Article

Chlorine-free Extractions of Mixed-Culture Polyhydroxyalkanoates Produced from Fermented Sewage Sludge at Pilot Scale

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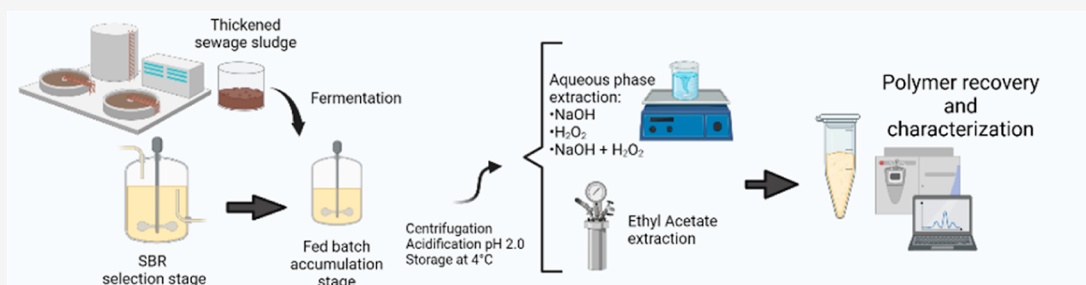
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ABSTRACT: In this study, various conventional and innovative methods were investigated for the recovery of polyhydroxyalkanoates (PHA) from a single batch of biomass produced at a pilot scale from mixed microbial cultures (MMCs) and fermented sewage sludge as a feedstock. Sustainable chlorine-free methods using NaOH and/or H_2O_2, as well as extraction in nontoxic ethyl acetate, were analyzed. Interestingly, the combined treatment of biomass with NaOH and H_2O_2 solutions demonstrated good recovery (70 wt %) and high purity (92 wt %) of the polymer in small-scale trials. Moreover, when the coupled treatment was performed on a larger biomass quantity (approximately 200 g), it achieved high purity and recovery yield (93 and 88 wt %, respectively), indicating the feasibility of this extraction method on a larger scale.

INTRODUCTION

Polyhydroxyalkanoates (PHAs) are an important class of bio-based polyesters, which can be produced by a wide range of prokaryotic microorganisms able to intracellularly accumulate carbon under stress conditions as energy and carbon reserve, electron sinks, regeneration of redox-equivalents, and stress protectants.[1] PHAs are constituted predominantly by repeating units of 3-hydroxyalkanoic acids. The chemical structure, concentration and distribution of different monomers within copolymers influence the PHA characteristics such as melting point, glass transition temperature, crystallinity, and mechanical properties.[2] Although they offer clear advantages of complete biodegradability to CO_2 and H_2O, biocompatibility and thermal, physical and mechanical properties suitable for different applications, PHAs face difficulties in replacing oil-based polymers due to their higher production costs (1.18−6.12 €/kg of PHA vs < 1 €/kg of oil based polymers such as polyethylene and polypropylene).[3] Indeed, industrial processes for PHA production are based on the use of pure or genetically modified cultures of selected strains and of ad hoc designed unbalanced growth media.[4] Hence, the high overall costs are due to the culture maintenance, substrate formulation (e.g., refined glucose) and reactor sterilization.[5] In the past decade, the use of mixed microbial cultures (MMCs) has been suggested as a promising alternative to pure cultures. The

MMCs allow reduction of the production costs of PHAs because sterile conditions are not required. In addition, a variety of cheap carbon sources can be used as substrates, including organic fraction of municipal solid waste (OFMSW),[6] mixture of OFMSW and waste activated sludge (WAS),[7,8] and thickened sewage sludge.[9,10] As a confirmation of this, the conversion of wastewater and different available organic feedstocks by MMC represents key elements for the implementation of a three-step process through the integration of MMC-PHA production into a wastewater treatment plant (WWPT).[8] Although the use of organic fermentable substrates allows to reduce the upstream costs, the production of MMC-PHA is still at the pilot scale only and the downstream process is a crucial stage due to the impact on the economic sustainability.[4] In this view, PHA recovery and purification represent the bottleneck of the whole process, and the use of an optimal treatment for extracting the polymer is currently under wide investigation. A recent publication, which reviews

various established and advanced approaches for the down-stream processing, has highlighted that the PHA recovery methods which generate maximum recovery yields and product purity are based on the polymer extraction with halogenated solvents, predominately chloroform.[11] However, due to evident safety concerns, research has been increasingly focused on exploring alternative approaches to extracting PHA in recent years. Extraction of polymers from MMC has been reported[3,12,13] to be more challenging than from pure cultured biomass due to the stronger and more complex extracellular biomass matrix of PHA-accumulating microorganism consortia. Therefore, it represents a litmus test to evaluate the effectiveness of new extraction procedures. As far as the separation of the polymer by its dissolution, opting for the most environmentally friendly solvent allows for a considerable decrease in the overall environmental footprint. Then, various safe liquids were tested including dimethylcarbonate,[12−14] 1-butanol,[15,16] 1,2-methyl tetrahydrofuran and cyrene,[17] ethyl acetate (EA),[18] methyl isobutyl ketone, 2-butanol, and acetone.[16] The other procedure for the polymer recovery is based on chemical solubilization of non-PHA cell matrix (NPCM) with strong oxidant, typically NaClO and H_2O_2, or bases, mainly NaOH water solution, as well as enzymatic digestion.[14,19] However, the use of sodium hypochlorite also presents drawbacks due to the toxicity of potentially formed halogenated compounds and its difficult removal from the polymer.[20] The combination of parallel or sequential methods have been proposed in order to increase the recovery yields and PHA purity without compromising the original polymer features by harsh extraction conditions.[21,22] For instance, preliminary cell disruption has been obtained by bead milling, mechanical mastication, sonication, and supercritical carbon dioxide treatment.[23−26] Mixed NPCM digestion or PHA solubilization procedures, including treatment with NaOH solution and sodium dodecyl sulfate (SDS), NaClO solution and SDS, NaClO and DMC, have been reported.[27−30] Furthermore, depending on the desired purity of the PHAs for their intended application, additional purification stages may be implemented, such as the precipitation the solubilized polymer using a nonsolvent.[16] However, despite the considerable volume of literature encompassing these extraction methods, the comparison, evaluation, and selection of the most advantageous methodologies remain complex, challenging, and laborious. Additionally, considerations of cost-effectiveness further contribute to the complexity of these assessments. In fact, distinct feedstocks and feeding strategies can yield diverse biomasses characterized by different polymer contents and cell resistance to chemicals as well as different PHAs, in terms of molecular weight, structure, and copolymer composition, all of which profoundly affect the extraction of the polymer. Therefore, in the present research, the possibility to have a relatively high amount of biomass containing PHA produced in a full-scale wastewater treatment plant by using MMC and thickened waste active sludge (WAS) as feedstock gave the opportunity to directly compare the performance of extraction methods, conventional, uncommon, or innovative. Aliquots of the same biomass were separately subjected to extraction and the polymer recovery yield, purity, molecular weight, and thermal properties evaluated. The conventional extraction procedures involved the solubilization of PHA using chloroform and the bleaching of NPCM with a NaClO and NaOH aqueous solution. Less common chlorine-free methods included polymer solubilization in EA and NPCM solubiliza-

tion through H_2O_2 oxidation. A new sequential biomass treatment using NaOH and H_2O_2 was also tested on a large amount of biomass (200 g) in order to reduce the potential loss of polymer during the extraction and collection processes and thus increase the recovery yield. Moreover, the tested extraction procedures were subjected to preliminary economic analysis.

■ MATERIALS AND METHODS

Materials. Chemicals used for the extractions and characterization were all provided by Sigma-Aldrich: chloroform ($CHCl_3$; ≥99.8%), EA ($CH_3COOC_2H_5$; ≥99.9%), hydrogen peroxide solution (H_2O_2; 35%), and sodium hydroxide (NaOH; pellets ≥ 97.0%).

PHA Production. PHA was produced in a full-scale wastewater treatment plant (WWTP), located in Treviso (northeast Italy), by using thickened WAS as the feedstock.

The PHA-rich biomass production (described in previous works[10,31]) was obtained through a three stage process consisting in (i) a first anaerobic fermentation in a 380 L reactor for the production of volatile fatty acids (VFAs), used as feedstock; (ii) a biomass selection into PHA-accumulating consortium (enriched-MMC) in a second 100 L aerobic sequencing batch reactor (SBR) and (iii) a PHA accumulation in a third 70−90 L fed-batch aerobic reactor. At the end of the accumulation stage, the biomass was stabilized with sulfuric acid (pH 2), in order to preserve the PHA content and properties,[32] centrifuged, and stored at 4 °C until the downstream processing. The original biomass contained 62 wt % of PHA with respect of total dry solids, consisting in a P(3HB-co-3HV) copolymer with a 3HV content of 12.9 wt %.[10]

PHA Extraction. Six different extraction experiments on aliquots of the same PHA-rich biomass were compared.

The results of the conventional extraction procedures, namely, chloroform extraction in Soxhlet and NaClO oxidation, were obtained from previous study on the same biomass.[10] These results are herein considered as a reference of consolidated extraction methods.

Alkaline and Oxidative Chlorine-free Treatments. The wet acidified biomass (1.5 g with an approximate water content of 80 wt %) was subjected to chemical digestion with NaOH or oxidative treatment with hydrogen peroxide. The stabilized biomass was suspended in 180 mL of a 0.2 M NaOH solution or in 4.5 mL of a H_2O_2 (1.5% w/v) solution. The suspensions were kept under magnetic stirring for 6 and 4 h, respectively. Then, the insoluble fraction was separated by centrifugation (Multispeed centrifuge PK 131, ALC) at 8500 rpm for 15 min, washed three times with distilled water, and finally dried in an oven at 60 °C. Additionally, a coupled treatment was conducted on 1.5 and 200 g of wet biomass, by applying the chemical digestion treatment with NaOH for 4 h, followed by centrifugation and one washing step. Then, oxidation with H_2O_2, was carried out for 1 h on the thickened biomass. At the end of the coupled procedure, the same steps of centrifugation, washing, and drying were used.

EA Extraction. EA does not solubilize PHA at room temperature and, hence, a dissolution temperature above the EA boiling point (77 °C) is used under pressure exceeding 1 atm, as reported in a previous paper.[18] Briefly, PHA solubilization was carried out at 115 °C and at a pressure of about 300 kPa (3 atm). An amount of 1.5 mg of dry biomass, roughly ground in a mortar, was placed in a 15 mL thick wall

Table 1. Properties and Parameters of Extraction Methods Used to PHA Recovery

extraction agent	time (h)	T (°C)	PHA purity (wt %)	recovery yield (wt %)	3HV (wt %)	M_v (kDa)	references	PHA-storing microorganism	carbon sources
CHCl$_3$	10	70	100.9 ± 1.3	91.5	14.3 ± 0.1	405	10	MMC	WAS
	24	70	92.9 ± 6.1	80.5 ± 5.3	19.2 ± 0.1	not reported	33	MMC	OFMSW–WAS mixture
	48	80	95.1 ± 7.3	not reported	31.2	not reported	19	MMC	fermented fruit waste
NaClO	5	25	99.8 ± 0.41	98.3	13.5 ± 0.1	396	10	MMC	WAS
	3	85	>80	50	not reported	not reported	4	MMC	synthetic effluent
	24	25	98 ± 5	100 ± 5	11 ± 0.3	not reported	5	MMC	synthetic mixture of VFA
	3.4	30	99.4 ± 4.2	not reported	30.9	not reported	19	MMC	fermented fruit waste
NaOH	6	25	79.4 ± 0.1	85.1	13.5 ± 0.2	461	this study	MMC	WAS
	4.8	30	56.8 ± 0.8	not reported	18.3	not reported	19	MMC	fermented fruit waste
	24	25	56 ± 5	80 ± 6	13 ± 0.7	not reported	5	MMC	synthetic mixture of VFA
H$_2$O$_2$	4	25	77.4 ± 2.5	110.5	13.3 ± 0.5	436	this study	MMC	WAS
	10	not reported	99.5	not reported	not reported	not reported	37	*Cupriavidus necator*	not reported
NaOH + H$_2$O$_2$	5	25	92.3 ± 0.5	69.5	12.9 ± 0.2	571	this study	MMC	WAS
EA	1	115	86.6 ± 0.7	64.5	17.8	358	this study	MMC	WAS
	1	115	90–97	66–71	16–28	62–236	18	MMC	OFMSW–WAS mixture

glass pressure vessel with PTFE bushing and Viton O-ring (Precision Labware), with a magnetic stirrer and 4 mL of EA. The tube was tightly closed and placed in a silicon-oil bath preheated at 115 °C for 2 h. Then, the system was cooled, and the physical gel, formed from solution at room temperature, was separated from the biomass and dried at 60 °C.

Analytical Methods. The polymer fraction in the biomass, the purity of the extracted PHA and its composition were obtained by the gas chromatographic standard method (GC), described elsewhere.[33] In particular, the relative abundance of 3HB and 3HV monomeric units was determined using a commercial P(3HB-co-3HV) copolymer with a 3HV content of 5 wt % (Sigma-Aldrich, Milan, Italy) as reference standard. The 3HV content in PHA was reported as the weight ratio (wt %) of 3HV and (3HB + 3HV) monomers as (wt %).

PHA Recovery Yield and Purity. The recovery yield, defined as the ratio between the weight of extracted polymer ($w_e \times p$) to the weight of PHA initially within the dry biomass before the extraction procedure ($w_b \times f_i$), was calculated using eq 1

$$\text{recovery yield} = \frac{w_e \times p}{w_b \times f_i} \times 100 \qquad (1)$$

where w_e and p are the weight and purity of extracted polymer, w_b is the weight of the biomass and f_i the initial content of PHA in biomass. Both p and f_i were evaluated by GC analysis.

Viscosity Measurements. The viscosity average molecular weight of the extracted PHA samples was determined by viscosimetry of diluted solution in chloroform at 30 °C using a SCHOTT AVS 350 viscometer equipped with an AVS/SHT sensor, a LAUDA CD15 thermostatic bath, and a SCHOTT GERÄTE Ubbelohde capillary viscometer (ID = 0.46 mm). A volume of 20 mL of solution with a concentration of 0.6 g dL^{-1} was transferred into the Ubbelohde capillary viscometer, and at least five dilutions were made directly into the viscometer. From the measurement of the flow times, the

intrinsic viscosity values [η] were determined and correlated to the viscosity average molecular weight (M_v) by the Mark–Houwink eq 3

$$[\eta] = k \times M_v^{\alpha} \qquad (3)$$

The constants used were $k = 7.7 \times 10^{-5}$ $\alpha = 0.82$[34]

Thermogravimetric Analysis. The thermal stability of extracted PHA samples was investigated by thermogravimetric analysis (TGA) using a Mettler TG 50 thermobalance equipped with a Mettler TC 10 A processor. All measurements were carried out under nitrogen flow from 25 to 500 °C, at a heating rate of 10 °C min^{-1}. About 5–8 mg of dried samples were used for the analysis.

Differential Scanning Calorimetry. The thermal properties of extracted PHA samples were evaluated by differential scanning calorimetry (DSC) (Mettler Toledo DSC 822e). All the analyses were carried out under nitrogen flow (30 mL min^{-1}) on about 4–8 mg of polymer. The applied temperature program was as follows: (a) first heating scan at 10 °C min^{-1} from RT to 190 °C; (b) rapid cooling at 30 °C min^{-1} from 190 to −70 °C; and (c) second heating up to 190 °C at 10 °C min^{-1}.

The first heating was necessary to erase any previous thermal history of the samples.

The enthalpy of fusion recorded in the second heating (ΔH_m) was used to evaluate the sample crystallinity X_c according to eq 4

$$X_c = \frac{\Delta H_m}{\Delta H_m^0 \times p} \times 100 \qquad (4)$$

where $\Delta H_m^0 = 146$ J g^{-1} is the enthalpy of fusion of 100% crystalline poly(3-hydroxybutyrate) (P3HB). Although determined for the P3HB homopolymer, this value was used also for the P(3HB-co-3HV) copolymer with low 3HV content.[35,36]

C

■ RESULTS AND DISCUSSION

PHA Extraction and Recovery. Table 1 summarizes the results of the different performed extraction experiments together with those described in other studies,[4,5,10,18,19,33,37] where conditions similar to those used in the present study were employed. To ensure consistency, the same biomass harvest was used for all PHA extractions and characterizations reported in both the present study and in ref 10.[10]

The extraction methods based on the digestion of NPCM by hydrolysis or oxidation have the advantage of being applicable to wet biomass and, therefore, do not require neutralization when the biomass has been stabilized with sulfuric acid. Conversely, extraction by solubilization of the polymer in water-immiscible solvents requires neutralization of the biomass before drying to prevent PHA hydrolysis.

Although different conditions (temperature and time) are reported in the literature, the results of the conventional extractions by chloroform solubilization and NaClO oxidation usually show a high purity and recovery yield (Table 1). Similar results have been obtained from the biomass used in this research. Differently, the extraction carried out by using EA, a green solvent, at a temperature above the boiling point and, hence, at high pressure, resulted in a low recovery yield and, more significantly, a polymer with a composition significantly different from the PHA present in the biomass before extraction. These findings, already investigated in a previous paper,[18] are due to the preferential solubilization of polymer fraction with higher 3HV content.

Different treatment time and temperature were also used in the extraction involving oxidizing (H_2O_2, NaClO) or hydrolyzing (NaOH) agents (Table 1). In this research, a relatively short time and room temperature were investigated to favor a possible extraction cost reduction and process scaling-up. The results show that NaOH (6 h) and H_2O_2 (4 h) treatments brought about good (85 wt %) and very good (100 wt %) recovery yields, respectively, and an unchanged composition of the extracted polymers but relatively low purities of 79 and 77 wt %, respectively. Therefore, the effect of sequential extraction by the two methods was evaluated. The wet and not neutralized biomass was digested with NaOH solution for 4 h followed by oxidation with H_2O_2 for 1 h (total 5 h). A clear increase of extracted PHA purity was found, albeit with a reduction in recovery yield (70 wt %). It was presumably due to a high number of washing cycles, after the first treatment with NaOH and after the oxidation with H_2O_2, before the final recovery of the polymer.

Viscosity Average Molecular Weight (M_v). Viscosity measurements of the extracted samples, conducted in diluted $CHCl_3$ solutions, were used to evaluate the correlation between the extraction methods and the polymer molecular weight (viscosity average molecular weight, M_v). The obtained results are reported in Table 1. It can be observed that the extraction method based on polymer solubilization with EA resulted in the lowest M_v. This result, together with the polymer composition data (Table 1), suggests that under the adopted experimental conditions, a partial solubilization of PHA in the biomass as a function of composition and molecular weight reduction occurred. Indeed, it has been reported that the 3HV-rich polymer fractions showed higher solubility in EA and that the extraction at high temperature brought about partial degradation of the polymer.[18] For this

reason, a longer time period was not investigated. Nevertheless, polymer solubilization in EA resulted in high sample purity.

In contrast, oxidizing agents (NaClO and H_2O_2) and hydrolyzing agent (NaOH) act as cellular digesters by selectively solubilizing NPCM in water and releasing PHA, (insoluble in water). Therefore, by operating under controlled and mild conditions to prevent potential PHA degradation, the characteristics of the polymer can be preserved in terms of molecular weight and composition. As a result, the viscosity average molecular weights obtained by the dissolution of biomass were the highest.

Thermal Analysis. The PHA samples extracted in the current research and those obtained in the previous study (i.e., NaClO and $CHCl_3$ extraction)[10] from the same biomass, were subjected to thermal characterization using TGA and DSC. Figure 1 displays the TGA curves of the extracted polymers.
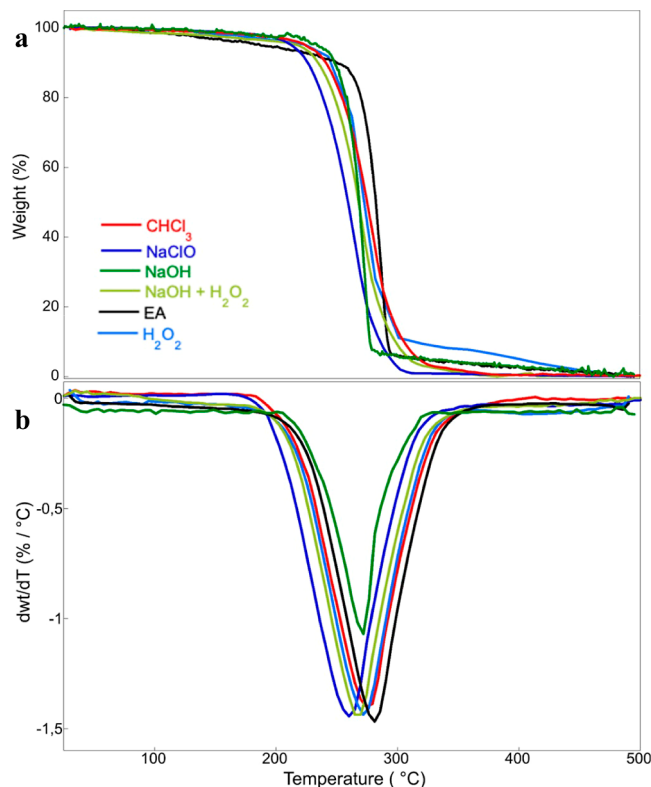

Figure 1. Weight loss (a) and its derivative (b) curves of PHBV samples extracted with different methods.

The thermal stability results, evaluated from the values of temperatures at 10% of weight loss ($T_d^{10\%}$) and at the maximum decomposition rate (T_d^{MAX}), are reported in Table 2.

The sample purity obtained by the thermogravimetric analyses, evaluated through the main PHA weight loss occurring between about 200 and 320 °C (Table 2), confirmed the data determined by the GC technique (Table 1).[38] The T_d^{MAX} and $T_d^{10\%}$ values fall within the typically observed range for PHAs with similar characteristics[39] and, as expected, no significant correlation can be observed between the thermal stability of the samples and the other properties of the polymers, such as purity, composition and molecular weight.[32,40]

The DSC thermograms of the second heating of all of the extracted PHAs are reported in Figure 2.

Table 2. Thermal Properties of the Whole Sample Set Obtained by TGA and DSC Analysis

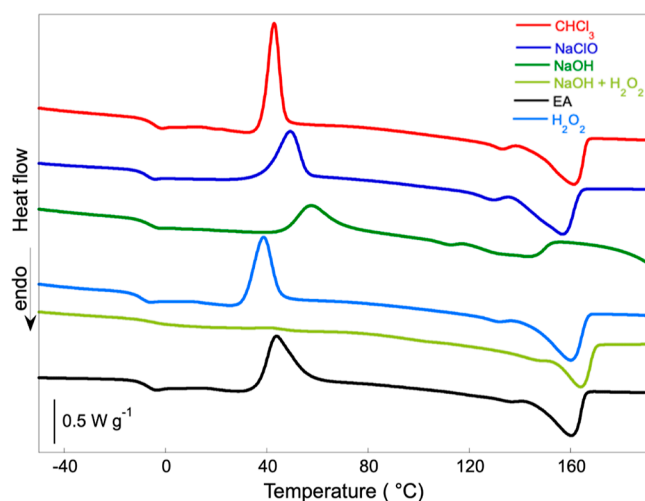| extraction method	TGA				DSC			
	$T_d^{10\%}$ (°C)	T_d^{MAX} (°C)	T_g (°C)	T_{cc} (°C)	ΔH_{cc} (J/g)	T_m (°C)	ΔH_m (J/g)	X_c (%)
HCl$_3$[a]	248	280	−5	43	58	161	53	36
NaClO[a]	232	266	−7	49	41	157	61	42
NaOH	250	273	−7	58	28	143	27	18
H$_2$O$_2$	248	278	−10	39	53	160	54	37
NaOH + H$_2$O$_2$	234	272	−3,5			164	67	46
EA	224	282	−8	45	60	161	45	31

[a]Data from ref 10.

Figure 2. DSC thermograms from second heating scan carried out from −70 to 190 °C at 10 °C min⁻¹ of PHBV samples extracted with alkaline and oxidative chlorine-free treatments. DSC curves of samples extracted with standard methods (chloroform and hypochlorite) are also reported for comparison.

Since the composition and molecular weight of extracted PHAs resulted quite similar, the different thermal behaviors of the whole sample set can be related to the nature of impurities. In fact, according to their chemical and physical properties, they can act as nucleating agents or plasticizers that can promote or hamper the melt or cold crystallization, respectively. All the samples showed the glass transition (T_g) in the −10 to −3.5 °C temperature range. PHA extracted by the double NaOH + H$_2$O$_2$ treatment showed a very low specific heat capacity change (ΔC_p) of the amorphous phase at T_g and lacked the cold crystallization, as it was the only sample that underwent an extensive melt-crystallization in the cooling scan. In contrast, the other samples either remained completely or partially amorphous after cooling and exhibited cold-crystallization between approximately 39 and 58 °C (T_{cc}). At higher temperature, the melting occurred in a wide temperature range. The sample obtained by EA extraction showed the lowest X_c value because of the high fraction of 3HV monomeric unit which, as is well-known, depress the polymer crystallinity.[33] On the other hand, PHA recovered from the NaOH and H$_2$O$_2$ double treatments crystallized from the melt in the cooling scan and resulted in a high crystallinity and order degree, as evidenced by the high melting temperature in the subsequent heating ramp. Encouraged by the good results obtained by the double treatment in terms of recovery yield, purity, polymer properties and mainly readiness, the extraction process was repeated on a larger amount of the same wet

biomass (200 g). The results of this extraction are reported in Table 3.

Table 3. Results of the extraction with the double treatment (NaOH + H$_2$O$_2$) carried out on 200 g of wet biomass

PHA purity (wt %)	93 ± 3
Recovery yield (wt %)	88 ± 4
3HV (wt %)	13.4 ± 0.5
M_v (kDa)	565
T_m (°C) (2nd scan)	160
X_c (%) (2nd scan)	36

The extraction resulted in polymer purity, composition, and overall thermal properties that were comparable to those obtained using a lower amount of biomass. Most importantly, it led to a significant increase in the recovery yield, from 70 to 88 wt %. Using larger quantities of biomass reduces the potential loss of polymer during extraction and collection procedures. These results support the viability of the coupled treatment as an alternative PHA recovery strategy and indicate the feasibility of scaling up this extraction method.

Economic Analysis. In order to evaluate feasibility and affordability of the extraction procedures presented in this study, an economic analysis has been conducted based on the calculation reported in the paper by Pagliano et al.[3] Table 4 shows the main sources of costs for the studied extraction treatments, hence, both aqueous-phase and solvent extractions. In detail, the overall cost for each procedure was evaluated as the operational costs (OPEX) combined with the fixed cost (CAPEX) derived from a potential industrial scale extraction. In this view, a production rate of 100 kg$_{PHA}$/h has been considered. Furthermore, based on the equations reported by Pagliano and co-workers,[3] the different contributions to OPEX and CAPEX (Eur/kg$_{PHA}$) determination have been defined and calculated. Indeed, one of the most relevant costs for OPEX determination of the oPEX is the material input (MI) which considers the commercial price of the reagents (Table S1), related to the required amounts, and the maximum solvent/reagent recovery. This latter can be represented by a factor which typically ranges between 0.005 (highest solvent/reagent recovery) and 1 (single use reagent) for PHA extraction. In this regard, for each inorganic reagent, which are typically single use, a 1 factor was considered, while for water 0.0005 was used and for EA and CHCl$_3$ 0.005 (considering the higher recovery by solvent evaporation).[3] Moreover, the OPEX related to the extraction reactor (ER) is mainly influenced by heating, and then it has been calculated only for solvent extractions since only these treatments required high temperatures. In detail, ΔT equal to 95 K and to 50 K, in adiabatic conditions, were considered for EA and

Table 4. Sources of costs for the studied extraction treatments[a]

extraction	MI	ER		SRU		Tot		total costs
	OPEX (Eur/kg$_{PHA}$)	OPEX (Eur/kg$_{PHA}$)	CAPEX (Eur/kg$_{PHA}$)	OPEX (Eur/kg$_{PHA}$)	CAPEX (Eur/kg$_{PHA}$)	OPEX (Eur/kg$_{PHA}$)	CAPEX (Eur/kg$_{PHA}$)	
CHCl$_3$	0.71	0.1	0.06	0.51	2.41	1.32	2.47	3.79
NaClO	2.36	0.00	0.023	0.28	1.34	2.64	1.36	4.00
NaOH 0.2 M	1.71	0.00	0.058	0.33	1.54	2.04	1.60	3.64
H$_2$O$_2$ (1.5%)	0.35	0.00	0.04	0.28	1.31	0.63	1.35	1.98
NaOH + H$_2$O$_2$	2.56	0.00	0.06	0.40	1.89	2.96	1.95	4.91
EA	0.33	0.19	0.02	0.32	1.52	0.84	1.54	2.38

[a]MI (material imput); ER (extraction reactor); and SRU (solvent recovery or drying unit).

CHCl$_3$ extractions, respectively. On the other hand, the main contribution to the CAPEX related to ER is given by the operating pressure. For this calculation, room pressure has been considered for all of the extractions, except for EA solubilization, which is conducted under pressure (3 bar). The last contribution to the OPEX and CAPEX comes from the solvent recovery or drying unit (SRU) which are related to the amount of evaporated solvent at the end of the extraction and then to the required thermal energy (OPEX) and to the number of drying steps (CAPEX). In the case of EA and CHCl$_3$ extractions, SRU contribution to both OPEX and CAPEX has been calculated by considering the complete evaporation of the solvent, as reported by Pagliano et al.[3] On the other hand, in the case of aqueous-phase extraction only the residual water content in the humid pellet should be considered (since the highest volume of water was previously separated by centrifugation). In this regard, a 20 wt % content of solids in the humid pellet recovered after centrifugation and a complete solids recovery in adiabatic conditions were considered. As a result, hydrogen peroxide is the less expensive treatment (1.98 Eur/kg$_{PHA}$), followed by the EA solvent extraction (2.38 Eur/kg$_{PHA}$). However, in this case, the relatively high operative pressure, the high molecular weight reduction, and the PHA composition change must be taken into account. On the other side, higher costs are related to the aqueous phase treatments, as a consequence of the higher MI due to the single use inorganic reagents. In detail, chemical disruption by NaOH and NaClO treatment are characterized by intermediate costs (3.64 and 4.00 Eur/kg$_{PHA}$, respectively), comparable with that obtained for CHCl$_3$ extraction (3.79 Eur/kg$_{PHA}$). As reported in Table 1, the combined treatment resulted in the highest total costs (4.91 Eur/kg$_{PHA}$). However, the costs should decrease if the combined treatment is applied to a biomass characterized by an even higher PHA content, increasing the extraction performance but limiting the amount of reagents needed. Hence, the most suitable extraction method should be evaluated by considering both the costs and the extraction performance. In conclusion, the combined NaOH and H$_2$O$_2$ treatment seems to meet the criteria for choosing the best performing extraction procedure since it allowed us to obtain a very high purity of the final product (92.3 ± 0.5 wt %) and the highest M_v among the tested PHA (571 ± 25 kDa) with competitive total costs. As an added value, the proposed procedure reduces the amount of chemicals involved in the PHA recovery if compared with CHCl$_3$ extraction used on an industrial scale.

CONCLUSIONS

The coupled chlorine-free treatment (4 h of NaOH digestion and 1 h of H$_2$O$_2$ oxidation) on wet acidified biomass yielded PHA high purity (92.3 ± 0.5 wt %) and molecular weight (571 ± 25 kDa), with a recovery yield of approximately 70 wt %. Furthermore, this innovative extraction method resulted in the stabilization and crystallization of PHA granules, showing an improved thermal stability. Additionally, it had no adverse effect on the molecular weight of the resulting polymer, which was higher than that achieved through other analyzed standard methods. When the coupled treatment was applied to a larger quantity of PHA-rich biomass (approximately 200 g), the recovery yield increased to 88 ± 4 wt %, while the purity (93 ± 3 wt %) remained comparable to that of the sample extracted from a smaller mass. Moreover, the elimination of two significant steps, namely, neutralization and drying of wet biomass, contributed to a more efficient downstream process, with the total contact time for the coupled treatment being 6 h.

These results highlight the high potential of the coupled treatment as an alternative strategy to the most commonly cited PHA recovery methods, such as chloroform solubilization or sodium hypochlorite (NaClO) oxidation. Furthermore, the competitive cost, quantified in the economic evaluation (4.91 Eur/kg$_{PHA}$), adds value and should be considered in the perspective of a technology scale up, also taking into account the possibility to improve the extraction performance. Indeed, it is well-known that PHA content on enriched biomass can be significantly improved (i.e., higher than 70 wt.%), depending on the operative conditions of the production process (e.g., exploiting an uncoupled carbon−nitrogen feeding strategy[41]), leading to a more efficient extraction and recovery of the polymer reducing the use of chlorinated solvents.

Taken as a whole, the coupled treatment herein proposed can improve the environmental and economic sustainability of the entire PHA production process, by reducing operative steps, amount of reagents, and total operative time involved in the extraction operations.

ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.iecr.3c02684.

Price list of the reagents used for the extraction procedures and utilized for the economic evaluation (PDF)

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Notes

The authors declare no competing financial interest.

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