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Polyhydroxyalkanoates production by an advanced food-on-demand strategy: The effect of operational conditions

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ABSTRACT

Despite the increasing number of studies related to the polyhydroxyalkanoate (PHA) production from sewage sludge of wastewater treatment plants, there is still a gap in the correlation between the operational conditions, such as the organic loading rate (OLR), and the intracellular polyhydroxyalkanoate (PHA) content, productivity and final recovery of the polymer. Therefore, this work aims to provide experimental data on PHA productivity and purity in view of scaling up the process to an industrial level taking into account process parameters. In view of that, three OLR were applied during the selection of PHA-accumulating bacteria in sewage sludge. Then, the biomass was harvested and subjected to batch accumulation experiments at two organic loads per dosage by employing a tailor-made software to adopt an automated feed-on-demand strategy, which allowed for 30–56 h of accumulation tests in stand-alone mode. Finally, an improved protocol for PHA extraction has been applied. Experimental results show that the maximum PHA content (60% w/w) was achieved using the highest organic load per dosage during the accumulation test with the biomass selected at the highest OLR (1.8 g COD L^{-1} d^{-1}). Also, the extraction protocol efficiency was proven with four samples with different PHA content, achieving recovery yield as high as 78 ± 3 % with a purity of 89 ± 2 %, thus demonstrating that the adopted strategy might be beneficial for industrial use.

1. Introduction

Being nowadays globally dependent on fossil-fuel-based plastic materials, biodegradable polymers are assuming an increasingly significant role in the challenge to replace conventional plastics by decoupling their production from fossil fuel extraction [1]. Among the different types of polymers, polyhydroxyalkanoates (PHA) have garnered growing attention as they possess similar properties to conventional fossil-fuel-based plastics and they can be bioproduced by various microorganisms [2]. In addition, PHA production by mixed microbial cultures (MMC) can be envisaged using the organic matter present in wastewater as a substrate. The utilisation of MMCs and wastewater are crucial factors that have contributed to the rising popularity of PHA, particularly when considering the circular economy approach, since sludge disposal is the main economic cost within the management of wastewater treatment plants, which is still based on a linear economy model [3]. The implementation of the PHA production is generally based on a four-step process: i. the acidogenic fermentation of sewage sludge to produce volatile fatty acids that serve as a carbon source for MMC; ii. the selection of PHA producing microorganisms through the application of a feast-famine process; iii. the enhancement of the amount of PHA produced, commonly referred to as "accumulation" and iv. the PHA extraction by disrupting the cells employing tailored protocols [3–8]. Despite the recent research on PHA production using sewage sludge [4,5], few works still focus on improving the PHA accumulation and downstream process. However, biopolymer content in the biomass, productivity and purity are pivotal parameters for achieving sustainability in the overall process [5]. In this context, Bengtsson et al. [8] stated that the minimum PHA content in the biomass necessary for a commercially sustainable production process (referred to as the cut-off point) was equal to 0.4 g PHA g^{-1} VSS (Volatile Suspended Solids). However, as far as the authors are aware, no existing protocol has been found to consistently enable the production of such a

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significant content of PHA from sewage sludge. [3,7]. The lack of consistency in achieving a substantial quantity of PHA, coupled with the manual execution of the accumulation step, arguably the primary stage in PHA production, significantly hampers the potential scalability of the process. [8]. Usually, when pilot plant experiments are performed [9–11], there is often a lack of emphasis on other parameters such as the PHA productivity, that can be prominent for the industrial PHA production process.

Finally, the PHA extraction is another crucial step of the process since it can contribute to more than 50% of the polymer production cost. The main current recovery processes are energy-intensive and rely on the use of chemicals, such as chloroform, which are efficient but still potentially hazardous to the environment, particularly at the industrial level [12–19]. Numerous studies have been published focusing on research of less expensive and more environmentally friendly downstream processing methods for industrial implementation [20–24]. On the one hand, Colombo et al. [19] successfully implemented a non-ionic surfactant for dissolving the non-polymeric cellular mass (NPCM), followed by extraction using dimethyl carbonate. Results showed increased recovery when the non-ionic surfactant pre-treatment was employed. Nevertheless, despite the high polymer purity (>90%), the recovery efficiency was less than 60%, which highlights how challenging it is to perform an environmentally friendly PHA extraction without compromising the overall performance. On the other hand, as suggested by Kurian et al. [16], the use of green solvents such as ethylene carbonate on large-scale production can be regarded as another alternative since this environmentally friendly approach achieved excellent product recovery and purity, reaching 98% and 99%, respectively.

As far as authors are aware, studies have yet to be conducted on the role played by the organic loading rate (OLR) in the biomass selection step and by the organic load per dosage during the accumulation step.

Building upon the identified research gap, the objective of this study was to evaluate the impact of biomass selection under three different OLR and the influence of using two different organic loads per dosage employed during the accumulation step. To achieve this, a two-stage process was implemented, consisting of PHA accumulation batch tests conducted automatically using a tailor-made software, to fully automate the process, followed by PHA extraction. An advanced extraction protocol previously described by Mannina et al. [17] was modified to boost the PHA obtention. One of the gaps in current PHA production at the industrial scale is the automation of the accumulation process as few studies [20,21] have been published.

2. Materials and methods

2.1. Selection step for enriching the sewage sludge in PHA-accumulating bacteria

The reactor used for the selection step was part of the new pilot plant established at the Water Resource Recovery Facility at Palermo University [22,25]. The strategy adopted for biomass selection was aerobic dynamic feeding [13,25] and a synthetic substrate was used to comprehensively compare three OLR and two organic loads per dosage. The carbon source was composed of acetic acid (70%) and propionic acid (30%) (Sigma Aldrich), as reported in the literature [10], while KH_2PO_4 and NH_4Cl (Sigma Aldrich) were added as a mineral medium. Table 1 reports the ORL applied during the selection step.

2.2. Accumulation step for improving PHA contents in selected biomass

The PHA-accumulation tests were run in duplicated in fed-batch mode with pulsating substrate feeding. Fig. 1 shows a schematic representation of the experimental set-up. Two 1.5 L glass reactors were used, which were magnetically stirred and aerated. The reactors were equipped with probe ports to monitor the temperature and the dissolved oxygen (DO, WTW). The temperature was controlled using a thermostat

Table 1
Details of selection step and accumulation tests.

OLR applied during the selection step (g COD L^{-1} d^{-1})	Accumulation tests	Organic load per dosage (g COD L^{-1} $dosage^{-1}$)
0.8	T1	7.5
	T2	15.0
1.3	T3	7.5
	T4	15.0
1.8	T5	7.5
	T6	15.0

*COD: Chemical Oxygen Demand.

(Corio CD-CB6) maintained at 20 °C. The biomass (3 L) was harvested from the selection reactor, washed with tap water, and let to settle down to discard 1 L of supernatant. Then, it was mixed with 1 L mineral medium without NH_4Cl. Finally, the biomass was left aerated and stirred overnight before starting the experiments to ensure endogenous conditions. The carbon source used was the same as the one used during the selection step and the feeding was automatically controlled by a tailor-made software (PHACC). For each OLR used in the selection step, two different accumulation experiments (1.5 L each) were carried out to evaluate the influence of two different organic loads per dosage (Table 1). Tests were stopped when no substrate consumption was detected after about 2 h from the last injection. Depending on the effect of the different organic loads per dosage evaluated in this work, the tests lasted 24 to 56 h. During the experiments, samples (ca. 20 mL) were collected and immediately mixed with formaldehyde (36% w/v in H_2O, Sigma Aldrich) to stop the biological activity. All the samples were centrifuged (40 min, 4046 RCF) and the obtained pellets were stored overnight at −80 °C and then lyophilised.

2.3. Automatically controlled pulsate-feeding

The accumulation tests were run with an automatic pulsate feeding based on a feed-on-demand strategy [21] controlled by tailor-made software implemented in a Raspberry Pi 4 model B. The automatic control was adapted from the literature [20] and implemented in the software. The feeding inputs were controlled by continuously monitoring the DO. At the same time, two pumps (Watson-Marlow Qdos 30, Falmouth, United Kingdom) were used to feed the synthetic substrate in the two reactors simultaneously. The software functioning is described by Eq. (1):

$$\delta = \frac{DO_t - DO_m}{DO_r - DO_m} \geqslant T_f \quad (1)$$

- DO_r is the initial background respiration rate of the source biomass.
- DO_m is a weighted average of the minimum DO level monitored.
- DO_t is the current DO value read by the probe.
- δ is the trigger value to feed the substrate.

After performing different tests and analysing data available from Mannina et al. [17] two new conditions were added:

- DO_m threshold which is the difference DO_t - DO_m; to perform the feeding, this value must be $\geq T_f$.
- T_f is the threshold value for feeding or not, this value range from 0.01 to 0.3 and it is related to the type of microorganisms.
- The number of samples allows deciding the number of DO values to build the weighted average DO_m.

The DO_m threshold allows identifying the slope in the DO shifting, thus letting the software acknowledge when an increase/decrease of the biomass respiration rate is happening. Introducing this condition prevented an error in feeding the substrate when the biomass has not yet consumed all the substrate. The number of samples used to build the

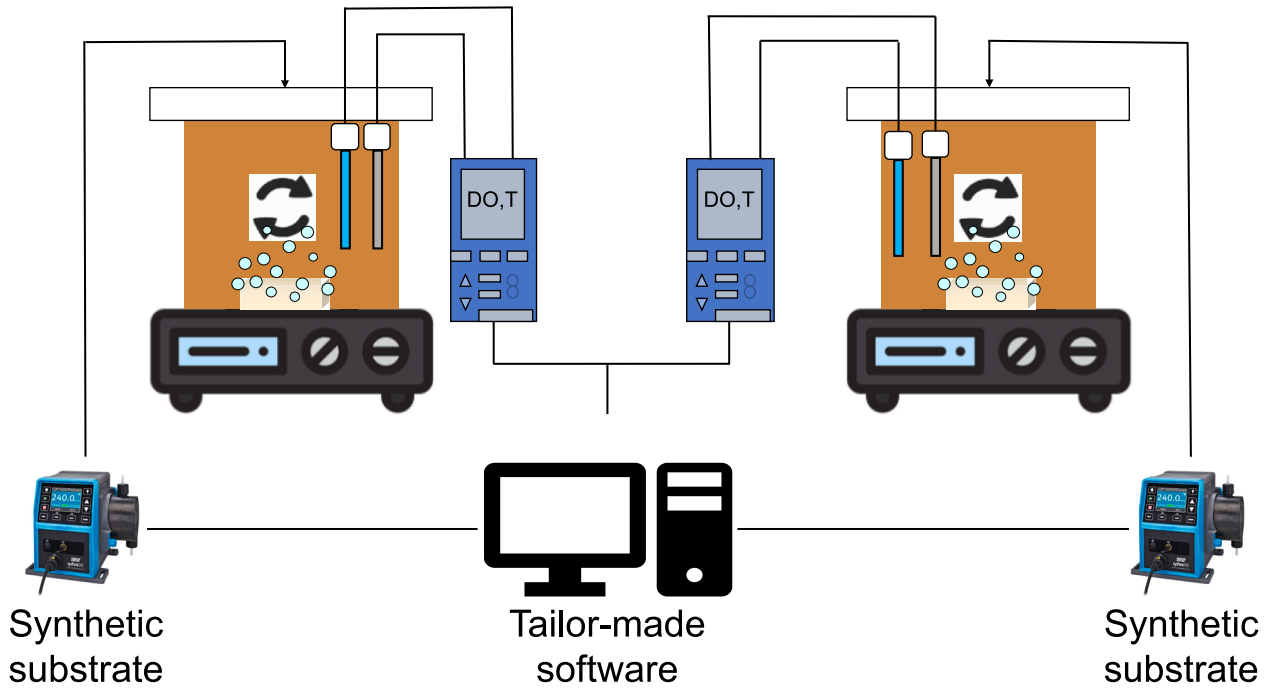

Fig. 1. Schematic representation of the experimental set-up for the accumulation tests.

weighted average allows a more precise calculation when the software identifies the end of the feast. These conditions and values were found to fit more than ten different accumulation tests performed with a synthetic substrate under different conditions.

2.4. PHA extraction protocol from biomass

The extraction protocol proposed by Mannina et al. [17] was adopted using four samples collected during T6 at 4.6, 7.2, 25.5 and 56.2 h (T6-S1, T6-S2, T6-S3 and T6-S4, respectively). The extraction was performed in triplicate. Briefly, 50 mg of lyophilised biomass was weighted in a glass tube and mixed with 5 mL of NaOCl (15 % in H_2O). The tube was then mixed (11 RCF) and heated (75 °C, 1 h) in an Eppendorf block heater (ThermoStat™ C). After centrifugation (1694 RCF, 10 min), the pellet was washed twice with MilliQ Grade water (5 mL). Afterwards, the pre-treated biomass was mixed (11 RCF, 75 °C, 3 h) with an ammonium laurate solution prepared by adding 0.75 mL of ammonia (1 M, Sigma Aldrich, Merck, Darmstadt, Germany) to a 100 mg lauric acid (Sigma Aldrich) suspension in 3 mL of water. The pellet obtained after the centrifugation was dried at 60 °C overnight.

2.5. Analytical methods

The total PHA concentration was calculated as the sum of poly(3-hydroxy)butyrate (PHB) and poly(3-hydroxy)valerate (PHV) monomers concentrations inside the lyophilised biomass, measured by adopting the protocol described by Werker et al. [23]. Briefly, butanol (1.5 mL) and hydrochloric acid (0.5 mL) were added to the lyophilised samples, previously weighted, and the tubes were incubated at 100 °C for 8–20 h. After, 2.5 mL of hexane and 4 mL of MilliQ Grade water were added to the test tubes and then vortex mixed. The organic phase was transferred to the centrifuge tube and another aliquot of MilliQ Grade water was added. After the centrifugation (350 RCF, 10 min), the organic phase was extracted, filtered through 0.22 μm filters and finally collected into gas chromatography (GC) vials. The analysis of the samples was carried out as reported by Montiel-Jarillo et al. [24].

2.6. Calculations

The calculation of PHA amount in microbial cells, yield, purity and recovery were calculated as reported [17]:

$$PHA\_amount\_in\_microbial\_cells = \frac{Mass_{PHA}}{Mass_{lyophilized\_biomass}} \quad (3)$$

$$PHA\_yield = \frac{Extracted\_polymer\_weight}{Biomass\_weight} \quad (4)$$

$$PHA\_purity = \frac{Mass_{PHA}}{Mass_{extracted\_polymer}} \quad (5)$$

$$PHA\_recovery = \frac{PHA\_yield \times PHA\_purity}{PHA\_amount\_in\_microbial\_cells} \quad (6)$$

PHA concentration as g PHA g^{-1} VSS and PHA storage yield were calculated as reported by Conca et al. [13].

2.7. Biopolymer characterisation

The biopolymer structure and percentage of each monomer were confirmed by ^{1}H NMR analysis performed on a Bruker magnetic resonance spectrometer operating at 400 MHz. Extracted biopolymers (10 mg, section 1.4) were dissolved in $CDCl_3$ to carry out the above analysis. NMR spectra were recorded at 25 °C and solvent residual peak was used as reference. Molecular mass (Mw), molecular mass distribution (Mn), and polydispersity index (PDI; Mw/Mn) were determined by means of SEC-MALS performed on an HPLC-SEC Agilent 1200 series using a Phenogel organic SEC column (104 Å with cut-off 5 to 500 kDa), isocratic runs were performed for 25 min with 100% THF as eluent and a flow rate of 0.5 mL/min. Biopolymers (1 mg) were dissolved in 1 mL of THF and filtered before injection.

3. Results and discussion

3.1. Accumulation experiments

The accumulation experiments were conducted until the substrate

fed was no longer consumed (generally after 30 h). To prevent PHA accumulation inhibition by excessive COD injection [16], 5 and 10 mL of substrate were fed in 3 min for T1, T3, T5 and T2, T4 and T6, respectively.

Fig. 2 shows the final PHA content achieved for each accumulation test and the PHB:PHV ratio calculated by analysing the lyophilised biomass, as reported in the analytical methods (section 2.5). Simple main effects analysis showed that both ORL and the organic load per dosage shows a statistically significant influence on PHA content ($p = 0.018$, $p = 0.036$, respectively). T5 and T6 (ORL of 1.8 g COD L^{-1} d^{-1} during the selection step) led to the highest amount of PHA concentration (42 and 60 % w/w, 0.54 and 0.77 g PHA g^{-1} VSS respectively) within the biomass cells compared to the other tests. The biomass harvested from the selection reactor working at an ORL of 1.3 g COD L^{-1} d^{-1} reached 20 (T3) and 31 (T4) % w/w (0.24 and 0.36 g PHA g^{-1} VSS, for T3 and T4, respectively) while the accumulation performed with the biomass selected at an ORL of 0.8 g COD L^{-1} d^{-1} exhibited the lowest PHA contents (17 and 27 % w/w, 0.19 and 0.29 g PHA g^{-1} VSS for T5 and T6, respectively). When comparing accumulation tests with the same organic load per dosage (T1, T3 and T5, T2, T4 and T6), the higher the OLR applied during the selection step, the more effective is the enhancement of the microorganisms' PHA storage and production capability [23].

Moreover, high OLR in the selection step enhances PHA production mostly by favouring PHV synthesis, as can be seen by the PHB:PHV ratio [13]. The amount of PHV produced was affected by several factors, such as the feeding strategy or the organic load per dosage [25]. A continuous regime of increase and decrease of feed results in higher HV content (8% of the increase) compared to an hourly pulse feeding regime when a synthetic substrate is applied [26–28]. Also, higher organic load per dosage, due to the use of propionic acid in the substrate, enhanced the PHV production [15]. Also, Vazquez et al. [29] obtained a similar HB:HV ratio since the carbon source composition (acetate, butyrate:propionate, valerate) was similar to the one used in this work. Instead, when a more complex substrate is used [28], the synthesised PHV amount drastically decreases. The PHV:PHB ratio in T6 could suggest that this polymer may be a promising alternative to PHB homopolymers, especially regarding its industrial applications [16,29,30].

Fig. 3 shows the DO profile and PHA contents during T5 and T6. In more detail, over the course of the initial 30 h, T5 exhibited a total of 17 DO peaks (Fig. 3A), while T6 had 11 DO peaks (Fig. 3B). This outcome highlights the significant influence of the organic load per dosage on the accumulation process. It indicates that a higher organic load per dosage resulted in a greater amount of PHA produced within the same time frame, even with fewer substrate injections being performed. The software implementation allowed to continuously run and monitor the accumulation test up to 56 h during T6. The feed-on-demand strategy with an automatic control increased the final PHA content up to 20% w/w compared to the regular 6–10 h accumulation test performed without the stand-alone injection [31]. These results prove the feasibility of implementing this software in a scaled-up process: the accumulation could be easily managed and monitored while maintaining its reliability due to the ability to acknowledge biomass' respiration rate shift. Also, thanks to the number of parameters that can be set up and the flexibility to make changes in them (trigger value, DO_m threshold and several samples for DO_m), the software can be used regardless of the substrate and the selection strategy adopted, both at bench and pilot plant scale.

Fig. 4 shows the PHA productivity, expressed as g PHA $L^{-1}h^{-1}$, and the storage yield in COD equivalents of PHA per COD substrate equivalents. Fig. 4A points out the key role played by the OLR applied in the selection step and the organic load per dosage used in the accumulation step, which strongly influences the PHA productivity peak as well as the final PHA content in the biomass. Specifically, at the three OLRs employed (0.8, 1.3, and 1.8 g COD L^{-1} d^{-1}) during the biomass selection stage, the peak productivity values were of 0.40 (T1), 0.20 (T3), and 0.72 (T5) g PHA $L^{-1}h^{-1}$, while the corresponding PHA biomass contents were 18%, 20%, and 42% w/w as shown in Fig. 2 for T1, T3, and T5, respectively. Regarding the organic load per dosage, results indicate productivity values of 0.4 and 0.6 g PHA $L^{-1}h^{-1}$, with PHA biomass contents of 18% and 28% w/w (Fig. 2) for T1 (7.5 g COD L^{-1} $dosage^{-1}$) and T2 (15.0 g COD L^{-1} $dosage^{-1}$) at the first OLR used in the selection (0.8 g COD L^{-1} d^{-1}). Similar trends were observed for the other OLR: T4 and T6 reached productivity peaks of 0.23 and 0.26 g PHA $L^{-1}h^{-1}$, respectively, with final PHA contents of 31% and 60% w/w (Fig. 2).

Also, increasing the OLR enhanced the microorganism storage capability as reported in Fig. 4B. Moreover, simple main effects analysis showed that both, ORL and the organic load per dosage have a statistically significant effect on storage yield ($p = 0.004$ and $p = 0.184$, respectively). This means that the biomass could increase its PHA contents from less than 1% (T5) up to 32% w/w (T6), but at the expense of decreasing the productivity peak by 43% comparing T5 (0.7 g PHA $L^{-1}h^{-1}$) to T6 (0.3 g PHA $L^{-1}h^{-1}$) and of employing more time for

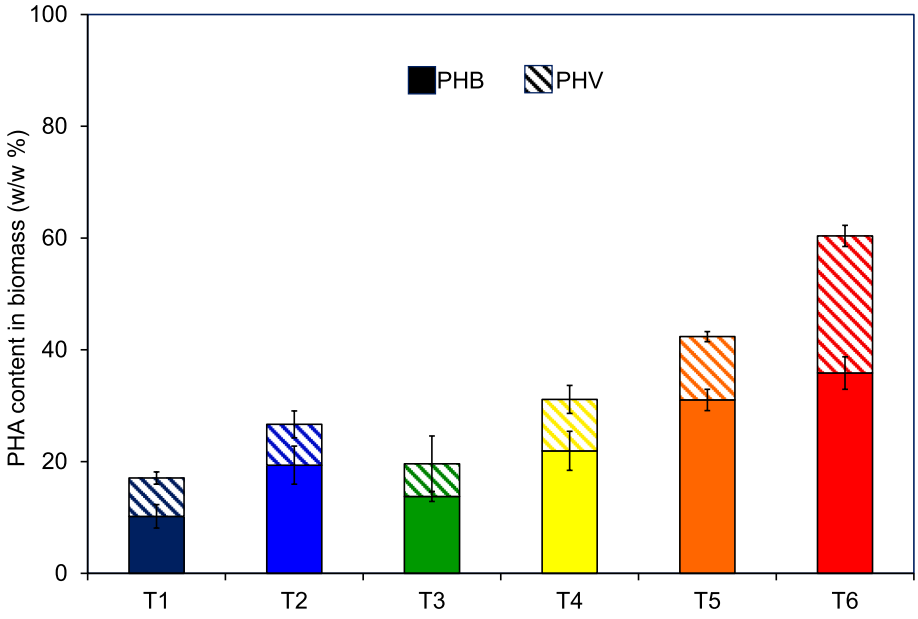

Fig. 2. Final PHA content and HV:HB polymer ratio. Filled bars correspond to PHB. Striped bars correspond to PHV.

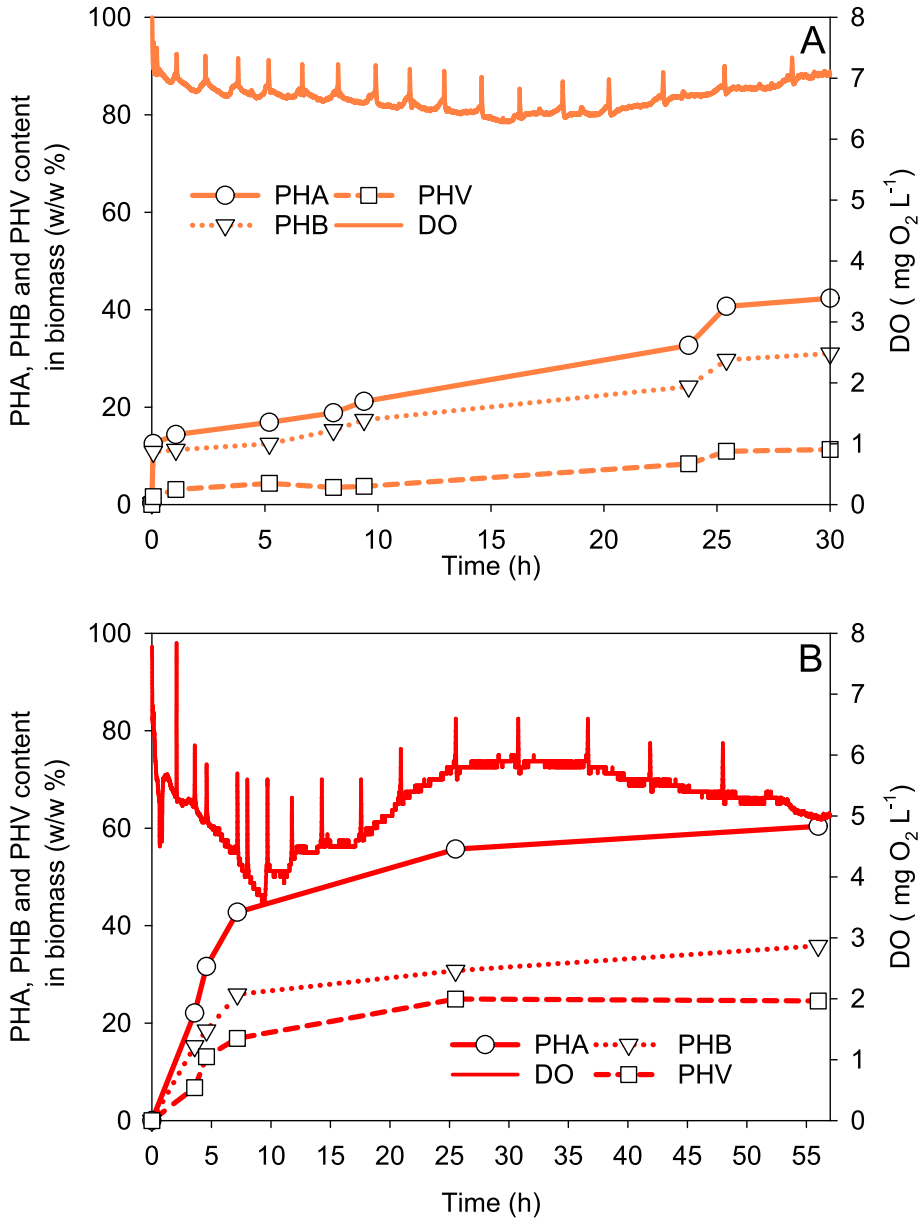

Fig. 3. PHA content and DO profile during T5 (A) and T6 (B).

accumulating PHA (Fig. 4A) [32]. Considering the same OLR used, when a higher organic load per dosage is applied, the productivity peak is always higher [33], the same as the amount of PHA produced. Therefore, if the target is to achieve the highest productivity, it is advised to use low OLR in the selection step, otherwise, high OLR should be used if the target is to achieve the highest amount of PHA. Regarding the organic load per dosage, results indicate that using high values is recommended but, at the same time, substrate inhibition must be avoided [34].

These results highlight crucial parameters to consider when conducting a pilot or full-scale accumulation test. Still, few works focus on optimising the organic load per dosage in the accumulation test while considering the PHA productivity. These parameters could be used in an energy-mass balance to express, at an industrial level, where it is more convenient to stop the accumulation process and extract the stored PHA.

3.2. *Extraction protocol and biopolymers characterisation*

The extraction protocol [15] was applied to four samples collected during T6 (Table 2). As described in the materials and methods section, PHA content, purity and recovery were calculated. The protocol used herein comprises a two-step solvent extraction: NaClO pre-treatment and NPCM destruction using an organic surfactant (ammonium laureate). Table 2 shows an overall high purity (89 ± 2 %) and recovery (78 ± 3 %), demonstrating that the protocol's efficiency is not influenced by the PHA content in the lyophilised samples if higher than 32 % w/w (cut-off value).

Table 3 shows the extraction efficiency obtained in this work compared to other literature studies. As it can be observed, high-purity results were obtained through the NPCM dissolution, as reported by other studies [17]. Compared to the literature, Lorini and colleagues [5] reported high PHA purity and recovery percentages (>80%) applying alkaline treatment with NaOH, however, they obtained lower recovery yields using SDS surfactant (<50%) as a solvent for the extraction. Although the sample preparation and extraction process can seem highly energy-consuming compared to other works [4,5,9,25,35,36], the results consistently demonstrate the feasibility of applying this protocol for samples containing different amounts of PHA with different

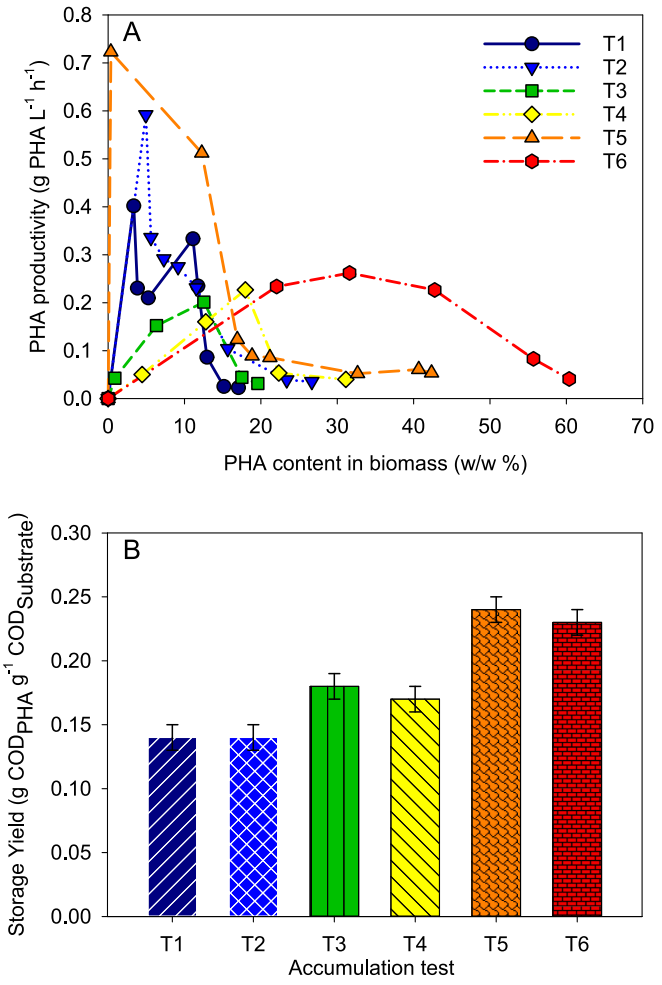

Fig. 4. PHA productivity as a function of PHA content (A) and storage yield for all the experiments (B).

Table 2
PHA content, recovery and purity percentage for samples collected from T6.

Sample	Sampling time (h)	PHA content (% w/w)	Purity (%)	Recovery (%)
T6-S1	4.6	31.6 ± 0.3	90 ± 1	81 ± 2
T6-S2	7.2	42.8 ± 0.6	90 ± 1	74 ± 1
T6-S3	25.5	55.7 ± 0.8	87 ± 1	78 ± 2
T6-S4	56.2	60 ± 1	89 ± 1	78 ± 1

Table 3
Extraction efficiency compared to other literature studies.

Sample	PHA content range (%w/w)	Purity range (%)	Recovery range (%)
Present study	32–60	87–90	74–81
[15]	35–54	42–100	57–82
[17]	50	57–92	26–64
[35]	42–60	75–100	52–100
[36]	55–60	56–82	48–74

proportions between PHB and PHV monomers.

To confirm the polymer obtainment and composition, PHA recovered after extraction in T6 was dissolved in deuterated chloroform for characterisation through 1H NMR measurements. Fig. 5 shows the representative spectrum of recovered PHA compared to a commercial standard.

Signals corresponding to the protons of the PHB units are labelled as 1–3 while signals corresponding to PHV units are labelled as 4–7. Thus, deshielded peaks above 5 ppm (labelled as 2 and 5) are indicative of C-H protons directly linked to an electronegative nucleus such as O. The multiplicity of the signals indicates that the peak at 5.25 ppm is a sextuplet integrating for 1 proton, so this peak can be assigned to the C-H of the PHB (labelled as 2) as it is linked to a CH_2 and CH_3. On the other hand, the signal at 5.15 ppm is a quintuplet and it can be assigned to the C-H of the PHV (labelled as 5) as it is linked to two CH_2. Signals around 2.5 ppm are assigned to CH_2 at the alfa position with respect to a carbonyl group. These signals are overlapped in PHB and PHV. In the aliphatic region, a signal around 1.3 ppm (labelled as 3) is assigned to CH_3 linked to C-H of the PHB unit and therefore is a doublet, while two signals at 1.6 ppm and 0.9 ppm (labelled as 6 and 7) were assigned to the ethyl group of the PHV units and their multiplicities, as expected, are (broad) quintuplet and triplet, respectively. Obtained PHA signals are in good agreement with the literature [37].

The HB-HV monomers percent present in the obtained PHA samples were calculated by comparison of the intensity, hence of integrals' area, of diagnostic C-H peaks 2 and 5, respectively, as the integrals area of these two peaks is directly related to the percentage of PHB and PHV present in PHA sample.

The NMR analysis is performed in the extracted polymer samples and not on the lyophilised biomass. In particular, the commercial PHA confirmed a monomer composition of 92% in PHB and 8% in PHV, while the composition of extracted polymers for T6-S4, T6-S3, T6-S2 and T6-S1 are stated in Table 4. In general, the PHV content of extracted PHA is larger than the one observed in the commercial sample as can be inferred from the higher intensity of PHV signals (4–7) compared to the commercial one (Fig. 5). From NMR analysis, sample purity around 90% was confirmed and agrees with previously reported data [38–40]. Moreover, from the observed peaks, could be assessed that impurities are ascribed to the presence of residual lauric acid from the extraction process.

Molecular weight data obtained via SEC-MALS analysis revealed the presence of two peaks in each sample, the first one with larger molecular weight indicative of the polymer and the second one with smaller molecular weight probably indicative of the impurity of lauric acid quantified by NMR analysis (Table 5). Molecular weight data agree with previously reported biopolymers even if the value is lower than recent studies with different extraction procedures [9]. PDI was close to 1, indicating a homogeneous polymer, a pivotal feature for other polymer's applications [15]. These results, despite the low molecular weights are a further demonstration of the reliability of the extraction protocol which can be further optimised and implemented it for pilot-scale experiments towards automation.

4. Conclusions

High OLR in the selection step (1.8 g COD L^{-1} d^{-1}) and high organic load per dosage (15.0 g COD L^{-1} $dosage^{-1}$) in the accumulation test were the best conditions, resulting in the highest PHA content (60% w/w) and the highest HV:HB ratio (0.66). These results were obtained by using a newly tailor-made software which applies a feed-on-demand strategy to perform the accumulation tests in stand-alone mode. This allowed to perform 56 h long accumulation tests, thus achieving high final PHA content (up to 60% w/w). Also, it was shown how high OLR and organic load per dosage decreased the PHA productivity during the test, despite increasing the biomass storage capability. Finally, an extraction protocol was tested with different PHA content samples demonstrating that the recovered polymer purity (89 ± 2 %) and recovery yield (78 ± 3 %) are not affected by the PHA content in the sample, thus proving the reliability and consistency of the overall strategy which can be beneficial for industrial use.

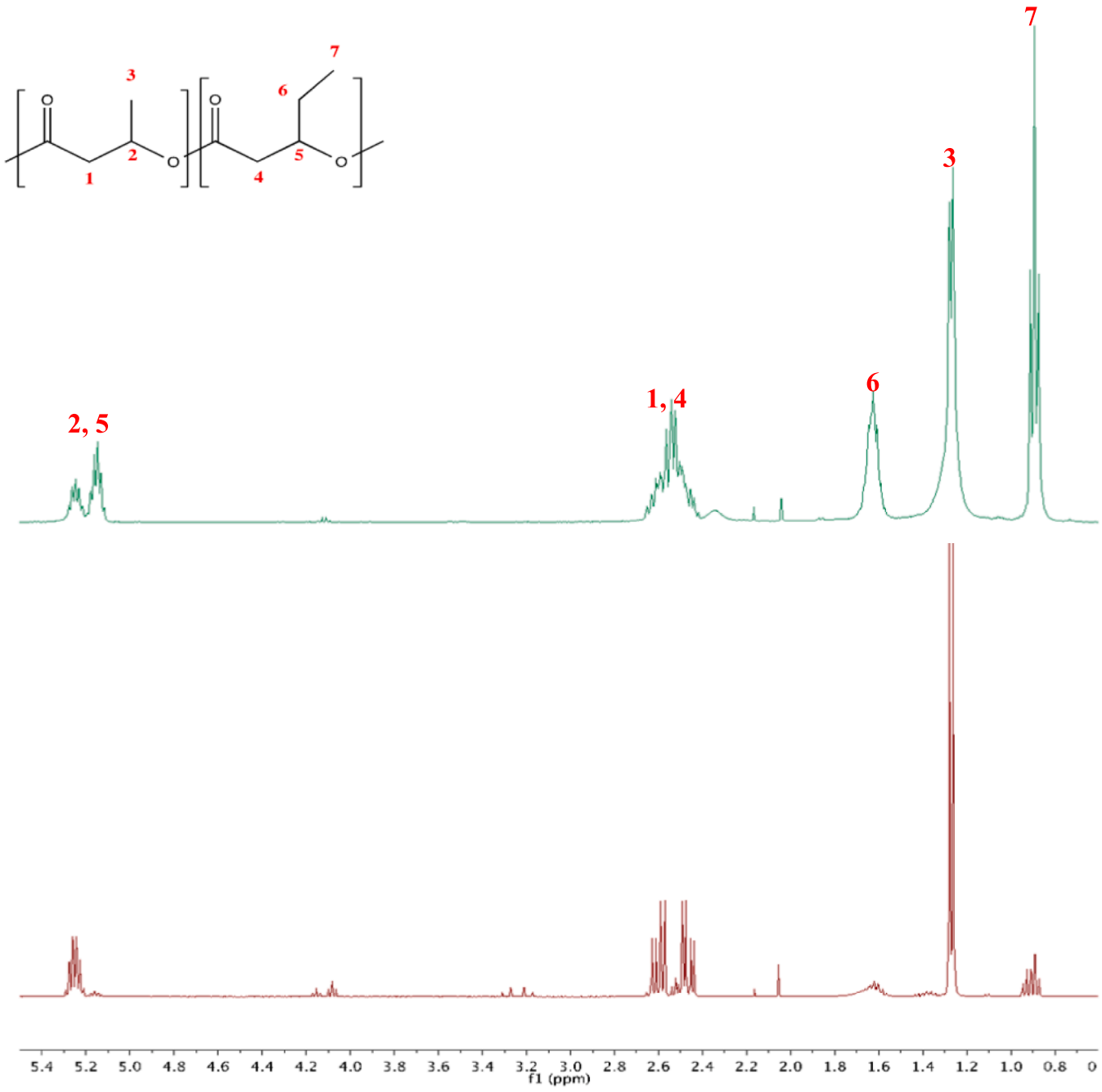

Fig. 5. ^{1}H NMR spectra in $CDCl_3$ of commercial PHA (bottom in red) and extracted PHA (upper in green). Representative PHA structure and peak assignment were reported in the inset (sample T6-S4).

Table 4
PHA composition, as determined using ^{1}H NMR.

Sample	%PHB	%PHV
T6-S4	39	61
T6-S3	38	62
T6-S2	50	50
T6-S1	31	69

Table 5
Retention time (RT), M_w, M_n and PDI.

Sample	RT time (min)	M_w (Da)	M_n (Da)	PDI
T6-S4	17.13	18,998	14,871	1.278
	21.43	765	763	1.002
T6-S1	18.95	53,166	32,494	1.636
	21.48	1737	1733	1.002

Declaration of Competing Interest

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

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cej.2023.145007.

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