Mixed culture polyhydroxyalkanoates accumulation with synthetic and real feedstocks

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Abstract

Over last years, bioplastics have been attracting considerable attention as environmentally sustainable alternatives to fossil-based plastics. Among the others, polyhydroxyalkanoates (PHAs) are particularly interesting being both biobased and completely biodegradable in the environment (Reis et al. 2011; Villano et al. 2014). Commonly, the production of PHAs is carried out using pure microbial cultures, which require sterile conditions and specially formulated substrates, thus increasing the cost of production and making this family of polymers less competitive on the market with respect to conventional plastic materials. A possible strategy to cut production costs consists in the use of mixed microbial cultures (MMCs), that provide an ecological benefit giving new life to waste streams, which can be used as feedstocks instead of simply being disposed of (Li and Wilkins 2020). MMC-PHA production implies multistage processes which includes the acidogenic fermentation of waste organic feedstocks to obtain volatile fatty acids (VFA), the selection stage of PHA-storing microorganisms from an activated sludge, as well as the polymer accumulation stage aimed at maximizing the intracellular PHA content (Zeng et al. 2018). In particular, the performance of the microbial selection stage plays a pivotal role on the overall process performance. The microbial selection is favored by the establishment of the feast and famine (FF) regime, consisting in the alternance of excess (feast phase) and deficiency (famine phase) of external carbon substrates, inducing growth kinetics limitations in favor of PHA accumulation. In this way, a selective pressure is imposed on the microbial culture triggering competition between bacteria that can store the external carbon source in the form of polymer granules and bacteria that are unable to do so.
In this work, the fermentation stage was carried out using a lab scale Continuous Stirred Tank Reactor (CSTR, 1.1 L working volume) to obtain VFA, that are direct precursors for PHA production by MMC (Kourmentza et al. 2017). The CSTR was fed with a food-industry by-product (i.e., regrind pasta) and operated at the temperature of 30°C through a thermostatic bath. The selection stage was performed in a lab-scale sequencing batch reactor (SBR) operated at an applied organic loading rate (OLR) of 4.25 gCOD (Chemical Oxygen Demand)/Ld, made of a synthetic mixture of acetic (HAc) and propionic (HPr) acids (accounting for 65% and 35% of the overall COD), and at a fixed cycle length of 6 h, corresponding to 4 cycles per day. Fully aerobic FF conditions were imposed to perform the microbial selection. The SBR (1 L working volume, T = 25 °C) was inoculated with activated sludge collected from a municipal wastewater treatment plant, and the hydraulic retention time was equal to the sludge retention time (1 day), since no settling phase was performed**.** An uncoupled carbon and nitrogen feeding was applied, with a COD/N ratio equal to 35 gCOD/gN-NH_4^+ (Silva et al. 2017). Regarding the accumulation stage, this was made in a fed-batch reactor using the biomass selected in the SBR and was fed with a concentrated carbon source poor in nutrients. In general, the composition of the feeding solution can affect the PHA production and composition. For this reason, here, the influence of two different carbon sources on the accumulation stage was investigated. The feeding solutions consisted of either a synthetic mixture of acids (as the one used in the SBR) or the fermented real feedstock. The latter composition was mainly composed of acetic (ca. 32%, on COD basis), isobutyric (19%), propionic (ca. 18%), butyric (16%), and valeric (14%) acids. The obtained results with both feedstocks have been compared and are shown in Figure 1. With the synthetic mixture, the intracellular PHA and hydroxyvalerate content in the stored polymer were higher than with the fermented feed. This was likely since the fermented feedstock contained acids (around 70% of the overall COD) along with other compounds, such as sugars. Concerning the PHA composition, HAc is involved in the synthesis of both the 3-hydroxybutyrate (HB) and 3-hydroxyvalerate (HV) monomers, while HPr and valeric acids are mainly involved in the production of the HV monomer (Dionisi et al. 2004). Therefore, the acids that contribute to the formation of this monomer in the fermented feedstock accounted for about 30% of the total acids, that is lower than the content of HPr in the synthetic mixture. This could explain the lower HV content achieved in accumulation tests with the fermented feedstock than with the synthetic one. However, a significant increase in the intracellular PHA content from the SBR to the accumulation reactor was reached with both feedstocks (accounting for 175% and 80% with synthetic and fermented substrates, respectively).

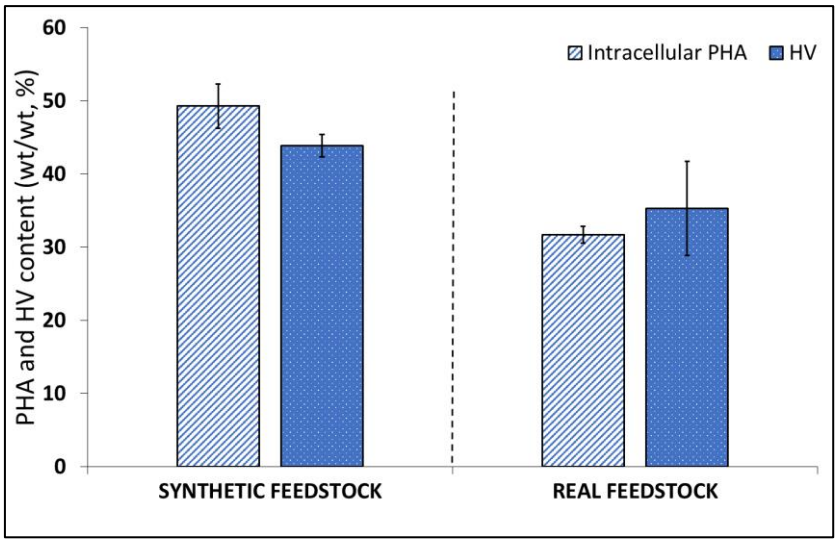

Figure 1. PHA and HV content in accumulation PHA tests, with synthetic and real feedstock (tests performed with biomass selected from SBR at OLR 4.25 gCOD/Ld).

In conclusion, the obtained results show that with both feedstocks there was an increase in the intracellular PHA during accumulation tests. Although the greatest increase was achieved with the synthetic mixture, these results open new possibilities for using food industry by-products, which can be valorized through biopolymers production, fitting with the concept of circular economy.

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