Organic load affects carbon and electron allocation in OFMSW photobiorefinery of purple phototrophic bacteria for PHA accumulation.

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Introduction

Polyhydroxyalkanoates (PHA) are promising materials for bioplastic production due to their physical properties and biodegradability and can mitigate global environmental pollution resulting from petroleum-based plastics (Silva et al., 2022). However, large-scale PHA production predominantly uses pure bacterial cultures and expensive substrates. The high cost of the substrate and sterilization make PHA uncompetitive compared to traditional plastics. To reduce costs, mixed cultures such as purple phototrophic bacteria (PPB) are being investigated (Almeida et al., 2021). PPB assimilate different carbon sources, including sugars and organic acids, in anaerobic conditions, making them perfect candidates for treating heterogeneous materials.

The organic fraction of municipal solid waste (OFMSW) is a renewable and abundant resource. One of the most suitable pretreatments to improve biodegradability is a steam explosion due to the high solubilization of organics and the possibility of energy recovery. Previous studies showed the feasibility of producing PHA from this hydrolysate but with low yields (5% dry mass) (Allegue et al., 2020). Therefore, a thermophilic acidogenic fermentation step is a potential intermediate step, as it has proven to be appropriate to produce short-chain carboxylic acids (SCCA) with high yields using both food and lignocellulosic waste (Allegue et al., 2021), which are the ideal substrate for PHA production (Almeida et al., 2021).

This work analyses PHA production from OFMSW through four cascading processes: steam explosion and thermophilic fermentation pretreatments and afterward anaerobic digestion and a photoheterotrophic process with PPB in a membrane photobioreactor (MPBR), modifying the OLR, which exerted a substantial effect on the PPB mechanisms for the allocation of excess of carbon and electrons.

Materials and methods

The OFMSW samples came from the Mercamadrid market (Spain). The steam explosion was conducted in a 6L batch reactor at 150 ºC and 40 min. The acidogenic fermentation was performed in a 2 L continuous reactor at an OLR of 4 gCOD L^{-1} d^{-1}, an HRT of 5 days, and 55º. The sludge obtained is separated by centrifugation: the solid phase undergoes an anaerobic digestion process (BMP test), and the liquid fraction is derived to a photoheterotrophic on a 2L MPBR. The MPBR had a submerged hollow fiber membrane and a LED lamp emitting at 805nm, with a volumetric irradiance of 2.2 W L^{-1} and a 2-day HRT. The different operating conditions are summarized as: Start-up (S0), operation under stable biomass growth (S1 and S2), first carbon overload (S3), biomass recovery (S4 and S5) and second carbon overload (S6). Bacterial communities were analyzed by DNA extraction and PacBio sequencing and statistical analysis by PAC and RDA. The rest of the analyses were performed using standard methods.

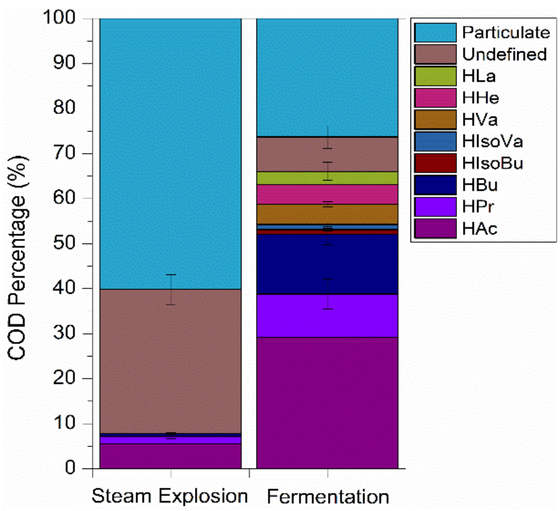

Figure 1. Performance of the steam explosion and subsequent acidogenic fermentation in terms of COD mass balance.

Results and conclusions

Steam explosion performance was measured via COD solubilization and solids destruction, 40%, and 44%, respectively. Figure 1 shows the overall COD balance of the pretreatments. Thermophilic fermentation yielded around 0.66 $gCOD_{SCCA}$ $gCOD_{feed}^{-1}$ and an acidification rate of 90%. The three most prevalent SCCAs were acetic (46%), propionic (14%), and butyric (20%). Even more, it produced on average 345 mlH_2 L^{-1} d^{-1} as a co-product.

The start-up of the MBPr lasted 4 d when the reactor was fed with synthetic media to obtain a highly active phototrophic community. Then, the liquid fraction of the fermentate was fed to the MBPR. As shown in Figure 2, stable biomass growth and PHA accumulation up to 42% was achieved in S1 and S2. This PHA was composed of the usual monomers (PHB and PHV) and PPH (up to 34% in dry mass of the total PHA). This is the first instance to show PHH production in a PPB mixed culture. This result entails polymers with lower crystallinity or lower melting temperature, enlarging their industrial interest (Silva et al., 2022). These results are remarkable since the PPBs are fed continuously in "permanent feast" mode instead of the usual "feast and famine" process of aerobic processes. So, it allows for continuous production of PHA, exploiting 100% of the substrate for this purpose.

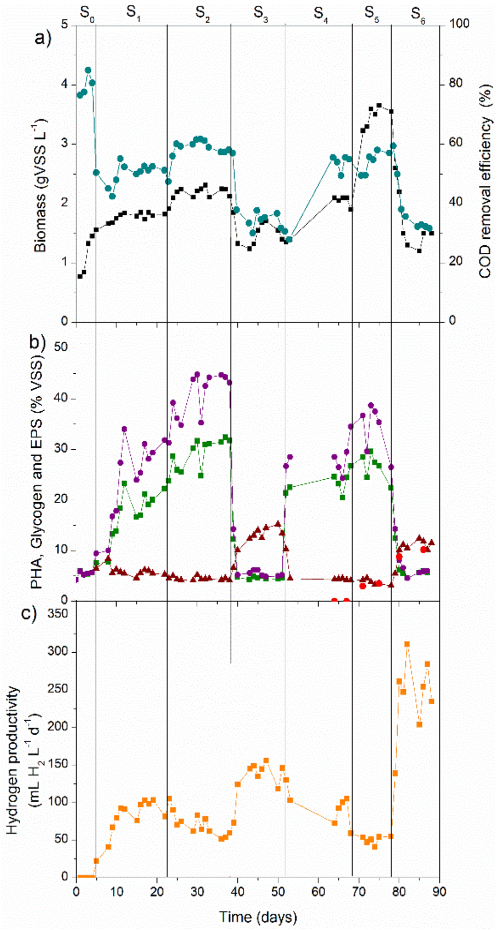

Figure 2. MPBR operation profile. a) Black squares represent biomass and blue square represent COD removal efficiencies. b) Green squares represent PHB/PHV percentage and purple dots total percentage of PHA (PHB/PHV/PHH). Brown triangles represent glycogen and red dots EPS percentage. On c) Orange squares represent hydrogen productivity.

When the organic loading rate rose, PHA production collapsed, and the mixed cultures preferentially accumulated glycogen and extracellular polymeric substances (EPS). The production of hydrogen in the reactor is also substantially increased. In the S4 and S5, the MBPR was submitted to lower OLR (Same as S1). The system recovered its initial performance, achieving COD removal efficiencies of around 55% and PHA accumulation close to 40%, reducing glycogen and hydrogen again. After a new organic overload (S6), the collapse of PHA accumulation and the accumulation of glycogen and EPS and high hydrogen productions are observed again. Therefore, the mixed culture modified the strategy of carbon allocation during the overload to drive glycogen and ALE production, electron allocation drifts from PHA to hydrogen production, and this process was reversible

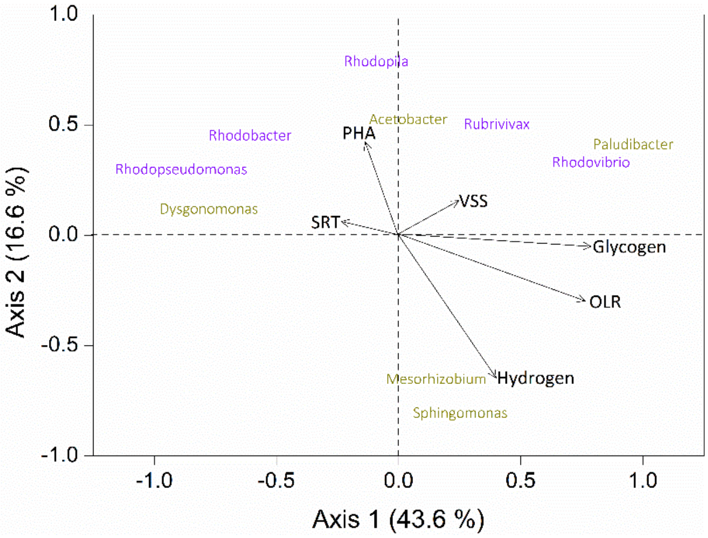

Figure 3. Redundancy discriminate analysis (RDA) ordination diagram of bacteria in relation to environmental variables. The nodes show the 10 genera with the most weight in both axis (PPB in purple, other genera in yellow), and the arrows indicate the weight of each environmental variable.

We also studied the effect of environmental variables on microbial communities (Figure 3) through an RDA that indicates a high correlation between PHA accumulation and PPB (mainly *Rhodopseudomonas sp*., *Rhodobacter sp.,* and *Rhodopila sp*.). Furthermore, an inverse relationship is observed between PHA, glycogen, and hydrogen, and the increase of OLR is negatively related to the presence of PPB in the culture. This result evidence the PPB mixed culture's robustness to recover when submitted to organic overload and provides insights on how to optimize PHA production.

Overall, this study demonstrates the possibility of obtaining high yields of PHB-PHV-PHH PHA with a PPB mixed culture by treating fermented OFMSW. In addition, it revealed the metabolic versatility of PPB that shifts carbon and electron allocation between PHA, glycogen, EPEstoS, and hydrogen, allowing for the recovery of the system after organic overload. Finally, it also provides insights into the concept of multi-purpose photo-biorefinery, where the operating conditions can be tuned to get a broad portfolio of final products from a single organic waste source.

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