

# Downstream processing for Polyhydroxyalkanoates from mixed microbial cultures: study of microbial activity inhibition, polymer recovery and characterization

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Keywords: polyhydroxyalkanoates, MMC, quenching, extraction, characterization

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Polyhydroxyalkanoates (PHA) are completely biodegradable polyesters with thermoplastic properties and it is well known the ability of mixed microbial cultures (MMC) to produce them by using waste organic streams as renewable feedstock resources. These biopolymers are synthesized as intracellular carbon and energy source by numerous species of microorganisms. The interest on PHA is progressively increasing also in consideration of reducing the consumption of fossil fuels and valorising waste stream in a profitable way. The possibility to integrate MMC-PHA production into infrastructures typically adopted for bio-waste residuals and wastewater treatment could make this technology economically and environmentally sustainable. MMC technology provides for the use of activated sludge as inoculum, which microbial composition needs to be enriched in functionalized PHA-storing bacteria. This usually occurs in sequencing batch reactors (SBR) operated under aerobic dynamic feeding (ADF) regime. After their synthesis, it is necessary to apply an efficient technique of microbial quenching, in order to inhibit the microbial activity and preserve the intracellular accumulated PHA prior to its recovery and purification in the downstream processing, which is still the bottleneck of the whole process. Industrially, high usage of precarious solvents and excessive requirement for energy input still are common features in PHA recovery, somewhat antagonizing sustainability and economic feasibility (Koller, *et al.* 2013). The aim of this work was to investigate various methods of microbial inhibition, applied on PHA-rich cells. In the quenching trials, several chemical or oxidizing agents (NaOH, H<sub>2</sub>SO<sub>4</sub>, H<sub>2</sub>O<sub>2</sub>, NaClO), or an excess of substrates itself were added on the not aerated biomass. A thermal pre-treatment (70°C) was also applied, with or without a previous thermal shock (170°C) (Phario project, 2017). On the dried PHA-rich biomass, obtained as hinted previously, different recovery tests were conducted: extraction in Chloroform (solubilization of the polymer), oxydation with NaClO (disruption of the cellular material) and extraction in Ethyl Acetate. Finally, extracted samples were characterized through several techniques: FT-IR, DSC, TGA, GC-FID, capillar viscosimetry.

Lab scale SBR (1 L) has been operated for MMC enrichment/selection (OLR 4.25 g COD/L.d, with synthetic VFA mixture. Different accumulation tests were conducted in a batch reactor (0.5 L), inoculated with the selected MMC and fed with a high concentrated VFA solution. At the end of the batch tests, the aeration has been stopped and then different quenching agents have been added: NaOH 1M until pH 12; H<sub>2</sub>SO<sub>4</sub> until pH 4; 80 mL of NaClO solution (7% of activated chlorine); 7.7 mL of H<sub>2</sub>O<sub>2</sub> (34.5%), equivalent to NaClO; 2 mL of substrates solution (850 gCOD L<sup>-1</sup>). PHA content has been monitored for 3 h after the beginning of the inhibition test and a final sample has been taken after 24 h. Two kind of thermal treatment have been applied on centrifuged biomass produced at Treviso pilot scale platform: 70°C in an oven until complete drying; 170°C for 30 min and then 70°C until complete drying. On PHA-rich dry biomass samples (2-5 g), three extraction methods have been used: direct extraction with CHCl<sub>3</sub>, (Soxhlet extractor) for 24 h, followed by solvent evaporation under fume hood; suspension in NaClO solution (1.5% Cl<sub>2</sub>), magnetic stirred overnight, centrifuged at 8500 rpm for 20 min and washing with distilled water, then oven dried at 70°C; direct extraction with Ethyl Acetate at 100°C and 125°C (pressurized steel reactor) for 1h, followed by solvent evaporation under fume hood and extraction with CHCl<sub>3</sub> of residual biomass. Extracted samples were subsequently dissolved in CHCl<sub>3</sub> obtaining solution at 0.041 %w/v to determine the viscosimetry average molecular weight (M<sub>v</sub>).

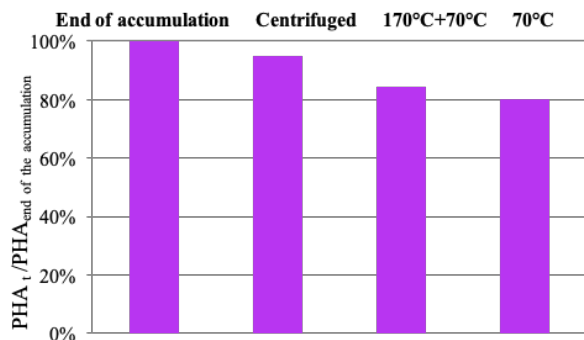
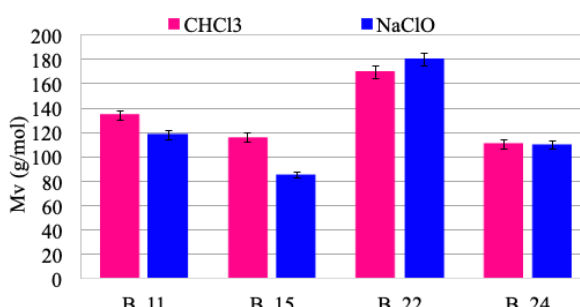


Figure 1 Comparison between 2 thermal treatments

PHA decrease (20 %). Moreover, that one with a previous thermal shock allowed to obtain a final PHA content

The comparison of PHA content data, collected for each inhibition trial, shows that there's not a significant decrease of the PHA for three hours following the start, demonstrating that every agent used is suitable for microbial activity quenching, except for sulfuric acid that reduced PHA content more than 60%. However, it's important to consider that, by using concentrated NaOH or strong oxydizing agents (NaClO or H<sub>2</sub>O<sub>2</sub>), a significant reduction of molecular weight can occur. Therefore, as shown in [Figure 1](#), thermal treatments were chosen as the best performing quenching methods thanks to the little

over 80 %w/w. This treatment has then been applied on PHA-rich biomass obtained by using mixed urban wastes (food waste and secondary sludge) in Treviso pilot platform (Valentino *et al.*, 2018). Four different batches (B11, B15, B22, B24), with PHA content more than 40 %w/w, have been selected to perform polymer extraction and characterization. Chloroform extraction, in this study considered as a benchmark, and NaClO oxydation allowed to reach satisfying performances in terms of purity (average value of 95.3 %w/w and 85.2 %w/w, respectively) and recovery (83.2% and 87.4%). Thermogravimetric analysis confirmed GC-FID data of purity and showed an average degradation temperature of 264°C. [Figure 2](#) shows that both recovery treatment gave the same results in terms of viscosimetry average molecular weight, therefore oxydation with NaClO can be considered a valid alternative to alogenated solvents. Most important is the evaluation of Ethyl Acetate as an innovative solvent for PHA recovery. Preliminary tests of Ethyl Acetate extraction have been conducted on



**Figure 2** Viscosimetry average molecular weights of extracted samples

Treatment	Purity (PHA %w/w)	M <sub>v</sub> (g/mol)	% HV	χ <sub>c</sub> %	T <sub>m</sub> (°C)
CHCl <sub>3</sub>	92.9 ± 6.1	133947	19	40	164
Ethyl Acetate 100°C	75.7 ± 1.8	71667	31	28	164
Ethyl Acetate 125°C	100 ± 4.5	48880	20	36	163
Residue 100°C CHCl <sub>3</sub>	99.5 ± 7.9	126250	12	48	167
Residue 125°C CHCl <sub>3</sub>	90.7 ± 1.1	75000	25	31	161

**Table 1** Characteristics of extracted samples and residual biomass of recovery trial on Batch 11

Batch 11 samples (B11). [Table 1](#) reports Ethyl Acetate extraction results, in comparison with standard CHCl<sub>3</sub> treatment (considered as B11 raw polymer). Data in table highlight that Ethyl Acetate extraction at 100°C allowed to reach a purity of 76 %w/w, instead of that one operated at 125°C which gave a higher-purity polymer (100 %w/w). On the other hand, molecular weights are quite different, in fact extraction at 125°C caused a partial degradation of both the extracted and the residual polymers, resulting in a lower M<sub>v</sub>. What is important to underline is that Ethyl Acetate extracted selectively at 100°C low-molecular-weight chains with a higher 3-hydroxyvalerate (31 %HV) content than in the residual one. In this sense, it would be possible to consider Ethyl Acetate as a selective solvent for low-molecular-weight PHA that could be used for specific

application in plastic industry. Moreover, DSC analysis showed that melting temperatures (T<sub>m</sub>) are quite similar for all the analyzed samples, also for residual biomasses. [Table 1](#) shows also a negative linear correlation between HV content and percent crystallinity (χ<sub>c</sub> %), so it's important to consider that monomeric composition influences the crystallinity, with effects in subsequent polymer processing. Thermal treatment has been successfully applied at pilot scale PHA production, demonstrating that it can be a valid inhibition method which could be considered as a fundamental step in polymer downstream processing. In fact, a strategy for PHA preservation allows to develop polymer extraction and processing outside of the production site, taking account a possible cooperation with plastic industries. On the other hand, it would be possible to develop an optimal recovery method using Ethyl Acetate to directly solubilize PHA. Ethyl Acetate is a good candidate as extractive agent thanks to its environmental compatibility and non-toxicity, contrary to alogenated solvents, as Chloroform and dicholoromethane, currently used as consolidated PHA-solvents. However, other extractive agents and conditions have to be investigated in order to find the best solution to PHA recovery. In this way, our research group is currently studying Ethyl and Butyl Acetate extraction under variable conditions of temperature and time of extraction.

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The financial support from the H2020 EU project RES URBIS (GA 730349) is gratefully acknowledged