INTRODUCTION

The depletion of the C-fossil resources, traditionally used as the main resource to obtain energy and chemicals, has pushed the scientific community to look for alternative feedstocks. New strategies, technologies and renewable resources are being investigated to favor the shift of the society toward a bio-based and circular economy. The biorefinery concept has gained attraction in the last decade to efficiently produce energy and high value-added products from renewable feedstocks (Ubando et al., 2019) while decreasing the dependence of C-fossil resources and increase demand for bio-based commodities that replace those analogs synthesized by fossil resources. Therefore, in order to optimize their environmental and economic efficiency, robust multi-platform biorefineries must ensure a wide portfolio of products ideally from the agricultural, forest, and municipal waste using multiple conversion processes (FitzPatrick et al., 2010). One of the most promising resources for a biorefinery

Abstract

Purple phototrophic bacteria (PPB) showed an enormous potential for polyhydroxyalkanoates (PHA) production using the hydrolysate from lignocellulosic waste as feedstock. Thermal hydrolysis pretreatment was carried out at three different temperatures (120, 150, and 180°C) aiming to improve the biodegradability of the lignocellulosic waste. After this pretreatment, two streams are collected: a solid and a liquid fraction. The anaerobic digestion process of the pretreated solid fraction at 180°C increased its biodegradability extent by 31% compared to the untreated biomass, yielding 210 ± 10 L CH₄ kg⁻¹ VS even when the liquid fraction was removed. On the photoheterotrophic of the liquid fraction, PPB were able to remove up to 55% of COD during batch operation, showing peaks of PHA production yield up to 21 wt.%. The treatment of the liquid fraction obtained at high temperatures (T > 120°C) reduces biomass growth and COD consumption efficiency, and thus potential productivity, with no effect over the specific activity and the biomass yield, indicating growth limitation by lack of nutrients (N and P). A preliminary economical assessment indicates suitable economic viability based on an energetic autarchy process. This study pioneered a PPB-based biorefinery for PHA production from lignocellulosic residues and aligned within the bioeconomy strategy of the European Union.

KEYWORDS
anaerobic digestion, circular economy, photo-biorefinery, photo-fermentation, polyhydroxyalkanoates, purple phototrophic bacteria, thermal hydrolysis

1 | INTRODUCTION

The depletion of the C-fossil resources, traditionally used as the main resource to obtain energy and chemicals, has pushed the scientific community to look for alternative feedstocks. New strategies, technologies and renewable resources are being investigated to favor the shift of the society toward a bio-based and circular economy. The biorefinery concept has gained attraction in the last decade to efficiently produce energy and high value-added products from renewable feedstocks (Ubando et al., 2019) while decreasing the dependence of C-fossil resources and increase demand for bio-based commodities that replace those analogs synthesized by fossil resources. Therefore, in order to optimize their environmental and economic efficiency, robust multi-platform biorefineries must ensure a wide portfolio of products ideally from the agricultural, forest, and municipal waste using multiple conversion processes (FitzPatrick et al., 2010). One of the most promising resources for a biorefinery
is lignocellulosic biomass, since it is the most abundant natural renewable resource, with an annual worldwide production of \(200 \times 10^9\) t year\(^{-1}\) (Kumar et al., 2020), and does not compete with food production (Liguori & Faraco, 2016).

Lignocellulosic waste is traditionally processed via composting or incineration, which are low-added value pathways that additionally produce residual matter that must be landfill-filled (Kumar et al., 2020). Thereby, several alternatives have been proposed to increase the added value of this abundant biomass source, including thermochemical processes, catalytic transformations, acid and basic hydrolysis, enzymatic processes, ionic liquid-based treatments, solvent extraction, gasification, torrefaction, liquefaction, and biotechnological transformations, or a combination thereof (Kurian et al., 2013). Among them, biological processes offer a good alternative due to low operation costs, high specificity, and a wide portfolio of potential products that are not just limited to energy and simple organics production (Iqbal et al., 2013). However, biological processes usually need pretreatment stages to improve the accessibility of microorganisms to the reactive substrates.

Anaerobic digestion is a suitable technology for the conversion of diverse complex feedstocks, such as lignocellulosic biomass, into energy-rich biogas with low production costs (Sawatdeenarunat et al., 2015). However, the hydrolysis step often becomes the rate-limiting process during anaerobic digestions (Amiri & Karimi, 2018). Lignocellulosic materials form strong native hierarchical structures of recalcitrance nature yield relatively low digestibility (Kainthola et al., 2019). A pretreatment step is required to disrupt the crystallinity of lignocellulose, reduce the degree of polymerization of cellulose, and increase the accessible surface. Several pretreatments have been studied including thermal, biological, chemical, physical, or physicochemical and they should be appropriately integrated with the rest of the process to increase the potential of lignocellulosic biomass (Menon & Rao, 2012). Thermal pretreatments are known to break some structural bonds and induce hemicellulose solubilization, improving cellulose biodegradability (Sun et al., 2016). These processes also produce a liquid stream rich in soluble carbohydrates that can be further processed to produce high added value chemicals, such as polyhydroxyalkanoates (PHAs) (Chen et al., 2017).

PHAs are interesting and compostable bio-based polymers with similar properties compared to non-biodegradable plastics. These biopolymers are produced by a variety of microorganisms under unbalanced nutrition conditions, such as nitrogen and phosphorus limitation or excess of reducing power, as carbon and energy storage materials (Sudesh et al., 2000). PHA is usually produced through a two-stage aerobic process known as the feast–famine approach. PHA-accumulating bacteria are enriched in a first stage (feast stage that promotes growth), which is subsequently submitted to high organic load with nutrient limitation in a second stage that drives the PHA accumulation (famine stage) (Fernández-Dacosta et al., 2015). Most PHAs are currently produced primarily from food crops, sugar cane, and vegetable oils, being its industrial production still expensive (Rodriguez-Perez et al., 2018). In recent years, the lignocellulosic biomass wastes have been proposed as feedstock for bioplastics production through the use of the aforementioned aerobic process (Dietrich et al., 2019). However, the two-step aerobic process is costly due to the high aeration needs, and also the overload of biomass has not been considered to be recovered. Therefore, further advances and breakthroughs are needed to improve the resource recovery yield and reduce production costs.

Purple phototrophic bacteria (PPB) are potential candidates to use hydrolysates from lignocellulosic biomass. PPB use natural infrared light to drive PHA accumulation under anaerobic conditions in a single stage (permanent feast regime), which simplifies the operation of the plant and reduces the production costs (Fradinho et al., 2016). PPB can metabolize most of the organic compounds extracted from thermal decomposition of lignocellulosic residues, such as mono- and oligosaccharides, organic acids, and volatile fatty acids (Ballesteros et al., 2011; Dietrich et al., 2019; Hädicke et al., 2011; Tang et al., 2011). These compounds can be processed to produce PHA photo-anaerobically via Acetyl-CoA or Propionyl-CoA, depending on the central metabolic pathway and side branches (Amara et al., 2008; Petushkova et al., 2019). Despite the metabolic versatility of PPB, up to now, they have never been proposed for organic carbon recovery through PHA production in a lignocellulosic waste-based biorefinery.

In this work, the integrated approach for the transformation of lignocellulosic wastes into PHA and biogas within the biorefinery concept is proposed and tested in laboratory batch tests. The hydrothermal pretreatment (HTP) can create two useful streams: solid and liquid. The solid stream is used for biogas production by anaerobic digestion and the liquid fraction is driven toward PHA production by using mixed microbial cultures (MMC) of PPB. By applying such an integrated approach, biorefineries can potentially reduce PHA production costs, diversify their product portfolio, and valorize an abundant waste stream.

## 2 | MATERIALS AND METHODS

### 2.1 | Source of biowastes

Samples were collected from an urban waste treatment facility located in Madrid (Spain). The samples correspond to urban lignocellulosic waste, mostly composed of prune and gardening residues. This waste is usually collected and
submitted to incineration or landfilling, though some wet fractions can be composted to produce low-cost organic fertilizers (Madrid Consistory, 2020). The biowaste was blended and homogenized with a blade mill and screened to achieve a size of less than 10 mm particle size. As shown in Table 1, the waste has less than 10% moisture and seems to be very ligneous due to a N composition lower than 1%, which also indicates low contamination with another urban biowaste.

2.2 | Hydrothermal pretreatment

Hydrothermal pretreatments (HTP) were performed in a 1 L autoclave (Parker, Autoclave Engineers) under different temperatures: 120, 150, and 180°C stirring at 150 rpm with a solid-to-liquid ratio of 1:5 (w/w). The autoclave was heated to the target temperature with an average heating rate of 6°C min⁻¹ under autogenous pressure conditions and held for 5 min when the temperature was reached. Thereafter, the autoclave was cooled with ice until room temperature before opening the lid. In order to obtain comparably and reliably effects due to time and temperature for the hydrolysis pretreatment, HTP severity can be monitored based on the severity factor, $R_0$ (Overend & Chornet, 1987) (Equation 1), and although it is subject to some limitations, enables an easier comparison among different tested conditions.

$$R_0 = \int_0^t \exp \left( \frac{T(t) - T_{\text{Ref}}}{14.75} \right) dt,$$

where $t$ is heating time (min), $T$ is the target temperature (°C), $T_{\text{Ref}}$ is the reference temperature of 100°C, and 14.75 is based on the activation energy when assuming pseudo-first-order kinetics. Upon the hydrolysis, half of the raw slurry was conserved, and a half was centrifuged at 12,074 g for 10 min and then separated into two fractions: solid and liquid. Both samples were stored at 4°C until further use. The organic matter and nutrient solubilization extent were calculated according to previous work (Allegue et al., 2020).

| Parameters  | Raw    | TH-120°C  | TH-150°C  | TH-180°C  |
|-------------|--------|-----------|-----------|-----------|
| Slurry      |        |           |           |           |
| TS (g kg⁻¹) | 920 ± 8 | 130 ± 105 | 130 ± 10  | 116 ± 9   |
| VS (%TS)    | 94 ± 1 | 97.1 ± 0.2| 96.9 ± 0.3| 97.3 ± 0.4|
| COD (g kg⁻¹ TS) | 1120 ± 40 | 1060 ± 20 | 1070 ± 20 | 1050 ± 20 |
| Carbon (%)   | 47.2 ± 0.1 | 48 ± 1    | 48 ± 2    | 49.6 ± 0.6|
| Nitrogen (%) | 0.8 ± 0.2 | 0.8 ± 0.2 | 0.9 ± 0.2 | 1.1 ± 0.1 |
| Hydrogen (%) | 5.9 ± 0.4 | 5.8 ± 0.1 | 5.8 ± 0.2 | 5.8 ± 0.1 |
| Oxygen (%)   | 45.9   | 45.3      | 45.1      | 43.5      |

| Liquid fraction |        |           |           |           |
| SCOD (g L⁻¹)    | 19.6 ± 0.9 | 22 ± 1    | 45 ± 2    |
| Monosaccharides (%) | 2.96   | 2.74      | 1.23      |
| NVFA (%)        | 0.26   | 1.43      | 1.75      |
| VFA (%)         | 0.89   | 1.61      | 1.66      |
| Oligosaccharides, alcohols, and proteins (%) | 95.90 | 94.22 | 95.35 |
| TKN (g N L⁻¹)   | 1.2 ± 0.1 | 1.2 ± 0.2 | 1.4 ± 0.1 |
| TOC (g L⁻¹)     | 11.3 ± 1.2 | 12.8 ± 2.2 | 26.2 ± 1.8|
| TIC (g L⁻¹)     | 0.4 ± 0.1 | 0.5 ± 0.2 | 1.1 ± 0.4 |
| NH₄⁺ (mg N L⁻¹) | 610 ± 10  | 710 ± 10  | 930 ± 20  |
| PO₄³⁻ (mg P L⁻¹) | 100 ± 10  | 122 ± 8   | 140 ± 10  |
| pH              | 5.8     | 5.2       | 4.8       |

*TCOD/SCOD: total/soluble; NH₄⁺: ammonium; PO₄³⁻: phosphates.

b% of oxygen was calculated from the difference of C, H, and N since the proportion of S was negligible compared to the rest of the major elements.

Percentage calculated based on SCOD. The percentage of oligosaccharides, alcohols, and proteins was calculated form the difference of VFA, NVFA, and monosaccharides.
2.3 Anaerobic digestion for biogas production

The HTP effect on the digestibility and methane yield on both the raw slurry and the isolated solid fraction was examined through standard biochemical methane potential (BMP) tests. BMP tests were conducted in a series of 160 ml serum bottles (working volume was adjusted to 100 ml by milli-Q water), performed by triplicate at mesophilic conditions (37 ± 0.5°C) following Angelidaki et al. (2009). Biogas volume was corrected to STP conditions and production was calculated as ml CH₄ g⁻¹ VS. A control test of the untreated lignocellulosic waste was analyzed for comparison with the same solid-to-liquid ratio. The anaerobic inoculum was collected from a mesophilic anaerobic digester from a WWTP located at Mostoles, Madrid (Spain). An inoculum to substrate (I/S) ratio of 2:1 and an initial inoculum concentration of 10 g VS L⁻¹ were set up. A triplicate control of the inoculum was used to subtract methane production coming from its endogenous digestion. Kinetic parameters were obtained by fitting first-order models to the data following Segura et al. (2016), thereby calculating the first-order hydrolysis constant k_H (day⁻¹) and the biodegradability extent, B_0 (ml CH₄ g⁻¹ VS). Parameter uncertainty surface was obtained as described in Batstone et al. (2003), and confidence intervals (at 95%) were also calculated based on two-tailed t-tests from parameter standard error for statistical representative comparison. All statistical analyses were performed using Matlab.

2.4 Photoheterotrophic polyhydroxyalkanoate production

The activity of the phototrophic biomass on the liquid phase resulted from the HTP was determined by specific phototrophic activity (SPA) batch tests following the indications in Puyol et al. (2017). An active mixed culture of PPBs was used as inoculum for the experiments. The phototrophic biomass was collected from the photo-an aerobic membrane bioreactor (PAnMBR) described elsewhere (de las Heras et al., 2020). The liquid phase from HTP was used as a substrate fixing a concentration of 1 g COD L⁻¹. More details are shown in the supplementary material. The specific substrate rate, k_M (g soluble COD (SCOD) g⁻¹ VSS h⁻¹), and the biomass yield, Y_XS (g VSS g⁻¹ SCOD), were estimated dynamically using Aquasim 2.1d as specified in Puyol et al. (2017). Confidence intervals (at 95%) were calculated based on a two-tailed t-test from parameter standard error and used for statistical representative comparisons. After the photo-heterotrophic step, biomass samples were treated to measure PHA content, thereby calculating the PHA production yield (Y_PHA, expressed as wt.% on dry basis). The biomass samples for PHA measurements were extracted when the cultures had consumed around 80% of the soluble COD, and upon reaching the stationary stage. It has been previously described that PHA accumulation is a strategy for redox balancing and is a process that is linked to substrate assimilation. Thereby, it is associated with growth. A maximum of PHA accumulation is therefore expected before reaching the stationary stage (Bayon-Vicente, Wattiez, et al., 2020; Bayon-Vicente, Zarbo, et al., 2020; Fradinho et al., 2013). A more extensive and comprehensive description of the process can be found in the supplementary material.

2.5 Analytical methods

Analytical determination of pH, total and volatile solids (TS, VS), total and volatile suspended solids (TSS, VSS), TKN and COD of the solid and liquid fraction was carried out following Standard Methods for the Examination of Water and Wastewater. NH₄⁺, PO₄³⁻, and total phosphorus (TP) were measured with Merck kits (Merck). Elemental analysis (C, H, N, and S) of the solid fraction (including raw waste material) was performed by an elemental analyzer (Vario EL III, Elemental Analysis System GMHB). Volatile fatty acids (VFA), non-volatile fatty acids (NVFA), and monosaccharides were analyzed using an ion-exclusion Razex™ ROA-Organic Acid H⁺ HPLC column (Phenomenex), coupled to a refractive index detector (Agilent) and operated at 65°C and 1 ml min⁻¹, with 0.005 M H₂SO₄ as the eluent. Liquid samples were filtered through a cellulose-ester filter of 0.45 μm of pore size prior to the analysis (Advantech).

Gas pressure in BMP and SPA tests was measured using a Boyle-Mariotte apparatus, and biogas composition was quantified by gas chromatography coupled to a thermal conductivity detector, as reported in Segura et al. (2016), thereby calculating the CH₄ production. Quantification of the PHA content was performed on the fixed PPB samples, according to previous work (Allegue et al., 2020).

The biowaste samples were dried at 100°C in an oven, before mid-infrared spectroscopic (FTIR) and X-ray diffraction (XRD) tests. For XRD, samples as flat as possible were correctly distributed on a glass holder that does not produce diffraction effects. A X'Pert PRO diffractometer (Malvern Panalytical) with θ/2θ geometry was used, using Cu-Kα radiation. The data were collected from 5 to 90° (2θ) with a resolution of 0.02°.

The crystallinity index was calculated as shown in Equation (2).

\[
CrI = \frac{I_{002} - I_{amorphous}}{I_{002}} \times 100.
\]
where CrI is the crystallinity index, $I_{002}$ is the maximum intensity of the 002 peak at $2\theta = 22.5^\circ$, and $I_{amorphous}$ is the intensity at $2\theta = 18.7^\circ$.

The FTIR spectra were recorded between 400 and 4000 cm$^{-1}$ using a Spectrum 100 system (Perkin Elmer). Samples were prepared by grinding the solid fractions on an agate mortar and pressed at 10 MPa.

### 2.6 Energy assessment

An energy evaluation was performed to check the scalability of the proposed biorefinery. The energy balance of the process was assessed to ensure the sustainability and economic viability of the process (Avellar & Glasser, 1998). Energy balances have been carried out simulating a commercial Cambi® CHP plant and following Allegue et al. (2020). Energy integration and parameters chosen have been previously described in Scenario two in Cano et al. (2014). A calculation basis of 1 ton of feedstock (20% TS) was chosen as a reference. A more extensive overview of the assessment and all parameters is presented in the Supplementary Material.

### 3 RESULTS

#### 3.1 Hydrothermal pretreatment

Table 1 shows the main characteristics of the raw waste and the slurry and liquid fractions upon the HTP. Thermal conditions at 120, 150, and 180°C including the temperature gradient correspond to a severity factor ($log R_0$) of 2.1, 3.1, and 3.9, respectively. The elemental analysis carried out before and after the pretreatment shows that there are almost no variations in the percentage of carbon, nitrogen, or hydrogen. This means that the HTP process did not entail the loss of matter by an oxidative process.

To study the changes in greater depth over the solid fraction, XRD and FITR analyses were performed. XRD experiments were mainly performed to determine the crystallinity indexes of the samples before and after HTP (see Figure S1). Raw lignocellulosic waste had a CrI of 68.6%, which increased up to 73.5%, 76.8%, and 76.7% upon the HTP at 120, 150, and 180°C, respectively. The FTIR measurements were also used to characterize the solid fraction after HTP. The structural changes of hemicelluloses, cellulose, and lignin after the thermal treatment can be observed in the FTIR spectra. The effect of HTP on the change of functional groups of the biomass studied can be accessed in Supplementary Material (see Figure S2).

The increase of the severity factor of the HTP also affected the composition and characteristics of the liquid hydrolysate. The solubilization of COD accounts for 9%, 14%, and 24% for 120, 150, and 180°C HTP, respectively. This means a 69% increment in COD solubilization for the 180°C HTP compared to the 120°C one. A similar trend happens with the destruction of solids, yielding 13%, 19%, and 29% destruction efficiencies for 120, 150, and 180°C HTP, respectively. As shown in Table 1, the pH of the hydrolysate decreased significantly with increasing HTP temperature, due to the presence of VFAs such as acetic and propionic acids that are usually formed during HTP pretreatment of wood biomass (Wang et al., 2018). VFAs concentrations range from 0.89% to 1.66% of the SCOD, and up to 570 ± 60 mg L$^{-1}$ of acetic acid were achieved on the 180°C HTP, an increase of 338% from the 130 ± 20 mg L$^{-1}$ at 120°C HTP. Also, a small quantity of propionic acid is formed from 150°C THP. NVFAs make up 0.26%–1.75% of the SCOD (mainly succinic and oxalic acid) while monosaccharides ranged from 2.96% to 1.23%. Values strongly suggest the predominance (about 95% of SCOD) of oligosaccharides, proteins, and alcohols in the liquid phase. Indeed, the liquid fraction obtained after centrifugation had variation in color according to the HTP temperature and turned darker at high HTP pretreatments likely due to the solubilization of sugars and their subsequent caramelization.

#### 3.2 Biochemical methane potential tests

Results from BMP tests over the isolated solid fractions after HTP and the raw waste showed an increase in the cumulative methane yield as HTP temperature increases (Figure 1). The HTP at 180°C resulted in the highest cumulative methane yield, representing an increase in methane production of 27% compared to the raw sample ($145 ± 14$ L CH$_4$ g$^{-1}$ VS$_{a}$). This increment is noteworthy despite the removal of liquid fraction, which may reduce the sample’s methane potential significantly. Indeed, the anaerobic digestion of raw slurry yielded up to 53% higher methane potential than the solid fraction (see Figure S3), indicating that the removal of the liquid fraction decreased significantly the methane potential. Thereby, the increase of the BMP due to the HTP is counteracted with the removal of the liquid fraction. This fact has important implications on the global energy balance of the proposed biorefinery concept, as will be furtherly analyzed.

The experimental results from BMP tests have been fitted to a simple nonlinear first-order model following Batstone et al. (2003). The 95% confidence surfaces for the hydrolysis constant ($k_H$) and the biodegradability extent ($B_0$) were calculated. Results are presented by using the real feedstock added as a basis (kg VS added), thereby the direct (non-relative) kinetic parameters are representative of the anaerobic digestion process. Estimated values generally predicted accurately the experimental data and confirm the positive relation of the HTP temperature with
the increase of the biogas production. As an example, the $B_0$ of the experiment using the solid fraction upon HTP at 180°C is 210 ± 10 ml CH$_4$ kg$^{-1}$ VS, which represents an improvement of 31% with respect to the value estimated for the raw waste (146 ± 10 ml CH$_4$ kg$^{-1}$ VS).

The estimated value for the $k_H$ of the untreated sample was 0.060 ± 0.003 day$^{-1}$, while treated samples reached 0.078 ± 0.002, 0.113 ± 0.004, and 0.057 ± 0.005 day$^{-1}$ for 120, 150, and 180°C HTP, respectively. This means that the HTP increased the process rate at 150°C but maintained at 180°C despite the increment of the BMP. This trend is even more clear observing the results when using the slurry (both solid and liquid fraction) as feedstock (see Figure S3): in the 180°C HTP experiment, the $B_0$ increased by 128% (320 ± 10 ml CH$_4$ kg$^{-1}$ VS) but $k_H$ decreased by 33% (0.045 ± 0.002 day$^{-1}$) with respect to the untreated biowaste.

### 3.3 Specific phototrophic activity tests

Figure 2 shows the time course of the SCOD consumption and biomass concentration in the SPA tests. A control test under Ormerod media and acetate as organic carbon served as a reference for the maximum theoretical SPA value. Active biomass assimilated 95% of the SCOD in the control test, which was reduced to 55% and 48% in the 120 and 150°C HTP test, respectively. This caused an increase of the biomass concentration at the end of the test up to 1046 ± 30 mg VSS L$^{-1}$ in
the control test and 649 ± 20 and 640 ± 30 mg VSS L⁻¹ in the 120 and 150°C HTP. A considerable reduction occurred for the 180°C HTP, where just 37% of SCOD was consumed and biomass concentration reached 490 ± 30 mg VSS L⁻¹. In parallel, close to 100% of the main nutrients (N and P) were consumed, likely due to their low concentration compared to the organic carbon concentration (see Figure S4). Therefore, the limitation of nutrients that leads to the accumulation of PHA can be due to both N and P. Some biohydrogen was produced on the experimental tests, which accounted for 8, 10, and 17 mg COD L⁻¹ at the end of the 120, 150, and 180°C HTP tests, respectively. Biohydrogen production was coincident with ammonium depletion, and the control experiment did not produce biohydrogen due to an excess of nutrients. The highest COD/N ratio found at the 180°C HTP test was coincident with the lowest SCOD consumption and the highest biohydrogen production. This indicates that the nutrients limitation was the main cause of the lower COD consumption efficiency during the phototrophic tests, which was further analyzed through model-based analysis.

The kinetic parameters of the tests were determined (Figure 3). The HTP temperature caused a negligible effect on both the specific substrate rate, $k_M$, and the biomass yield ($Y_{X/S}$). The estimated $k_M$ of the control was significantly higher (at 95% level) than those from the experimental tests, but the values for the experimental tests were not significantly different from each other. This is likely due to a higher biodegradability of the synthetic media in comparison with the complex hydrolysate. In any case, $k_M$ values for the experimental tests averaged 0.078 g SCOD g⁻¹ VSS h⁻¹. The differences between the biomass yields calculated for the experiments at the three temperatures were also not statistically relevant (95% confidence level), averaging 0.74 ± 0.02 g VSS g⁻¹ SCOD, which indicates that the solubilization of organic compounds does not affect the biomass yield. Taking these results, it can be concluded that the hydrolysate is highly biodegradable by the mixed culture of PPB. As the cultures were not previously acclimated to these effluents, these data strongly support the viability of the proposed photobiorefinery. Subsequently, a detailed analysis of the PHA accumulation has been conducted.

The PHA production yield ($Y_{PHA}$) was measured midpoint, 30 h after the experiment began, when the SCOD consumption in the experiments was around 80%, and also at the end of the experiments. The only type of PHA detected was poly-3-hydroxybutyrate (PHB). Although the presence of propionic acid has been related to the production of the copolymer (PHV), its concentration in the experiment after the substrate dilution to 1 g COD L⁻¹ was negligible. Data show that PHA production is slightly dependent on the thermal hydrolysis temperatures. In the measure taken at 30 h after the experiment began, the $Y_{PHA}$ values averaged 19%, 17%, and 15% for the 120, 150, and 180°C HTPs, respectively. These results are, to the best of our knowledge, the first instance to report the production of PHA in waste-lignocellulosic hydrolysates using PPB.

4 | DISCUSSION

4.1 | Hydrothermal pretreatment

Severity factors were contrasted with literature values. Previous studies have shown the optimal severity for maximum sugar yield between 3.0 and 4.5 (Silva-Fernandes et al., 2015). In lignocellulosic biomass, the combination of cellulose, hemicellulose, and lignin makes it highly recalcitrant and hinders the accessibility of hydrolytic enzymes to cellulosic components (Duque et al., 2016). The extension of hemicellulose solubilization is directly proportional to the severity factor, while cellulose and lignin are usually retained. Xylan and arabinan-based sugars have the highest solubilization on a severity factor of 4 (Carvalheiro et al., 2009).

The crystallinity indexes may indirectly indicate the amorphous phase composition of the biomass related to hemicellulose, cellulose, and lignin domains (see Table S1). The cellulose in lignocellulosic biomass is composed of crystalline and amorphous structures, which have a great influence on the enzymatic hydrolysis (O’Dwyer et al., 2007). This influence also anaerobic digestion, as it uses an analogous enzymatic mechanism, which is derived from the extracellular hydrolysis caused by the release of enzymes by hydrolytic bacteria (Azman et al., 2015). XRD studies on pretreated and untreated samples to analyze crystallinity through crystallinity index (CrI) were assessed, and the comparison of XRD spectra can be seen in
the supplementary material (Figure S1). The composition of the biomass influences the CrI since hemicellulose and lignin are amorphous while cellulose is crystalline (Jeoh et al., 2007). The increase in CrI was caused by the removal of amorphous substances in the biomass, mostly hemicellulose, which exposed the crystalline cellulose core and increased the glucan content in the pretreated solid fraction. Increased CrI values after biomass pretreatment have been observed in previous studies (Zhang et al., 2020). As crystallinity of cellulose is incremented by the hemicellulose removal, an increase in the xylose removal, from amorphous or crystalline hemicellulose is observed (Evans et al., 1995).

The FTIR spectra showed that the wave number at 3252 cm$^{-1}$ shifted to 3759 cm$^{-1}$ with the HTP, which indicated the change in the characteristic of crystalline cellulose. The change in the signal was mainly due to less water and hydrogen bonding in the treated biomass (Li et al., 2015). The aromatic ring vibrations of C=C (between 1600 and 1545 cm$^{-1}$) changed as temperature increased, which meant the lignin of the biomass was affected (Wen et al., 2020). There was no lignin destruction, as no signal is associated with a single compound as guaiacyl, but the intensity of the signal at 1545 cm$^{-1}$ increases. Besides, a clear peak at 1877 cm$^{-1}$ was found in the treated biomass, being more intense as temperature increases. This is related to the acetyl groups of hemicelluloses or the ester linkages of ferulic and p-coumaric acids with lignin (Li et al., 2015). The results observed in the FTIR measurements show clear changes in the crystallinity of cellulose, which agrees with the XRD analyses, partial solubilization of hemicellulose, and slight changes in the structure of lignin, as it was expected. These conclusions are correlated to the severity factor of the HTP: the greater the severity factor the clearer the three conclusions: more changes in the crystallinity, solubilization of hemicellulose, and changes in lignin structure.

Regarding the solubilization of the organic matter, previous works agree with our results and consistently showed that the liquid fraction resulting from the HTP of lignocellulosic wastes similar to the feedstock used in the present paper is mainly composed of oligosaccharides (higher than 95% on mass basis). Xylose and glucose, followed by galactose, were found as the major components for severity factors similar to the reported in the present paper, at a temperature of 180°C. In these conditions, the resulting VFAs concentrations were found as the major components for severity factors similar to the reported in the present paper, at a temperature of 180°C. This reduction fits with the formation of inhibitory furan components, which appear at HTP temperatures close to 180°C (severity factor around 4) (Steinbach et al., 2019). Indeed, after removing the liquid fraction, $B_0$ decreases but $k_H$ increases, which clearly points toward the solubilization of a large portion of the organic fraction, but also the presence of potentially inhibitory organics. The increase of the HTP severity may amplify the formation of these compounds, making the organic matter recalcitrant and less biodegradable. Thereby, HTP at temperatures higher than 180°C are not recommended for this biorefinery platform.

### 4.2 Anaerobic digestion of the solid fractions

First of all, it is important to clarify that after solid–liquid separation by centrifugation, the solid fraction was not washed. The effect of thermal pretreatment on the solid fraction of lignocellulosic residues has been extensively studied, for example, in Buitrón et al. (2019), and it is not the scope of this study that attempts to represent results more close to the industrial scale. For that reason, the effect of the pretreatment over the biodegradability of the solid phase alone has been ignored in this work. The raw material produces an important amount of methane. The physical pretreatments of the solid waste (which reduced the particle size below 10 mm) may increase the biodegradability of the feedstock by reducing the crystallinity and making more accessible the residue to the attack of the hydrolytic bacteria (Hendriks & Zeeman, 2009), and thereby the anaerobic consortia are able to produce methane from the raw feedstock.

A reduction of the hydrolytic activity has been reported for a temperature of 180°C. This reduction fits with the formation of inhibitory furan components, which appear at HTP temperatures close to 180°C (severity factor around 4) (Steinbach et al., 2019). Indeed, after removing the liquid fraction, $B_0$ decreases but $k_H$ increases, which clearly points toward the solubilization of a large portion of the organic fraction, but also the presence of potentially inhibitory organics. The increase of the HTP severity may amplify the formation of these compounds, making the organic matter recalcitrant and less biodegradable. Thereby, HTP at temperatures higher than 180°C are not recommended for this biorefinery platform.

### 4.3 Conversion of the liquid fraction of the hydrolysate by purple phototrophic bacteria

In general, the waste composition fits very well with the aim of PHA production by PPB. The main driver for PHA production is the scarcity of nutrients, especially N and P, which limits bacterial growth, pushing PPB to accumulate carbon excess in form of PHA (Monroy & Buitrón, 2020). As shown in Table 1, the raw lignocellulosic waste contains a low N proportion, resulting in a COD/N ratio of 100/0.71. Upon HTP, the liquid fraction resulted in COD/N/P ratios (considering SCOD, N as NH$_4^+$, and P as PO$_4^{3-}$) of 100/3.1/0.5, 100/3.2/0.5, and 100/2.1/0.31 for HTP temperatures of 120, 150, and 180°C, respectively. The increase of the N proportion in the liquid phase with respect to the raw waste indicated that proteins have been solubilized preferentially during the HTP. While the temperature is rising, more carbohydrates are being solubilized, resulting in higher COD/N ratios. As will be shown later, this had strong effects
on the photoheterotrophic growth. The optimum physiological COD/N/P ratio for mixed cultures of PPB is 100/7.2/1.8 (Puyol et al., 2017), though average values in literature are around 100/5/1 (Capson-Tojo et al., 2020). Increasing these values entails a preferential usage of COD for PHA accumulation, as the medium lacks key nutrients to allow for bacterial growth.

The kinetic parameters of the photoheterotrophic process have been compared with the literature values. The $k_m$ values reported (averaging 0.078 g SCOD g$^{-1}$ VSS h$^{-1}$) are in the same range as the values reported in the literature for simple organics (Puyol et al., 2017). In contrast, the biomass yield values are generally higher than other reported in the literature (Capson-Tojo et al., 2020). This is attributed to the partial release of COD in form of hydrogen (as commented previously) and a lower COD/VSS ratio of the biomass produced in this work. The accumulation of PHA had an important role here, as PHA ([C$_4$H$_6$O$_2$]$_n$) is more oxidized than PPB biomass (CH$_{1.8}$O$_{0.38}$N$_{0.18}$, Puyol et al., 2017) (1.62 g COD g$^{-1}$ VSS vs. 1.75 g COD g$^{-1}$ VSS, respectively).

The PHA yields are significantly higher than those found in the literature (5%–6.3%) when using PPBs MMCs with waste substrates (Monroy & Buitrón, 2020). This is remarkable taking into account that the inoculum is not adapted to this complex media, which contains a very low amount of VFAs. But it is still far from the 30%–40% achieved when PPBs MMCs have been used with synthetic substrates such as acetate (Fradinho et al., 2019), and up to 70% using acetate and pure cultures (Brandl et al., 1991). Lower $Y_{PHA}$ on the control tests was obtained (10%), which responds to the excess of nutrients in the synthetic medium, as PHA production is enhanced when growth is limited (Brandl et al., 1991). On the measurement taken at the end of the experiment, on the stationary phase, a slightly reduced $Y_{PHA}$ was noticed: 16%, 16%, and 14%, which may be related to the consumption of PHA for maintaining the redox balance, as has been recently suggested (Bayon-Vicente, Zarbo, et al., 2020). Therefore, the analysis of the mechanisms of PHA accumulation is critical to elucidate the process performance.

Most of the studies published on MMC agree that acetate and other VFAs are the predominant carbon source for PHA production (Lee et al., 2014; Reis et al., 2003). However, the presence of these volatile fatty acids in our liquid fractions was low (1.66% of the SCOD or up to 570 mg L$^{-1}$ of acetic acid and 43 mg L$^{-1}$ for 180°C HTP); therefore, most of the organic carbon must be derived from the solubilization of the lignocellulosic material (hemicellulose) into oligosaccharides majorly containing xylose, glucose, and arabinose, as previously discussed. Oligosaccharides can be catabolized by three main pathways to PHB: the Embden–Meyerhof–Parnas (EMP), the Entner–Doudoroff (ED), and the pentose phosphate shunt (PPS) (Jeffries, 1983). Previous studies have verified symbiotic interaction between species of xylan fermentative bacteria (which contains xylanase) and phototrophic bacteria (Hongyuan et al., 2016), where two main mechanisms may occur. First, fermentative bacteria can produce the xylan hydrolysis, followed by fermentation of simple sugars to VFA (Dziga & Jagiełło-Flasinska, 2015), and the consequent accumulation of PHA by PPB. A second mechanism may be simpler, as PPB can degrade glucose and arabinose (through the EMP pathway), and has also been shown to cause direct degradation of xylose, in fact, the genome of some species indicates the presence of xylose ABC transporter, which suggests that these bacteria actively degrade it via PPS pathway (Pattanamanee et al., 2012). Therefore, xylan hydrolysis by fermentative bacteria can occur, followed by the PPS of the resulting xylose by PPB bacteria, which internally derive the products of xylose degradation to the EMP, and hence, PHA accumulates from acetyl-CoA. The excess of electrons from this process can be assimilated by PPB through the Calvin–Benson–Batham cycle, where this pathway works as an electron sink for attaining redox homeostasis in PHB (McKinlay & Harwood, 2010). This idea agrees with the type of PHA found in all the experiments, which was PHB as reported previously. Future studies on the mechanism of xylan degradation in the photoheterotrophic process by PPB and PHA accumulation are encouraged but are beyond the scope of this study.

### 4.4 Energy assessment

A theoretical energy balance of a full-scale CHP plant was estimated using the experimental data from the batch HTP and BMP tests and other parameters from the literature, as specified in the supplementary material. Thermal pretreatments require a large amount of thermal energy to be carried out, and hence, it is one of the bottlenecks of the process to be economically feasible (Avellar & Glasser, 1998). As shown in Table 2, positive thermal and electrical balances were achieved on all HTP temperatures, confirming the energetic viability of the proposed process. Besides, the electrical balance of the process has been calculated, and the remaining electricity can be sold to the electrical market. One of the determining parameters of a THP process is the boiler efficiency of wet biomass that strongly depends on moisture content, we used 20% TS, but it should be thoroughly studied when scaling up the process and can be adapted for better integration of the process.

### 4.5 Prospects and industrial implications

PHAs have gained much attention both in research and industry, but its production cost is still its greatest disadvantage. Its production price in 2018 was 3.1 € kg$^{-1}$ (Aramvash
et al., 2018), which is still three times more expensive than fossil-based plastics polyethylene and polypropylene were around 1.05–1.15 € kg⁻¹ (S&P Global Platts, 2020). But the approach of the European Commission toward a circular economy strategy and the recommended use of biodegradable plastics through the EU Commission directive 2018/0172 and even more the recent adoption of the European Green Deal, open the door to new investments and research in this field, especially in Europe.

To improve the PHA production process, industrial producers are currently working toward decreasing the cost price of these biopolymers by increasing the volumetric production capacity of the fermentation systems and improving the process technology. We believe that the combination of lignocellulosic wastes hydrolysates and PPB can help to achieve this goal. Lignocellulosic biomass has been projected as an abundant and promising alternative to replace crude oil, while PPBs have several advantages over aerobic fermentation microorganisms, which are currently being used in commercial PHA. The use of PPBs eliminates the need for aeration, in addition to its effective enrichment through illumination with IR light in a single reactor. Furthermore, the theoretical maximum PHA production capacity achieved by PPBs is 0.9 mol PHA mol Acetate⁻¹ (Fradinho et al., 2019) vastly higher than any aerobic processes (Bengtsson et al., 2010), which could potentially increase the volumetric production of PHA.

The illumination requirement might be one of the biggest drawbacks when analyzing a photo-fermentation process using PPBs. According to recently analyzed data, the illumination costs to produce PPB are 1.68 € kgbiomass⁻¹ (Capson-Tojo et al., 2020), which would make the process economically unfeasible. Several strategies could be carried out in scaling-up the proposed process to reduce illumination costs. The production of PHA has been studied through light/dark cycles, obtaining good results (Fradinho et al., 2013). Thereby, the use of raceway reactors that take advantage of using natural light for PHA production may entail a cost reduction of 40%. Currently, this option is under investigation at a semi-industrial scale within the first photobiorefinery in Europe, constructed in the framework of the BBI-H2020 Deep Purple project focused on the extraction and recovery of high-value-added resources with PPB (https://deep-purple.eu/). However, it is possible that PHA productivity would be reduced as well, as the reactor would be in dark mode half of the operative time. If artificial illumination is used, membrane bioreactors (MBRs) with cell retention systems could circumvent the problem of low biomass production. MBRs have been extensively applied in wastewater treatment with PPBs (Hülser et al., 2016), but little research has been performed on the frame of PHA production. Another advantage of using this type of reactor is the possibility of the co-production of biohydrogen, which is not possible in open raceways. In any case, recent results have enlarged the economic capabilities of PPB-based technologies, as the minimum irradiation needed for wastewater treatment has been demonstrated to be much lower than suggested before, at around 1.4 W m⁻², corresponding to 0.33 kWh m⁻³ or 3.96 kWh m⁻³ day⁻¹ (Dalaei et al., 2020). Thereby, a more detailed analysis of the capabilities of natural-irradiated PPB technologies is needed to allow for the up-scaling of the process, which is currently ongoing.

In our opinion, economic viability can only be achieved through an integrated biorefinery process, where all process streams are valorized. The anaerobic digestion of the solid fraction is a good complement to achieve a self-sustained process, but the high C/N ratio of the lignocellulosic biomass is not suitable for a continuous process. Nutrients must be added either through the supplement of a synthetic mixture of nutrients or by mixing with another high nutrient content waste (e.g., domestic sewage or the organic fraction of municipal solid waste) in a co-digestion process. This will not only improve the continuous process but may favor the possibility of using the remaining digestate as an organic fertilizer.

Another possibility to increase the sustainability of the concept relies on the reuse of the remnant COD caused by its partial consumption during photoheterotrophic treatment. Lack of nutrients caused an important excess of the soluble substrate at the end of the photo-bioprocess. Thereby, an increase of nutrients would theoretically improve the biomass productivity of the process due to the consumption of the excess of SCOD. This in turn can affect everything related to the biomass, including PHA productivity. The co-substrate fed to the anaerobic digestion can also provide nutrients when

| Substrate | Total energy biogas (kWh t⁻¹) | Thermal output (kWh t⁻¹) | Electrical output (kWh t⁻¹) | Thermal energy required (kWh t⁻¹) | Electrical balance (kWh t⁻¹) | Thermal energy balance (kWh t⁻¹) | Electric output Euro (t⁻¹) |
|-----------|-------------------------------|--------------------------|-----------------------------|---------------------------------|-----------------------------|---------------------------------|-----------------------------|
| Raw       | 1343                          | 739                      | 443                         | 428                             | 739                         | 64                              |
| TH-120°C  | 1343                          | 739                      | 443                         | 647                             | 408                         | 92                              |
| TH-150°C  | 1398                          | 769                      | 461                         | 743                             | 426                         | 26                              |
| TH-180°C  | 1693                          | 931                      | 559                         | 882                             | 524                         | 49                              |

T A B L E  2 Energy integration balance. Results were simulated for a combined heat and power (CHP) system for electricity and thermal energy production. The thermal integration results in the recovery of heat from the exhaust gases from a CHP system (a waste stream) to produce the steam for thermal hydrolysis.
these are released into the digestate and recirculated into the photobioreactor. Another option to consider is to derive the excess of soluble organic matter for anaerobic digestion since it is a way to ensure the closure of the carbon cycle, important within a circular economy. As commented before, this liquid fraction increases the methane production, favoring the viability of the proposed integrated process. All these options must be studied to check their effect on the operational strategy and the capital costs. This biorefinery concept is taking its first steps and there are still big gaps in the knowledge for the scalability of the process. Nevertheless, this is also an opportunity for further research and optimization.

5 | CONCLUSIONS

This study shows for the first time the possibility of using the hydrolysate coming from the thermal hydrolysis of lignocellulosic residues as a substrate to feed PPB for the production of PHA (a peak of 20 wt.% of PHA after a 120°C HTP has been achieved). High HTP temperatures improve organic matter solubilization as well as digestibility of solids but also limit PPB growth due to low nutrients release. These can be sourced externally to enhance the productivity of the concept. The anaerobic digestion of the solid fraction complements the process by producing biogas that serves to achieve energetic autarchy. The proposed PPB integrated biorefinery concept shown in this work offers potential and several alternatives for the reduction of PHA production costs, inviting for future research.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section.

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