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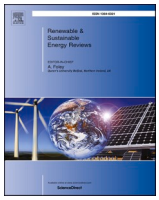

Unraveling PHA production from urban organic waste with purple phototrophic bacteria via organic overload

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ABSTRACT

The production of polyhydroxyalkanoates (PHA) with purple phototrophic bacteria (PPB) has been limited due to low yields and limited knowledge regarding the diverse routes used for carbon biosynthesis. The present study increases PHA accumulation yields using urban organic waste pretreated by steam explosion and acidogenic fermentation as substrate. Throughout the PPB-based photoheterotrophic process in an anaerobic membrane photobioreactor, the organic loading rate (OLR) was modified to increase the amount of PHA and biomass in the reactor. A maximum PHA accumulation of 42% ($g_{PHA}\ g^{-1}_{Biomass}$) on a dry basis was achieved and maintained for 10 d for an OLR of 1 gCOD L^{-1} d^{-1}, and hydraulic and sludge retention times of 2 and 6 d, respectively. This PHA accumulation capacity is the maximum obtained using a mixed culture of PPB fed with waste. Also, a medium-chain PHA (polyhydroxyhexanoate) has been quantified, enhancing the physicochemical properties and diversifying their industrial applications. Furthermore, we show novel alternatives to PHA accumulation: carbon storage as glycogen and extracellular polymers while deriving the excess electrons into hydrogen. Finally, a statistical study of microbial communities has settled the environmental variables with the most significant influence on these communities' variability. This work demonstrates the importance of acquiring a thorough understanding of carbon accumulation and electron allocation strategies of PPB under stressful conditions and shows promising results for a larger scale implementation of a PPB-based photobiorefinery, which could valorize urban organic waste to produce different high added-value products within the context of the circular bioeconomy.

1. Introduction

The management of MSW challenges the EU as a critical element to transition to a circular economy. The EU 27 has produced 505 kg of municipal waste per capita in 2020 [1]. However, only 30% of MSW is recycled, far from the EU target of 55% by weight settled by 2025. In the Waste Framework Directive and the Landfill Directive, high emphasis is placed on the organic fraction of municipal solid waste (OFMSW), a key element in planning the sustainable MSW management system [2]. OFMSW must be collected separately from 2023 onwards, a major step in establishing a circular bioeconomy in the EU [3]. The EU has defined a treatment hierarchy that prioritizes the valorization of organic wastes into biomaterials rather than compost or energy, thus defining the scope of the biorefineries for OFMSW [4]. Indeed, the fundamental contribution of biorefineries to the concept of circular economy is the 'pyramidal approach,' where the extraction and production of an extensive portfolio of high added-value products have greater priority than bioenergy production.

Plastics derived from petroleum-based industries have revolutionized modern life since their commercialization due to their low cost, durability, and good mechanical and thermal properties [5]. However, they can remain in the environment for more than a century without decomposing, and their recycling is a complicated process. Therefore, more sustainable alternatives must be sought, compelling the transition to the bioeconomy and circular economy. Bioplastics have emerged as a sustainable alternative in this context, as they can be produced from renewable resources, are biodegradable, and have similar thermal and mechanical properties to petroleum-based plastics [6].

Polyhydroxyalkanoates (PHA) are a family of environmentally sustainable polyesters produced by a wide variety of prokaryotes [7]. PHA are synthesized in excess of carbon and limitation of a major nutrient, or in situations of bacterial stress, in addition to acting as carbon and reducing power sink [8]. More than 150 monomers of PHA with a wide range of structures are known, and PHA can be divided based on the size of their monomers into short-chain PHAs, consisting of monomers of 2–5

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Abbreviations

Biochemical methane potential BMP
Chemical Oxygen Demand COD
Extracellular polymers EPS
Hydraulic retention time HTR
Membrane photobioreactor MPBR
Organic fraction of municipal solid waste OFMSW
Organic loading rate OLR
Polyhydroxybutyrate PHB
Polyhydroxyvalerate PHV
Polyhydroxyalkanoate PHA
Polyhydroxyheaxanoate PHH
Poly(hydroxybutyrate-co-hydroxyvalerate) PHBV
Purple phototrophic bacteria PPB
Total Kjeldahl nitrogen TKN
Total phosphorus TP

carbon atoms, and medium-chain PHAs, containing 6 to 14 carbon atoms [6]. The polyhydroxybutyrate (PHB) is the most studied polymer so far, being its thermomechanical properties very similar to those of polypropylene [9] but more stiff and brittle [10]. On the other hand, the PHB and polyhydroxyvalerate (PHV) copolymer, called poly (hydroxybutyrate-co-hydroxyvalerate), commonly known as PHBV, is less stiff and brittle than the PHB monomer but retains most other mechanical properties. In general, and depending on their composition, copolymers decrease the degree of crystallinity and melting temperature and increase the extension to break [11]. Still, in particular, the combination of short-chain PHAs (PHB and PHV) with medium-chain PHAs such as polyhydroxyhexanoate (PHH) give polymers elastic properties that increase their value in the industry as they can be used in different applications [12]. However, the production cost of PHA can be 3–4 times more expensive than conventional fossil plastics [13,14]. Pure cultures typically produce commercial PHA using synthetic substrates, which are responsible for 30–50% of production costs [15]. Mixed microbial cultures fed with organic and biodegradable wastes have been thoroughly investigated to reduce these costs [16]. These wastes are usually accommodated by pretreatments depending on their complexity and later treated by acidogenic fermentation, where short-chain carboxylic acids (SCCA), ideal precursors of PHA, are produced [17].

Among the potential feedstock, the organic fraction of municipal solid waste (OFMSW) is one of the most suitable options due to its high availability and low cost. Previous studies have shown that thermal pretreatments such as steam explosion solubilize a substantial amount of carbon, reduce the final quantity of residues and increase the rates and productivities in fermentation processes [18]. The coupling of the acidogenic fermentation process and PHA production has already been tested at a pilot scale in several studies using simpler substrates such as the solid phase of squeeze OFMSW plus biological sludge [19] or fruit waste [20], obtaining high fermentation yields (0.74 gCOD $gCOD^{-1}$). However, these studies have been carried out with aerobic mixed microbial cultures, which, in addition to the aeration costs, require the uncoupling of the famine and feast phases in two reactors. An exciting strategy to reduce costs is using light-dependent microorganisms like purple phototrophic bacteria (PPB), as they can grow under anaerobic conditions, allowing easy enrichment without aeration. The PPBs also allow PHA carbon accumulation ratios of up to 0.96 $Cmol_{PHB}$ $Cmol_{Hac}^{-1}$ [21], and unlike most chemoheterotrophs, PPB can grow and accumulate PHA simultaneously [22]. Thereby, PPB can perform PHA accumulation in a continuous feeding regime, eliminating the costly and non-productive periods associated with the famine phases used in aerobic systems [23]. Another strategy studied has been the combined production of PHA and hydrogen in several publications [24,25]. Several PPB species are strong PHA accumulators, most of them bearing to the families Chromatiaceae, Rhodospirillaceae, Rhodobacteraceae, and Bradyrhizobiaceae. However, the organic loading rate (OLR) strongly conditions the community composition, where *Rhodopseudomonas*, *Rhodobacter*, *Rhizobium,* and some representatives from the Hyphomicrobiaceae family stand out at high OLR [26]. Still, a study that examines the effect of operational factors over community transitions and how they affect PHA production and elucidates the relationship between electron and carbon sinks in PPB metabolism is lacking.

The production of PHA by PPB enriched mixed cultures has been studied primarily on synthetic substrates and ideal illumination conditions (light intensities higher than 40 W m^{-2}) [21]. Our group has studied PHA production using hydrolysates from the OFMSW and lignocellulosic wastes, reaching PHA accumulations of 5% [27] and 21% [28], respectively. Several works have also studied the PHA accumulation using acidogenic fermentation effluents as substrate, achieving accumulations of 24% [24] and 31% [29]. A previous study analyzed the effect of transient OLR derived from a mixture of domestic wastewater and fermented molasses on the performance of a sequencing batch reactor in light sufficiency [28]. The OLR tested in that study varied from 0.02 to 0.74 gCOD L^{-1} d^{-1}, and the substrate concentration inside the reactor was always below 0.2 gCOD L^{-1} and was rapidly consumed during the feeding sequence. Interestingly, the maximum PHA accumulation obtained in that study (around 700 mgCOD L^{-1}) coincided with the period where the excess of the substrate was longer maintained (around 7 h). Thereby, maintaining an excess of substrate on the culture seems to favor PHA accumulation. However, the analysis of how substrate overloading affects the overall performance of a photobioreactor and how much substrate concentration a mixed culture can tolerate is lacking, and they are critical questions that must be solved to allow scale-up.

PPB mainly obtains all the necessary energy through anoxygenic photosynthesis by absorbing IR light through its photosystem [30]. This energy is most commonly used for photoheterotrophic growth on SCCA and sugars and the accumulation of carbon in PHA and phosphorus in polyphosphates, which can also serve as energy reserves or electron sinks for the regulation of intracellular redox reactions [31]. In addition, they can also accumulate carbon to glycogen [32] and some extracellular polymeric substances (EPS), such as alginate [33], and can produce hydrogen via nitrogenase as well [34]. Furthermore, it is known that competition between hydrogen production and PHA accumulation can occur in some PPB species, as both functions constitute a way to dissipate the excess reducing power [35]. Still, the distribution of carbon in enriched mixed cultures between the different polymers that PPBs can accumulate (PHA, glycogen, and alginate) and the energy distribution between the different electron sinks (Hydrogen and PHA) needs dedicated research.

Hence, this study aims to optimize the PHA accumulation in a PPB enriched mixed microbial culture. The main research questions are how the organic overloading affects the PHA production and the competence for carbon and electron allocation, and how this overloading affects the microbial community transitions in a dynamic process. Furthermore, this study has been carried out in a novel membrane photobioreactor (MPBR) using for the first time an OFMSW eluent pretreated by steam explosion and fermentation as substrate. Finally, the holistic, organic waste treatment was studied to understand the overall impact of this multi-strategy approach for OFMSW valorization via a PPB-based biorefinery.

2. Material and methods

2.1. OFMSW and inoculums

The OFMSW was obtained and previously sorted by the solid waste treatment plant Centro las Dehesas (Madrid). The organic fraction was separated at the collection point. The macro-characteristics of the

organic fraction of municipal solid waste are 135 gVS Kg^{-1} with a ratio of VS/TS of 86%. The total COD (TCOD) was 118.5 gTCOD L^{-1}, while the soluble COD (SCOD) was 2.3 gSCOD L^{-1}. In addition, it had low concentrations of Total Kjeldahl nitrogen (TKN: 2.9 gN $kgTS^{-1}$) and total phosphorus (TP: 0.4 gP $kgTS^{-1}$). The OFMSW was manually shredded, homogenized, and stored at 4 °C until further use.

The inoculum to initiate the acidogenic fermentation reactor was a highly adapted inoculum from a thermophilic acidogenic fermenter fed with a complex synthetic substrate composed of starch, sucrose, peptone, and frying oil in a mass ratio of 1:1:1:0.1 g kg^{-1}, for more than two years. The inoculum used for the MPBR was a refrigerator-stored inoculum from an MBR described in Ref. [36]. The inoculum used in the BMP tests came from a mesophilic anaerobic digester placed at a WWTP located at Mostoles, Madrid, Spain.

2.2. Experimental set-up

The main steps for the proposed OFMSW valorization process are briefly summarized in Fig. 1, and each step is explained in detail in the following sections.

2.2.1. Steam explosion pretreatment

The OFMSW was pretreated with Steam Explosion at 150 °C and 38 min [37]. We aimed at avoiding the formation of melanoidins through Maillard reactions, which usually occur rapidly at temperatures above 160 °C by the interaction of free amino acids and carbohydrates [38]. The pilot plant consisted of a steam boiler (Certuss E56, Krefeld, Germany) and a 20 L total volume hydrolysis reactor ($V_{working}$ = 10 L) connected to a flash tank (V = 100 L). The reactor load was 6 kg of waste per batch. Upon the reaction, a sudden opening of the reactor caused a steam explosion in the flash tank, and the thermally treated wastes were extracted and cooled to ambient temperature.

2.2.2. Acidogenic fermentation of the hydrolyzed waste

The hydrolysate obtained after the steam explosion directly fed the acidogenic fermenter. This process was carried out in a CSTR reactor with a total volume of 2.5 L (working volume, $V_{working}$ = 2 L). An organic loading rate (OLR) of 4 gCOD L^{-1} d^{-1} was fixed and kept for 99 days. Twenty-two days were dedicated to the start-up process. The rest was considered steady-state, and the effluent was stored for its use as a substrate in the MPBR. The thermophilic temperature (55 °C) was controlled with an external water bath connected to an external jacket of the reactor. The HRT was 5 days, and the pH was set at 5.5 with a PLC. One outlet on the headspace was connected to a flowmeter (Ritter, Germany), while the other was sealed with a rubber stopper to take biogas samples. The anaerobic state of the reaction was ensured both in the reactor and in the feed and outlet bottle. Anaerobic conditions were established in the substrate by sparging the medium with Ar and reducing it with Na_2S + $9H_2O$ (1 mL per liter). No more than 0.5% O_2 was observed in the reactor headspace during the experimental period.

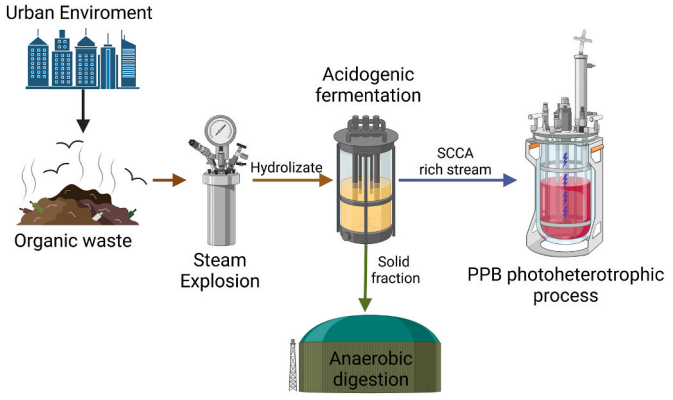

Fig. 1. Process configuration adopted in the present study.

The fermentate was stored at 4 °C for no more than one week. Before use, it was centrifuged for 10 min at 4000 rpm to separate the solid phase from the liquid phase. Then, the solid phase was derived to a biochemical methane potential (BMP) test to determine the methanogenic potential of this secondary stream, and the liquid phase was fed to the MPBR, as shown in Fig. 1.

2.2.3. The phototrophic process with PPB

The liquid fraction resulting from the fermenter effluent was treated in an MPBR for study in a photoheterotrophic process with a mixed culture of PPB. The scheme of the MPBR is shown in the supplementary material (Fig. S1). It consisted of a 2.5 L reactor ($V_{working}$ = 2 L) with a submerged LED lamp emitting at 805 nm (Idea Bioprocess technology, Italy) that provided a volumetric irradiance of 2.2 W L^{-1}. A submerged hollow fiber membrane (Zena s.r.o, Czech Republic) was also used to discharge the filtered output. A pH-meter with a control system was used to maintain the pH above 6.5 by dosing KOH (0.1 M). The reactor head had two gas outlets, one sealed with a rubber stopper dedicated to taking biogas samples and the other connected to a flowmeter (Ritter, Germany) to measure the volume of gas produced.

The MPBR head was opened every other day to clean the submerged LED lamp to avoid biofilm formation. Each time this happened, the reactor headspace was purged with Ar to ensure anaerobic conditions. It was also ensured that the substrate did not degrade by keeping the feed bottle in a cooler at 4 °C. During the first 5 days of operation, the MPBR was fed with a synthetic substrate consisting of HAc:HPr:HBu:EtOH on a 1:1:1:1 COD basis and nutrients from the Ormerod growth medium described elsewhere [39] for acclimatization. Afterward, the liquid fraction of the fermenter effluent was fed, and the operating conditions were varied in 7 stages, as depicted in Table 1, which can be classified 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). Due to extreme weather conditions (snowstorm), access to the laboratory was not possible, and thus the reactor was not sampled from day 54–60. The biomass yield is calculated as the COD equivalent of the biomass in the culture (a theoretical 1.75 gCOD $gVSS^{-1}$ is taken as a reference) divided by the COD consumed.

2.2.4. BMP test

Anaerobic digestion performance of the solid fraction obtained after centrifugation of the acidogenic fermenter output was carried out through standard biochemical methane potential (BMP) tests [40]. In brief, the tests were performed by triplicate, yielding $mLCH_4$ gVS^{-1}, in 160 mL serum bottles at mesophilic conditions (37 ± 0.5 °C). The organic substrate used was the solid fraction after centrifugation of the fermentate. An inoculum to substrate (I/S) ratio of 2:1 (as VS) and an initial concentration of 10 gVS L^{-1} were set up. A triplicate control of the inoculum was used to subtract the methane production coming from its endogenous digestion.

2.3. High-throughput 16S rRNA gene sequencing

For the DNA extraction and microbial community analysis by PacBio sequencing, representative samples of the MPBR were taken twice a week to analyze the development of the bacterial communities, ensuring that each phase studied had at least three samples each, except for the S0 acclimation phase, where only one sample was collected. Samples were stored at −4 °C until use. Total DNA was extracted using a Microbial DNA isolation kit (Soil DNA Isolation Kit, CANVAX, Cordoba, Spain) to perform the molecular identification of prokaryotes and frozen to −20 °C for 4 days until use. The quantification, amplification (PCR), 16S rRNA gene measurements, and the amplicon taxonomic annotation and comparative analysis were outsourced to Instituto ISABIAL-FISABIO, Hospital General Universitario de Alicante, Alicante, Spain. SMRT

Table 1
Description of the operational stages of the membrane photobioreactor.

Parameters	Stage 0	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	Stage 6
Substrate	Synthetic	Liquid fraction of the fermentate					
Time (days)	5	18	15	15	15	10	11
HRT (days)	2	2	2	2	2	2	2
OLR (gCOD L^{-1} d^{-1})	1	1	1	3	1	1	3
SRT (days)	4	4	6	4	4	6	6

sequencing was performed on a PacBio RS II instrument. A more detailed description can be found in the Supplementary Material.

2.3.1. Statistical analysis

The evolution of the bacterial composition determined by PacBIO was used to perform a principal component analysis (PCA) to explore the variation of the communities over time. In addition, distance-based redundancy (RDA) was conducted to reveal potential associations and multivariate relationships between environmental factors (OLR, sludge retention time (SRT), PHA, Glycogen, volatile suspended solids (VSS), and hydrogen production) and the relative abundance of phylotypes at the genus level. Both PCA and RDA were carried out using the "Vegan" Package in RStudio (R Development Core Team, 2022.02.0 version). Relationships between samples, bacterial communities, and environmental parameters were examined using multiple linear regression analysis to rule out linear variables (>95%).

2.4. Analytical procedures

TS, VS, total suspended solids (TSS), volatile suspended solids (VSS), chemical oxygen demand (COD), ammonium, phosphate, total Kjeldahl nitrogen (TKN), NH_4^+, PO_4^{3-} were determined according to Standard Methods [41]. Liquid samples were filtered through a cellulose-ester filter of 0.45 μm of pore size (Advantech, Japan). The SCOD is considered to be the COD measured in the liquid fraction after filtration. TCOD is the COD measured before any filtration. SCCA and monosaccharides are analyzed using an ion-exclusion RazexTM ROA-Organic Acid H^+ HPLC column (Phenomenex, USA), coupled to a refractive index detector (Agilent, USA) and operated at 65 °C and 1 mL min^{-1}, with 0.005 M H_2SO_4 as the eluent. Gas samples were quantified using the 7820A gas chromatography (GC-TCD) system (Agilent Technologies, Santa Clara, CA, USA). The mobile phase is Argon at a 5 mL min^{-1} flow rate. The temperature of the oven and the detectors are 45 and 220 °C, respectively.

Samples were taken directly from the reactor using a purge valve to quantify PHA, glycogen, and EPS. Immediately after extraction, the biomass was fixed with 0.2% (by volume) formaldehyde and freeze-dried overnight. Commercial standards for PHB, PHV, and PHH (Sigma-Aldrich, USA) were used to perform the calibration curves. The methodology used for the digestion, extraction, identification (through GC-MS), and quantification (through GC-FID) of PHA can be found in the Supplementary Material. For the measurement of glycogen, a method was adapted from Ref. [42]. In brief, after overnight lyophilization, 1–2 mg of dry biomass are weighed, digested, supplemented with 2 mL of 0.9 M HCl, and dissolved in a thermoblock for 3 h at 100 °C. Then, they are quickly cooled on ice and filtered with a 0.45 cellulose-ester filter. Finally, glycogen was quantified by HPLC measurement using the same method described for SCCA quantification (see Supporting Information). The calibration curve was performed with standard glycogen from oysters (Sigma-Aldrich, USA). EPS quantification strictly followed the method explained in Ref. [43] (see Supplementary Material).

3. Results

3.1. Steam explosion and acidogenic fermentation pretreatments

The steam explosion disruption apparatus and experimental conditions provided reproducible conditions between the different batches performed. Fig. 2 shows the release of soluble organic matter with a final result of 40% soluble COD on average. Considering that the soluble COD before the steam explosion was 12%, there is an increase in soluble COD of 43%. An average of 7% SCCA is measured, mainly Hac, HBu, and HPr. The majority of soluble COD is unidentified organic matter (33%), while the remainder is particulate COD. This pretreatment reproducibly solubilizes a substantial percentage of the COD present in the initial waste. This solubilization is essential to reduce hydrolysis, a limiting factor in most biological processes, including acidogenic fermentation.

A thermophilic acidogenic fermentation was carried out for nearly 100 days as a complementary pretreatment. This process significantly increases the soluble COD, especially the percentage of SCCA produced, as shown in Fig. 2. Soluble COD increased by 82.5%, up to 74% of the total COD. 65.6% of all COD was transformed into SCCA, representing acidification of 90%. HAc and HBu are the two major acids produced, accounting for 63% of the COD equivalent of all SCCAs. Also, Fig. 3 shows the operation profile of the fermenter. Fig. 3a shows a stabilized yield of about 0.66 $gCOD_{SCCA}$ $gCOD_{feed}^{-1}$. The combination of both pretreatments on the OFMSW for the production of SCCA achieves high yields and high stability over time. In addition, hydrogen is co-produced at high yields during the acidogenic fermentation process.

Fig. 3b shows the hydrogen production and VSS amount present in

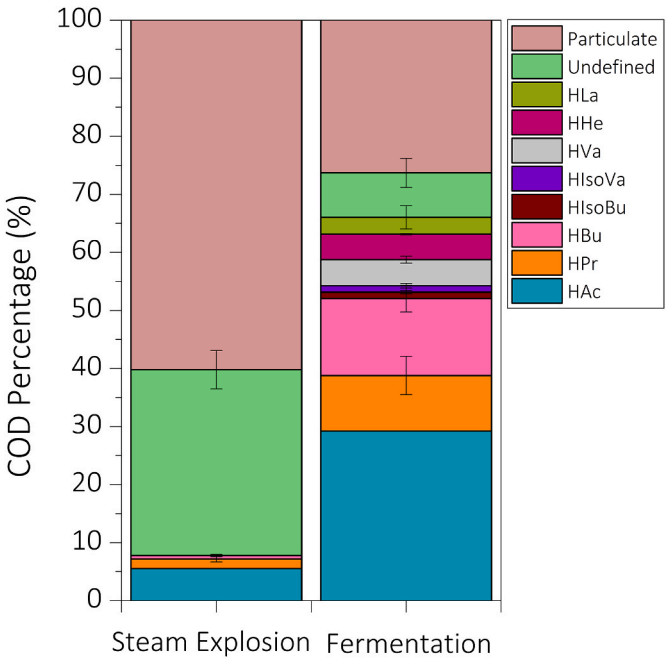

Fig. 2. Performance of the steam explosion (n = 4) and subsequent acidogenic fermentation (average of 70 days) in terms of COD mass balance. Average values with 95% confidence intervals.

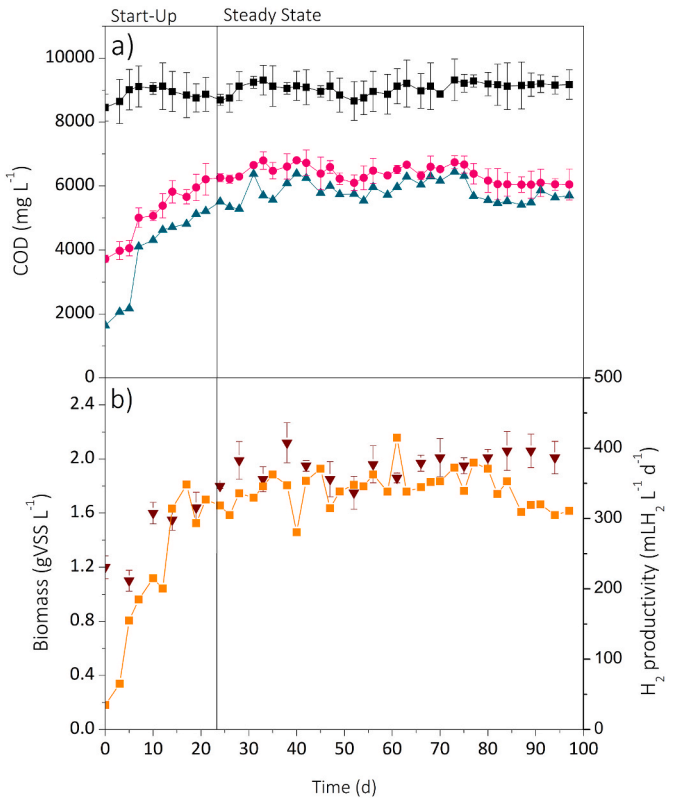

Fig. 3. Acidogenic fermentation reactor operation profile for 98 d. a) Shows TCOD (■), SCOD (●), and COD equivalent of all SCCA (▲). b) shows H_2 productivity (■) and biomass (▼) within the reactor. Error bars are 95% confidence intervals.

the fermenter. The stabilization of both parameters is similar to the COD and hydrogen, which could serve as an online indicator of fermentation performance. Average hydrogen productivity was 345 $mLH_2\ L^{-1}\ d^{-1}$, with a hydrogen percentage of 57% (43% CO_2), and biomass stabilized near 1.8 $gVSS\ L^{-1}$. Based on the total COD balance, hydrogen production never exceeded 3% of the total COD equivalent, confirming that it is a high-value co-product but represents a small percentage of the total organic matter in the OFMSW valorization.

3.2. The photoheterotrophic treatment of the liquid fraction with PPB

3.2.1. Start-up (S0)

The capacity of a mixed culture enriched in PPBs to accumulate PHA has been evaluated at different stages. Fig. 4 shows the main results. During the first 5 days (Stage 0), the culture underwent an adaptation period to promote PPB's growth using a synthetic substrate. The biomass went through an exponential growth phase from the second to the third day and increased to 1.5 $gVSS\ L^{-1}$, with COD consumption reaching 85%. Correct acclimatization is confirmed by a bacterial community study with a sample from day 4 (D4). Fig. 5 shows a culture highly enriched in PPBs (>80%). The most prominent genus was *Rhodopseudomonas* sp. with more than 60% relative abundance, followed by *Rhodobacter* sp. with 15%. *Rhodovibrio* sp., *Rhodopila* sp., and *Rubrivivax* sp. were also identified with abundances lower than 1.2%. Glycogen was not measured in the first 4 days of acclimatization, and the average percentage of PHA accumulation was 5% in dry mass, mainly composed of PHB monomer (98% PHB and 2% PHV). From the beginning of the operation, NH_4^+ consumption is complete (Fig. S4), but no hydrogen production is detected. The acclimatization stage was considered finished on day 5 when COD removal efficiency stabilized and hydrogen production began. Then, the liquid fraction of the fermentate was fed as a substrate.

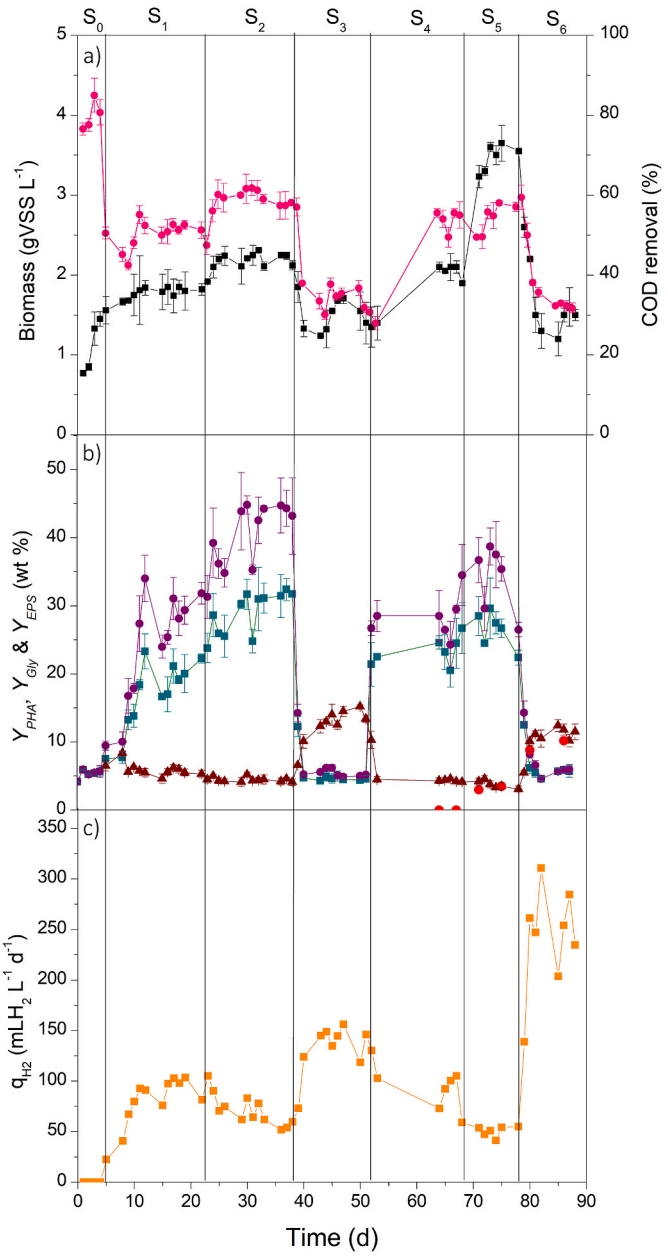

Fig. 4. Membrane photobioreactor operation profile for 88 d. Panel a) shows biomass (■) and COD removal (●). Panel b) shows PHB + PHV percentage (■), total percentage of PHA (PHB + PHV + PHH) (●), glycogen percentage (▲) and EPS percentage (●). Panel c) shows hydrogen productivity (■). Error bars are 95% confidence intervals.

3.2.2. Operation under stable biomass growth (S1, S2)

Stable biomass growth and high PHA (up to 34% dry weight) accumulations were obtained in stages 1 and 2. The low organic load and a substrate composed mainly of SCCA but with limited nutrients first favored the growth of biomass and PHA accumulation, as shown in Fig. 4. In Stage 1, COD consumption decreased compared to Stage 0, to an average of 50%. However, biomass continues to grow to an average of 1.7 $gVSS\ L^{-1}$. This COD consumption was limited by the availability of nutrients (as shown in Supplementary Material, Fig. S4). As a result, PHA accumulation increased significantly, and then it stabilized at around 31% dry weight during the following 12 days. This PHA comprised PHB, PHV (20% and 5% by dry weight), and PHH that was

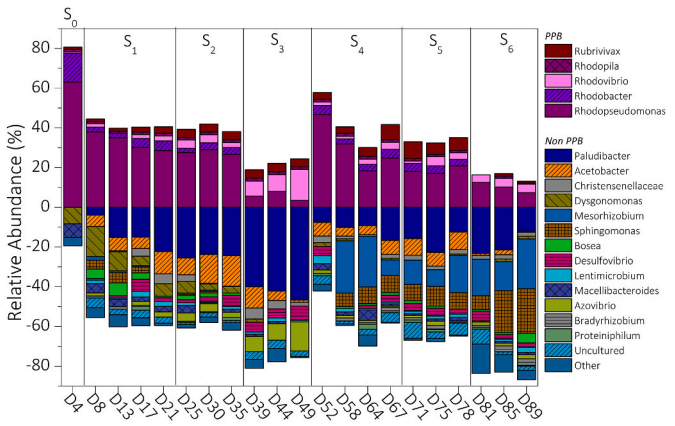

Fig. 5. Evolution of the microbial community structure of the membrane photobioreactor at the genus level during the different operative stages.

also detected, accounting for 6% by dry weight. Average production of 6% glycogen was also observed in a very stable pattern. Interestingly, hydrogen evolved with a similar pattern to that of PHA.

In Stage 2, the SRT increased the sludge age, thus keeping the biomass longer in the reactor. In this Stage, the biomass concentration slightly increased to 2.2 gVSS L^{-1} while the COD consumption rose to 60%. PHA accumulation took 4 days to stabilize at an average of 44%, with PHH being about 30% of total PHA by weight. Glycogen accumulation slightly dropped to 5% by weight. Hydrogen, unlike Stage 1, seems to have an inverse relationship with PHA accumulation. As expected, upon adding the liquid fraction of the fermentate, the pressure of cross-contamination on the culture increased, and the relative abundance of PPB dropped to just over 40%, with *Rhodopseudomonas* sp. being still the predominant genus (Fig. 5). In both Stages, a stable PPB-enriched culture was achieved, with high PHA accumulations and hydrogen yields, with inverse trends. Given these promising results, the organic load was increased to examine if the productivity of PHA can be further improved.

3.2.3. First carbon overload (S3)

In Stage 3, the OLR was increased from 1 to 3 gCOD L^{-1} d^{-1} to analyze the culture behavior under an overload episode. The culture immediately fails to accumulate PHA. However, glycogen accumulation and hydrogen production increased. As shown in Fig. 4, biomass collapsed, reducing to 1.3 gVSS L^{-1}, although after 5 days, it stabilized at 1.6 gVSS L^{-1}. The COD consumption fell to 36%, though the limitation of COD consumption was not driven by nutrient availability, as both NH_4^+ and PO_4^+ were not 100% consumed, as shown in Table 2. The most drastic reduction was PHA accumulation, reduced to 5%. This minimal accumulation remained stable and did not increase during the 14-day phase. As PHA accumulation dropped, glycogen increased to 12%, and hydrogen production doubled. In addition, a decrease in PPBs to less than 20% was observed, with *Rhodovibrio* sp. unusually increasing to 15% (see Fig. S4). The next step would be to recover the biomass and a more PPB-enriched culture, thereby studying whether PHA accumulation is regained.

Table 2
MBPR performance parameters on each phase. Average ± standard deviation.

Parameters	S1	S2	S3	S4	S5	S6
$Y_{x/s}$ (gCOD $gCOD^{-1}$)	0.83 ± 0.07	0.97 ± 0.05	0.62 ± 0.08	0.86 ± 0.25	0.94 ± 0.02	0.62 ± 0.07
$-q_S$ (mgCOD L^{-1} d^{-1})	541 ± 45	638 ± 32	677 ± 62	688 ± 28	1055 ± 66	665 ± 49
q_{PHA} (gPHA L^{-1} d^{-1})	0.22 ± 0.05	0.45 ± 0.05	0.05 ± 0.02	0.28 ± 0.02	0.61 ± 0.06	0.06 ± 0.04
q_{H2} (mlH_2 L^{-1} d^{-1})	88 ± 12	71 ± 15	140 ± 25	83 ± 26	51 ± 5	256 ± 34
q_{GLY} (gGLY L^{-1} d^{-1})	0.10 ± 0.03	0.09 ± 0.01	0.19 ± 0.04	0.09 ± 0.00	0.13 ± 0.01	0.17 ± 0.02
NH_4^+ comsuption (%)	100 ± 1	100 ± 1	94 ± 2	100 ± 2	100 ± 1	93 ± 1
PO_4^+ comsuption (%)	81 ± 9	85 ± 7	71 ± 3	84 ± 2	68 ± 7	60 ± 4

3.2.4. Recovery of the biomass (S4, S5)

The organic load and other parameters were reduced to mimic those studied in Stage 1. Subsequently, the culture gradually recovered its performance, demonstrating the resilience of this technology. In Stage 4, biomass stabilized at 1.8 gVSS L^{-1}, and COD consumption rose to 55%. The PHA accumulation was recovered, achieving 28% dry weight (5% PHH), and glycogen again lowered to 5%. The hydrogen production trend was also negative, decreasing its productivity. In Stage 5, the SRT was increased and followed the same trend as Stage 2, increasing the biomass concentration considerably up to a maximum of 3.6 gVSS L^{-1} and slightly the PHA concentration. In both Stages, the percentage of PHH is slightly reduced compared to Stage 3, with an average of 25% of the total PHA dry mass. Glycogen accumulation and hydrogen production follow decreasing trends, as in Stage 4. Since Stage 3 showed slight granulation in the culture (see Fig. S6), EPS were measured to see if it was producing them. At Stage 4, the culture did not show as much granulation, and no EPS was detected. However, at Stage 5, EPS reached 5% dry mass. As for the communities, up to 55% of PPB were recovered in Stage 4 (Fig. 5) but stabilized at around 40% in Stage 5. The only difference was a higher abundance of *Rubrivivax* sp. In short, biomass could be restored, significantly increasing its concentration and PHA accumulation, while glycogen accumulation and hydrogen production decreased again. We proceeded again to perform an organic overload to see if the tendencies observed were reproducible.

3.2.5. Second carbon overload (S6)

Similar to Stage 3, the organic overload of Stage 6 destabilized the culture, reducing biomass concentration, COD consumption, and PHA accumulation. On the other hand, glycogen accumulation and hydrogen production had the opposite tendency to achieve maximum hydrogen productivity at 310 mL L^{-1} d^{-1}. The culture showed evident granulation, and the EPS accumulation increased to more than 10% dry weight. PPB abundance was again reduced to less than 20%, confirming that an organic overload destabilizes the PPB-enriched culture. The trends observed in Stage 3 are repeated in Stage 6, confirming the reproducibility of the results and the culture's behavior under organic overload.

The trends observed at each Stage depicted in Fig. 4 are also corroborated when we observe the performance parameters in Table 2. The highest PHA productivities occurred at Stages 2 and 5, while higher glycogen and hydrogen productivities occurred at Stages 3 and 6, matching the organic overload. Biomass yields in the best performing Stages (2 and 5) of 0.97 and 0.94 gCOD $gCOD^{-1}$ were obtained, respectively. It is assumed that when the biomass yield drops, the non-consumed COD is being diverted to hydrogen production. This fact agrees with the experimental data where we see the highest hydrogen yields and the lowest biomass yields at the same Stages. A maximum q_s of 1 gCOD L^{-1} d^{-1} was achieved on Stage 5, but, interestingly, this parameter was not significantly reduced in the organic-overloaded Stages. As discussed in previous sections, NH_4^+ is 100% consumed in Stages 1, 2, 4, and 5. Although it was not wholly consumed in Stages 3 and 6, its assimilation was still very high, up to 94 and 93%, respectively. However, PO_4^+ was not fully consumed in any Stage, with Stages 5 and 6 having the lowest consumption, down to 60%. It was again confirmed that PHA is inversely related to glycogen accumulation and hydrogen production. The relationship of the environmental variables to the transitions in the microbial communities was examined in more depth.

3.3. Statistical analysis of the evolution of microbial community profiles

The development of the microbial communities was evaluated by PCA ordination based on Hellinger transformed rarefied abundance and shown in Fig. 6. This analysis showed an apparent clustering of the samples according to their Stage and temporal progression. The major statistical difference occurred in Stages 1, 3, and 6. It was due to a start-up in which the feed was synthetic, and the culture was highly enriched in PPB. Inversely, in Stages 3 and 6, an organic overload produced the opposite effect. In terms of genera, we can observe a relationship between *Rhodopseudomonas* sp., *Rhodobacter* sp., and *Dsyngomonas* sp., which had more significant weight in the first three Stages. *Rhodophila* sp. and *Rhodovibrio* sp. had a greater weight in Stages 2 and 5, respectively. *Paludibacter* sp. and *Dsulfovrio* sp. were correlated and had the highest weight in the first organic overload (Stage 3). Also, they had a higher weight in the second one (Stage 6), which could mean cross-contamination *Sphingomonas* sp. and *Mesorhizobium* sp. (see Fig. S3). The clustering of the Stages and how most PPB genera were negatively related to organic overloads were confirmed. To further analyze these results, an RDA was carried out to assess the contributions of environmental variables to variances in the microbial communities.

RDA analysis, shown in Fig. 7, confirms the relationship of PPB genera (*Rhodopseudomonas* sp., *Rhodobacter* sp., *Rhodopila* sp., and *Rubrivivivax* sp) with PHA accumulation and SRT and its inverse relationship with organic load (OLR). Also, *Acetobacter* sp. is highly related to PHA accumulation. Furthermore, the inverse relationship between PHA, glycogen, and hydrogen is again evident. A positive relationship is also observed between higher biomass concentration (VSS) and *Paludibacter* sp. and *Rhodovibrio* sp. To find more evident trends, another RDA analyzed only the statistical weight of time and OLR on the composition of the bacterial communities. The PPB *Rhodopseudomonas* sp., *Rhodobacter* sp., and *Rhodopila* sp., as well as *Acetobacter* sp. and *Dsyngomonas* sp., are not affected by the time or OLR (see Fig. S5 in Supporting Information). However, we can see that *Mesorhizobium* sp. and *Sphingomonas* sp. are positively related, indicating a possible development of contamination from the acidogenic fermenter developed over time. In a similar case, the PPBs that are gaining ground with time are *Rubrivivax* sp. and *Rhodovibrio* sp., but also *Paludibacter* sp., associated with time and higher organic loads. Finally, fermentative genera had a great weight on hydrogen production. Therefore, PHA and SRT are inversely related to hydrogen, glycogen, and OLR production, with different fermentative genera having the most weight for the latter environmental factors.

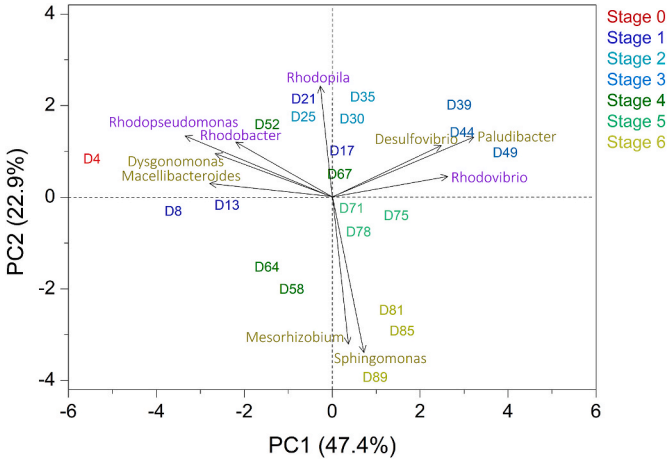

Fig. 6. PCA plot of the microbial communities. The samples are shown on day code (D4: day 4) and colored according to the Stage. The names indicate the 10 genera that contribute the most weight to PC1 and PC2, colored according to PPB (purple) or another genus (yellow). The arrows indicate the weight of each genus in each PC.

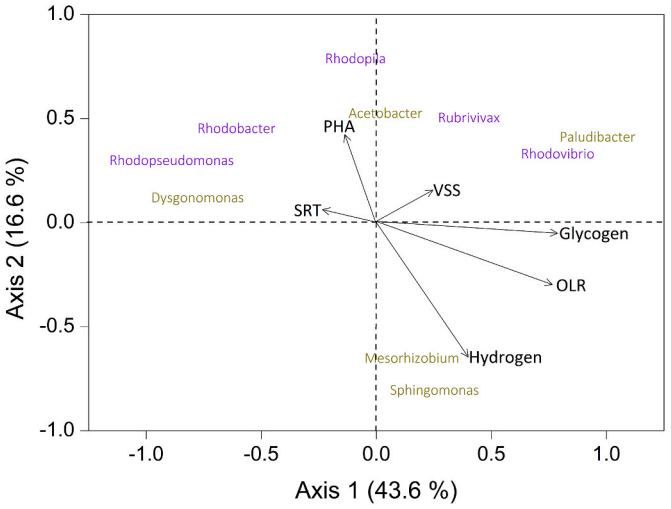

Fig. 7. Redundancy discriminate analysis (RDA) ordination diagram of bacteria related to environmental variables. The nodes show the 10 genera with the most weight on both axis (PPB in purple, other genera in yellow), and the arrows indicate the weight of each environmental variable.

4. Discussion

The results obtained in the combination of steam explosion and acidogenic fermentation pretreatments (0.66 $gCOD_{SCCA}$ $gCOD_{feed}^{-1}$) can be considered a very efficient step in the PHA production pathway. This result is slightly lower than that obtained recently in acidogenic fermentation of fruit residue, where a yield of 0.74 $gCOD_{SCCA}$ $gCOD_{feed}^{-1}$ was achieved [20]. Another work showed fermentation of OFMSW to obtain an overall yield of 0.65 ± 0.04 $gCOD_{VFA}$ $gVS_{(0)}^{-1}$ and acidification of 86% [44]. In our work, we obtained slightly higher acidification (90%), which we know from previous work is essential when working with PPB since the high presence of sugars in the substrate can negatively affect the accumulation of PHA [29]. The remainder of the undefined COD could be oligo- and monosaccharides derived from hemicellulose, lipids, proteins, complex carbohydrates, and amino acids [45]. Within the SCCAs produced, acetic acid corresponded to 43.4% of the COD-equivalent. Previous studies determined that it is the most suitable substrate for PHA production with PPB [46]. The second SSCA was butyric acid, corresponding to 25% of the COD-equivalent of SCCAs. Propionic, valeric, and hexanoic acids represented 17, 7, and 7%, respectively. It is well-known that the mixing of acids favors the production of copolymers; for example, propionic and valeric acids are oxidized to propionyl-CoA, then to β-hydroxyvalerate (PHV) to produce PHV [21]. Moreover, although it has not yet been demonstrated in a PPB mixed culture before, it has recently been discovered that the presence of hexanoic acid promotes the accumulation of PHH in *Rhodospirillum rubrum* [47]. If these results are maintained in the process scale-up, the SCCA productivity and the fast reaching of a steady-state, reproducibility, and the process stability, highlight the practicality and high suitability of these pretreatments for the production of PHA with PPB.

The PPB-enriched mixed culture accumulated high percentages of PHA by assimilating fermented effluent from OFMSW feed. As shown in Fig. 4, the maximum percentage of PHA accumulation achieved was 42%, which is the maximum achieved with mixed cultures of PPB using residual substrates [48], the closest result being the treatment of fermented domestic wastewater, where 30.8% was achieved [29]. Fig. 7 shows an unequivocal relationship between the abundance of PPB genera and PHA production. *Rhodopseudomonas* sp. is the most abundant PPB genus in all Stages except S3, reaching up to 90% of the total, and coincides with the most abundant genus in other studies performed in

our laboratory [36]. In other studies, the bacterial communities fluctuate in their percentage of PPB, for example, from the dominance of *Rhodopseudomonas* to *Rhodobacter* [49]. Although *Rhodobacter* sp. is the second most abundant PPB genus identified in our study, *Rhodopseudomonas* sp. always remained predominant among PPB. *Rubrivivax* sp. is the PPB genus with the highest reported PHA accumulation, with a percentage of 85% by weight in pure cultures [48], which is coincident with the high PHA accumulations in Stage 5. In addition, we can see a recurrent closeness between these PPB and the genera of *Dsyngomonas* sp. and *Acetobacter* sp., which may indicate symbiotic processes. Acetobacter, for example, can oxidize short-chain acids and ethanol to acetic acid [49], and PPBs can assimilate this substrate to accumulate PHA faster. A more comprehensive metaproteomics study could help to clarify these symbiotic processes in enriched mixed cultures since it is essential to know which PPB species have the highest capacity to accumulate PHA to improve this process. Among the different PHA monomers produced, PHB is the predominant one, but other less common ones, such as PHH, reach relevant percentages.

Most PHA accumulation studies with PPB only considered PHB and PHV accumulation. In our work, we found, in addition to PHB and PHV, the PHH in all stages except Stage 0. The rest of the Stages accumulated between 2% and 30% of the total PHA in dry mass. It was demonstrated that PHH accumulates when hexanoic acid is present in the medium, assimilates via β-oxidation, and incorporates into PHA chains [50]. In addition, the presence of hexanoic acid increases the accumulation of PHB by boosting the production of intermediates such as acetyl-CoA and butyryl -CoA [47]. The acetyl-CoA and butyryl-CoA produced as intermediates and end products are then assimilated using anaplerotic pathways, e.g., the ethylmalonyl-CoA and the methylbutanoyl-CoA synthesis and degradation pathways [51]. In Stage 0, PHH was not detected because the synthetic substrate did not contain hexanoic acid. In the rest of the Stages, PHH had similar behavior to the rest of the PHA. Only two papers reported a PHH production by phototrophic bacteria, one where C6 and C7 monomers were detected in *Rhodospirillum rubrum* [52] and another where PHH was detected in a mixed PPB culture [52]. In addition to the PHH, we detected another peak in the GC, and, considering its retention time, we assumed that it could be a 3-carbon monomer forming polyhydroxypropionate (PHP). This assumption was furtherly verified on GC-MS as explained in the supplementary material (Fig. S2). Both PHH and PHP, which are generally not accounted for, indicate that PHA accumulation is usually underestimated with mixed PPB cultures, increasing the interest in the technology.

One of the significant limitations of advancing this technology is the low productivity obtained so far. In this study, PHA productivity reached very high values (q_{PHA} up to 0.61 gPHA L^{-1} d^{-1}) compared to previous works. The highest data obtained to date in mixed cultures of PPB is 0.77 gPHA L^{-1} d^{-1} [21], but being fed with acetic acid. In a recent study by the same group using fermented wastewater, the highest productivity obtained with a permanent feeding regime was 0.23 gPHA L^{-1} d^{-1} [29]. Although the results presented in this work are promising, they are still far from the productivities obtained with aerobic cultures, as they report average productivities of 5–10 g L^{-1} d^{-1} [53]. The primary constraint is biomass growth, in this work, limited by nutrients. However, the higher the biomass concentration, the more this technology is limited by the volumetric irradiance in the reactor. In this work, a submerged LED lamp with a very low volumetric irradiance (2.1 W L^{-1} or 5 W m^{-2}) was used when most studies are usually above 30 W m^{-2} [31]. Using the biomass productivity of Stage 5 (1.8 gVSS L^{-1} d^{-1}), this would result in a biomass energy yield of 61 gCOD kWh^{-1}, which is slightly higher than the highest recorded so far of 59 gCOD kWh^{-1} [53], but still does not allow to obtain a process energetically more favorable than an aerobic process. New lighting systems and reactor configurations (especially systems to retain solids) should be investigated to produce more concentrated biomass. Another critical element is increasing PHA accumulation and understanding what other components, carbon, electrons, or both, can be diverted when PHA accumulation ceases.

The organic load's increase in the MPBR led to the destabilization of the culture and the collapse of PHA accumulation (Stages 3 and 6, Fig. 4). An increase in glycogen accumulation and hydrogen production was detected in both Stages. This evidences the competition between carbon allocation to growth and accumulation of PHA and glycogen, with electrons allocation between growth, PHA accumulation, and hydrogen production, as outlined schematically in Fig. 8. Glycogen accumulation by PPBs has been known for a long time. Still, only one study has examined the relationship between PHB and glycogen production [32], showing that PHB is an electron sink that works as an intracellular reserve for reducing power. However, it rapidly decreases when biomass growth is reduced, thus diverting carbon storage to glycogen accumulation. A variety of metabolic principles are linked to glycogen metabolism: the maintenance of (i) photosynthetic efficiency in light, (ii) viability in periods of starvation, such as in darkness or macronutrient depletion, and (iii) the acclimation to macronutrients deficiency [54]. It is noteworthy that after Stage 3 and the lowering of OLR, there was a rapid recovery of the culture, demonstrating that the PHA production is stable and resistant to severe organic stress, and although the community within the PPB mixed culture may change, its functionality remains consistent. However, once PHA is no longer available as an electron sink, hydrogen production appears as a possibility for the balance of reducing equivalents.

The competition between hydrogen production and PHA accumulation in *Rhodobacter sphaeroides* and *Rhodospirillum rubrum* strains has been observed [55], and the conversion of PHB accumulated in *Rhodovalum sulfidophilum* to hydrogen in the absence of other substrate was demonstrated [56]. In our experiment, this inversely proportional relationship is evident in Fig. 7. However, the mechanisms of electron transfer and how both metabolic pathways compete for electrons allocation are poorly understood. While the decreasing trend in PHA and increase in glycogen accumulation and hydrogen production is observed at Stages 3 and 6, biomass granulation was also evident, whereby at Stages 4, 5, and 6, the accumulation of EPS was measured. During organic overload of these stages, PPBs lower their cellular efficiency and divert excess electrons to EPS and hydrogen. Another hypothesis for the increased hydrogen production is that EPS, which are exopolymers composed mainly of carbohydrates, might be optimal substrates for the growth of fermentative bacteria. As explained in previous sections, these bacteria can come from cross-contamination between bioreactors, increasing their biomass over time and producing more hydrogen. In addition, the biomass's COD and the cell yield decreased considerably, which is linked to fermentative processes. Fermentative bacteria produce more oxidized compounds for PPBs, which can assimilate them for growth, causing a symbiotic relationship already evidenced in Figs. 6 and 7. The strategy of achieving a granular culture of PPB first and then deriving its metabolism to PHA production could lead to considerable cost savings downstream of the process, as granular sludge can decrease the Operational Expenditure (OPEX) of the process by up to 50%, which has been reported for other anaerobic bacteria [57].

The granulation capacity of PPB has been studied recently, and EPS accumulations of up to 35% in dry mass were observed [33]. At Stage 6, a 10% dry mass of EPS was reached. EPS are primarily composed of carbohydrates, proteins, and lesser amounts of other components and have multiple roles such as flotation and locomotion, feeding, protection against desiccation/UV/pollution, development of biofilms, and communication [58]. These compounds are widely employed in the cosmetic, pharmaceutical, and petroleum industries [59]. In the work where PPB granulation is analyzed, the relative abundance obtained is between 40 and 70% [33], slightly higher than obtained in this work, where the range moves from 22 to 80%. The relationship between PHA and EPS in PPB has not been studied so far, but the negative correlation between EPS and PHA was evidenced in other microorganisms [60], as shown in Fig. 4 of our study. However, it is known that the EPS confer protective effects upon the cell, allowing microorganisms to grow in attached mode and improving the settling. Although a study showed

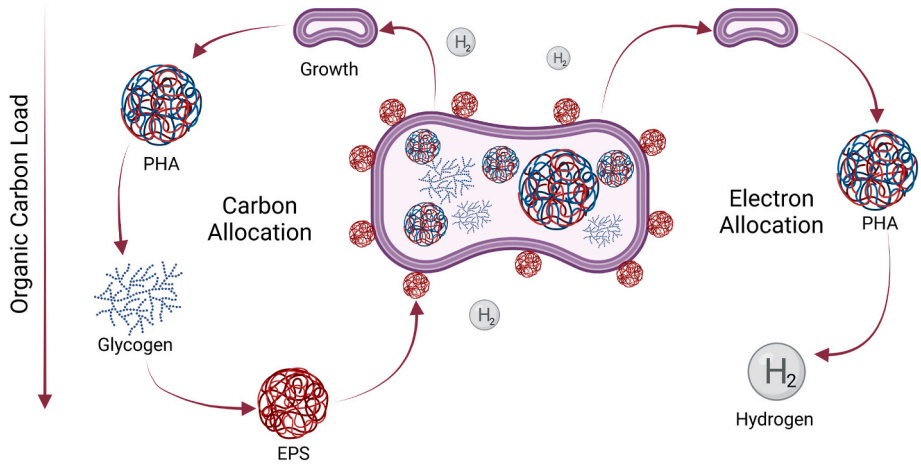

Fig. 8. Schematic description of the different biopolymers for carbon storage and consequent electron allocation between PHA, glycogen, EPS, and hydrogen, related to increasing organic load stress.

that the settling cohesion was enhanced and stabilized by purple phototrophic bacteria [61], little is known about the stabilization and sedimentation in PPB cultures, even less with high EPS content. Considering that the downstream processing, including settling and biomass extraction, is a bottleneck in PHA production [62], the granulation of mixed PPB cultures and the simultaneous production of PHA and EPS can reduce costs in the scale-up of the technology [63] and opens up exciting research opportunities for the future.

4.1. Implications for a future photo-biorefinery

Cascading biorefineries for waste treatment are indispensable for a sustainable future because they can turn a problem into an opportunity. The innovative biorefinery platform herby proposed integrates a steam explosion pretreatment that can significantly increase the fraction of fermentable organic carbon from the OFMSW with an acidogenic fermentation that produces high yields of SCCA, which are the perfect substrate for PHA accumulation in a PPB-enriched culture. This biorefinery is completed with the anaerobic digestion of the solid fraction obtained after fermentation, yielding 336 LCH_4 $kgVS^{-1}$ (see Fig. S7 in Supporting Information). This additional energy source would close the carbon and energy cycle of the biorefinery. As for the COD removed, more than 40% of the COD is not assimilated, mainly due to nutrient limitations. This residual COD flow from the MPBR could be combined with the final digestate from anaerobic digestion, a high nutrient side stream, in a possible second phototrophic reactor with PPB dedicated to protein production. This technology has already been tested several times [64,65]. Nonetheless, the biggest challenge of this technology is the industrial scalability of the MBPR.

The industrial production of PHA is currently carried out by pure aerobic cultures based on sugars or other similar substrates. Comparatively, the production of PHA with mixed PPB cultures has some advantages: (i) no need for sterilization of equipment, (ii) they can use a wide variety of waste as substrate, eliminating a large part of the production costs, (iii) they do not need aeration, (iv) higher yields of PHA are possible, and (v) they can accumulate and produce PHA in the same reactor by IR illumination, eliminating the requirement for sorting and an accumulation reactor. An intermediate approach is aerobic mixed cultures, which are already studied at a pilot plant scale [19,20]. This technology's best performance is 0.45 $gCOD_{PHA}$ $gCOD_{Feed}$ [20]. A preliminary mass balance of the overall novel photobiorefinery proposed here shows a total yield of 0.15 $gCOD_{PHA}$ $gCOD_{Feed}$. Moreover, although this is the highest biomass energy yield seen so far, as discussed in previous sections, for the time being, the production of PHA with PPBs and artificial illumination is considered to be economically unfeasible [53], the most logical solution being the scaling of the technology using the irradiance of the sun. Scaling up outdoors involves many uncertainties, such as contamination by other microorganisms, response to non-fully anaerobic systems, and intermittent solar illumination. These problems could be solved by sunlight collection and filtration systems [31]. Operational strategies adaptable to the different seasons of the year and the feedstock arriving in the biorefinery is a crucial point for its development.

The possibility of producing different products seasonally (hydrogen, glycogen, EPS) and PHA can provide the system with more economic resilience. This study demonstrates that if the reactor is operated at a higher organic load, the carbon accumulation is redirected to glycogen, or even granulation can occur in the culture due to stress and derived to EPS production. This links to higher hydrogen productivities through electron allocation in PPB metabolism or synergies generated with fermentative bacteria. In addition, this work opens the way for the production of longer chain biopolymers that could diversify their industrial applications, considering that the PHB-PHV-PHH polymers present rubber-like elastomeric properties and can therefore be used in a different set of applications than a polymer composed solely of PHB and PHV [12]. Thus, it would still be essential for the scale-up of this technology to optimize PHA production and to be able to tune the type of polymer obtained. Finally, we evidenced which PPB genera are more critical in the PHA accumulation process and how although the community evolves, its functionality remains the same. All this Information gives us tools for optimizing and controlling this technology and paves the way for future large-scale feasibility analyses.

5. Conclusions

This study demonstrates the possibility of increasing PHA production by combining different operating strategies. Firstly, the association of steam explosion pretreatment and thermophilic acidogenic fermentation leads to stable production of up to 0.74 $gCOD_{SCCA}$ $gCOD_{feed}^{-1}$ and 345 mLH_2 L^{-1} d^{-1} from OFMSW. Secondly, understanding the different metabolic pathways of carbon assimilation and consequent electron allocation during transient OLR is critical to enhancing PHA production. This work achieved a maximum PHA accumulation of 42% on a dry basis by controlling the OLR. This production is stable and resistant to severe stress caused by organic overload, and although the community within the PPB mixed culture may change due to this stress, its functionality remains consistent, and the stress is reversible after the overload. Finally, this comprehensive analysis elucidates critical mechanisms that allow the design of a PPB-based pho-biorefinery with seasonal PHA, EPS, and hydrogen production, depending on the culture

feed, contributing to urban solid waste management, and taking a step forward in the direction of a circular bioeconomy.

Author Contributions

LDA: Data curation; Formal analysis; Investigation; Software; Visualization; Writing – original draft; MV: Data curation; Investigation; Writing – review & editing; JAM: Funding acquisition; Project administration; Resources; Supervision; Validation; Writing – review & editing; DP: Conceptualization; Data curation; Formal analysis; Funding acquisition; Methodology; Project administration; Supervision; Validation; Writing – review & editing

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Juan Antonio Melero, Luis D Allegue, Daniel Puyol reports financial support was provided by Regional Government of Madrid. Daniel Puyol reports financial support was provided by Spanish Ministry of Economy.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.rser.2022.112687.

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