

Innovative Strategy for Polyhydroxyalkanoates Recovery from Mixed Microbial Cultures: Effects of Aqueous Phase and Solvent Extraction on Polymer Properties

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This work aimed to investigate various methods of polyhydroxyalkanoates (PHA) extraction and to optimize the recovery in the view of reducing solvents' use and waste. The extraction tests were applied on PHA-rich biomass collected at the end of the accumulation step, conducted both at pilot and lab scale. PHA-rich biomass obtained from the lab scale process was produced from synthetic feedstock (mixture of acetic and propionic acids), while fermented organic waste was used for PHA production at pilot scale. In the extraction tests, different solutions of NaOH, also in combination with a surfactant (sodium dodecyl sulfate, SDS), were used to recover the polymer from the non-polymeric cellular material (NPCM). Different times (3, 6 and 24 h) and concentrations (0.05 – 1 M) were tested, evaluating also the effect of adding SDS (0.1 % w/v). As a benchmark, solvent extraction with chloroform and oxidation with NaClO were also conducted. Finally, extracted samples were characterized through several techniques: DSC, TGA, GC-FID, capillary viscosimetry. Alkaline treatment at short times and low concentrations resulted to be more efficient in terms of purity (85 – 99 % w/w PHA) and recovery (higher than 80 % w/w), than higher concentration. On the other hand, the presence of SDS had dramatic effects on the recovery (lower than 50 % w/w) and also on the molecular weight, which was two folds lower than that obtained from alkaline extraction. Overall, extraction with aqueous phase reagents had no effects on thermal properties, which resulted to be in the range of those reported in literature.

1. Introduction

Polyhydroxyalkanoates (PHA) are among the most interesting and promising biopolymers due to their complete biodegradability and the possibility to be synthesized by several microbial species exploiting various organic wastes as feedstock. Indeed, PHA can be produced from renewable organic resources using mixed microbial cultures (MMC) instead of pure cultures (current industrial production process) (Reis et al. 2011; Valentino et al. 2017). MMC technology is generally coupled to the use of waste-activated sludge (available in municipal wastewater treatment plants; WWTPs) as inoculum, that is then enriched in PHA-accumulating microorganisms. These species are selected by applying aerobic dynamic feeding conditions (ADF) through the consolidated feast-famine regime (Valentino et al., 2017). In the last decade, MMC-PHA production using different types of waste has been the topic of several applied studies and more recent investigations have been conducted at pilot-scale to demonstrate the technical feasibility of the technology. More in detail, PHA production from the organic fraction of municipal solid waste (OFMSW) has been developed to quantify a reasonable range of PHA productivity and final polymer quality, also in consideration of a possible integration in the existing WWTP (Valentino et al. 2019; 2021). However, polymer extraction is still among the most important factors affecting the overall PHA production cost and needs to be optimized for pilot scale processes. Currently, the most studied methods for recovering PHAs can be grouped into two main categories: solvent extraction and digestion of the NPCM (non-PHA cellular material) (Kosseva et al. 2018; Pérez-Rivero et al. 2019). Up to now, regarding the MMC PHA-rich biomass, the use of several non-chlorinated solvents (Samorì et al. 2015) and, very recently,

the disruption of NPCM through chemical agents and surfactants (Colombo et al. 2020) have been investigated. In the view of MMC-PHA production at an industrial scale and a subsequent market scenario, the improvement and optimization of the extraction step are necessary. Hence, this study investigated the performances of extraction tests carried out using different NaOH solutions on PHA-rich biomass produced at lab scale from synthetic substrate and at pilot scale from organic waste. This work evaluated also the effects of extraction conditions on the polymer chemical and thermal properties.

2. Materials and Methods

2.1 Biomass selection and PHA accumulation

A lab-scale SBR was set up for the selection and enrichment of PHA-storing MMC. The reactor consisted of 1 L working volume and was fed with a synthetic mixture of organic acids, namely acetic (85% on chemical oxygen demand basis, COD) and propionic acid (15%), at an applied organic loading rate (OLR) of 8.5 gCOD/L d. The accumulation reactor (0.5 L working volume) was operated in batch mode and a small volume of a solution at a high volatile fatty acids (VFA) concentration was spiked to reach an initial VFA/biomass ratio of approximately 2.0 g COD_{VFA}/g VSS. This parameter was constant in all of the batch tests. Any subsequent substrate spikes (no more than one or two) were performed when the oxygen uptake rate (OUR) dropped to low values to prevent the complete VFA removal (Valentino et al. 2014). At the end of the accumulation, PHA-rich biomass was centrifuged and freeze-dried. At pilot scale, PHA was produced from fermented OFMSW as feedstock, in a pilot platform situated in the full-scale WWTP in Treviso (northeast Italy). The production process (described in a previous study (Valentino et al. 2019)) included three main steps: a first anaerobic fermentation reactor (380 L) for PHA precursor production (volatile fatty acid, VFA), a second aerobic reactor (sequencing batch reactor, SBR; 100 L) for biomass selection into PHA-accumulating consortium (enriched-MMC), and a third fed-batch aerobic reactor (70–90 L) for PHA accumulation. At the end of the accumulation stage the biomass was centrifuged and oven dried at 60 °C, until downstream processing, as reported elsewhere (Lorini, Martinelli, Pavan, et al. 2021).

2.2 PHA extraction

About 100 mg of freeze-dried PHA-rich biomass from the lab-scale batch tests were suspended in 60 mL of NaOH solution at different concentrations (0.05 – 0.1 – 0.2 – 0.3 – 0.5 - 1.0 M). The suspension was kept under magnetic stirring for different operative times (3 – 6 – 24 h). In parallel, a coupled treatment with NaOH (same concentration range) and 0.1 % w/v SDS was conducted for 3 h and 24 h. At the end of the extraction, the suspensions were centrifuged at 8500 rpm for 20 min (ALC multi speed centrifuge PK 131) and washed 3 times with distilled water. The wet biomass was finally transferred into a crystallizer and oven dried at 70 °C. Based on preliminary results, the best performing condition was applied on dried PHA-rich biomass produced at the pilot scale (NaOH 0.2 M and 0.1 %w/v SDS for 3 h). As a benchmark, oxidative treatment with NaClO solution (1.5 % active Cl₂) and solvent extraction with chloroform were conducted on PHA-rich biomass, both from lab and pilot scale, following the procedures extensively reported elsewhere (Lorini, Martinelli, Pavan, et al. 2021).

2.3 PHA characterization

PHA content (%w/w) in dry biomass and monomeric composition (3-Hydroxybutyrate, 3HB, and 3-Hydroxyvalerate, 3HV, %w/w) were determined by applying a consolidated procedure for GC-FID analysis (Lorini, Martinelli, Pavan, et al. 2021). Approximately 3.5 mg of dried biomass is suspended in 2 mL of acidified methanol solution (at 3% v/v H₂SO₄) in a screw-capped test tube and 1.0 mL of chloroform. The samples are then kept at 100°C for 4 hours. During this time, an acid-catalyzed methanolysis of the PHA occurs, and the methyl esters are solubilized in chloroform. These latter are determined according to the analytical method described in Braunegg et al. (Braunegg et al. 1978). The recovery was determined with Equation (1) that correlates the dry weight of microbial biomass (recovered at the end of extraction) and purity (%w/w PHA) of extracted polymer, with the PHA content inside raw biomass before extraction.

$$recovery (\%) = \frac{[recovered\ biomass\ (g) \times PHA\ purity\ (\% \frac{w}{w})]}{[raw\ biomass\ (g) \times PHA\ content\ (\% \frac{w}{w})]} \times 100 \quad (1)$$

Thermal properties of extracted PHA samples were evaluated by differential scanning calorimetry (DSC). 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: 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; c) second heating up to 190°C at 10°C min⁻¹. The first heating is necessary to erase all previous sample thermal history. The thermal stability of extracted PHA samples was investigated by thermogravimetric analysis (TGA). All measurements were carried out under nitrogen flow from 25 °C to 500

°C, at a heating rate of 10 °C min⁻¹. About 5-8 mg of dried sample were used for the analysis. Extended procedures for both thermal analyses are reported elsewhere (Lorini, Martinelli, Pavan, et al. 2021).

The viscosity average molecular weight (M_v) of the extracted PHA samples was determined by diluted solution viscosimetry in chloroform at 30 °C using a Ubbelohde capillary viscometer (ID = 0.46mm).

The flow time of different solutions was measured and M_v was finally determined by the Mark-Houwink equation (Lorini, Martinelli, Pavan, et al. 2021).

3. Results and discussion

3.1 Extraction performances

Solvent extraction by chlorinated solvents is currently the consolidated extractive method for PHAs from MMCs. Moreover, disruption of non-PHA cellular material (NPCM) obtained by using a NaClO solution in different conditions (concentration and retention time) is reported in the literature (Villano et al. 2014) showing good recovery and purity level. In this study, both Chloroform extraction and NaClO digestion have been used as consolidated methods for PHA recovery, to make a comparison with results obtained from the PHA recovery through NaOH digestion. The first set of extraction tests has been carried out on freeze-dried PHA-rich biomass produced at lab scale and concentration and retention time were varied in ranges between 0.05 – 1.0 M and 3 – 24 h, respectively. Results obtained by GC-FID analysis conducted on extracted samples allowed to determine purity, in terms of PHA content in the recovered biomass after extraction, and the recovery yield (as defined by equation 1). Data reported in Figure 1A show that, regardless of the NaOH concentration, there is a differently wide range of variability at each extraction time. More in detail, the purity of the PHA extracted after 3 hours of treatment varied between 75.0 ± 4.0 % and 85.0 ± 1.0 % w/w. On the other hand, purity was higher for the extractions lasting 6 h, ranging between 83.1 ± 2.0 % and 99.9 ± 0.2 % w/w in the specific case of NaOH 0.5 M. Data relating to the extractions conducted for 24 h were characterized by an even wider variability, with minimum values close to 70.4 ± 0.1 % w/w and maximum values of 95.1 ± 2.1 % w/w. Therefore, even if a high contact time could allow more efficient digestion of the NPCM, with consequent high purity, on the other hand, treatment for a longer time could lead to possible hydrolysis of the PHA itself, with consequent loss of monomers. Figure 1B compares values of recovery yield for the same set of extraction. In this case, the range of variation was higher for the 3 h (73.6 ± 0.9 % - 100 ± 2 % w/w) and 24 h (51.5 ± 0.9 % - 100 ± 3 % w/w) tests. Hence, the 6 h treatment seemed to be more efficient and reproducible, since the recovery yield variability ranged between 87.7 ± 0.1 % and 100.8 ± 0.2 % w/w (as observed also for purity) regardless of NaOH concentration in a range between 0.05 M and 0.5 M. Based on these first results, a set of extractions was conducted on the same biomass at 3 h and 24 h of treatment, combining the effect of NaOH (in the same concentration range) and SDS, to evaluate the possibility of improving the extraction performances.

Purity and recovery data of the extracted PHA are reported in Figure 1 A and B, respectively, in comparison with previously discussed results of the sole NaOH extractions. Tests conducted for low reaction times (3 h) showed a substantial increase in purity for NaOH concentrations higher than 0.1 M, with a maximum purity at the concentration of 0.2 M (97.8 ± 3.7 %w/w) in just 3 hours of treatment. Even for higher NaOH values (0.3M and 0.5M), there is a net increase of purity, thanks to the use of SDS. On the other hand, by extending the extraction time up to 24 h, the effect of SDS on the purity of the extracted PHA was less marked than in the tests conducted for 3 h, probably due to the greater effectiveness of NaOH during a longer contact time. However, the positive effects of SDS during the 3h tests were not encountered for recovery, which instead underwent a substantial decrease (below 90% w/w) for increasing NaOH concentrations (0.3M and 0.5M). Considering the whole set of extraction for 24 h, there is an overall continuous decreasing trend of recovery yield as the NaOH concentration increased (i.e. from 100 ± 3 % w/w for 0.2 M to 51.5 ± 0.9 % w/w for 1 M NaOH). The same trend was obtained also in the tests in which SDS was added, but recovery values were on average 10% lower than the respective tests without SDS.

As a main result, a reaction time equal to 3 h and combined treatment with SDS and NaOH 0.2 M was considered a good extraction method, and then it was applied also on PHA-rich biomass produced at the pilot scale. Moreover, chloroform and NaClO extraction were performed on biomass produced both at the lab and pilot scale. The main results of extraction on pilot-scale biomass are reported in Table 1, in comparison with the best results obtained from NaOH and benchmark extraction on lab-scale biomass. It can be highlighted that combined NaOH and SDS extraction on biomass obtained at lab scale was more efficient than that one conducted on pilot-scale biomass. This was reasonably due to different characteristics of the dry matter, indeed PHA-rich biomass coming from the pilot-scale contained a very high amount of solid residues derived from the OFMSW fermentation. The high concentration of solids led to a lower PHA content in the biomass at the end of the accumulation step, if compared to that one obtained at lab scale (42.1 ± 0.8 and 60.1 ± 0.9 %w/w, respectively). As a consequence, the hydrolytic effect of NaOH and NaClO was significantly lower on the dried biomass and it resulted in lower PHA purity (79.2 ± 0.4 and 85.7 ± 1.3 %w/w, respectively) and recovery (80.1

± 1.0 and 75.6 ± 0.5 %w/w, respectively). Hence, further investigation for improving extraction performance of NaOH are necessary, since good preliminary results were achieved.

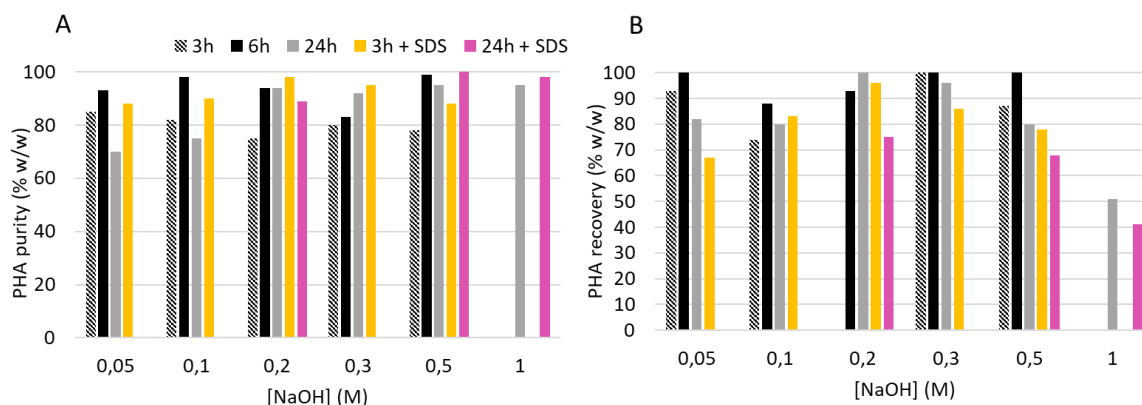


Figure 1: Lab scale NaOH Extraction Performances: (A) PHA purity; (B) PHA recovery.

3.2 PHA characterization

As discussed in the previous section, different extractions led to different results in terms of PHA purity. Notwithstanding, the HV monomer content was stable after every kind of extraction. Monomeric composition is reported in Table 1, in comparison with those determined in raw PHA-rich biomass (11.0 ± 0.5 and 7.2 ± 0.2 %w/w, respectively for lab and pilot scale).

Capillary viscosity analysis was conducted on PHA solutions in CHCl_3 for some representative samples: NaOH 0.05M + SDS 3h; NaOH 0.2M + SDS 3h; NaOH 0.5M 3h (lab scale) and NaOH 0.2M + SDS 3h (pilot scale). These results were also compared to those obtained from benchmark extractions, as reported in Table 1.

Indeed, chloroform extraction has been considered as a reference, since chloroform can permeate the cellular wall, solubilize the polymer and extract it from the cytoplasm, without affecting PHA structure. On the other hand, the use of NaClO and NaOH to digest NPCM could in parallel cause a partial degradation of the polymeric chains, leading to molecular weight decrease. As a main result, NaClO digestion did not affect the molecular weight for both lab and pilot scale samples. In fact, for both samples the values of the viscosity average molecular weight (\bar{M}_v) are comparable to those determined after chloroform extraction: 181 ± 13 and 197 ± 14 kDa respectively for lab and pilot-scale PHA. On the other hand, \bar{M}_v of PHA produced at lab scale was slightly affected by NaOH treatment at low concentration (0.05 and 0.2 M) coupled with SDS for 3h (162 ± 12 and 165 ± 23 kDa, respectively), while the increased NaOH concentration (0.5 M) caused the halving of the \bar{M}_v (95 ± 9 kDa). However, different results were found for PHA produced from waste, indeed all the considered extractions gave comparable values of \bar{M}_v , around 200 kDa, as shown in Table 1. Previous studies reported M_w of 340-540 kDa (Villano et al. 2014) for lab scale biomass and \bar{M}_v around 130 kDa for PHA produced from OFMSW and sludge at pilot scale (Lorini, Martinelli, Pavan, et al. 2021).

Thermogravimetric analysis allowed to determine the decomposition temperature derived at the maximum decomposition rate (T_d^{\max}). Results on all the samples reported in Table 1 show that PHA thermal stability was not affected by the extraction agent used. Indeed, all the determined T_d^{\max} were in the range of 271 – 301 °C (both lab and pilot scale samples). Overall, such values indicated high thermal stability of the polymers, comparable to those reported in the literature for MMC-PHA (Lorini, Martinelli, Capuani, et al. 2021; Morgan-Sagastume et al. 2015). Moreover, the percentage of weight loss, due only to the polymeric fraction of the solid sample, was quantified from the thermogram and the obtained values were comparable to PHA purity data measured by GC-FID analysis (Table 1). Finally, melting temperatures (T_m), determined by DSC analysis, are reported in Table 1 and they agree with the literature (ranging between 155 and 165 °C), both for PHA from synthetic feedstock (Bengtsson et al. 2010) and from organic waste (Lorini, Martinelli, Pavan, et al. 2021).

Table 1: Extraction Performances and PHA characterization results

Sample	Purity (PHA %w/w)	Recovery (%)	HV (%w/w)	M _v (kDa)	T _d ^{MAX} (°C)	T _m (°C)
Lab scale						
Raw PHA-rich biomass (freeze-dried)	60.1 ± 0.9		11.0 ± 0.5			
NaOH 0.05M + SDS 3h	87.2 ± 8.1	67.8 ± 6.0	11.6 ± 0.3	162 ± 12	271	162
NaOH 0.2M + SDS 3h	97.8 ± 3.7	95.8 ± 3.5	11.0 ± 0.3	165 ± 23	275	157
NaOH 0.5M 3h	99.9 ± 0.2	100.8 ± 0.2	14.9 ± 0.1	95 ± 9	281	158
NaClO	98.4 ± 0.1	88.3 ± 0.5	11.6 ± 0.2	181 ± 13	274	163
CHCl ₃	97.6 ± 0.1	98.3 ± 0.4	11.1 ± 0.3	194 ± 12	279	165
Pilot scale						
Raw PHA-rich biomass (oven dried)	42.1 ± 0.8		7.2 ± 0.2			
NaOH 0.2M + SDS 3h	79.2 ± 0.4	80.1 ± 1.0	7.2 ± 0.6	198 ± 10	289	157
NaClO	85.7 ± 1.3	75.6 ± 0.5	7.4 ± 0.1	197 ± 14	301	155
CHCl ₃	95.6 ± 0.7	89.3 ± 1.1	7.0 ± 0.3	200 ± 15	298	164

4. Conclusions

Results from extraction tests conducted on lab-scale samples have highlighted how the oxidative treatment, compared to traditional methods, could be considered as a valid alternative. Indeed, the performance obtained from some tests with NaOH can be considered as an interesting starting point for wider research.

More in detail, the use of SDS for short retention time (3h) had a positive effect on purity (comparable to that obtained in the tests of longer duration), but generally lower recovery yields were obtained compared to the tests conducted without SDS. Overall, the tests conducted with sole NaOH for 6 h of reaction, or with the combination of NaOH and SDS for 3 h of reaction, were the most promising in terms of extraction performance and effect on molecular weight, as it was highlighted by the viscosity analysis. Since the molecular weight and thermal properties of PHA produced from organic waste at the pilot scale were not affected by the type of extraction, further investigation on NaOH treatment and the following characterization are needed. Thanks to the very recent diffusion of pilot-scale processes involving solid wastes for PHA production, it is now important to demonstrate that thermal and mechanical properties can be modulated based on future applications, leading to the choice of the best performing extraction method.

References

- Bengtsson, S., A. R. Pisco, P. Johansson, P. C. Lemos, and M. A.M. Reis. 2010. "Molecular Weight and Thermal Properties of Polyhydroxyalkanoates Produced from Fermented Sugar Molasses by Open Mixed Cultures." *Journal of Biotechnology*. <https://doi.org/10.1016/j.jbiotec.2010.03.022>.
- Braunegg, G., B. Sonnleitner, and R Lafferty. 1978. *Rapid Gas-Chromatographic Method for Determination of Poly-Beta-Hydroxybutyric Acid in Microbial Biomass*. *Applied Microbiology and Biotechnology*. Vol. 6. <https://doi.org/10.1007/BF00500854>.
- Colombo, Bianca, Joana Pereira, Margarida Martins, Mario A. Torres-Acosta, Ana C.R.V. Dias, Paulo C. Lemos, Sónia P.M. Ventura, et al. 2020. "Recovering PHA from Mixed Microbial Biomass: Using Non-Ionic Surfactants as a Pretreatment Step." *Separation and Purification Technology*. <https://doi.org/10.1016/j.seppur.2020.117521>.
- Kosseva, Maria R., and Edy Rusbandi. 2018. "Trends in the Biomanufacture of Polyhydroxyalkanoates with Focus on Downstream Processing." *International Journal of Biological Macromolecules*. <https://doi.org/10.1016/j.ijbiomac.2017.09.054>.
- Lorini, Laura, Andrea Martinelli, Giorgio Capuani, Nicola Frison, Maria Reis, Bruno Sommer Ferreira, Marianna Villano, Mauro Majone, and Francesco Valentino. 2021. "Characterization of Polyhydroxyalkanoates Produced at Pilot Scale From Different Organic Wastes." *Frontiers in Bioengineering and Biotechnology* 9 (February): 1–13. <https://doi.org/10.3389/fbioe.2021.628719>.
- Lorini, Laura, Andrea Martinelli, Paolo Pavan, Mauro Majone, and Francesco Valentino. 2021. "Downstream Processing and Characterization of Polyhydroxyalkanoates (PHAs) Produced by Mixed Microbial Culture (MMC) and Organic Urban Waste as Substrate." *Biomass Conversion and Biorefinery* 11 (2): 693–703. <https://doi.org/10.1007/s13399-020-00788-w>.

- Morgan-Sagastume, F., M. Hjort, D. Cirne, F. Gérardin, S. Lacroix, G. Gaval, L. Karabegovic, et al. 2015. "Integrated Production of Polyhydroxyalkanoates (PHAs) with Municipal Wastewater and Sludge Treatment at Pilot Scale." *Bioresource Technology*. <https://doi.org/10.1016/j.biortech.2015.01.046>.
- Pérez-Rivero, Cristina, J. Pablo López-Gómez, and Ipsita Roy. 2019. "A Sustainable Approach for the Downstream Processing of Bacterial Polyhydroxyalkanoates: State-of-the-Art and Latest Developments." *Biochemical Engineering Journal*. <https://doi.org/10.1016/j.bej.2019.107283>.
- Reis, M.A.M., M. Albuquerque, M. Villano, and M. Majone. 2011. "Mixed Culture Processes for Polyhydroxyalkanoate Production from Agro-Industrial Surplus/Wastes as Feedstocks." *Comprehensive Biotechnology*, January, 669–83. <https://doi.org/10.1016/B978-0-08-088504-9.00464-5>.
- Samorì, Chiara, Federica Abbondanzi, Paola Galletti, Loris Giorgini, Laura Mazzocchetti, Cristian Torri, and Emilio Tagliavini. 2015. "Extraction of Polyhydroxyalkanoates from Mixed Microbial Cultures: Impact on Polymer Quality and Recovery." *Bioresource Technology*. <https://doi.org/10.1016/j.biortech.2015.03.062>.
- Valentino, F., F. Morgan-Sagastume, S. Campanari, M. Villano, A. Werker, and M. Majone. 2017. "Carbon Recovery from Wastewater through Bioconversion into Biodegradable Polymers." *New Biotechnology* 37: 9–23. <https://doi.org/https://doi.org/10.1016/j.nbt.2016.05.007>.
- Valentino, Francesco, Mario Beccari, Serena Fraraccio, Giulio Zanaroli, and Mauro Majone. 2014. "Feed Frequency in a Sequencing Batch Reactor Strongly Affects the Production of Polyhydroxyalkanoates (PHAs) from Volatile Fatty Acids." *New Biotechnology* 31 (4): 264–75. <https://doi.org/10.1016/j.nbt.2013.10.006>.
- Valentino, Francesco, Laura Lorini, Paolo Pavan, and Mauro Majone. 2021. "Development of a Biorefinery Platform for Urban Waste Valorisation into Biogas and Added-Value Products." *Chemical Engineering Transactions* 86 (August 2020): 13–18. <https://doi.org/10.3303/CET2186003>.
- Valentino, Francesco, Giulia Moretto, Laura Lorini, David Bolzonella, Paolo Pavan, and Mauro Majone. 2019. "Pilot-Scale Polyhydroxyalkanoate Production from Combined Treatment of Organic Fraction of Municipal Solid Waste and Sewage Sludge." *Industrial & Engineering Chemistry Research* 58 (27): 12149–58. <https://doi.org/10.1021/acs.iecr.9b01831>.
- Villano, M., F. Valentino, A. Barbetta, L. Martino, M. Scandola, and M. Majone. 2014. "Polyhydroxyalkanoates Production with Mixed Microbial Cultures: From Culture Selection to Polymer Recovery in a High-Rate Continuous Process." *New Biotechnology* 31 (4): 289–96. <https://doi.org/10.1016/j.nbt.2013.08.001>.