino, Novel insights in dimethyl carbonate-based extraction of polyhydroxybutyrate (PHB), Biotechnol. Biofuels 14 (1) (2021) 13, https://doi.org/10.1186/s13068-020-01849-y.

[24] G.A. de Souza Reis, M.H.A. Michels, G.L. Fajardo, I. Lamot, J.H. de Best, Optimization of green extraction and purification of PHA produced by mixed microbial cultures from sludge, Water 12 (4) (2020) 1185, https://doi.org/10.3390/w12041185.

[25] C. Samorì, F. Abbondanzi, P. Galletti, L. Giorgini, L. Mazzocchetti, C. Torri, E. Tagliavini, Extraction of polyhydroxyalkanoates from mixed microbial cultures: Impact on polymer quality and recovery, Bioresour. Technol. 189 (2015) 195–202, https://doi.org/10.1016/j.biortech.2015.03.062.

[26] A. Aramvash, F. Moazzeni Zavareh, N. Gholami Banadkuki, Comparison of different solvents for extraction of polyhydroxybutyrate from Cupriavidus necator, Eng. Life Sci. 18 (1) (2018) 20–28, https://doi.org/10.1002/elsc.201700102.

[27] P. Murugan, L. Han, C.Y. Gan, F.H. Maurer, K. Sudesh, A new biological recovery approach for PHA using mealworm, Tenebrio molitor, J. Biotechnol. 239 (2016) 98–105, https://doi.org/10.1016/j.jbiotec.2016.10.012.

[28] T. Brouwer, B. Schuur, Bio-based solvents as entrainers for extractive distillation in aromatic/aliphatic and olefin/paraffin separation, Green Chem. 22 (16) (2020) 5369–5375, https://doi.org/10.1039/D0GC01769H.

[29] N. Guajardo, P. Domínguez de María, Assessing biocatalysis using dihydrolevoglucosenone (Cyrene™) as versatile bio-based (co)solvent, Mol. Catal. 485 (2020) 110813, https://doi.org/10.1016/j.mcat.2020.110813.

[30] P. Ray, T. Hughes, C. Smith, M. Hibbert, K. Saito, G.P. Simon, Development of bio-acrylic polymers from Cyrene™: transforming a green solvent to a green polymer, Polym. Chem. 10 (24) (2019) 3334–3341, https://doi.org/10.1039/C9PY00353C.

[31] T. Marino, F. Galiano, A. Molino, A. Figoli, New frontiers in sustainable membrane preparation: Cyrene™ as green bioderived solvent, J. Membr. Sci. 580 (2019) 224–234, https://doi.org/10.1016/j.memsci.2019.03.034.

[32] T. Brouwer, M. Blahusiak, K. Babic, B. Schuur, Reactive extraction and recovery of levulinic acid, formic acid and furfural from aqueous solutions containing sulphuric acid, Sep. Purif. Technol. 185 (2017) 186–195, https://doi.org/10.1016/j.seppur.2017.05.036.

[33] T. Brouwer, B. Schuur, Dihydrolevoglucosenone (Cyrene), a biobased solvent for liquid-liquid extraction applications, ACS Sustain. Chem. Eng. 8 (39) (2020) 14807–14817, https://doi.org/10.1021/acssuschemeng.0c04159.

[34] A. Werker, S. Bengtsson, L. Korving, M. Hjort, S. Anterrieu, T. Alexandersson, P. Johansson, A. Karlsson, L. Karabegovic, P. Magnusson, F. Morgan-Sagastume, L. Sijstermans, M. Tietema, C. Visser, E. Wypkema, Y. van der Kooij, A. Deeke, C. Uijterlinde, Consistent production of high quality PHA using activated sludge harvested from full scale municipal wastewater treatment - PHARIO, Water Sci. Technol. 78 (11) (2018) 2256–2269, https://doi.org/10.2166/wst.2018.502.

[35] A. Werker, F. Maurer, P. Jannasch, P. Magnusson, P. Johansson, Method for the recovery of stabilized polyhydroxyalkanoates from biomass that has been used to treat organic waste, EP2606080 (A1) 2016.

[36] G. Odian, Principles of polymerization, fourth ed., John Wiley & Sons, United States, 2004. https://doi.org/10.1002/047147875X.

[37] M. Kunioka, Y. Doi, Thermal degradation of microbial copolyesters: poly(3-hydroxybutyrate-co-3-hydroxyvalerate) and poly(3-hydroxybutyrate-co-4-hydroxybutyrate), Macromolecules 23 (7) (1990) 1933–1936, https://doi.org/10.1021/ma00209a009.

[38] A. Ekamstam, The behaviour of cellulose in mineral acid solutions: kinetic study of the decomposition of cellulose in acid solution, Ber. Deutschen Chem 69 (1936) 553.

[39] P. Calvini, The role of the Ekenstam equation on the kinetics of cellulose hydrolytic degradation, Cellulose 19 (2) (2012) 313–318, https://doi.org/10.1007/s10570-011-9645-5.

[40] G. Braunegg, B. Sonnleitner, R.M. Lafferty, A rapid gas chromatographic method for the determination of poly-β-hydroxybutyric acid in microbial biomass, Eur. J. Appl. Microbiol. Biotechnol. 6 (1) (1978) 29–37, https://doi.org/10.1007/BF00500854.

[41] H. Xiang, X. Wen, X. Miu, Y. Li, Z. Zhou, M. Zhu, Thermal depolymerization mechanisms of poly(3-hydroxybutyrate-co-3-hydroxyvalerate), Progr. Nat. Sci.: Mater. Int. 26 (1) (2016) 58–64, https://doi.org/10.1016/j.pnsc.2016.01.007.

[42] Q. Liu, M. Zhu, W.-H. Wu, Z. Qin, Polym. Degrad. Stab. 94 (2009) 18–24, https://doi.org/10.1016/j.polymdegradstab.2008.10.016.

[43] K.J. Kim, Y. Doi, H. Abe, Effects of residual metal compounds and chain-end structure on thermal degradation of poly(3-hydroxybutyric acid), Polym. Degrad. Stab. 91 (4) (2006) 769–777, https://doi.org/10.1016/j.polymdegradstab.2005.06.004.

[44] M. Patel, D.J. Gapes, R.H. Newman, P.H. Dare, Physico-chemical properties of polyhydroxyalkanoate produced by mixed-culture nitrogen-fixing bacteria, Appl. Microbiol. Biotechnol. 82 (3) (2009) 545–555, https://doi.org/10.1007/s00253-008-1836-0.

[45] M. De bruyn, V.L. Budarin, A. Misefari, S. Shimizu, H. Fish, M. Cockett, A.J. Hunt, H. Hofstetter, B.M. Weckhuysen, J.H. Clark, D.J. Macquarrie, Geminal diol dihydrolevoglucosenone as a switchable hydrotrope: A continuum of green nanostructured solvents, ACS Sustain. Chem. Eng. 7 (8) (2019) 7878–7883, https://doi.org/10.1021/acssuschemeng.9b00470.