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DOI

[10.1016/j.jece.2022.108573](https://doi.org/10.1016/j.jece.2022.108573)

Publication date

2022

Document Version

Final published version

Published in

Journal of Environmental Chemical Engineering

Citation (APA)

Vermeer, C. M., Nielsen, M., Eckhardt, V., Hortensius, M., Tamis, J., Picken, S. J., Meesters, G. M. H., & Kleerebezem, R. (2022). Systematic solvent screening and selection for polyhydroxyalkanoates (PHBV) recovery from biomass. *Journal of Environmental Chemical Engineering*, 10(6), Article 108573. <https://doi.org/10.1016/j.jece.2022.108573>

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Systematic solvent screening and selection for polyhydroxyalkanoates (PHBV) recovery from biomass

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ARTICLE INFO

Editor: Fumitake Takahashi

Keywords:

Polyhydroxyalkanoates
Waste-to-resources
Biopolymers
Downstream processing
Solvent extraction
Mixed microbial communities

ABSTRACT

The biotechnological production of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) derived from organic waste streams by mixed microbial communities is well established at the pilot-level. However, there is limited research on the recovery of the biopolymer from the microbial biomass, while its impact on product quality and product costs is major. When applying solvent extraction, the choice of solvent has a profound influence on many aspects of the process design. This study provides a framework to perform a systematic solvent screening for PHBV extraction. First, a database was constructed of 35 solvents that were assessed according to six different selection criteria. Then, six solvents were chosen for further experimental analysis, including 1-butanol, 2-butanol, 2-ethyl hexanol (2-EH), dimethyl carbonate (DMC), methyl isobutyl ketone (MIBK), and acetone. The main findings are that the extractions with acetone and DMC obtained the highest yields (91–95%) with reasonably high purities (93–96%), where acetone had a key advantage of the possibility to use water as anti-solvent. Moreover, the results provided new insights in the mechanisms behind PHBV extraction by pointing out that at elevated temperatures the extraction efficiency is less determined by the solvent's solubility parameters and more determined by the solvent size. Although case-specific factors play a role in the final solvent choice, we believe that this study provides a general strategy for the solvent selection process.

1. Introduction

Polyhydroxyalkanoate (PHA) has attracted widespread attention as an alternative to petrochemical-based plastics [19]. PHA is completely biodegradable, biobased, and has thermoplastic properties. A wide range of bacteria are able to produce this biopolymer as an intracellular carbon and energy storage [34]. The substrate, the environmental conditions, and the microorganisms will determine the type of PHA monomer produced. The PHA monomer composition will influence the physicochemical properties of the final polymer product.

An opportunity to produce PHA cost-effectively is by using mixed microbial communities (MMC) and organic waste streams as feedstock. These technologies diminish the relatively high costs for raw substrates and sterilization of equipment [16], and as a consequence, avoid part of the waste disposal expenses [9]. To date, at least 19 pilot projects have been reported, using either industrial or municipal organic waste

streams as feedstock [8]. Here, the most common type of PHA produced is the random copolymer poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV).

While the biotechnological process to produce PHA from low-value feedstock is reasonably well-established, the knowledge to convert it into an affordable thermoplastic is still largely lacking [8,7]. Yet the downstream processing step is a crucial part of the process because of its large impact on the final product quality, and because of its significant contribution to the production costs (almost 50%) [29,31,35,38].

Currently, there are two main methods for the recovery of PHA-rich biomass, digesting the cellular matrix with chemical agents in a water-based process or solubilizing the PHA itself with organic solvents. In general, the cellular digestion method results in a slightly higher yield, while the solvent-based method results in a higher purity and higher molecular weight [27]. MMC are known to form biomass consisting of highly robust cells and extracellular substances, and have in most cases a

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somewhat lower PHA content than industrial pure culture processes. Patel et al., (\$year\$) [17,21,28]. From this point of view, it can be argued that digesting this relatively large fraction of strong cellular matrix becomes less favorable than solubilizing the PHA fraction with organic solvents [25]. In this work, we therefore focused on a solvent-based extraction of PHA from biomass obtained from a MMC process.

The choice of solvent is important for SHE (safety, health, and environment) considerations, solvent pricing, and certainly for the obtained product yield and quality. However, the solvent selection also has a major impact on the overall process design. For economic and sustainability reasons, it is key that the solvent is efficiently recyclable (removal of contaminants and water), and readily recoverable from the depleted biomass and the PHA product. Furthermore, the selected solvent determines what kind of PHA precipitation strategy can be employed (cooling, addition of antisolvent, evaporation). These latter factors can be equally important as product yield, or solvent pricing in determining the final PHA production costs, and are not considered in existing PHA downstream processing literature.

The number of publications focusing on solvent selection for extraction of PHA from MMC is low, especially when waste-based pilot plants are involved. The majority of the research has been done with chlorinated solvents, however, recent studies focused on assessment of greener solvents. Regarding MMC, dimethyl carbonate has been tested on lab-scale biomass produced with a synthetic feed [30,33]. Furthermore, Alfano et al. [1] tested three different ethyl esters on pilot plant biomass derived from an actual waste source, where ethyl acetate revealed the most promising results. The available research has in common that the focus is on one solvent or one solvent family. Due to a wide variation in the specifications of the PHA-rich biomass between the publications (microbial composition and structure, PHA content, monomer composition, molecular weight, type of contaminants), it is difficult to compare the solvents. Therefore, we argue that for a good comparison a diverse range of solvents must be tested under similar conditions with the same input biomass.

To this end, we conducted an extensive solvent screening for the extraction of PHBV derived from a pilot plant fed with leachate from the organic fraction of municipal solid waste (OFMSW). First, a solvent database was constructed containing all solvents described in academic literature in relation to PHA extraction, and containing a few additional solvents with interesting properties. Then, six solvent selection criteria were formulated, and the initial database of 35 solvents was assessed according to these criteria. Subsequently, six solvents (1-butanol, 2-butanol, 2-ethyl hexanol, DMC, MIBK, and acetone) were selected for experimental assessment, comprising: 1) the determination of the precipitation strategy with cooling and antisolvent tests using extracted PHBV, 2) small-scale biomass extractions, where yield, purity and molecular weight were monitored as output. PHBV mass balances were constructed to validate the reliability of the obtained values of the biomass extractions. With the obtained data, we tried to increase the understanding about the factors affecting PHBV extraction. Lastly, the best solvent choice is discussed, including the role of case-specific factors, such as the properties of the input biomass and the intended application of the polymer. To our knowledge, this is the first time that such a systematic solvent screening was conducted for PHA extraction, and the first time that a diverse range of solvents was tested on waste-derived pilot plant PHA-rich biomass.

2. Materials & methods

2.1. Pilot plant production and thermal drying of biomass

The PHBV-enriched biomass used in the experiments was produced at a pilot plant (Orgaworld, Lelystad, the Netherlands) where OFMSW is used as a raw material for PHBV production. The upstream production process of this pilot plant consists of a hydrolysis and acidification stage,

a PHBV producing biomass enrichment stage, and a PHBV accumulation stage. The operating conditions of the enrichment reactor and the accumulation reactor are described by Mulders et al. [26]. The first part of the downstream processing was conducted on-site and consisted of centrifugation and oven-drying (18 h at 120 °C) of the PHBV-enriched biomass. For this research, three different biomass batches were used, one for the medium-scale extraction (Section 2.2), one for the biomass extraction experiments (Section 2.7), and one for the determination of the molecular weight without an oven-drying step (Section 2.8). The three batches were chosen in such a way that the fraction of HV monomers approached the average value for all batches produced by the pilot plant.

2.2. Medium-scale extraction for production of 'extracted' PHBV

Purified or so-called 'extracted' PHBV was produced to study the dissolution and precipitation without having interference of the biomass components (see Fig. 1). To this end, the dried PHBV-enriched biomass was processed further under laboratory conditions. The PHBV was extracted using 1-hexanol as a solvent. An amount of 200 g PHBV-enriched biomass was heated together with 1.5 L of 1-hexanol to 140 °C. The material was incubated at this temperature for 30 min under continuous stirring (100 rpm). Subsequently, the mixture was filtered to remove non-dissolved biomass, using a 1.2 µm filter paper that was placed in a Büchner funnel. Before filtering, the Büchner funnel and the filter paper were preheated to 105 °C in an oven. After filtering, the filtrate was allowed to cool down to room temperature under continuous stirring (100 rpm). The PHBV precipitated during the cooling procedure. In a second filtration step, the PHBV was filtered from the solution, using a cotton cloth. The purified PHBV was subsequently oven dried at 60 °C for 48 h to remove traces of 1-hexanol.

2.3. Solvent selection procedure

A database was constructed of 35 solvents including all solvents described in literature in relation to PHA extraction, retrieved from a recent review by Pagliano et al. [27] and from another recent research article by Alfano et al. [1]. Out of the 35 solvents, 9 solvents were added which are, to our knowledge, not yet described in academic literature in relation to PHA extraction (i.e. 2-propanol, 1-pentanol, 1-hexanol, 1-octanol, 2-ethylhexanol, DIBK, toluene, xylene, pyridine).

Six solvent selection criteria were formulated which were deemed important for a PHBV downstream processing design (Table 1). An explanation of the six criteria can be found in the appendix A. Here, it was assumed that the downstream process consisted of the following elements: biomass dewatering, solvent extraction, solid/liquid separation 1, PHBV precipitation, solid/liquid separation 2, PHBV drying, and solvent-(antisolvent) recovery and regeneration. From the 35 solvents, 15 were instantly eliminated because of the suggested rejection boundary (i.e. DMF, pyridine, n-hexane, MTBE, chloroform, 1,2-dichloroethane because of the unfavorable ACS SHE recommendation; 1-octanol, 1,2-propylene carbonate, ethylene carbonate, DMSO, γ-butyrolactone because of the high boiling point; 1-hexanol, ethyl propionate, ethyl butyrate, phenetole because of the high solvent price).

The 20 remaining solvents were assessed according to the selection criteria and the corresponding parameters in Table 1, and are shown in Table B.1. The results of this assessment and background information are available in the appendices (A and Table B.1). Six solvents were chosen which were deemed promising based on the assessment in Table B.1, 1-butanol, 2-butanol, 2-ethyl hexanol (2-EH), dimethyl carbonate (DMC), methyl isobutyl ketone (MIBK), and acetone. 2-EH was mainly chosen because of the combination of its affordability and its low water solubility, and therefore high solvent regeneration potential, as explained in appendix A.

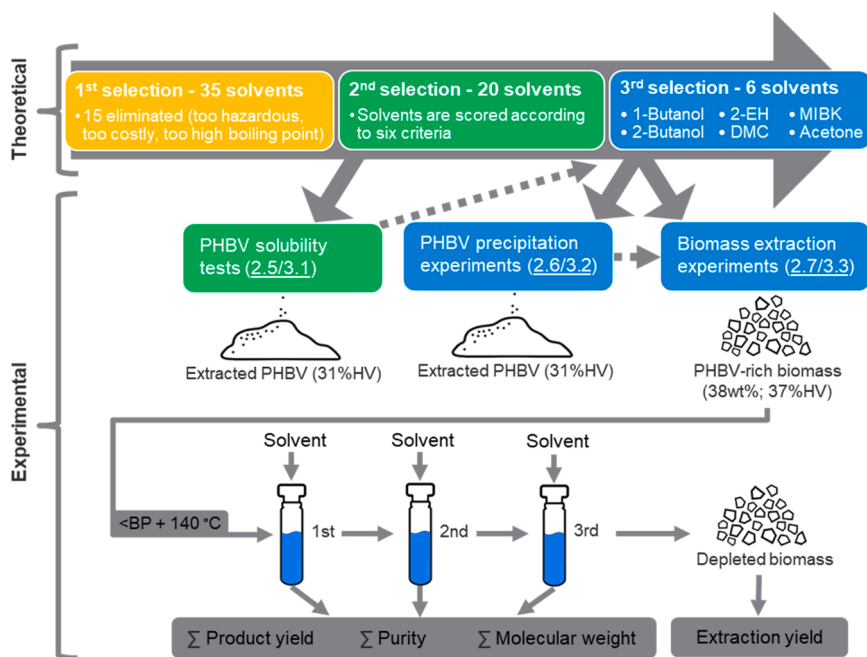


Fig. 1. Flow-chart of all actions in this study. The dashed line represents the influence of the result of one step on the strategy of another step. The underlined numbers refer to the sections where this step is described. The term '<BP' refers to an extraction temperature of 5% below the boiling point.

Table 1

Solvent selection criteria with corresponding parameters, and the suggested rejection boundaries. The solvents that were not eliminated by the suggested rejection boundary were assessed according to these criteria. An explanation of the criteria and the outcome of the assessment can be found in the appendices (A and Table B.1).

Solvent selection criteria	Corresponding parameters	Rejection boundary
1. Safety, health & environment	SHE score ACS ^a , Recommendation ACS ^a	ACS adjusted ranking = (Highly) Hazardous
2. PHBV solubilization potential	Molar volume solvent, Hansen distance ^b , PHBV solubility at RT (exp.) ^c	No boundary
3. Solvent recovery from biomass/product	Density, Boiling point (BP), Vapor pressure at 60 °C	BP > 185 °C
4. Solvent regeneration	Solubility water in solvent, boiling point difference with water, azeotrope with water	No boundary
5. Precipitation strategy	PHBV solubility at RT (exp.) ^c , Boiling point, Miscibility with water	No boundary
6. Costs solvent/antisolvents	euro/L ^d	Price > 2 euro/L

Note:

^a Solvent selection tool on website of American Chemical Society (ACS)

^b Distance between Hansen parameters of PHBV (31 wt% HV) and different solvents

^c Data of experiments with extracted PHBV, see materials & methods for details

^d Estimation of price, retrieved from zauba.com

2.4. Antisolvent selection procedure

The PHBV solubility of PHBV-solvent mixture can be lowered by adding an anti-solvent. Because the PHBV polymer is semi-polar, substances that are very polar or very non-polar can act as antisolvent to precipitate the PHBV. This is reflected in the solubility tests where water, alcohols and alkanes revealed a low PHBV solubility (see Section 3.1). As water is only miscible with acetone, another antisolvent was

required for DMC and MIBK. From the alcohols, 2-butanol was chosen as anti-solvent because of its affordability and low PHBV solubility (Fig. 2 and Table B.1). From the alkanes, n-pentane was chosen because it is less toxic than hexane. Heptane was not chosen because it has no boiling point difference with DMC, and forms an azeotrope with MIBK. For comparison, the combination of acetone with pentane was also included in the precipitation experiments.

2.5. PHBV solubility tests

After the first selection, 20 solvents were subjected to a PHBV solubility test. In addition, the antisolvents water, pentane, and heptane were tested, and chloroform and acetonitrile were added as a reference solvent. Extracted PHBV as described in Section 2.2 was used for this test. First, 250 mg of extracted PHBV was added to a tube with 2.5 ml of solvent to reach a concentration of 100 g PHBV/L. The tube was vortexed and incubated at room temperature (RT) for 1 h. Then, the contents of the tube were filtered (0.4 mm glass fiber filter). The filtrate was captured in pre-weighed tubes, and subsequently oven dried until a constant weight was reached. The precipitate was weighed to calculate the PHBV solubility. The PHBV on the filter was also oven dried and weighed to construct a mass balance of every experiment (data not shown).

2.6. PHBV precipitation experiments

2.6.1. Cooling to RT

Temperature dependent dissolution and precipitation tests were conducted to measure the precipitation yield of the solvents. The same batch of extracted PHBV was used as in Section 2.5. First, 250 mg of extracted PHBV was added to a tube with 5 ml of solvent to reach a concentration 50 g PHBV/L. The same concentration was used as for the biomass extraction experiments (Section 2.7). The tubes were heated for 1 h at a temperature of 5% below the boiling point of the solvent. Acetone formed an exception, because 72 °C was required to dissolve all the PHBV. Then, the tubes were incubated for 1 h at RT, after which the precipitated PHBV was collected by centrifugation (5 min at 4000 g). The remaining solvent was decanted and the precipitated PHBV was

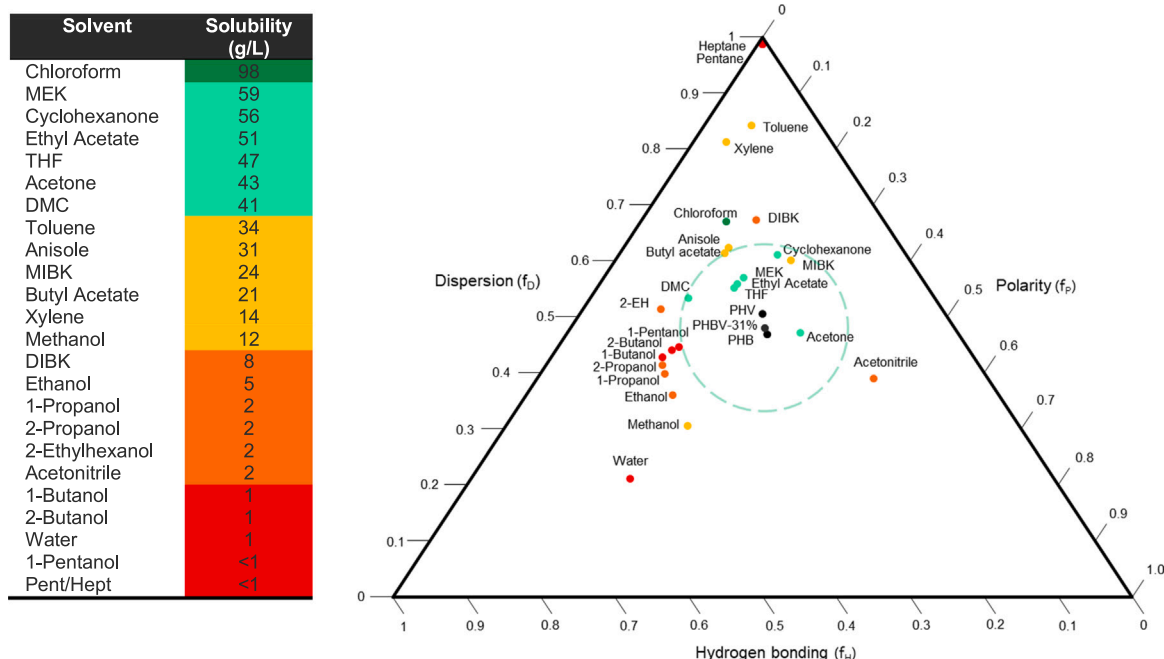


Fig. 2. The table on the left shows the outcome of the PHBV-31% solubility tests after 1 h at RT. The ternary graph on the right is a Teas diagram which displays all solvents plotted against their three fractional solubility parameters (f_D , f_P , f_H) [11]. The color scale in the table is linked to the colors of the markers in the Teas diagram. The location of PHB, PHV, and PHBV-31% (used for the solubility tests) in the Teas diagram is represented by a black marker [37]. The green dashed circle is drawn with PHBV-31% in the center. It represents the so-called solubility window where solvents can be found with a high PHBV solubility. MEK = methyl ethyl ketone, THF = tetrahydrofuran, and DIBK = diisobutyl ketone.

oven dried until no weight loss was detected. The dried PHBV was weighed to calculate the precipitation yield. The experiment was conducted in duplicate.

2.6.2. Antisolvent

DMC, MIBK, and acetone required an antisolvent to precipitate the PHBV. Two antisolvents were tested in different ratios for these solvents. 2-butanol was tested on DMC, and MIBK and water were tested on acetone, all with a ratio of 1/1, 3/1, and 5/1 (v/v). Pentane was tested on DMC, MIBK and acetone with a ratio of 1/1 and 3/1 (v/v). Extracted PHBV was dissolved as described in Section 2.6.1. Then, the different antisolvents in different ratios were added. The tubes were vortexed and incubated at RT for 1 h, after which the precipitated PHBV was collected by centrifugation (5 min at 4000 g). The acetone tubes with water formed an exception and were centrifuged for 1 h. The remaining solvent was decanted and the precipitated PHBV was oven dried until no weight loss was detected. The dried PHBV was weighed to calculate the precipitation yield. This experiment was also conducted in duplicate.

2.7. Biomass extraction experiments

Small-scale biomass extractions were conducted to compare the six selected solvents. First, 1.2 g of biomass was dried for 2 h at 60 °C to remove traces of water. Then, the biomass was incubated for 5 min at 120 °C. This was directly followed by the addition of the biomass to the extraction tubes with 10 ml solvent. The extractions were executed in a glycerol heating bath at two different temperature regimes, at 5% below boiling point and at 140 °C. For extractions below boiling point, the solvent was pre-heated. Extractions with 2-EH were only conducted at 140 °C, because the boiling point is above 140 °C. Extractions with acetone were only conducted at 125 °C, because the boiling point is too low (56 °C) for conducting an effective extraction below this value, and because 140 °C generated too much pressure for the used equipment. Three consecutive extraction cycles of 1 h were performed, with every cycle a new batch of solvent (see Fig. 1). The tubes were manually

shaken every 5 min.

After every extraction cycle, the PHBV-containing solvent was poured in a small Büchner funnel with a 1 µm filter while the biomass remained in the extraction tube. A tube was placed below the funnel to collect the PHBV-containing solution. The filter set-up was placed in an oven at a temperature of 5% below boiling point of the solvent to prevent premature PHBV precipitation. For the extractions above boiling point, the tubes were first cooled in a water bath at RT until atmospheric pressure was achieved.

Pentane was chosen as antisolvent for DMC, MIBK, and acetone (3/1 ratio). In addition, water was tested as antisolvent for acetone (1/1 ratio), and solvent evaporation was tested for acetone. First, the PHBV-containing solution was cooled for 3 min, then 3 volumes of pentane were added or 1 vol of water was added. Subsequently, the tubes were vortexed and incubated at RT for 1 h. For poor solvents, the PHBV-containing solution was taken out of the filtration set-up and incubated at RT for 2 h. After precipitation, all tubes were centrifuged (5 min at 4000 g), and the supernatant was decanted and collected. The PHBV was dried at 50 °C for 2 days in a convection oven. The PHBV extracted with 2-EH was dried for several weeks to achieve a constant weight. All experiments were conducted in triplicate. For the acetone evaporation experiment, the tubes with PHBV-containing solution were after filtration directly placed in the oven at 50 °C for 2 days. As a reference, an extraction with chloroform and acetonitrile at 140 °C, and methanol at 125 °C were added to the experiments. Because the main objective was to obtain the extraction yield (Section 2.11), the solvent was evaporated in a fume hood directly after filtration.

Special pressure resistant tubes (Sigma-Aldrich, USA) were used for extractions above boiling point. For DMC and acetone, the vacuum of the Büchner filter set-up resulted in premature PHBV precipitation due to low boiling point. To close the mass balances for these two solvents with a low boiling point, a syringe-filter combination was used instead.

2.8. Extraction of freeze-dried biomass

A small portion of wet biomass from the pilot was freeze-dried and compared to a portion which was oven dried. For both portions, the PHBV of 1 g of dried biomass was extracted with 10 ml of chloroform at 60 °C for 4 h. The solution was filtered, precipitated with 3-volumes of n-hexane, incubated, centrifuged and dried as described in Section 2.7. At last, M_w was determined by GPC (Section 2.12.2).

2.9. Hansen solubility parameters and Teas diagram

The cohesive interactions between a solvent and a polymer largely determine the solubility. A polymer tends to dissolve better in a solvent with similar types of cohesive energies, also known as the 'like dissolves like' principle. An approach to predict polymer solubility is by quantifying this cohesive energy in the form of three Hansen solubility parameters (HSP), as in Eq. 2.1 [13].

$$\delta^2 = \delta_D^2 + \delta_P^2 + \delta_H^2 \quad (2.1)$$

Here, δ_D is the energy density from dispersion bonds between molecules, δ_P is the energy from dipolar intermolecular force between molecules and are produced by permanent dipole-dipole interactions, and δ_H is the energy density from hydrogen bonding, expressed in $\text{MPa}^{1/2}$.

The disadvantage of the HSP is that a three-dimensional (3D) graphical representation is required, while for practical applications, a two-dimensional (2D) method is to be preferred. The Teas diagram is a plotting technique to display the three HSP in a 2D plot [36]. The fractional solubility parameters were calculated with the equations in 2.2, and were subsequently plotted in a ternary graph.

$$f_D = \delta_D / (\delta_D + \delta_P + \delta_H)$$

$$f_P = \delta_P / (\delta_D + \delta_P + \delta_H) \quad (2.2)$$

$$f_H = \delta_H / (\delta_D + \delta_P + \delta_H)$$

2.10. Mass balance construction

PHBV mass balances were constructed for every biomass extraction to confirm the reliability of the obtained data. Moreover, the mass balances gave insight into the PHBV flows of the process. Here, one ingoing stream was identified ($PHBV_{X,in}$), and three outgoing PHBV streams were identified, including PHBV in the depleted biomass ($PHBV_{X,out}$), PHBV as product ($PHBV_{Product}$), and PHBV remaining in the solvent (-antisolvent) ($PHBV_{SA}$).

$$PHBV_{X,in} = PHBV_{X,out} + PHBV_{Product} + PHBV_{SA} \quad (2.3)$$

The terms in Eq. 2.3 can be further specified.

$$\begin{aligned} X_{in} (g) * f_{PHBV,in} &= X_{out} (g) * f_{PHBV,out} \\ &+ \sum_{i=1}^3 (Product_i (g) * Purity_i) \\ &+ \sum_{j=1}^3 (Precipitate_j (g) * \frac{SA_{total}(g)}{SA_{sample,j}(g)} * Purity_j) \end{aligned} \quad (2.4)$$

Here, X_{in} and X_{out} represent the weighed biomass before and after extraction respectively, and f_{PHBV} represent the corresponding weight fraction of PHBV determined by gas chromatography (GC) analysis.

The third part of the formula represents the sum of the obtained PHBV products from the three consecutive extractions. The dried product of each extraction ($i = 1, 2, \text{ or } 3$) was weighed and multiplied by its corresponding purity determined by GC analysis.

The fourth part of the formula represents the sum of the PHBV remaining in the solvent (-antisolvent) liquid over the three consecutive extractions. An additional precipitation experiment was done to quantify this fraction. To this end, a sample (SA_{sample}) of the total solvent

(-antisolvent) liquid (SA_{total}) was taken and 10 volumes of heptane were added to precipitate the remaining PHBV. After an incubation period of 1 week, the sample was centrifuged (5 min at 4000 g). The solvent-antisolvent liquid was decanted and the precipitated PHBV was oven dried until no weight loss was detected. As the amount of precipitated PHBV was not sufficient to measure both the PHBV purity and the molecular weight, a PHBV purity fraction of 0.8 was assumed. This value was based on multiple medium-scale extraction where the same method was applied to precipitate the remaining PHBV (data not shown). For the acetone experiment with water as antisolvent, heptane precipitation was not possible due to immiscibility with the solvent-antisolvent liquid. Therefore, a sample of the solution was evaporated, and the PHBV content of the precipitate was determined by GC analysis.

2.11. Output variable definition

For the precipitation tests (Section 3.2), the precipitation yield was defined as the fraction of PHBV precipitated from the total amount of extracted PHBV dissolved.

$$Precipitation \text{ yield (wt\%)} = \left(\frac{PHBV_{Precipitated}}{PHBV_{in}} \right) * 100\%$$

For the biomass extractions (Section 3.3), solvent comparison was performed by calculating and analyzing four output variables, including: purity, molecular weight (M_w), product yield, and extraction yield.

The purity and molecular weight (M_w) were calculated as a weighted average of the obtained PHBV in the individual extractions ($PHBV_{product,i}$).

$$Purity \text{ (wt\%)} = \sum_{i=1}^3 \left(Purity_i * \frac{PHBV_{product,i}}{PHBV_{product}} \right) \quad (2.5)$$

$$M_w = \sum_{i=1}^3 \left(M_{w,i} * \frac{PHBV_{product,i}}{PHBV_{product}} \right) \quad (2.6)$$

$Purity_i$ was determined by GC analysis, while $M_{w,i}$ was determined by gel permeation chromatography (GPC), both analytical techniques are described in the Section 2.12.

The product yield was defined as the fraction of PHBV ending up as product, and was determined by summing up the products of the three individual extraction. It was defined by using the terms in Eq. 2.3.

$$Product \text{ yield (wt\%)} = \sum_{i=1}^3 \left(\frac{PHBV_{Product,i}}{PHBV_{X,in}} \right) * 100\% \quad (2.7)$$

The extraction yield was defined as the fraction of PHBV which was extracted from the biomass, and was only determined after three consecutive extractions. This variable focusses only on the extraction step, by measuring the PHBV that remained in the biomass (i.e. the non-extracted PHBV). It was defined by using the terms in Eq. 2.3.

$$Extraction \text{ yield (wt\%)} = \left(1 - \frac{PHBV_{X,out}}{PHBV_{X,in}} \right) * 100\% \quad (2.8)$$

2.12. Analytical methods

2.12.1. GC analysis for PHBV quantification

The PHBV after extraction was analyzed for purity and monomer content (hydroxybutyrate (HB) or hydroxyvalerate (HV)) using gas chromatography (GC). The method is described in detail by Johnson et al. [15]. In brief, the PHBV was hydrolyzed and esterified in the presence of concentrated HCl, 1-propanol, and dichloroethane with a ratio of 1/4/5 (v/v/v) for 3 h at 100 °C. The formed esters, which accumulated in the organic phase, were analyzed by a gas chromatograph (model 6890 N, Agilent, USA). A mixture of methyl-3-hydroxybutyrate and methyl-3-hydroxyvalerate (Sigma Aldrich, USA) was used as standard due to a reduced purity of the commonly used standard (P(3HB-co-3HV) with 12 mol% HV (Sigma

Aldrich, USA), as shown by Burniol-Figols et al. [4].

The purity and the HV or HB content were calculated with the GC data and the Eqs. 2.9 and 2.10 respectively.

$$\text{Purity}_i \text{ (wt\%)} = \frac{\text{HB}_{GC} \text{ (mg)} + \text{HV}_{GC} \text{ (mg)}}{\text{Initial GC sample (mg)}} \quad (2.9)$$

$$\text{HV or HB content (wt\%)} = \frac{\text{HV}_{GC} \text{ or } \text{HB}_{GC} \text{ (mg)}}{\text{HB}_{GC} \text{ (mg)} + \text{HV}_{GC} \text{ (mg)}} \quad (2.10)$$

2.12.2. GPC analysis for molecular weight analysis

A gel permeation chromatography (GPC) measurement was performed to measure the molecular weight distribution of the PHBV product using a Shimadzu Prominence GPC system equipped with a Shodex LF-804 column. For almost all PHBV samples, tetrahydrofuran (THF) was used as eluent, but for PHBV samples extracted with acetone dimethylformamide (DMF) was used as the eluent, both at a flow rate of 1 ml/min at 40 °C. A small comparison study was done to compare both eluents, and a correction factor (1.06) was implemented for the samples measured with DMF (See Fig. C.1 for more information).

Before injection, the PHBV sample was dissolved in chloroform and then mixed with eluent at a ratio of 1/9 (v/v) and a final concentration of 3 mg PHBV/ml, and subsequently filtered. Data of the refractive index detector was quantified with a universal calibration of monodisperse polystyrene standards with the help of LabSolutions software.

3. Results & discussion

3.1. PHBV solubility tests

The theoretical and experimental actions performed in this study are shown in the flow-chart of Fig. 1. First, PHBV-enriched biomass was produced at pilot-scale and processed at laboratorial scale to obtain extracted PHBV with a purity of 95 wt%, an HV content of 31 wt%, and an M_w of 30 kDa. This material was used to measure the PHBV solubility in the 20 selected solvents and three selected antisolvents at room temperature (RT). Fig. 2 shows the values obtained from the solubility tests, and at the same time shows the relation between measured PHBV solubility and the Hansen solubility parameters (HSP), displayed in a Teas diagram.

A clear inverse relation between the solubility of the (anti-)solvents at RT and the distance to PHBV-31% in the Teas diagram can be observed. The antisolvents have a very low PHBV solubility, and reveal the largest distance to PHBV-31% in the Teas diagram. 2-butanol, which was used both as solvent and as antisolvent in this study, also showed a very low solubility at RT but the distance to PHBV-31% is much smaller. In general, all alcohols showed a very low PHBV solubility at RT, pointing out their high PHBV precipitation potential. Methanol showed the highest solubility, although its distance to PHBV-31% is the largest. This could be explained by the small molar volume of methanol compared to the other alcohols. According to Hansen [13] and according to Flory-Huggins theory of polymer solutions [10], smaller solvents can have improved solubilization rates and improved solubility, even though the solubility parameters are identical.

A green dashed circle was drawn with PHBV-31% in the center. All solvents with a high PHBV solubility (light green marker) are located within this so-called solubility window. Chloroform forms the only exception. It has a superior PHBV solubility at RT, while the solvent is not close to the solubility window and its molar volume is larger than acetone. According to Shephard et al. [32], chloroform's extreme ability to dissolve a large range of substances at high concentrations can be explained by the phenomenon of polar stacking. MIBK shows a somewhat lower PHBV solubility, but is also the solvent with the highest molar volume within the solubility window.

Thus, predicting PHBV solubility of a given PHBV polymer in a solvent is more complicated than only measuring the distance in the Teas

diagram. Moreover, the PHBV solubility at RT is not linearly related to the PHBV solubility at elevated temperature during extractions, as shall be discussed in Section 3.4. Nevertheless, the Teas diagram can be applied as a useful tool to evaluate which solvents require an anti-solvent and which antisolvents are suitable. Furthermore, the Teas diagram reveals that the HV content of the polymer has only a minor effect on the location of the solubility window in the Teas diagram, and has from this perspective presumably a minor influence on the choice of solvent.

The observed link between the solubility results and the HSP, which are based on thermodynamic considerations, suggest that the maximal PHBV solubility is approached in this experiment. However, it is not excluded that kinetic effects also play a role in the obtained solubility values. On the other hand, it is important to realize that the M_w of the PHBV used was low (30 kDa), which had a positive effect on polymer solubility due to a larger entropy change and had a positive effect on solubilization rates due to less entanglements [14,24].

3.2. PHBV precipitation experiments

Six solvents were selected from Table B.1: 1-butanol, 2-butanol, 2-EH, DMC, MIBK, and acetone. Two different methods for precipitation were tested with extracted PHBV, cooling and antisolvent addition. Cooling was tested for all solvents. The precipitation yield was determined and plotted in Fig. 3a. It shows that the alcohols have a high precipitation yield, where the bulky molecules of 2-EH obtained the highest precipitation yield of $96 \pm 2\%$. The alcohols can be applied in a process without the addition of antisolvents, and are referred to as poor solvents. DMC, MIBK, and acetone have a much lower precipitation yield. They require the addition of an antisolvent or the evaporation of the solvent for an economically feasible process, and are referred to as good solvents. In general, the precipitation yields in Fig. 3a reveal a clear inverse relation with the PHBV solubility in Fig. 2.

The antisolvent experiment was only conducted for the good solvents, where two different antisolvents in different ratios were tested for each good solvent. For DMC and MIBK, addition of 2-butanol resulted in precipitation, but ten volumes were required to precipitate only $54 \pm 12\%$ and $72 \pm 6\%$ of the dissolved PHBV respectively (Fig. 3b-c). Pentane appeared to be a much more effective antisolvent. Only three volumes were enough to precipitate $98 \pm 3\%$ in DMC and $97 \pm 1\%$ in MIBK, thereby reaching a similar precipitation yield as the best performing poor solvent, 2-EH. Water appeared to be very effective in the precipitation of PHBV in acetone. One volume of water was already enough to precipitate $95 \pm 1\%$ (Fig. 3d). For acetone, the combination with pentane was slightly less effective (precipitation yield of $87 \pm 1\%$), but was selected for the subsequent biomass extractions experiments for comparative purposes. The bars with a red outline in Fig. 3 are the precipitation strategies that were chosen for the biomass extractions in Section 3.3.

The molecular weight of the polymer also had an influence on the results. While a low molecular weight of a polymer in general facilitates the dissolution process, it impedes the precipitation process [14]. This means that if higher molecular weight PHBV would have been used for this experiment, presumably higher precipitation yields would have been obtained or lower amounts of antisolvent would have been required.

3.3. Biomass extraction experiments

Extraction experiments with PHBV-enriched biomass were conducted with the six selected solvents and the precipitation strategy determined in Section 3.2. The PHBV-enriched biomass contained $38 \pm 0.1\%$ PHBV of the TSS with an HV content of $37 \pm 0.1 \text{ wt\%}$. The outcomes of the experiments by means of the different output variables are described one by one in the following subsections.

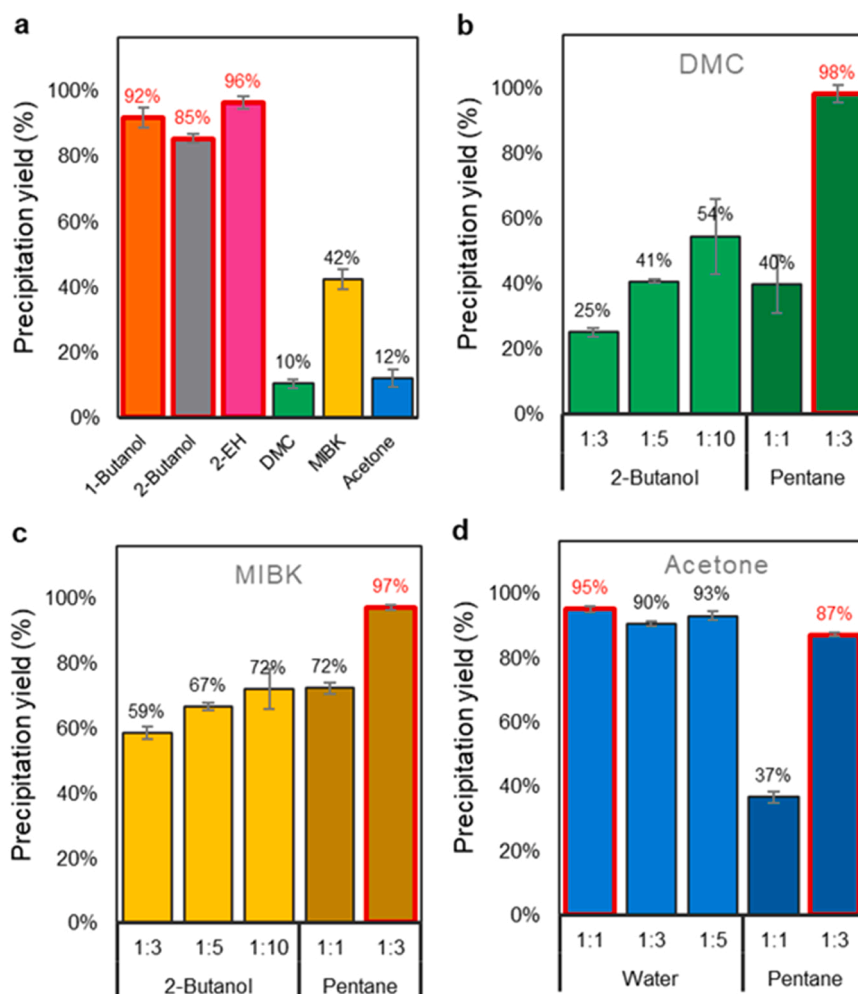


Fig. 3. Precipitation yields of cooling and antisolvents experiments performed with extracted PHBV. Fig. 3a shows the results of the cooling to RT experiment. b-d show the results of the antisolvents test which were conducted with the good solvents (DMC, MIBK, and acetone). The bars with a red outline represent the precipitation method chosen for each solvent during the biomass extractions in Section 3.3.

3.3.1. Purity

Most experiments obtained PHBV with a high purity, above 95% (Fig. 4a). The experiments below boiling point resulted in the highest purities, implying that a lower extraction temperature results in the extraction and precipitation of less contaminants. The usage of water as antisolvent compared to pentane appeared to reduce the purity slightly although the difference was not significant. The acetone evaporation experiment is a clear exception. Here, all contaminants that were not removed in the filtration step remained in the solvent and therefore ended up in the final product, resulting in a significantly lower purity. Furthermore, no significant difference in purity was observed over the consecutive extraction cycles of all solvents (data not shown).

From literature, it is known that solvent extractions result on average in a high purity (above 90%), regardless of solvent type and cultivation strategy (pure culture or MMC) [27]. This is in line with the data obtained in this study and underlines the robustness of solvent extraction in terms of purity.

3.3.2. Molecular weight

The molecular weight (M_w) of the PHBV product was measured for all experiments (Fig. 4b). A clear difference was observed between the molecular weight values obtained for extractions conducted below boiling point and the extractions conducted at 140 °C for the individual solvents (blue bars versus orange bars). A higher temperature results in a higher random scission rate, and therefore a lower molecular weight

[22,23].

For experiments below boiling point, it is hard to discriminate between the effect of the solvent and the effect of temperature, because the temperature varies for every solvent. It was observed that the PHBV obtained from extraction with DMC and MIBK had the highest molecular weight. For experiments at 140 °C, solvents could be compared. Again, it appeared that DMC and MIBK had the highest values, indicating that these solvent had a relatively low reactivity towards PHBV. The alcohols appeared to have a higher reactivity, although the effect slightly decreased as the carbon chain lengthened (2-EH), or when the alcohol group was at a secondary position (2-butanol) [40]. The experiments with acetone revealed very little variation in molecular weight, showing that the precipitation strategy had a minor effect on this variable. Surprisingly, the molecular weight of acetone was lower than for MIBK, the other ketone, while the extraction temperature was lower. This implies that the ketone group also acted as a reactive group in the transesterification process, and that an increasing length of the carbon chain reduces the reactivity of ketones, similar as with the alcohols.

Interestingly, the molecular weight showed little variation over the different extraction cycles (data not shown). For 7 out of 12 experiments, there was a small decrease when comparing the first with the third extraction ($17 \pm 4\%$). Four experiments did not show a significant decrease or increase, and one experiment, 2-butanol below boiling point, showed an increase in molecular weight (16%). As discussed before, high molecular weight PHBV has a lower extraction rate than

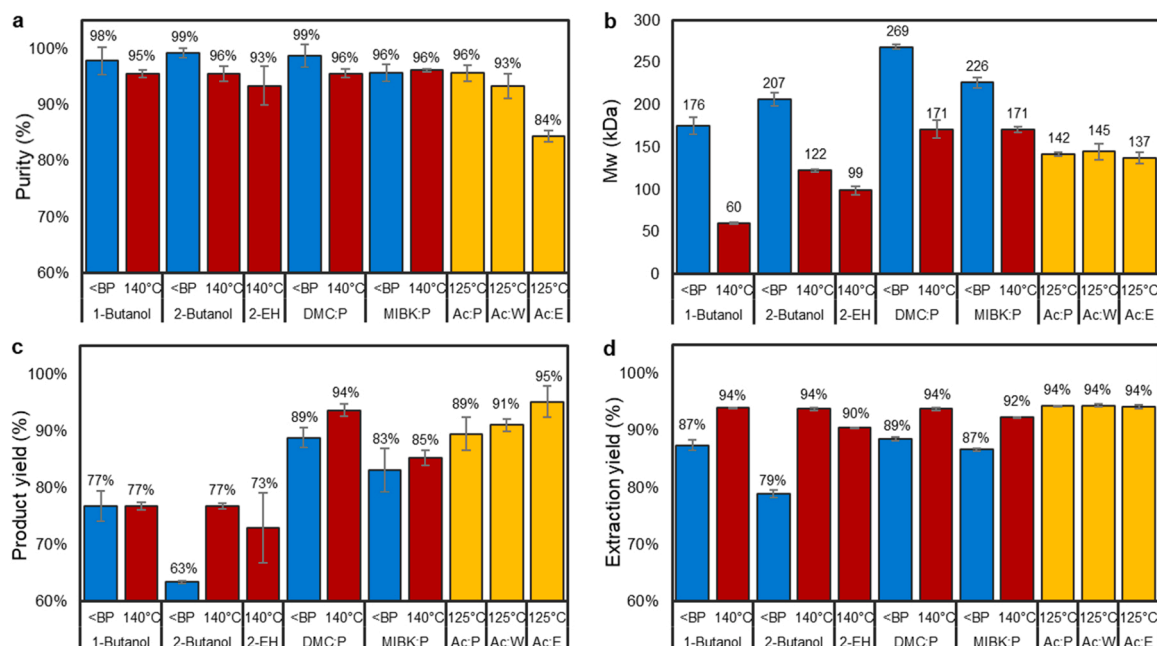


Fig. 4. Overview of the four output variables for the biomass extraction experiments including purity (a), molecular weight (b), product yield (c), and extraction yield (d). The term '<BP' refers to an extraction temperature of 5% below the boiling point (i.e. 112 °C for 1-butanol, 95 °C for 2-butanol, 86 °C for DMC, and 110 °C for MIBK). Ac = Acetone, P = Pentane, W = water, and E = evaporation.

low molecular weight PHBV. Therefore, it seems that the final molecular weight of each extraction cycle was the result of two seemingly opposing forces, random scission and selective extraction rates. For the same reason, it is very challenging to measure the molecular weight of the input material, because for short extraction times, only short molecular weight polymer is extracted, and for long extraction times, the polymer is significantly degraded. For this research, it is assumed that the input molecular weight after oven drying was around 270 kDa. This is the value obtained for the extraction with DMC below boiling point.

In most experiments the molecular weight was lower than the typical values reported in literature (between 200 kDa and 3000 kDa) [18]. To understand the reason behind this, PHBV-enriched biomass from the same pilot plant was freeze-dried and compared to oven dried biomass. Here, it appeared that the M_w was 1470 kDa and the PDI was 2.7, while for the oven dried counterpart the M_w was 258 and the PDI 3.7, thereby clarifying that the strong decrease in M_w is a result of the long oven-drying step. The molecular weight distribution was quantified in

terms of the polydispersity index (PDI). The obtained values were high and showed substantial variation between the different solvents and temperature regimes. The weighted average of the product fractions of all biomass extractions was 4.2 ± 1.1 , while the maximum was 6.5 and the minimum was 2.3.

According to Cox [6], the mechanical properties of the polymer start to degrade when the M_w is lower than 400 kDa. For this reason, optimizing the biomass drying step can be a valuable topic for future research. On the other hand, low molecular weight PHBV will probably facilitate the extraction process. Moreover, research showed that low molecular weight PHBV can be particularly interesting for certain applications, such as in slow-release fertilizer formulations [3].

3.3.3. Product yield

The product yield represents the fraction of PHBV ending up as a purified product per amount of PHBV entering the extraction procedure, a variable with important economic consequences. The product yield, as

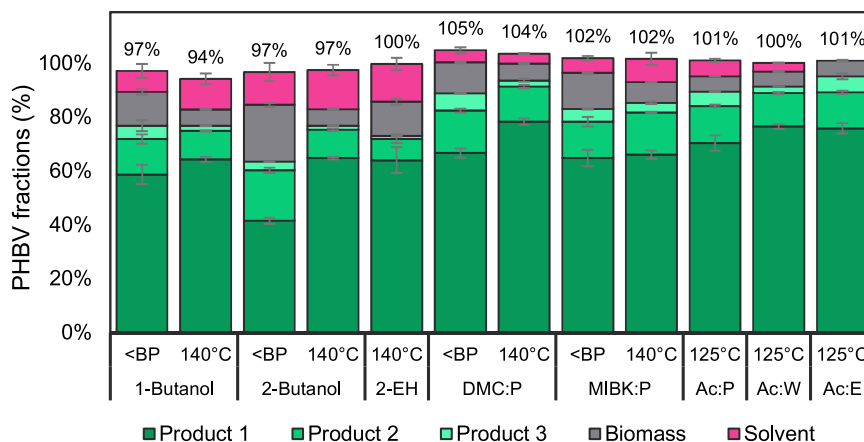


Fig. 5. PHBV mass balances constructed for all biomass extraction experiments. The three green fractions resemble the product fraction obtained in the consecutive extraction cycles, and together form the product yield. The term '<BP' refers to an extraction temperature of 5% below the boiling point (i.e. 112 °C for 1-butanol, 95 °C for 2-butanol, 86 °C for DMC, and 110 °C for MIBK). Ac = Acetone, P = Pentane, W = water, and E = evaporation.

a sum of three extractions, is shown in Fig. 4c, while the individual fractions are displayed as a part of the mass balance in Fig. 5. Fig. 5 reveals that the third extraction has a minor contribution to the product yield (1–6%), indicating that under the applied conditions the maximal PHBV yield is approached. A potential fourth extraction will yield an even smaller fraction, and will most likely not be economical.

For most solvents, a higher extraction temperature results in a higher product yield. Remarkably, the differences between extraction below boiling point and at 140 °C were small for 1-butanol and MIBK. For these solvents, it will likely not pay-off to increase the extraction temperature, considering the increase in cost of working under pressure and the extra loss in molecular weight. It should be noted that this might change for higher M_w polymers or polymers with a different HV content.

A higher product yield was obtained with the good solvents than with the poor solvents, with DMC (89–94%) and acetone (90–95%) having the highest values. For DMC, an increase in extraction temperature leads to an increase in product yield ($94 \pm 1\%$ compared to $89 \pm 2\%$). For acetone, the type of antisolvent does not influence the product yield significantly ($89 \pm 2.9\%$ for pentane and $91 \pm 1\%$ for water), whereas the solvent evaporation strategy does increase the product yield ($95 \pm 3\%$). These values are higher than the values obtained in other studies where MMC PHBV produced from synthetic substrates was extracted. For DMC, de Souza Reis et al. [33] obtained a maximal value of 81%, and Samorì et al. [30] obtained a maximal value of 82% after adding a hypochlorite pretreatment. For acetone, Chan et al. [5] was able to extract 51% of the PHBV at the same temperature.

The poor solvents suffer from a low precipitation yield compared to the good solvents. As can be seen in Fig. 5, the amount of PHBV remaining in the solvent is relatively high for the poor solvents for both temperature regimes. Therefore, the product yield of the poor solvents (77% at maximum for 1-butanol and 2-butanol) is lower than the values for the good solvents, and lower than values reported in literature. For 2-butanol, Werker et al. [39] obtained an product yield of 83% when extracting PHB from MMC. The higher product yield for the alcohols was probably related to the higher molecular weight PHBV used by these authors.

3.3.4. Extraction yield

The extraction yield is the fraction of the PHBV extracted from the biomass per amount of PHBV entering the extraction procedure. This variable can contribute to a better understanding of the critical factors affecting PHBV extraction by zooming in on the extraction step while disregarding the precipitation step.

Fig. 4d reveals that a higher extraction temperature resulted in a higher extraction yield for each individual solvent (blue bars versus orange bars). Here, the largest increase is observed for 2-butanol. A closer look at the extractions below boiling point reveal that DMC had among the highest extraction yields while it had the lowest extraction temperature. Therefore, solvent properties also played a role in establishing the extraction yield. From the PHBV solubility tests (Section 3.1), it was already known that DMC has a higher solvency for PHBV than MIBK and especially than the alcohols.

The extractions above boiling point (140 and 125 °C) showed a very homogenous outcome in six out of eight experiments, reaching an average extraction yield of $94.1 \pm 0.2\%$. Only 2-EH and MIBK had a somewhat lower extraction yield. Acetone also reached an extraction yield of approximately 94% while its extraction temperature is only 125 °C. No clear relationship seems to exist between the obtained values and the HSP or the solubility tests in Section 3.1. This issue will be further discussed in Section 3.5.3. As expected, the three acetone extractions had a very similar extraction yield, because there were no differences in extraction conditions only in precipitation conditions, which emphasizes the reproducibility of the experiments.

The extraction yield was measured by measuring the PHBV remaining in the biomass after three extractions. It must be realized that part of the remaining PHBV came from the solvent surrounding the

biomass after decantation. This PHBV was dissolved into the solvent, but the solvent remained in the biomass matrix.

Based on the PHBV and solvent mass balances, it was calculated that in all experiments on average $1.1 \pm 0.4\%$ of the total amount of PHBV added could be traced back as this type of PHBV. This is a small value compared to the amount of PHBV remaining in the biomass (i.e. between 5.6% and 21%). Another point of attention is that it is not certain if the maximal extraction yield was reached in every experiment. Therefore, different extraction rates could also have influenced the measured extraction yield. On the other hand, the small fraction of product obtained in the third extraction cycle (Fig. 5) suggests that the maximal obtainable extraction yield under the applied conditions is roughly approached.

3.3.5. PHBV mass balances

PHBV mass balances were constructed to validate the obtained values. Fig. 5 reveals that nine out of twelve mass balances closed with a maximal deviation of 3%, the other three mass balances closed with a maximal deviation of 6%. On average, the mass balances closed for $99.9 \pm 3\%$ indicating that there are no large systematic errors in the measurements and calculations. It is believed that these values are acceptable considering the large number of process steps and analyses executed for the construction of each mass balance. However, the mass balances also reveal that the product yields of some experiments could be a few percent higher or lower than described earlier.

3.4. Critical factors for PHBV extraction

3.4.1. Time, temperature, and HV content

An apparent observation from Fig. 5 is that time is a noteworthy factor in PHBV extraction. A longer extraction time results in more PHBV extracted, where a maximum is approached after 3 or 4 extractions of 1 h. The number of extraction cycles required to maximize PHBV yield is defined by the extraction kinetics, which are a function of temperature. Fig. 5 reveals that for extractions below boiling point a larger fraction of the PHBV is extracted in the second and third cycle compared to extractions at 140 °C. Therefore, extractions at 140 °C will presumably need less cycles or shorter extraction durations than extraction below boiling point to maximize PHBV yield.

Temperature does not only have an effect on extraction kinetics but also on the total amount of extracted PHBV. A higher extraction temperature results in a higher extraction yield (Fig. 4c), assuming that the maximal extraction yield is approached under the applied conditions. An explanation for this phenomenon is that the polymer melting temperature (T_m) is a function of HV content, where a higher HV content results in a lower T_m [2]. Assuming that the HV monomers are not uniformly distributed over the polymer, there are regions with a high HV content (and low T_m) and regions with a low HV content (and high T_m). For this reason, a higher extraction temperature will result in the melting and dissolution of more HV-poor regions of the polymer, thereby increasing the extraction yield.

Support for the theory described above was found in the data. In all biomass extractions, the PHBV in the biomass after extraction showed a significantly lower HV content than the PHBV in the biomass before extraction ($27 \pm 0.9 \text{ wt\%}$ compared to $37 \pm 0.1 \text{ wt\%}$), verifying that HV-rich regions are more effectively extracted.

3.4.2. Solvent properties

In Section 3.1, a relationship was observed between the distance in the Teas diagram and the solubility of PHBV at RT. Besides, it was noted that the molar volume of the solvent also had a modest effect on the solubility or solubilization rate at RT. The biomass extractions above boiling point (Section 3.3) revealed a very distinct outcome than the solubility test conducted at RT. The results suggest that the physico-chemical solvent properties described by the HSP have an insignificant influence on the extraction yield at elevated temperatures, and the effect

of the molar volume of the solvent on extraction yield is more pronounced.

Despite the large distances between the solvents in the Teas diagram, the extraction yield showed a surprisingly constant outcome of $94.1 \pm 0.2\%$, apart from MIBK and 2-EH. Although the solubility of acetone and DMC at RT was among the highest, its extraction yield at elevated temperatures reached a virtually identical value as for 1-butanol and 2-butanol, while their solubility at RT was among lowest. Once the extraction yields are plotted against molar volume of the solvent, a relationship seems to appear (Fig. 6). This relationship offers an explanation why MIBK and 2-EH have a lower extraction yield, and why acetone, operated at lower temperature, still has a high extraction yield.

Additional extractions were conducted to strengthen the above-described relationship. One solvent with an extremely high solvency at RT was tested, chloroform, and two solvents with a smaller molar volume were tested, acetonitrile and methanol. As predicted, the chloroform extraction at 140°C reached an almost identical value as the other solvents with similar molar volume ($94.1 \pm 0.1\%$). In addition, the acetonitrile and methanol extraction at 140°C revealed that the extraction yield can be increased ($95.2 \pm 0.3\%$ and $98.0 \pm 0.1\%$ respectively) when a solvent with a lower molar volume is chosen (Fig. 6). Interestingly, for methanol and acetonitrile, the solvency at RT was among the lowest, and their distance in the Teas diagram among the highest. These additional results further underline that the physico-chemical solvent properties as described by the HSP have a very limited influence on the extraction yield at elevated temperatures, while the molar volume has a significant influence. Moreover, for methanol, the HV content in the remaining biomass ($22 \pm 1.0 \text{ wt}\%$) appeared to be significantly lower than the average of all experiments ($27 \pm 0.9 \text{ wt}\%$), which points out the idea that the bottleneck to extract all PHBV are regions of the polymer with a high HB content.

In literature, molar volume of the solvent has been suggested as an important fourth parameter in polymer solubility [12,20], where a small molar volume result in a high solubility or in high solubilization rates of the polymer. This idea is also supported by the Flory-Huggins solution theory, which comprises a thermodynamic expression for the Gibbs energy change (ΔG_m) accompanying the mixing of polymers in solvents. It states that ΔG_m decreases if the molar volume of the solvent decreases, in particular at elevated temperatures, as explained in detail in appendix D. A more negative ΔG_m presumably results in a higher extraction yield.

3.5. What determines the best solvent choice?

3.5.1. Conclusion based on this study

The experimental results showed a comparison between six solvents

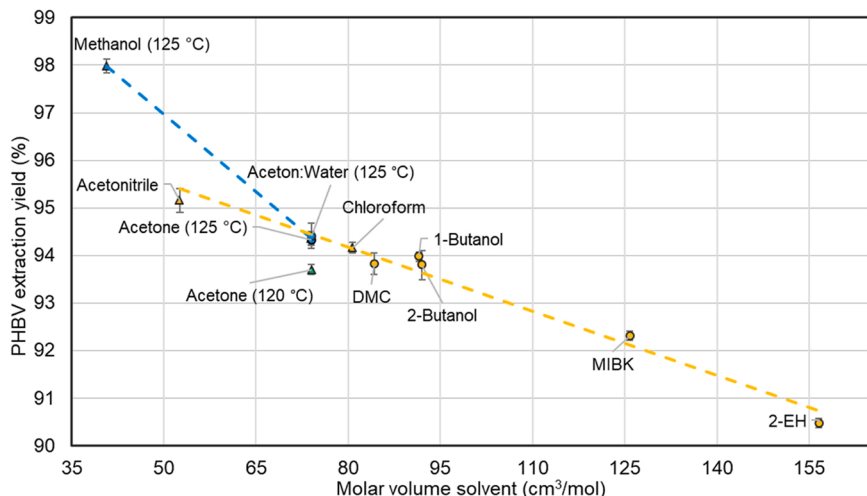


Fig. 6. PHBV extraction yield of biomass extractions versus the molar volume (M_v) of the solvent of all biomass extractions above boiling point ($\bullet$), complemented with additional experiments ($\blacktriangle$). A trendline was added to all data points obtained from extractions at 140°C (yellow markers and line), and a trendline was added to data points obtained from extractions at 125°C (blue markers and line). A test run with acetone at 120°C in duplicate was added to the figure to underline the significance of the observed trends.

from multiple solvent families at two different temperature regimes, thereby revealing evident strengths and weaknesses of each solvent. The highest purities were obtained for 1-butanol, 2-butanol, and DMC, and the highest molecular weights were obtained for MIBK and DMC, both below boiling point. The highest yields were obtained at 140°C or 125°C for DMC and acetone respectively. The results indicated that solvents with a small molar volume, such as acetone, are more effective in PHBV extraction seemingly independent of their solubility parameters. Acetone has the advantage that water instead of pentane can be used as an effective anti-solvent. Alternatively, acetone can be evaporated directly without substantial polymer degradation, thereby merging the PHBV precipitation step, the solid/liquid (PHBV/solvent) separation step, and the solvent regeneration step. These advantages make a potential process simpler and more affordable. If it is assumed that polymer quality requirements are for many applications not very stringent, then we argue that based on this study acetone is a very interesting option considering its high product yield and the avoidance of the usage of a costly, hazardous, and unsustainable anti-solvent.

3.5.2. Other considerations

The question which solvent is the best choice for PHBV extraction does unfortunately not end in an unambiguous, universal answer. First, the specifications of the input biomass play a role. The PHBV-enrich biomass of this pilot had a high HV content (31–37 wt% HV) compared to other pilot plants [8] and a relatively low molecular weight due to a harsh thermal pre-treatment, which both make the polymer particularly suitable for solvent extraction. Therefore, the results presented in this study should be applied with care when working with other PHBV polymers (e.g. higher M_w or lower HV content).

Second, the intended application of the PHBV contributes to the solvent choice. In general, high-value applications require PHBV with higher specifications (high purity, high M_w), while other applications require lower specifications (low purity, low M_w). Extractions with 2-butanol or DMC below boiling point will result in higher specifications, while extraction with DMC at 140°C or acetone at 125°C will result in lower specifications but in a higher product yield.

In addition, it should be noted that the other process conditions such as PHBV loading rate, mixing regime, cooling rate, and precipitation time were not optimized in this study and might improve the obtained values. Furthermore, besides the four output variables defined in this study, other variables might be important, such as gel formation during precipitation or compactness of the final product. Then, scale-up efforts should identify potential bottlenecks for a full-scale. Finally, a detailed process design including an economic analysis needs to be created to give decisive answers about the solvent choice.

3.5.3. Outlook

Although the discovery of a universal solvent for PHBV extraction is unrealistic, the development of a universal solvent selection procedure will not be. The number of solvents produced on industrial scale is limited, therefore the solvent choice for PHBV extraction is also limited. By applying a systematic approach and formulating the right selection criteria, the number of solvents to be tested experimentally can be limited even more. In this study, we aimed to provide a framework for guidance through the solvent selection process. The framework includes selection criteria that represented a wider process perspective. Although improvements can be implemented, for example, by adding solvents to the database, by optimizing the selection criteria, by testing additional types of PHBV-enriched biomass, we believe that this framework can form a starting point for the solvent selection process of other PHBV extraction processes.

CRediT authorship contribution statement

Chris M. Vermeer: Investigation, Conceptualization, Verification, Methodology, Writing – original draft. **Maaïke Nielsen:** Conceptualization, Verification, Investigation. **Vincent Eckhardt:** Methodology, Verification, Investigation. **Matthijs Hortensius:** Investigation. **Jelmer Tamis:** Conceptualization, Writing – review & editing. **Stephen J. Picken:** Conceptualization, Supervision, Writing – review & editing. **Gabrie M. H. Meesters:** Conceptualization, Supervision, Writing – review & editing. **Robbert Kleerebezem:** Funding acquisition, 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

The authors do not have permission to share data.

Acknowledgements

This work was supported by the Netherlands Organization for Scientific Research (NWO) (ALW GK.2016.021). The authors are grateful to Alan Werker for his valuable feedback on the manuscript.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.jece.2022.108573](https://doi.org/10.1016/j.jece.2022.108573).

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